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LATEXES FROM *EUPHORBIA CADUCIFOLIA* - ISOLATION AND CHARACTERISATION OF RUBBER HYDROCARBON. *PART - I*

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Latex was collected by incising the stem of *E.caducifolia* plant which contains 26.90 - 28.60% solid material. The chemical composition of solid material shows resinous mass 68.30 - 72.70%, where as rubber hydrocarbon is around 6.20 - 7.60 and inorganic matters lie in the range 19.00 - 22.90% and 1.50 - 1.90%, respectively. The rubber hydrocarbon was characterised by different chemical and instrumental methods. Iodine value of hydrocarbon (310.91 - 350.80%) percentage of unsaturation (83.40 - 94.10%), elemental analysis (C = 87.48 - 88.04%, H = 11.20 - 11.82%), solubility, IR spectrum (840 cm^{-1}), ^{1}H-NMR spectrum (olefinic proton at 5.15 - 5.20%), ^{13}C-NMR spectrum (olefinic region 120 - 140 ppm), molecular weight (15275 - 88405), the value of T_g (- 63.02 - 60.81°C), refractive index (1.49200 to 1.49325) and specific gravity (0.93102 to 0.93628) identify rubber hydrocarbon as polyisoprene. The material is sticky and poor in strength and burning gives a smoky flame.

Key words: Latex, *Euphorbia caducifolia*, Hydrocarbon, ^{13}C-NMR spectrum.

Introduction

The chief source of natural rubber is the milky latex obtained by secretion from the bark of certain type of plants like *Hevea*, *Manihot giaziievii*, *Castillea elastica*, *Ficus elastica*, *Landolphia*, *Euphorbia* etc. One of the plant *Euphorbia* has been found in the lower region of Sindh, Pakistan. It is a widely grown plant abounding in a milky latex, a source of rubber. Until the synthetic material became reality, rubber had been obtained only from such plants/trees.

Euphorbia is a tropical and warm temperature genus of the world mainly grown in dry places and resemble with *Cacti* in appearance but are distinguished from others by the presence of milky latex. About 68 species, including few exotics are found in India (Sastri 1953). Giner *et al* (2000) have studied nonpolar components in the latex of *Euphorbia peplus*. In Pakistan, a large number of latex producing species occur but potentially tappable are *Euphorbia neriifolia* Linn, *Euphorbia nivulia* Bush Ham, *E. royleane* Hosia, *E. caducifolia*, *E. antiguorum* etc. But *E. caducifolia* rather is the only wild tree among the tall shrubby species of this genus in southern Pakistan. Some biological activity of latex of *Euphorbia sp* were also studied (de Vasconcellos and de Amorim 2003) It is a tall, armed, dense and stout bush with thick cylindrical leafless green branches. Leaves, when present are ovate or broadly elliptic. *E.caducifolia* grows abundantly. On rocky and sandy grounds from Karachi to Jungshahi and Hyderabad. Coagulated latex of this plant contains terpene, resins, rubber hydrocarbon and proteins alongwith inorganic matter.

The present work deals with the isolation and characterisation of rubber hydrocarbon from *E. caducifolia* latex.

Experimental

The latex of the plant was collected by incising the stem of the plant in sterilized bottles already containing preservative like formaline solution ammonia or acetone (Mc Gavack 1959). The preservation made by first two methods did not show any deterioration even after month whilst in acetone coagulation takes place within 2-3 days. Ammonia was finally chosen for preservation of latex. It makes the system alkaline which prevents putrefaction. Ammonia not only makes the system alkaline but also hydrolyzes the fatty acids esters, naturally occuring in the latex and form soaps. These soaps act as stabilizer.

The latexes were first strained to remove dirt, diluted with water (20%) and then coagulated with 2% formic acid. After filteration, the coagulated material was vaccum dried. It was first soxhelated with acetone for 14 to 15 h to remove resin contents of the latex and the soxhelated with hexane for 13 h to separate rubber hydrocarbons. Residual portions were estimated for proteins and inorganic material. Solid contents of the latexes were determined by heating at 105°C to a constant weight. Results of various estimates are shown in respective Tables.

*Author for correspondence

Table 1
Composition of various constituents of latexes obtained from *Euphorbia caducifolia*

Sample	pH	Coagd mass	Solid content	Compostion of coagulated mass			
				Resin	Rubber	Protein	Inorg. matter
		(g)	%	%	Hyc	%	%
R1	6.9-7.0	700	28.60	72.70	6.50	19.00	1.80
R2	6.9-7.0	330	27.20	68.90	6.90	22.50	1.70
R3	6.9-7.0	285	26.90	69.10	6.20	22.50	1.90
R4	6.9-7.0	338	27.40	68.30	7.60	22.60	1.50
R5	6.9-7.0	340	27.10	68.60	7.20	22.70	1.50
R6	6.9-7.0	255	27.20	68.50	6.90	22.90	1.70
R7	6.9-7.0	350	28.00	68.90	6.80	22.70	1.60
R8	6.9-7.0	350	28.00	69.60	6.60	22.10	1.70
R9	6.9-7.0	202	28.20	72.10	6.90	19.20	1.80
R10	6.9-7.0	128	27.90	69.50	6.60	22.30	1.60
Literature value			27.65	69.62	6.82	21.85	1.68

Characterization of rubber hydrogen of latex. All samples of rubber hydrocarbon were softened at room temperature. Rubber hydrocarbons were characterised by determining unsaturation, elemental analysis, solubility, molecular weight, refractive index etc. Instrumental techniques like IR, NMR, DSC, GPC were also used for this purpose.

Iodine value was determined by Wij's method (Kemp 1927). Elemental analysis were carried out on Carto Erba Mod.1106 analyser. Molecular weight (M) was determined by measuring viscosities of dilute solutions at 30°C using an Ostwald type viscometer. Molecular weight was calculated by following relation, (Carter and Magat 1946; Scott *et al* 1949; Marks 1989).

$$[\eta] = 5.02 \times 10^{-4} M^{-0.667}$$

The intercept of the plot η_{sp} Vs C determined the value of intrinsic viscosity $[\eta]$ in dl/g, where η_{sp} is the specific viscosity and C is the concentration of solution.

Results and Discussion

Table 1 includes composition of various constituents of latex obtained form *Euphorbia caducifolia*. These results indicate that solid content obtained was found in the range 26.90 - 28.60%. The chemical composition of the solid content shows resinous mass as 68.30 - 72.70%, whereas rubber hydrocarbon is around 6.20 - 7.60%. The proteins and inorganic matters lie in the range 19.00 - 22.90% and 1.50 - 1.90%, respectively. It shows that the latex contain major quantity of resinous material and rubber contains are low. Besides, the proteinous material is also higher 19.00 - 22.90% than rubber hydrocarbon which causes putrefaction, if preservative is not added at the time of collection of latexes. pH of the latexes at the time of tapping was found to be 6.90 - 7.00% whilst on standing in open air it becomes 5.45% resulting in spontaneous coagulation. The dropping of pH from neutral to acidic is the action of lactic acid bacteria. Latex particles carry negative electronic charges and coagulation usually occurs when the pH reaches about 4.50 - 4.20%. The charged particles repel each other but below to the iso-electric charge approaches zero and coagulation takes place. To prevent coagulaion of latex at the time of collection, the pH may be increased from 6.90 to 11.00 by the addition of ammonia which not only enhance the pH of system but also hydrolyse the fatty acid esters and form soaps. These soaps act as stabilizing bodies.

Table 2
Iodine values and % unsturation of rubber hydrocarbon obtained from the latexes of *E. caducifolia* at 25°C (Reaction time one hour)

Sample no.	Iodine value	Unsaturation (%)
R1	313.33	84.05
R2	350.80	94.10
R3	310.91	83.40
R4	336.28	90.20
R5	349.80	83.83
R6	348.98	93.61
R7	250.00	93.88
R8	349.90	93.86
R9	348.00	86.80
R10	323.59	86.80
Literature value	350.90	94.12

The rubber hydrocarbon was characterised by different chemical and instrumental methods. These studies reveal the following findings:

Iodine value of rubber hydrocarbon. Rubber hydrocarbon extracted with toluene/hexane from the latexes of *E. caducifolia*, was characterised from extent of unsaturation by determination of iodine value of ten samples of rubber hydrocarbon which are summarized in Table 2. Iodine value of these hydrocarbon samples were determined which lies in the range 313.33 - 350.80%. Percentage of unsaturation has been calculated from the iodine value of rubber hydrocarbon which were found to be 83.40 - 94.10%. The determined value of unsaturation is in the vicinity of literature value for poly-isoprene as 94.12% (Kemp 1927; Kemp and Mueller 1934).

Element analysis. The elemental analysis of 10 samples of rubber hydrocarbon alongwith standard sample of natural rubber were accomplished. The results are summarized in Table 3. The analysis of standard sample shows its composition as

Table 3
Elemental analysis of rubber hydrocarbon obtained from the latexes of *E. caducifolia*

Sample no.	%C	%H
Standard sample	88.02	11.75
R1	87.50	11.82
R2	88.01	11.20
R3	88.40	11.25
R4	87.92	11.80
R5	88.00	11.50
R6	87.90	11.85
R7	87.45	11.83
R8	87.90	11.82
R9	87.60	11.70
R10	87.90	11.80

C = 88.02% and H = 11.75%, whereas the composition of 10 rubber hydrocarbon samples inidicates the composition as C = 87.45 - 88.40% and H = 11.20 - 11.85%. Furthermore, the sample were also tested for oxygen and halogens. It shows that the samples do not contain either oxygen or halogens. The absence of oxygen and halogens indicates that these samples do not contain protein and other carboxyl and hydroxyl containing materials and are therefore, simple. They are simply hydrocarbons. By comparing the composition of standard samples of natural rubber with the samples of rubber hydrocarbon, it is suggested that the chemical nature of both samples is same. Hence, rubber hydrocarbon extracted from *E. caducifolia* may be natural rubber containing 5 carbon and 8 hydrogen units (C_5-H_8).

Solubility. It is an important tool which helps in the identification of elastomers and polymeric materials. Unvulcanized samples of rubber hydrocarbon (masticated) get dissolved in all the solvents of natural rubber like benzene, toluene, xylene, hexane, cyclohexanone ether, carbon tetrachloride and carbon disulfide. On the other hand, vulcanized samples were found insoluble in these solvents and swollen in some solvents bearing the solvent uptake (>100%) like cyclohexanone (15%), CS_2 (800%), $CC1_4$ (1810%), benzene (1967%), ether (252%) and mineral oil (145%). The samples of rubber hydrocarbons show little solvent uptake in water (17%), butyl alcohol (24%), MEK (57%), chloroform (45%), acetone (22%) etc. These observations support the assumptions and the rubber hydrocarbon extracted from the latexes of *E. caducifolia* is polyisoprene.

Infrared examination. IR spectra of standard sample of natural rubber (from *Hevea*) and rubber hydrocarbon were examined and shown in Table 4. In this spectrum for *Hevea* (*cis*-1,4), the CH stretching modes are found in the 3000 cm^{-1} region and methylene (CH, CH_2-) and methyl (CH_3-) bands fall at about 1450 and 1380 cm^{-1}, respectively. On the other hand, the spectra due to rubber hydrocarbon obtained from *E. caducifolia* show C-H stretching vibration at about 2900 or 2950 cm^{-1} and methylene and methyl absorption bands lie respectively at about 1440 or 1435 and 1370 cm^{-1}. In the region between 700 and 990 cm^{-1}, the *cis*-1,4 (*Hevea*) shows single medium strong band at about 840 cm^{-1}, whereas, in the spectra due to rubber hydrocarbon, a strong broad band lies at about 840 or 820-680 cm^{-1}. These bands speculated the material is *cis*-1,4 rubber hydrocarbon -C(CH_3)=CH-. Furthermore, the absorption bands in the spectra of both hydrocarbon obtained from *Havea* and *E. caducifolia* fall at 1650 and 1640, 1650 or 1675 cm^{-1} respectively, indicating the characteristic C=C stretching vibration. The broad band (820 - 680 cm^{-1}) might be due to impurities present in the sample of rubber hydrocarbon

Table 4
Infrared studies of the rubber hydrocarbon *Euphorbia caducifolia*

	C-H stretch (cm^{-1})	C=C strectch (cm^{-1})	C-H, CH_2 C-H_3 bands (cm^{-1})	C-H band specifically (cm^{-1})	CH_2 and C-C C-H bands (cm^{-1})	C-H band -C(CH_3) = CH (*cis* 1,4) (cm^{-1})
Heyea 1	3000	1650	1450	1380 - 1370	1130, 1325,1070,935	840
E. cad 1	2950	1660	1440	1370	1300, 1240, 1040, 1080, 1120	830
E. cad 3	2900	1660	1440	1370	1300, 1120, 1080, 1030	840
E. cad 4	2950	1660	1440	1380	1350, 1120, 1090, 1020	840
E. cad 5	2900	1650	1440	1375	1300, 1090, 1000	840-645
E. cad 6	2900	1640	1435	1360	1290, 1110, 1080, 1000	820-645
E. cad 7	2900	1650	1440	1375	1300, 1110, 1090, 1020	840-660
E. cad 8	2900	1660	1440	1375	1300, 1130, 1110, 1020	840-650
E. cad 9	2950	1675	1440	1350	1300, 1080, 980	820-680

Table 5
Intrinsic viscosity [η] and molecular weight of rubber hydrocarbon

Sample No.	[η]dl/g	Molecular weight (M)
R1	1.00	88405
R2	0.66	48980
R3	0.90	75490
R4	0.83	66860
R5	0.67	48500
R6	0.33	16773
R7	0.31	15275
R8	0.35	18320
R9	0.31	15275
R10	0.91	76750

from *E. caducifolia.* Dobholkar *et al* (1991) separated rubber hydrocarbon from *E. nivulia* and reported a broad and intense absorption bands at 838 - 840 and 1130 cm^{-1} respectively which identifies the material to be (*cis*-1,4) polyisoprene. IR examiantion of various polyisoprene and identified (*cis*-1,4) polyisoprene at 840 cm^{-1}. Hence, in the light of these findings, it can be safely assumed that the rubber hydrocarbon obtained from *E.caducifolia* may be *cis*-1,4 polyisoprene.

NMR spectra. Two of the samples of rubber hydrocarbon obtained from *Euphoribia caducifolia* have been selected for NMR examination. These samples were analysed by ^{1}H and ^{13}C-NMR spectra.

^{1}H-NMR spectra. In ^{1}H-NMR spectrum of one of the samples, most of the intensities lie in the saturation region (0 - 2.85). The peak apprears at δ 1.20 - 1.30 and relates to doublet from CH_3- C - C=CH at δ 1.60 - 1.80, corresponds to a multiplet for CH_3- C=C/CH_2- C=C- and at δ 1.90 - 2.20. There is a multiplet for CH_2- C=C/CH_2- C=C-. The olefinic proton (-C - CH=C/ CH=CH_2) appears at δ 5.10-5.20. In another samples, most of the intensities lie in the saturated region δ 0-2.40 (Chen 1962).

The peak appearing at δ 1.20-1.29 represents the doublet CH_3- C - C=CH, where as at δ 1.46-1.70, it corresponds to a multiplet for CH_3- C=C/CH_2- C=C-. The peak at δ 1.85-2.25 shows a multiplet for CH_2- C=C/CH_2- C=C -. The olefinic proton (- C-CH=C-CH=CH_2) appears at δ 5.15. The ^{1}H-NMR spectra clearly indicates that the rubber hydrocarbon is polyisoprene.

^{13}C-NMR spectra. In ^{13}C-NMR spectrum of one of the sample, the most of the intensities lie in the saturated region 0.80 ppm. The peaks at 23.1, 26.3 and 32.0 ppm corresponds to CH_3, CH_2 carbons, respectively. There are two peaks in the olefinic region 120-140 ppm. One is at 124.5 ppm correspondence to = CH olefinic carbon and other is at 134.5 ppm relates to = C-CH_3 olefinic carbon. In the spectrum of the other sample, the intensities lie in the saturated region 0.80 ppm. The peaks at 23.20, 26.50 and 32.10 ppm relates to CH_3 and CH_2 carbons, respectively. The olefinic peaks lie in the region 120-140 ppm which correspond to = CH and = C-CH_3 olefinic carbons. These evidences suggest that the samples are *cis*-1,4 polyisoprene.

Table 6
Molecular weight of rubber hydrocarbon by GPC method.

Sample No.	Mn	Mw	Mw/Mn	Mn by viscosity
R1	150488	427547	2.84	88405
R2	52350	834978	15.95	48980
R3	64034	39684	6.20	75490
R7	11234	38183	3.40	15275
R8	15592	79728	5.11	18320

Mn, is number average molecular weight; Mw, is weight average molecular weight

$$- CH_2 - CH = C(CH_3) - CH_2 -$$

Both ^{1}H and ^{13}C NMR spectra suggests the rubber hydrocarbon of the polyisoprene.

Molecular weight. Intrinsic viscosities [η] in dl/g for rubber hydrocarbons were determined at 30°C in Ostwald type viscometer using toluene as solvent. Each sample showed different intrinsic viscosity (Table 5). It ranges from 0.31 to 1.00 dl/g, which determine molecular weight as 15275 - 88405. The difference in molecular weight and [η] show that as the stem of the plant is incised the hydrocarbon present in the latex starts polymerization and with the passage of time chain length of the resulting polymer gets increased. Isoprene molecules present in the latex in the prepolymer stage (Yousufzai and Khan 1984), on contact with atmospheric oxygen, it starts polymerizing, resulting in polyisoprene of different chain length. Besides, the molecular distribution of samples were determined by means of GPC. The chromatograms of rubber hydrocarbons show more than one peak which suggests that the samples should be fractionated for getting more dispersed polymers. The values of M obtained by GPC methods are found higher than those obtained by viscosity method. For sample R1 of rubber hydrocarbons, the value of Mn and Mw are 150488 and 427547, respectively. The value of Mw/Mn = 2.84 indicates a narrow molecular weight distribution. On the other hand, the value of M for this sample determined by viscosity method was found as 88405 (Table 6).

Similarly, the samples R2 , R3, R7 and R8 show the values of M by GPC as 52350, 64034, 11234 and 15592, whilst the values of Mw are 834978, 39684, 38183 and 79728 respec-

Table 7
Some physical parameters of rubber hydrocarbon obtained from the plants of *E. caducifolia*

Sample No.	Refractive index	Specific gravity	Consistency	Strength	Combustion
R 1	1.49281	0.93240	Sticky	Poor	Smooky flame
R 2	1.49300	0.39102	Sticky	Poor	Smooky flame
R 3	1.49282	0.39420	Sticky	Poor	Smooky flame
R 4	1.49250	0.93628	Sticky	Poor	Smooky flame
R 5	1.49325	0.93108	Sticky	Poor	Smooky flame
R 6	1.49250	0.93240	Sticky	Poor	Smooky flame
R 7	1.49200	0.93246	Sticky	Poor	Smooky flame
R 8	1.49282	0.93240	Sticky	Poor	Smooky flame
R 9	1.49325	0.93108	Sticky	Poor	Smooky flame
R 10	1.49288	0.93428	Sticky	Poor	Smooky flame

tively. On the other hand intrinsic viscosity for R2, R3, R7 and R8 samples determines Mn as 48980, 75490, 15275 and 18320, respectively. The values of Mw/Mn for R2, R3, R7 and R8 samples are calculated as 15.95, 6.20, 3.40 and 5.11% respectively. The sample R2 show broad molecular weight distribution, whereas rest of the samples, show narrow molecular weight distribution.

Differential scanning calorimetry (DSC). DSC studies of rubber hydrocarbon from *E. caducifolia* latex was also carried out.

The samples R2 and R5 were selected for this study. The sample R2 was heated from - 80 to - 10˚C/min. Glass transition temperature (T_g) appears to be an initial heating scale. The curve shows T_g as - 63.02˚C . Sample R5 was first quenched from 100 to - 100˚C and then heated from -100 to +100˚C at the rate of 10˚C/ min, the curve obtained shows the region of T_g as - 66.96 to 57.11 and middle point is - 60.81˚C. The values of T_g obtained for these samples are very close to literature value of polyisoprene i.e. - 67˚C which clearly evidences that the samples are polyisoprene.

Refractive index of various samples of rubber hydrocarbon are measured. It was found in the range 1.49200 to 1.49325. This value of refractive index is very close to literature value 1.5919 for confirms polyisoprene. It means the rubber hydrocarbons separated from latexes of *E.caducifolia* is polyisoprene. Specific gravity of rubber hydrocarbon samples were measured, which ranges from 0.93102 to 0.93628 and it is in the vicinity of literature value (0.92). Besides, all samples of rubber hydrocarbons are poor in strength and sticky. On flame, these samples are first converted into fluid and then are ignitiated. They burns freely with a smoky flame. These physical parameters also confirmed that the extracted material was natural rubber (Table 7).

In brief, on incising the stem of the plant, the latex was collected in sterilized bottles already containing preservative. The preservative not only makes the system alkaline but also hydrolyses the fatty acids esters naturally occuring in the latex and form soaps. These soaps serve as stabilizer. The latex was coagulated with formic acid and this coagulated material was first soxhelated with acetone to remove resin contents of the latex and then with hexane to separate rubber hydrocarbons. The rubber hydrocarbons was obtained and characterised by different chemical and instrumental methods. Iodine value of hydrocarbon (310.91-350.80) percentage of unsaturation (83.40-94.10%), elemental analysis (C = 87.48 - 88.04%), H = 11.20 - 11.82%). Solubility (soluble in the solvents of natural rubber), IR spectrum (840 cm^{-1}), ^{1}H-NMR spectrum (olefinic proton at δ 5.15-5.20), ^{13}C-NMR spectrum (olefinic region 120-140 ppm), molecular weight (15275 - 88405), molecular weight distribution (both broad & narrow molecular weight distribution), value of T_g (- 63.02/-60.81˚C), refractive index (1.49200 to 1.49350), specific gravity (0.93102 to 0.93628), consistency (sticky), strength (poor) and combustion (smoky flame) identify the rubber hydrocarbon to be certainly *cis*-1,4 polyisoprene.

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PREPARATION, CHARACTERIZATION AND MOLECULAR MODELS OF THE COMPLEXES OF QUADRIDENTATE TRIPODAL LIGAND TREN [TREN = TRIS(2-AMINOETHYL)AMINE] WITH Ag(I), Cd(II), Hg(II), Zr(IV) AND U(VI)

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The tripodal ligand tren, has been reacted with Ag(I), Cd(II), Hg(II), Zr(IV) and U(VI) in the EtOH medium. The solid product obtained immediately after the reaction, were formulated by comparing C, H, N, and S analysis data. Ag(I) and Cd(II) form simple five coordinated compounds and the others produce double salt like compounds. The bonding patterns of the compounds have been discussed on the basis of IR an UV visible spectral analysis. The geometry of the coordination sphere of the complexes are suggested on the basis of molecular models.

Key words: Tripodal ligand, Coordination sphere, Molecular model.

Introduction

The tripod like ligands produce five coordinated complexes rather than six coordinated one, with transition metals, due to their quadridentate property and to avoid steric hindrance (Ciampolini and Nardi 1966 a & b; Ciampolini *et al* 1968; Di Vaira and Mani 1983). In our previous communications (Ehsan 2000; Ehsan and Islam 2000), it was shown that the ligand tren reacts with first row transition metals in 2^+ and 3^+ oxidation state, to give five coordinated complexes with Cr(III), Mn(II), Fe(III), Co(II), Ni(II), Cu(II) and Zn(II). The divalent metal ions produce five simple coordinated complexes whereas, the trivalent metal ions produce double salt like compounds. In this communication, we report the preparation and characterization of the tren complexes with heavy metals Ag(I), Cd(II), Hg(II), Zr(IV) and U(VI). The molecular geometry of the complexes are reported here on the basis of molecular models.

Experimental

Materials and methods. The ligand tren was procured from Wako Pure Chemical Company, Japan and was used without further purification. Other chemicals and solvents, metal salts used in all preparative and analytical works were of analytical grade (BDH and Aldrich). Micro analytical data for C, H and N were obtained from the Institute for Chemical Reaction Science, Tohoku University, Japan. IR spectra of the compounds were recorded on a Shimadzu IR-Spectrophotometer, Model IR 470 of Japan in the region of 4000-400 cm^{-1} using KBr pellets and the IR spectrum of the ligand (liquid) was recorded as thin film between NaCl cells. The electronic spectra of the complexes were recorded with nujol in a Shimadzu UV-Visible recording Spectrophotometer, Model UV-160A, in the wave length range 200-1100nm. The molecular model of the compound was made using a Hyperchem Molecular Modeling programme (version 5.1), Hyperchem Inc, USA.

Preparation. All the compounds were prepared by direct reaction between metal salt and the ligand in the EtOH medium. The ligand (1 mmol) and the corresponding metal salt (1 mmol) solutions in EtOH were prepared separately and then mixed together on a magnetic stirrer at room temperature. Solid powdered product separated out immediately after mixing. In case of Hg and UO_2- complexes, NH_4SCN was mixed with metal salt before adding to the ligand solution. The solid products were separated by filtration, washed with EtOH and dried over silica gel. The compounds were stable in light and air. The empirical formulas of the compounds which were determined by comparing the elemental analysis data in Table 1 are

A $[Ag(tren)NO_3]$
B [Cd(tren)NCS]SCN
C $[Hg(tren)Cl]Cl.HgCl_2$
D $[ZrO(tren)Cl]Cl.ZrOCl_2$ and
E $[UO_2(tren)NCS]SCN.UO_2(CH_3COO)_2$

Results and Discussion

The complexes were prepared by straight forward reaction between ligand and the metal salt in nonaqueous solvent

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Table 1
Elemental analysis of the complexes

Compounds	Colour	Decomp Temp. ˚C	% C Found (Calc.)	% H Found (Calc.)	% N Found (Calc.)	% S Found (Calc.)
A [Ag(tren)NO_3]	White	380 - 384	22.60 (22.79)	5.37 (5.73)	21.77 (22.15)	
B [Cd(tren)NCS]SCN	White	294 - 300	25.58 (25.63)	4.93 (4.84)	22.41 (22.42)	17.20 (17.08)
C [Hg(tren)Cl]Cl.$HgCl_2$	White	365 - 370	10.72 (10.46)	2.62 (2.63)	7.97 (8.12)	
D [ZrO(tren)Cl]Cl.$ZrOCl_2$	Greenish white	374 - 378	14.07 (13.84)	4.14 (3.87)	10.30 (10.76)	
E [UO_2(tren)NCS]SCN.$UO_2(CH_3COO)_2$	Yellowish	455 - 460	15.21 (15.72)	3.01 (2.64)	9.81 (9.14)	7.63 (7.01)

Table 2
Assignment of important IR bands.

Complexes	NH Stretch cm^{-1}	CH Stretch cm^{-1}	NH Bend cm^{-1}	NO_3^- cm^{-1}	SCN cm^{-1}	MN Stretch cm^{-1}
A [Ag(tren)NO_3]	3295	2910	1600	ν(1434) ν(888)		578
B [Cd(tren)NCS]SCN	3120	2935	1579		ν(2065) CN ν(855) CS	592
C [Hg(tren)Cl]Cl.$HgCl_2$	3295	2930	1583			529
D [ZrO(tren)Cl]Cl.$ZrOCl_2$	3400 - 2910		1570			
E [UO_2(tren)NCS]SCN.$UO_2(CH_3COO)_2$	3215	2910	1587		ν(2070) CN ν(880) CS	515

(EtOH). In all cases the product was obtained immediately after mixing the ligand and the metal salt. The experimental and calculated values of C, H, N, and S of complexes are in good agreement which demonstrate that the compounds are pure enough and support the proposed formulations. The assignment of important IR bands of the ligand and complexes have been done on the basis of standard references and are listed in Table 2 (Cross 1964; Bellamy 1975; Nakamoto 1978; Ehsan 2000).

The IR spectra of all the metal complexes except **D** are very sharp and well resolved. The spectrum of the ligands is broad and that of the **D**, is very much broad. All the complexes absorb for NH stretching of primary amino groups of the ligands moiety, as doublet about 3120-3400 cm^{-1} frequency, which is at slightly lower field than that of the free ligand, indicating the bond formation between metal and N-atoms of the ligand. The complexes absorb for CH stretching (of CH_2 group) at 2910-2935 cm^{-1} range. The position of stretching vibration of CH are more or less at same position to that of the ligand, since these are not affected by the presence of metal in the system. In case of compound **D**, both NH and CH stretching bands appear as a broad band at 2910-3400 cm^{-1} region. The complexes **B** and **E** show strong absorption for C≡N stretching of the SCN group at about 2000 cm^{-1}, which is relatively at lower position than that of the normal position. This is an indication that SCN group is attached to the metal ion through the N-atom and S atom remains free, producing isothiocyanato complexes. The relatively high position of CS stretching bands further supports that the S atom of SCN group remains free. In case compound **A**, there is NO^-_3 ion at the fifth position of the trigonal bipyramid (tbp). The presence of the NO^-_3 ion in the compound is well indicated in it's IR spectrum. It shows very strong NO^-_3 stretching band at 1434 cm^{-1} and bending

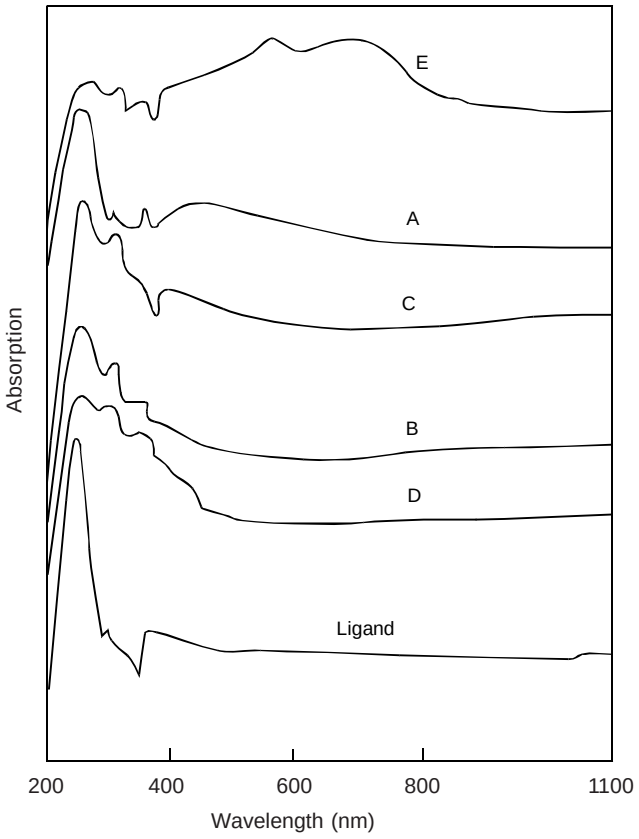


Fig 1. Electronic spectra of the ligand, tren and its metal complexes.

absorption at 888 cm^{-1}. The absorption bands of the complexes in the range 515-592 cm^{-1} are suggested for M-N stretching.

The electronic spectra of the complexes are compared with that of ligand in Fig 1. The signals of eletronic spectra of the ligand and the complexes were analyzed on the basis of standard references and are tabulated in Table 3 (Lever 1968; Roberts and Casserio 1977; Ehsan 2000, Ehsan and Islam 2000). The ligand tren shows very strong absorption at 247 nm for

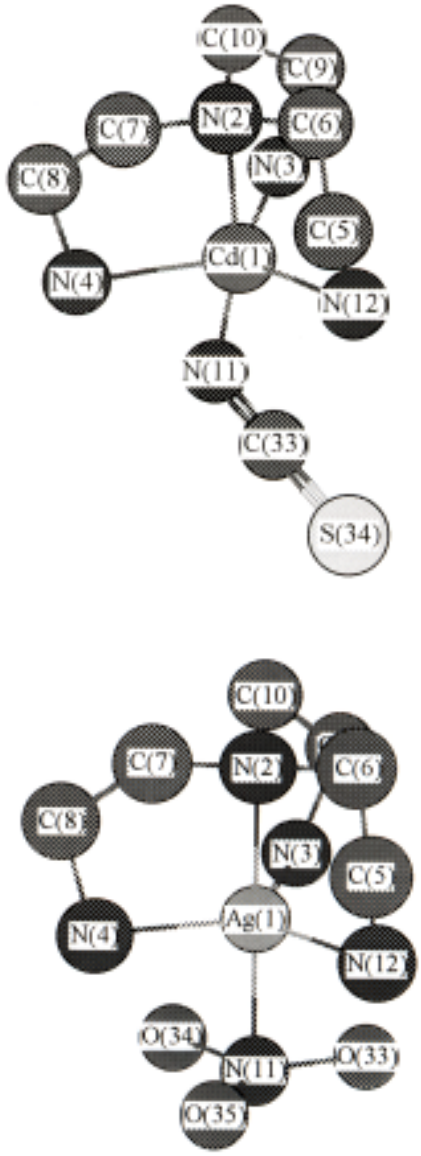


Fig 2(a). Slightly distorted trigonal bipyramidal coordination environment around Cd-atom (b) Spherically symmetric tbp coordination environment around Ag-atom.

Table 3
Assignments of electronic spectra of the complexes

Compounds		Electronic absorption bands (nm)				
Ligand		247	300	343		
$[Ag(tren)NO_3]$	**A**	245	300	342		
[Cd(tren)NCS]SCN	**B**	245	299	323	368	
$[Hg(tren)Cl]Cl.HgCl_2$	**C**	245	298		361	
$[ZrO(tren)Cl]Cl.ZrOCl_2$	**D**	252	299	340		420
$[UO_2(tren)NCS]SCN.UO_2(CH_3COO)_2$	**E**	252	299	341		514, 625



Fig 2. Molecular models of the tren -metal complexes showing almost spherically symmetrical trigonal bipyramidal coordination environment in the Ag and Hg-compounds, slightly distorted trigonal bipyramidal structure of Cd-compound and slightly distorted octahedral structure of U-compound.

n-σ* transition of lone pair of amino nitrogens (4N) to the antibonding σ orbital of the C-N bond. The ligand shows two more very weak absorptions at 300 and 343 nm which could not be identified (Table 3).

The spectra of all the complexes in the 200-350nm range are almost similar to that of the ligand, which demonstrate that after complexation no structural change of the ligand has occurred. Strong absorption in the range 245-252 nm are due to n→σ* transition of the nitrogen lone pair of the ligand. Like the lignad, the complexes show unidentified and very weak absorptions at 300 and 343 nm range. In case of first row transition metals complexes of tren (Ehsan 2000), these two absorption peaks (near 300 and 343 nm) were more intense than that of the n→σ* transition. Complexes **B** and **C** show

Table 4a

Bond lengths and bond angles in the coordination sphere of the complexes of Ag(I), Cd(II), and Hg(II)

Parameters	Ag - complex		Cd - complex		Hg - complex	
Bond length(Å)	Ag(1)-N(2)	2.066	Cd(1)-N(2)	2.182	Hg(1)-N(2)	2.150
	Ag(1)-N(3)	2.095	Cd(1)-N(3)	2.215	Hg(1)-N(3)	2.191
	Ag(1)-N(4)	2.095	Cd(1)-N(4)	2.215	Hg(1)-N(4)	2.191
	Ag(1)-N(11)	2.034	Cd(1)-N(11)	2.157	Hg(1)-Cl(11)	2.465
	Ag(1)-N(12)	2.097	Cd(1)-N(12)	2.210	Hg(1)-N(12)	2.191
Bond angle (◦)	N(2)-Ag(1)-N(3)	89.152	N(2)-Cd(1)-N(3)	87.131	N(2)-Ag(1)-N(3)	92.726
	N(2)-Ag(1)-N(4)	89.266	N(2)-Cd(1)-N(4)	87.295	N(2)-Ag(1)-N(4)	92.759
	N(2)-Ag(1)-N(11)	180.000	N(2)-Cd(1)-N(11)	161.076	N(2)-Ag(1)-Cl(11)	180.000
	N(2)-Ag(1)-N(12)	89.275	N(2)-Cd(1)-N(12)	87.544	N(2)-Ag(1)-N(12)	92.838
	N(3)-Ag(1)-N(4)	119.424	N(3)-Cd(1)-N(4)	117.062	N(3)-Ag(1)-N(4)	119.297
	N(3)-Ag(1)-N(11)	90.827	N(3)-Cd(1)-N(11)	82.803	N(3)-Ag(1)-Cl(11)	87.344
	N(3)-Ag(1)-N(12)	120.717	N(3)-Cd(1)-N(12)	121.301	N(3)-Ag(1)-N(12)	120.155
	N(4)-Ag(1)-N(11)	90.792	N(4)-Cd(1)-N(11)	83.120	N(4)-Ag(1)-Cl(11)	87.295
	N(4)-Ag(1)-N(12)	119.802	N(4)-Cd(1)-N(12)	120.989	N(4)-Ag(1)-N(12)	119.851
	N(11)-Ag(1)-N(12)	90.687	N(11)-Cd(1)-N(12)	111.378	Cl(11)-Ag(1)-N(12)	87.036

Table 4b

Bond lengths and bond angles in the coordination sphere of the complexes of Zr(IV) and U(VI)

Parameters	ZrO - compound		UO_2 - compound	
Bond length (Å)	Zr(1)-N(2)	2.156	U(1)-N(2)	1.856
	Zr(1)-N(3)	2.189	U(1)-N(3)	1.910
	Zr(1)-N(4)	2.189	U(1)-N(4)	1.934
	Zr(1)-N(11)	2.090	U(1)-N(11)	1.750
	Zr(1)-N(12)	2.183	U(1)-N(12)	1.901
			U(1)-N(15)	1.770
Bond angle (◦)	N(2)-Zr(1)-N(3)	87.885	N(2)-U(1)-N(3)	94.483
	N(2)-Zr(1)-N(4)	88.019	N(2)-U(1)-N(4)	92.670
	N(2)-Zr(1)-O(11)	167.320	N(2)-U(1)-O(11)	175.014
	N(2)-Zr(1)-N(12)	88.229	N(2)-U(1)-N(12)	94.784
	N(3)-Zr(1)-N(4)	117.921	N(2)-U(1)-O(15)	96.646
	N(3)-Zr(1)-O(11)	85.538	N(3)-U(1)-N(4)	103.207
	N(3)-Zr(1)-N(12)	120.867	N(3)-U(1)-O(11)	80.990
	N(4)-Zr(1)-O(11)	85.885	N(3)-U(1)-N(12)	105.099
	N(4)-Zr(1)-N(12)	120.867	N(3)-U(1)-O(15)	168.796
	O(11)-Zr(1)-N(12)	104.450	N(4)-U(1)-O(11)	86.367
			N(4)-U(1)-N(12)	150.017
			N(4)-U(1)-O(15)	75.005
			O(11)-U(1)-N(12)	88.453
			O(11)-U(1)-O(15)	87.839
			N(12)-U(1)-O(15)	75.302

CT transition at 368 and 361 nm respectively, but there is no d→d transition since they are d^{10} system. Complexes **A**, **D** and **E** show d→d transitions at 430, 420, and 514, 625 nm, respectively. These complexes may show CT transitions which are buried in the d→d transition bands.

Molecule modeling of the compounds were done by molecular mechanics with MM^{+} force field, (Hyperchem 1996) using commercially available computer programme of Hyperchem Inc, USA The structure was completely minimized to get the most stable state. The model of Ag and Cd-compounds and the numbering scheme are shown in Fig 2, and the bond angles and bond lengths are shown in Table 4a.

On the basis of models, the geometry of the coordination sphere of the complexes are suggested as trigonal bipyramidal (tbd). In case of compounds **A** and **C** the ligand at the 5th position of tbp are symmetrical (NH_3 in **A** and Cl in **C**), as a result the coordination geometry around the metal atom is almost spherically symmetric trigonal bipyramidal (tbp). In both cases equatorial bond lengths and bond angles are same. Axial bonds are in same straight line but the axial bond lengths are different. In both the compounds, the metal-5th ligand bond is longer than the other. In case of compound **B**, the unsymmetrical group (SCN) at the 5th position of tbp, make a slightly distorted (tbp) coordination environment around the metal atom shown in Fig 2a and 2b. Each bonds and bond angles in the coordination sphere (axial and equatorial) are slightly different from each other. The values of bond angles and bond lengths and their trends, of all the compounds are fairly comparable to crystallographic data for the compounds $[Cu(tren)NH_3](ClO_4)_2$ and [Cu(tren)NCS] SCN (Jain and Lingafelter 1967; Dugan *etal* 1980).

The coordination phenomena of the ZrO^{-} and UO^{-2} compounds are little different. No secondary ligand except 'tren' is coordinated to the metal atom. As a result we get slightly distorted tbp geometry around Zr-atom with one O-atoms linked to U-atom. The bond lengths and bond angles in the coordination sphere of these two compounds are listed in Table 4b.

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EXTRACTION AND CHEMICAL QUALITY CHARACTERISTICS EVALUATION OF ORANGE PEEL PECTIN

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Optimum conditions for the extraction and precipitation of pectin from orange peels were investigated. Changes in pH, temperature and extraction time, significantly, affected the extraction of pectin. Maximum pectin yield was 17.7%, which was obtained on soaking finely ground orange peels in sulphuric acid solution of pH 2.5 at 80°C for 120 min. Maximum pectin was precipitated from the extract by adding 95% ethanol at the rate of 200ml/l. Anhydrogalacturonic acid and methoxyl contents of pectin obtained under these optimum extraction conditions were 72.80% and 9.77% respectively, while equivalent weight value was 943. These chemical characteristics values were within the accepted limit of good quality pectin.

***Key words*:** Orange peel, Pectin, Extraction yield, Quality.

Introduction

Pectin is a complex heteropolysaccharide which mainly consists of long unbranched chains of polygalacturonic acid with carboxylic group partially esterified with methyl alcohol (Awan 1993). It is extensively utilized by the food processors especially for the conversion of low grade fruits into good quality products like jam, jelly, marmalade and candies. Low methoxyl pectin is used to increase firmness of canned fruits while high methoxyl pectin helps to prepare stable fruit beverages.

There are various methods for the extraction of pectin from fruit and vegetable wastes but the literature regarding optimum conditions for extraction of pectin is scanty. The quality of pectin depends mostly upon the source as well as the method employed for its extraction. Pectin is usually extracted by suspending chopped fruit and vegetable wastes in different mineral acids and salts solutions (Baltaga 1962; Srirangarajan and Shrikhande 1979; Jain *et al* 1984). Pectin from grape fruit orange and lemon peels was extracted with acidic solution of nitric acid and precipitated with ethanol (Rouse and Crandall 1976). Pectin extracted by soaking different plant materials in aqueous sodium nitrate solution containing amino acids (Golubev *et al* 1991). Pectin extract from soybean okara with sodium hexametaphosphate solution is reported by Yamoguchi *et al* (1996) and pectin from potato waste using solution of aluminium sulphate as precipitating agent obtained by Chen *et al* (1999). Muralikrishna and Tharanatha (1994) obtained only 1.43 - 5.37% pectin by soaking pulse husks in hydrochloric acid and EDTA solution at 70°C, whereas, Karpovich *et al* (1990) and Turquios *et al* (1999) were reported the yield 9-10% of good quality pectin from potato waste using solution of aluminium sulphate as precipitating agent. Microbial enzymes were also used for the production of pectin and pigment from orange peels (Elian *et al* 1984). Industrial pectin of good gelling property was prepared from peach pomace by Pagan *et al* (1999). Fresh Kiwi fruit peels were also used for the extraction of pectin by Wu Hui-Fang *et al* (1999). These methods for extraction of pectin are lengthy, complicated and laborious. Moreover, yield of pectin by most of the previous methods was very low. Therefore, present work was undertaken to optimize the conditions (pH, temperature, time period of extraction) for the development of a simple and efficient pectin extraction method from orange peels. Besides yield, chemical characteristics of pectin were also studied.

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Materials and Methods

Fresh orange peels were collected from a local commercial fruit processing plant. The peels were passed through shredding machine to separate albedo (Pectin rich) and flavedo (oil + pigment) portions. The albedo portion was then minced mechanically and washed with cold water to remove any adhering juice. The washed and minced albedo was then dried in a cabinet dryer at 65°C to reduce moisture content to 5-6%. The dried material was finally ground to 80 mesh size before extraction of pectin.

Extraction of pectin. Ground material was mixed well with water of different pH (1.5, 2.5, 3.5) keeping substrate to water ratio 1:40 (w/v). The desired pH of the mixture was adjusted

Table 1
Effect of pH, temperature and time period of extraction on yield (% age) of pectin from orange peels

Extraction temp. (°C)	pH - 1.5 Extraction time period (min) 30	60	120	pH - 2.5 Extraction time period (min) 30	60	120	pH - 3.5 Extraction time period (min) 30	60	120
60	7.20^{a}±0.2	7.80^{a}±0.2	8.50^{a}±0.4	9.80^{a}±0.4	11.20^{a}±0.5	14.00^{a}±0.6	8.20^{a}±0.2	8.30^{a}±0.3	8.90^{a}±0.5
70	8.00^{b}±0.4	9.30^{b}±0.6	10.80^{b}±0.5	12.00^{b}±0.7	14.20^{b}±0.3	15.90^{b}±0.4	10.00^{b}±0.5	10.60^{b}±0.1	11.00^{b}±0.5
80	9.20^{c}±0.6	10.70^{c}±0.6	11.60^{c}±0.3	14.00^{c}±0.5	15.50^{c}±0.5	17.70^{c}±0.7	11.20^{c}±0.7	11.60^{c}±0.4	12.00^{c}±0.3
90	8.10^{b}±0.5	8.00^{a}±0.5	8.70^{a}±0.4	11.50^{b}±0.3	11.00^{a}±0.3	12.70^{a}±0.2	10.00^{b}±0.6	10.20^{b}±0.2	10.40^{b}±0.4

Mean value ± S.D. triplicate determination
Mean values within a column with different superscripts are significantly different at $p < 0.05$

with 0.1N sulphuric acid on pH meter (PYE UNICAM model-1292) and then incubated at temperatures (60, 70, 80 & 90°C) for various time intervals (30, 60, 120 min) with frequent stirring. After incubation, the contents were filtered through cheese cloth and pectin from the filtrate was precipitated with 95% ethanol. The obtained pectin was dried in a vacuum oven at 40°C to obtain constant weight and ground finally to study chemical quality characteristics.

Yield was calculated as dried pectin g/100g dried orange peels. Anhydrogalacturonic acid content, equivalent weight and methoxyl contents were determined as quality characteristics of orange peel pectin by the standard methods of Owens *et al* (1952). Triplicate determinations were performed for all parameters studied and standard deviations (SD) were calculated according to the method of the Steel and Torrie (1980).

Results and Discussion

Optimum conditions for extraction of pectin. Data presented in Table 1 showed that pH extraction, temperature and time distinctly affected the extraction of pectin from finely ground orange peels. pH of the solution played a significant role in the extraction of pectin. The % yield of pectin was 11.60, 17.70 and 12.00 at pH 1.5, 2.5 and 3.5 respectively, when orange peel (albedo) powder was suspended at 80°C for 120 min (Table 1). Results revealed that maximum yield of pectin was obtained by soaking the raw material in a solution of pH 2.5 which was significantly, ($p < 0.05$) higher than the pectin yield obtained at pH 1.5 and 3.5. However, decline in pectin yield was observed at pH 3.5 at all studied temperatures and extraction time.

Variable amounts of pectin was obtained from orange peels at temperatures ranging from 60-90°C (Table 1). Maximum amount of pectin was obtained by soaking albedo portion of orange peels in acidic solution at 80°C. However, significant reduction in pectin yield was observed when temperature was raised from 80 to 90°C. At 80°C, pectin yield was 15.50% which was reduced to 11.00% at 90°C on soaking orange peels

Table 2
Effect of different mineral acids solution on the yield of pectin

Extractants	Extraction conditions pH	Temp.(°C)	Time (min)	Yield %
Hydrochloric acid	2.5	80	120	13.45^{a}±0.7
Sulphuric acid	2.5	80	120	17.80^{c}±0.5
Nitric acid	2.5	80	120	15.11^{b}±0.5

Mean value ± S.D. triplicate determination
Mean values within a column with different superscripts are significantly different at $p < 0.05$

Table 3
Effect of different precipitation agent on the yield of pectin

Precipitating agent	Extraction conditions Amount added ml/l	pH	Temp (°C)	Time (min)	Yield %
Ethanol	50	2.5	80	120	7.20^{a}±0.5
	100	2.5	80	120	11.00^{b}±0.6
	200	2.5	80	120	17.80^{d}±0.8
Acetone	50	2.5	80	120	5.40^{a}±0.1
	100	2.5	80	120	9.00^{a}±0.3
	200	2.5	80	120	14.44^{c}±0.5

Mean value ± S.D. triplicate determination
Mean values within a column with different superscripts are significantly different at $p < 0.05$

Table 4
Chemical quality characteristics of orange peels pectin extracted at 80°C

Chemical characteristics	pH - 1.5 Extraction time period (min)			pH - 2.5 Extraction time period (min)			pH - 3.5 Extraction time period (min)		
	30	60	120	30	60	120	30	60	120
Anhydrogalacturonic acid (%)	$66.73^{b}\pm1.6$	$67.45^{b}\pm1.4$	$68.72^{b}\pm1.4$	$70.88^{c}\pm1.4$	$71.45^{c}\pm1.3$	$72.80^{c}\pm1.3$	$8.20^{a}\pm0.2$	$8.30^{a}\pm0.3$	$8.90^{a}\pm0.5$
Methoxyl content (%)	$8.11^{a}\pm0.10$	$8.15^{a}\pm0.10$	$8.33^{a}\pm0.11$	$9.20^{c}\pm0.10$	$9.67^{c}\pm0.12$	$9.77^{c}\pm0.12$	$8.03^{a}\pm0.14$	$8.24^{a}\pm0.15$	$8.72^{b}\pm0.11$
Equivalent weight	$815^{b}\pm2.0$	$820^{b}\pm2.5$	$835^{b}\pm2.5$	$932^{c}\pm2.6$	$939^{c}\pm2.6$	$943^{c}\pm2.1$	$798^{a}\pm2.4$	$801^{a}\pm2.3$	$803^{a}\pm2.0$

Mean value ± S.D. triplicate determination
Mean values within a row with different superscripts are significantly different at $p < 0.05$

in acids solution of pH 2.5 for 60 min. Similar results were also obtained at other two pH values i.e. 1.5 and 3.5. Decrease in pectin yield at higher temperature 90°C could be attributed to the break down of pectin molecules as already observed by Chang *et al* (1994).

Extractability of pectin was also affected by extraction time ranging from 30 to 120 min. At pH 2.5, pectin yield was 14.0, 15.50 and 17.7% respectively at 80°C after 30, 60 and 120 min extraction time respectively, Table 1. It is also apparent from Table 1, that maximum pectin yield was also obtained at pH 1.5 and 2.5 after 120 min. These results are consistent with the findings of other workers who reported that prolonged extraction and higher temperatures adversely affected the yield of pectin from different sources (Turmucin *et al* 1983; Chang *et al* 1994). Rouse and Crandall (1976) extracted 11.00, 8.15 and 6.35% pectin from lemon, orange and grape fruit peels at pH 1.6 respectively, while 20% pectin yield was obtained from orange peels by precipitation with ferric salt (Zhao 1995). The differences between our results and reported in literature may be due to variations in raw material, particle size and extraction methods.

Extraction of pectin using different mineral acids. It is evident from Table 2 that maximum amount of pectin 17.80% was obtained with sulphuric acid solution of pH 2.5 at 80°C after 120 min extraction time while minimum amount of pectin (13.45%) was obtained with hydrochloric acid solution under the same extraction conditions. However, nitric acid solution extracted 15.11% pectin from orange peels, which is comparatively more than hydrochloric acid solution of pH 2.5. Earlier workers also obtained variable amounts of pectin from citrus peels using different mineral acids (Snyder 1970; Huang 1973; Braddok *et al* 1976). Better extraction of pectin with sulphuric acid might be due to the presence of sulphate ions in soaking solution.

Precipitation of pectin. Pectin was precipitated from the filtrate by the addition of variable amount of ethanol and acetone. Maximum pectin yield was 17.80% when ethanol at the rate of 200 ml/l was added slowly with stirring in the filtrate, while 14.44% pectin was recovered with acetone 200 ml/l (Table 3). Therefore, ethanol was found to be better precipitating agent as compared to acetone.

Chemical quality characteristics of pectin. Table 4 summarizes the chemical characteristics of orange peel pectin. It is apparent from these results that pH, temperature and extraction time affected the chemical quality characteristics of orange peel pectin. However, chemical quality of pectin obtained at pH 2.5 was comparatively better than the pectin obtained at pH 1.5 and 3.5. Anhydrogalacturonic acid and methoxyl contents were 72.80% and 9.77% respectively, while equivalent weight value was 943 for pectin extracted at pH 2.5, 80°C after 120 min. These results were within the range of reported values for anhydrogalacturonic acid (68.5 - 75.0%) and methoxyl contents (8.4 - 9.7%) of good quality orange peel pectin (Zafiris and Oreopoulou 1992).

Therefore, it is concluded that good quality pectin with maximum yield can be obtained by soaking finely ground albedo portion of orange peels in sulphuric acid solution of pH 2.5 at 80°C for 120 min. Ethanol can be successfully used as precipitating agent for maximum recovery of pectin from the extracted filtrate.

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CHEMICAL POLYMORPHISM IN ASTERACEAE

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The qualitative and quantitative composition of the essential oils of seven plants of asteraceae family were investigated. The essential oils were obtained by hydro-distillation of plants and analysed by Gas Chromatograph. The major essential oil components identified, broadly belong to monoterpenoids, sesquiterpeniods and caryophyllene were found to be common in all the investigated species.

Key words**:** Chemical polymorphism, Asteraceae, Essential oil, Hydro-distillation, Monoterpinoids, Sesquiterpenoids, Caryophyllene.

Introduction

Asteraceae are chemically characterised by synthesis and accumulation of many classes of natural products. The essential oils are comprise of the volatile steam - distillable fraction, responsible for the characteristic scent, odour or smell found in many plants. They are commercially important as the basis of natural perfumes, and also of spices and flavouring in the food industry. A considerable number of quite different functions have been ascribed to plant terpenoids. Their growth regulating properties are very well documented, two of the major classes of growth regulators are the sesquiterpenoid abscisins and the diterpenoid based gibberellins. Less is generally known of the role of terpenoids in the more subtle interactions between plants and animals as agents of communication and defence among insects, but this is now an area of active research. Many studies have demonstrated that terpenoids display a wide range of pharmacological activities. Some of these compounds have been identified as the active principles of crude drugs used in folk medicines. The important class of sesquiterpenes, sesquiterpene lactones, which have a wide distribution in the family Asteraceae (Herout and Sorn 1969).

Even though a considerable amount of work has been done on the various members of Asteraceae, on the chemistry of essential oils, however, they have focussed only on a few economically or commercially valuable species. The screening of a large number of lesser oil yielding plants of great medicinal importance, has not been attempted so far. It appears from the previous literature that only few species of the family Asteraceae has so far been phytochemically screened (Riaz *et al* 1995; Chandra *et al* 1996; Wandji *et al* 1996; Chuihua 1998). The data on the chemistry of essential oils of Asteraceae too are very meagre. Considering the richness of this family in the vegetation of India, it has been thought that a systematic work with regard to its essential oil constituents and their significance may lead to a better understanding of these plants. As most of the plants of Asteraceae are used in folk medicines or grand mother's remedy for common diseases.

The present study is therefore, undertaken to find out the quantity of essential oil in medicinal plants of Asteraceae and also to identify the major components in these oils. A chemo taxonomical evaluation based on volatile oils in various taxa and on the range of various oil constituents suggested making use of both studies for understanding the variation and formation of taxa in nature as well as in the natural classification of plants.

Materials and Methods

Plant materials. For the extraction of essential oils, the raw materials were collected from seven aromatic members of Asteraceae. The collected plant materials were cleaned and shade dried at room temperature, as shade drying reduces the weight of herb to 1/3 of the fresh weight and maximizes the oil yield with out affecting the quality of the essential oil.

Isolation of essential oil. Separation of the volatile oils from the dried, flaked and powdered plant tissue was conducted by hydro-distillation in a Clevanger apparatus (Clevanger 1928) for 4 to 5 h, as prolonged extraction normally increases the yield (Gildemeister and Hoffman 1961). Extraction was carried out at 80 to 230˚C temperature. The percentage of essential oils was calculated on a dry weight basis to avoid faulty estimation that may arise due to the different water content of the tissue analysed each time (Von Rudloff

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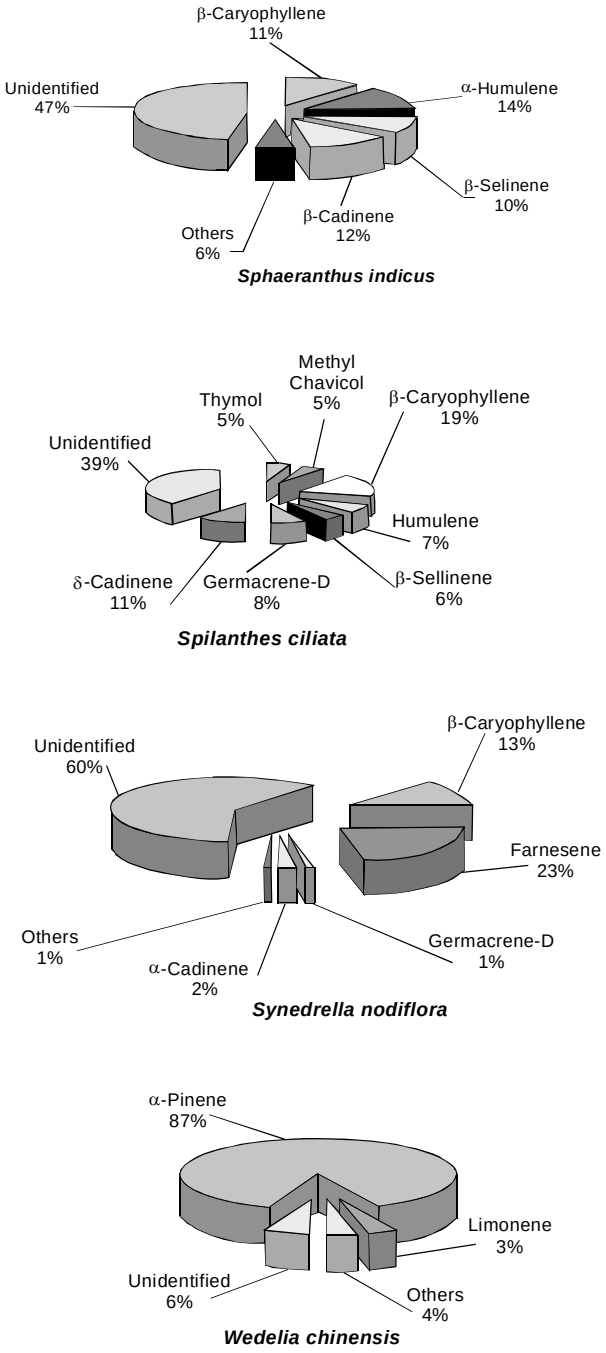


Fig 1. Composition of major essential oil components of *S. indicus,S.ciliata, S. nodiflora and W. chinensis.*

1972). The isolated oil was then dried over anhydrous sodium sulphate and stored at 4 to 6˚C.

Qualitative analysis. Qualitative estimation of the essential oils is done by capillary Gas Chromatography (GC) (Bahl and Bahl 1983). For each plant, GC analysis was performed by using a Chemito Gas Chromatograph (model CHEMITO 8510) equipped with a flame ionisation detector (FID). The capillary

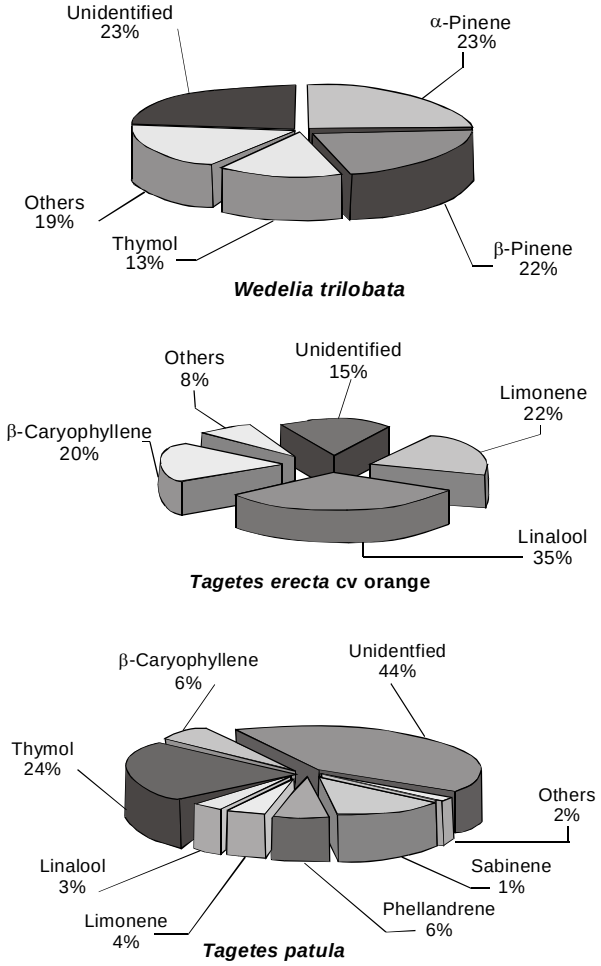


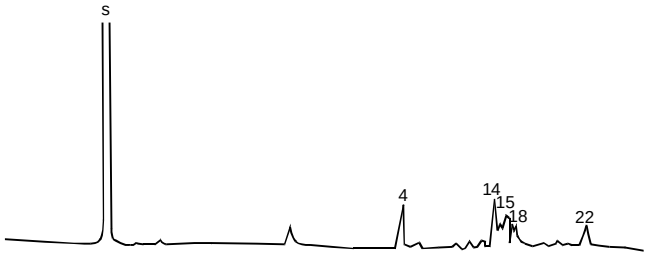
Fig 2. Composition of major essential oil components of *W. trilobata, T. erecta* cv orange and *T. patula.*

GC was carried out on 5% SE – 30 packed column with the following dimensions mesh size – 80/100; length - 8 feet, diameter - 1/8 inch. Nitrogen was used as a carrier gas at 10 psi (inlet pressure) with a flow rate of 30 ml/min. Temperature programming was performed from 80 to 230˚C at the rate of 6˚C/min. Major components were identified by retention time (RT) analysis (Finar 1975) and peak enrichment by co-injection with authentic standards (Jefferys *et al* 1989). Relative retention times of these reference standards were obtained by taking gas chromotograms of their mixtures in similar conditions.

Quantitative analysis. In GC, the quantification of the peak areas were done by the Shimadzu integrator having a built in computer (model CR3A - CHROMATOPAC). The quantitative data, obtained thereby, were based on computer integrated peak area calculations. Since the essential oils in most cases were diluted with a solvent (toluene), corrections have been made accordingly and accurate quantitative values were determined for each component separately. The corrected values are shown in the pie chart, Fig 1 & 2.

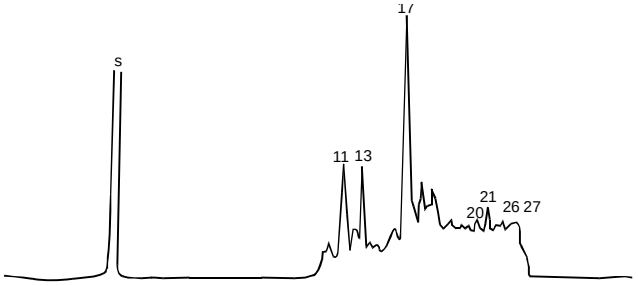
Table 1
List of various taxa investigated for their major essential oil components and chemotypes

S.No.	Name of taxa	Essential oil components	(%)	Chemotype
1.	*Sphaeranthus indicus*	α-Humulene	14.10%	
		δ-Cadinene	12.50%	Mixed
		β-Caryophyllene	10.80%	
		β-Selinene	9.85%	
		β-Ionone	5.50%	
2.	*Spilanthes ciliata*	β-Caryophyllene	19.36%	
		δ-Cadinene	10.97%	Mixed
		Germacrene-D	8.00%	
		α-Humulene	6.60%	
		β-Selinene	5.60%	
		Methyl chavicol	5.30%	
		Thymol	5.00%	
3.	*Synedrella nodiflora*	Farnesene	22.75%	
		β-Caryophyllene	12.70%	Mixed
		δ-Cadinene	2.35%	
		Germacrene-D	1.27%	
		Limonene	0.27%	
		β-Pinene	0.20%	
		α-Pinene	0.03%	
4.	*Wedelia chinensis*	α-Pinene	87.60%	α–pinene
		Limonene	3.33%	
		β-Pinene	2.70%	
		β-Caryophyllene	0.38%	
		Borneol	0.23%	
5.	*Wedelia trilobata*	α-Pinene	23.70%	
		β-Pinene	21.99%	Mixed
		Thymol	12.70%	
		β-Caryophyllene	5.26%	
		Limonene	5.21%	
		β-Phellandrene	3.65%	
6.	*Tagetes erecta* cv orange	Linalool	34.50%	
		Limonene	21.95%	
		β-Caryophyllene	20.36%	Mixed
		Piperitone	3.13%	
		β-Pinene	2.96%	
		Sabinene	1.29%	
		α-Pinene	0.36%	
7.	*Tagetes patula*	Thymol	23.65%	
		Sabinene	11.29%	Mixed
		β-Caryophyllene	6.19%	
		β-Phellandrene	5.96%	
		Limonene	4.18%	
		Linalool	3.38%	
		β-Pinene	0.97%	
		Piperitone	0.64%	
		α-Pinene	0.37%	



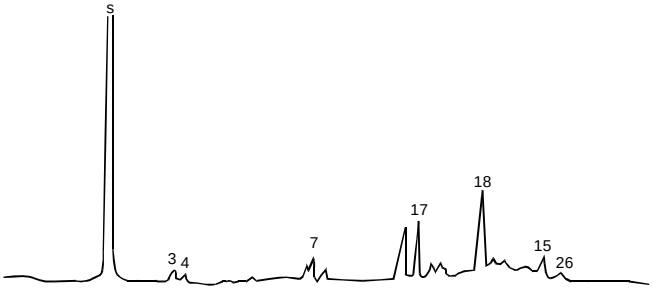
(a) Sphaeranthus indicus

S-solvent, 4-β-caryophyllene, 14-humulene, 15-selinene,18-ionone, 22-δ-cadinene.



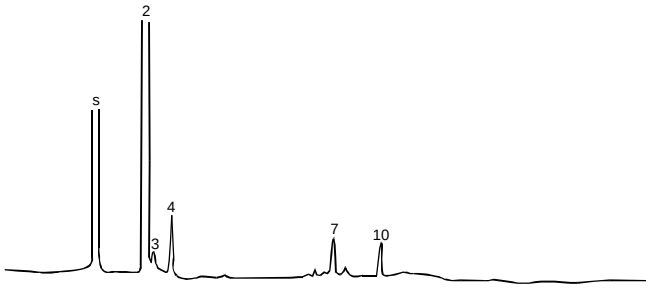
(b) Spilanthes ciliata

S-solvent, 11-thymol, 13-methyl chavicol, 17-β-caryophyllene, 20-humulene, 21-β-selinene, 26-germacrene-D, 27-δ-cadinene.



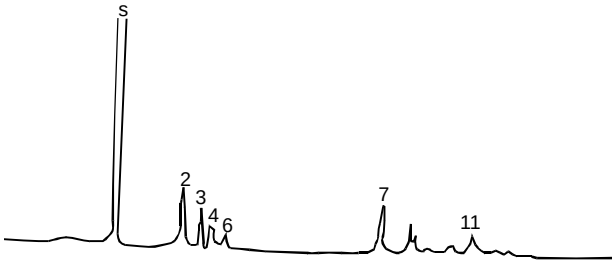
(c) Synedrella nodiflora

S-solvent, 3-α-pinene, 4-β-pinene, 7-limonene, 13-β-caryophyllene, 18-farnesene, 25-germacrene-D, 26-δ-cadinene.



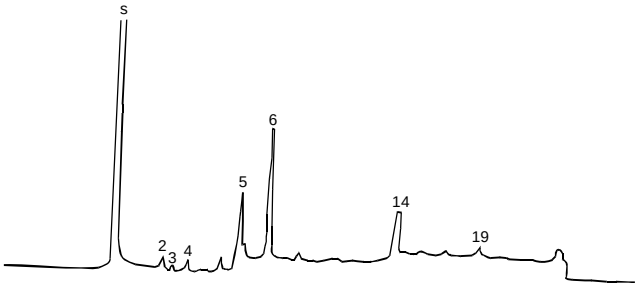
(d) Wedelia chinensis

S-solvent, 2-α-pinene, 3-β-pinene, 4-limonene, 7-borneol, 10-β-caryophyllene.



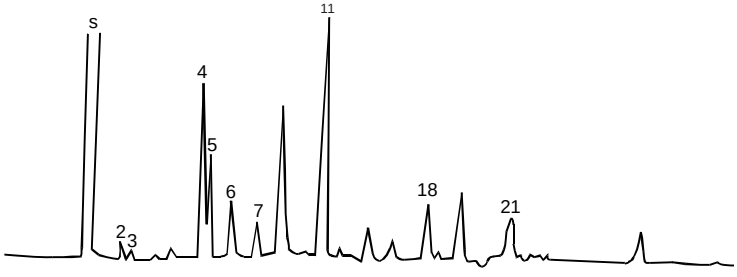
(e) Wedelia trilobata

S-solvent, 2-α-pinene, 3-β-pinene, 4-β-phellandrene, 6-limonene, 7-thymol, 11-β-caryophyllene.



***(f) Tagetes erecta* cv orange**

S-solvent, 2-α-pinene, 3-β-pinene, 4-sabinene, 5-limonene, 6-linalool, 14-β-caryophyllene, 19-piperitone.



(g) Tagetes patula

S-solvent, 2-α-pinene, 3-β-pinene, 4-sabinene, 5-β - phellandrene, 6-limonene, 7-linalool, 11-thymol, 18-β - caryophyllene, 21-piperitone.

Fig 3. Gas chromatograms of various essential oil yielding plants of (a) *Sphaeranthus indicus* (b) *Spilanthes ciliata* (c) *Synedrella nodiflora* (d) *Wedelia chinensis* (e) *Wedelia trilobata* (f) *Tagetes erecta* cv orange (g) *Tagetes patula*.

Evaluation of ecological role of terpenoids. The role of terpenoids in the ecology of different plant is evaluated, based on the field survey and observations noted during the collection of plants from original localities and cultivation of different plants in the botanical garden. The available literature data is also being used to correlate these information to discuss the role of these chemical components in the well being of these plants.

Results and Discussion

The investigation results of seven following taxa for their major essential oil components and chemotypes have been summarised in Table 1. A mixed chemotype is noted in *Sphaeranthus indicus* and sesquiterpene predominate over monoterpenes in the oil sample. α-Humulene (14.10%), δ-cadinene (12.50%), β-caryophyllene (10.80%), β-selinene (9.85%) and β-ionone (5.50%) are the major components present. The medicinal properties and other value added properties reported on this plant are attributed to these chemical compounds (Fig 3a - g).

The medicinal plant *Spilanthes ciliata* belong to mixed chemotype. The main components are β-caryophyllene (19.36%), δ-cadinene (10.97%), germacrene-D (8.00%), α-humulene (6.60%), β-selinene (5.60%), methyl chavicol (5.30%) and thymol (5.00%). Previous reports of Lemos confirmed the presence of these components in *S.ciliata* (Lemos *et al* 1991). The presence of these components seems to be responsible for the medicinal properties reported for *S.ciliata*. In *Synedrella nodiflora* mixed chemotype is noticed, α-farnesene (22.75%) and beta-caryophyllene (12.70%) are the major components identified. Previous chemical reports of Aalbersberg and Sing (1990) also agreed with the presence of these components.

The two species studied under the genus *Wedelia* show clear interspecific variations with regard to the chemical constituents. The chemotype of *W. chinensis* is seemed to be an α-pinene chemotype. More than 50% of the oil contains monoterpenes. The major essential oil constituents, identified are α-pinene (87.60%), limonene (3.33%) and β-pinene (2.70%). While in the oil rich taxa *W.trilobata* is a mixed chemotype. Major components are α-pinene (23.70%), β-pinene (21.99%), thymol (12.70%), β-caryophyllene (5.26%), limonene (5.21%) and β-phellandrene (3.65%). Previous reports show the existence of these components in *Wedelia* (Craverio *et al* 1993).

The two species studied under the genus *Tagetes* show marked similarity. *Tagetes erecta* cv orange and *T. patula* are characterised with a mixed chemotype. The identified major components of the oil of *T. erecta* are linalool (34.50%), limonene (21.95%) and β-caryophyllene (20.36%). In the case of *T. patula,* the main components were found are thymol (23.65%), sabinene (11.29%), β-caryophyllene (6.19%), β-phellandrene (5.96%), limonene (4.18%), and linalool (3.38%) along with traces of β-pinene (0.97%), piperitone (0.64%) and α-pinene (0.37%). Thymol is a reported chemical compound well known for its medicinal, aromatic and flavouring properties (Sobti *et al* 1977). This might have been the major factor responsible for the value added properties reported on *Tagetes.* Tagetes oil is commercially produced from *T.patula.* It is mainly used for the compounding of high grade perfumes. The oil, with its floral note and strongly waxy character, could find some special applications in perfumery.

Sesquiterpenes have a common occurrence in the essential oil of Asteraceae. Sesquiterpene lactones have an important role in Asteraceae members as chemical defence agents. They are often present in high concentration and relatively complex mixtures of different structures are found in many species. Usually bitter tasting, they are obviously deterrent to many kinds of herbivore, including man himself (Harborne 1991). Their effects on insects are very damaging and they are also poisonous to live stocks. Other properties possessed by these sesquiterpene lactones include their pungent taste and their ability to act as allergens (Mitchell *et al* 1970).

The biological activities associated with sesquiterpenes, are many and varied, from plant growth regulation (abscissic acid) to interfere with insect metamorphosis. The sesquiterpene lactones likewise have a wide range of demonstrated biological activities, as cytotoxic compounds, vertebrate poisons insect feeding deterrents, schistosomicidal substances, and allergenic agents (Rodriguez *et al* 1976). Some sesquiterpenes protect plants from insect attack because of their antimicrobial properties. This appears to be true of caryophyllene and caryophyllene epoxide which are repellent to the leaf cutting ants *Attacephalotes* and *Acromyrmex octopinosus*. Caryophyllene is present in all investigated species as revealed in Fig 3.

Conclusion

The natural purpose of the synthesis and accumulation of terpenoids in the plant kingdom still possess a considerable question mark to phytochemists. As discussed above, they have some role in plant growth regulation. In pollination biology, in various interactions with other plants, animals or insects, in defence against diseases and in other attacks. However, the studies on the chemical defensive mechanism of terpenoids are still at an early stage. Hence, it is high time to collect sufficient data and to duly emphasis the ecological role of terpenoids as primarily defensive agents against over grazing. This topic therefore, offers ample scope for future research programmers.

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SOME PHYSICAL PARAMETERS OF THE SANDSPIT BACKWATERS, KARACHI COAST

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Certain physical parameters like, salinity, temperature, dissolved oxygen, pH, rainfall, grain size analysis and organic content of the sediment from the Sandspit backwater, Karachi have been studied. The sampling was done on a fortnightly basis for a period of two years i.e. June 1987 to May 1988. The salinity of the backwater ranged between 16.99 to 45.00 ppt with an average value of 37.98 ± 4.32 ppt (S.D.) (n = 48). The open sea salinity varied from 34.30 to 39.48 ppt, with an average salinity value of 36.91 ± 0.97 ppt. (S.D.) (n = 48). The average temperature of the backwater was found to be 28.9 ± 3.9°C (S.D.) (n = 48) with a range of 19.5°C to 34.5°C. The average temperature of the open sea was found to be 27.2 ± 2.5 °C (S.D.) (n = 48). The lowest temperature was 22.0°C, whereas the highest temperature was 33.0°C. The monthly mean of daily maximum air temperature ranged between 25.6 to 36.4°C, while the monthly mean of daily minimum air temperature ranged between 10.5 to 28.4°C. Mean monthly temperature ranged between 18.1 to 32.3°C. The maximum rainfall was 85 mm in the month of August 1988, while the minimum rainfall recorded was 0.5 mm in the month of January 1989. The mean value of dissolved oxygen in the backwater was found to be 5.01 ± 0.97 ml/l (S.D.) (n = 48). The lowest value was 3.00 ml/l, whereas the highest value was 6.55 ml/l. The average value of dissolved oxygen for the open sea was found to be 5.25 ± 0.85 ml/l (S.D.) (n = 48). The average pH of the backwater was found to be 7.61 ± 0.46 (S.D.) (n=48), whereas, it ranged from 6.34 to 8.05. The samples collected from intertidal pool had greater percentage of sand fraction (79.70-89.84%) as compared to those collected from mud flat, which had 63.33 to 79.86 % of sand fraction. The organic content was found to vary from 3.19 to 8.32 %.

Key words: Salinity, Temperature, pH, Dissolved oxygen, Rainfall, Organic carbon, Karachi coast.

Introduction

Sandspit backwater is known as a nursery ground for various species of fish and shellfish. It is located between Manora and Hawks Bay at 24°50' N and 66°56' E i.e. southwest of Karachi. The backwater is connected to the Arabian Sea through Manora Channel (Fig 1). During high and low tides, water enters the Sandspit backwater area and drains back periodically to the Arabian Sea through Manora channel. It is estimated that an average volume of about 3.4 million m^3 of seawater enters and leaves the backwater area during the tidal cycle (Haq 1976). The backwater also receives discharge from the Lyaria river, which is not a perennial river. The Lyari river discharges freshwater during the rainy season, which lasts for two to three months (July through September), while during the rest of the year, it discharges mostly industrial and domestic effluents. It has been estimated that the Lyari river brings 120 million gallon per day of municipal and industrial wastewater with an organic load of 2000 tons of BOD per day. (Beg *et al* 1984).

Our knowledge regarding physical parameters of the Sandspit backwater is very limited. Some information is available through the work of Nooruddin and Khursheed-uddin (1968) Beg *et al* (1984), Qasim *et al* (1986), Khan and Saleem (1988), Siddiqui and Qasim (1988), Barkati and Tirmizi (1990), and Saifullah and Rasool (1998). While investigating the bionomics and population structure of the juvenile shrimps occurring in the Sandspit backwater it was felt necessary that a survey of the range of habitat in which these juvenile shrimps dwell should be examined. Hence data on the distribution and fluctuation of certain physical parameters like, salinity, temperature, dissolved oxygen, pH and rainfall were recorded for a period of two years. Grain size analysis and organic content of the sediment inhabited by the juvenile shrimps were also studied on some occasions.

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Materials and Methods

For the determination of salinity, surface seawater was collected in screw-capped amber glass bottles, from the Sandspit backwater (station 1 in Fig 1), on a fortnightly basis, for a period of two years (June 1987 to May 1989). For comparison, another sample of seawater was collected from the open sea of the Sandspit beach (station 2 in Fig 1). The bottles were sealed immediately with parafilm. The salinity was measured in the laboratory by a conductivity salinometer (Model E-2,

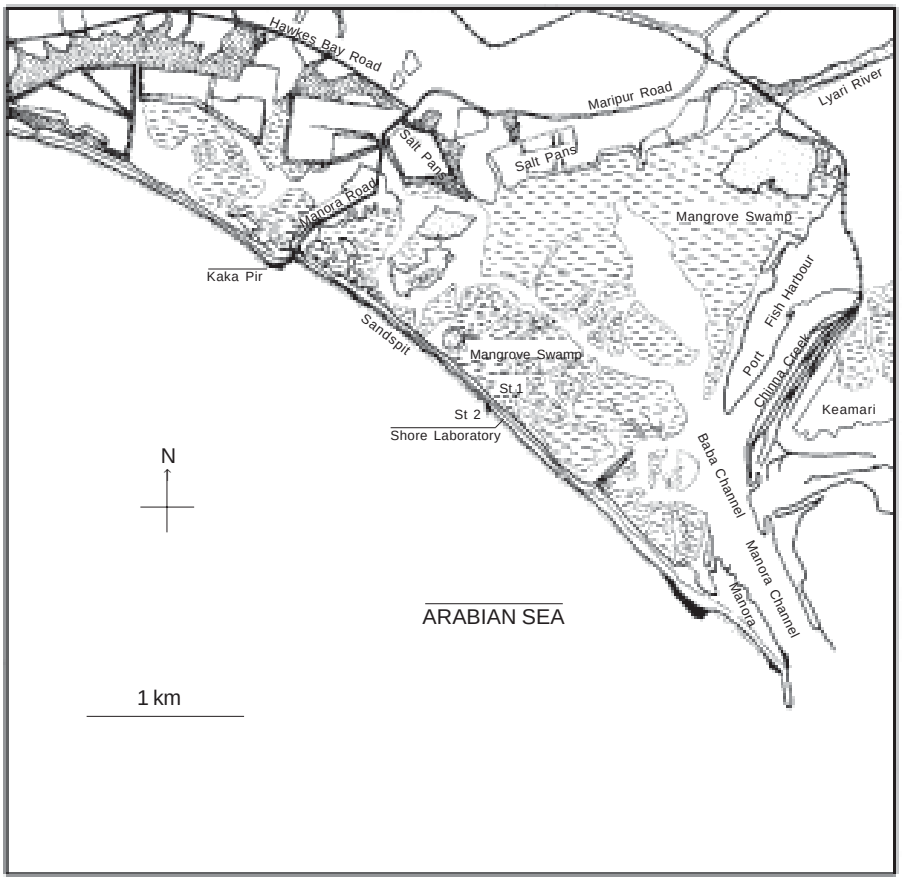


Fig 1. Map of the Karachi coast showing collection site: St. 1 Sandspit backwater; St.2, Open sea.

Ogawa Sieki Incorporation). The salinometer was calibrated with standard seawater and had a maximum range of 40 ppt. A portable salinity refractometer (Model S/Mill-E, Atago Co. Ltd.) was used for those samples whose salinity was found greater than 40 ppt. Temperature was recorded by a mercury thermometer. Dissolved oxygen and pH were recorded *in situ* by the use of a portable oxygen meter (Model 820, Orion Research Incorporated) and a portable pH meter (PICCOLO ATC), respectively.

For grain size analysis and organic content, sediment samples were collected on four occasions; 29th October, 1987, 13th April, 1988, 24th October, 1988 and 16th April, 1989 from the backwater area. The samples collected in the month of October represent the post-monsoon (southwest) period and the samples collected in the month of April, represent the pre-monsoon (southwest) period. On each occasion, two samples were collected. One of the samples was collected from the intertidal pond (hereafter referred as sub-station 1-A), while the other sample was collected from the nearby mud flat exposed during low tide (hereafter referred as sub-station 1-B). The sediment from each sub-station was collected with a plastic corer, having a diameter of 10cm. The corer was pushed about 15cm into the sediment and the core so obtained was placed in a plastic bag. For grain size analysis, the method described by Buchanan (1984) was followed. Based on the data cumulative curves were drawn and statistical parameters such as graphic mean (Mz), inclusive standard deviation (s), inclusive graphic skewness (Ski) and Graphic curtosis (K_G) were determined. The samples were categorized according to scheme proposed by Folk (1968). For estimation of the organic content, a modification of Schollenberger chromic acid oxidation technique was used. The analytical procedure is mainly taken from Walkley and Blake (1934). The data for air temperature and the rainfall were obtained from the Meteorological Department, Government of Pakistan.

Results and Discussion

Salinity. Figure 2 shows the fluctuation of salinity of the backwater and of the open sea from June 1987 through May 1989. The salinity of the backwater ranged between 16.99 ppt on 17th July, 1988 to 45.00 ppt on 14th December, 1987. The average salinity for the study period (24 months) was found

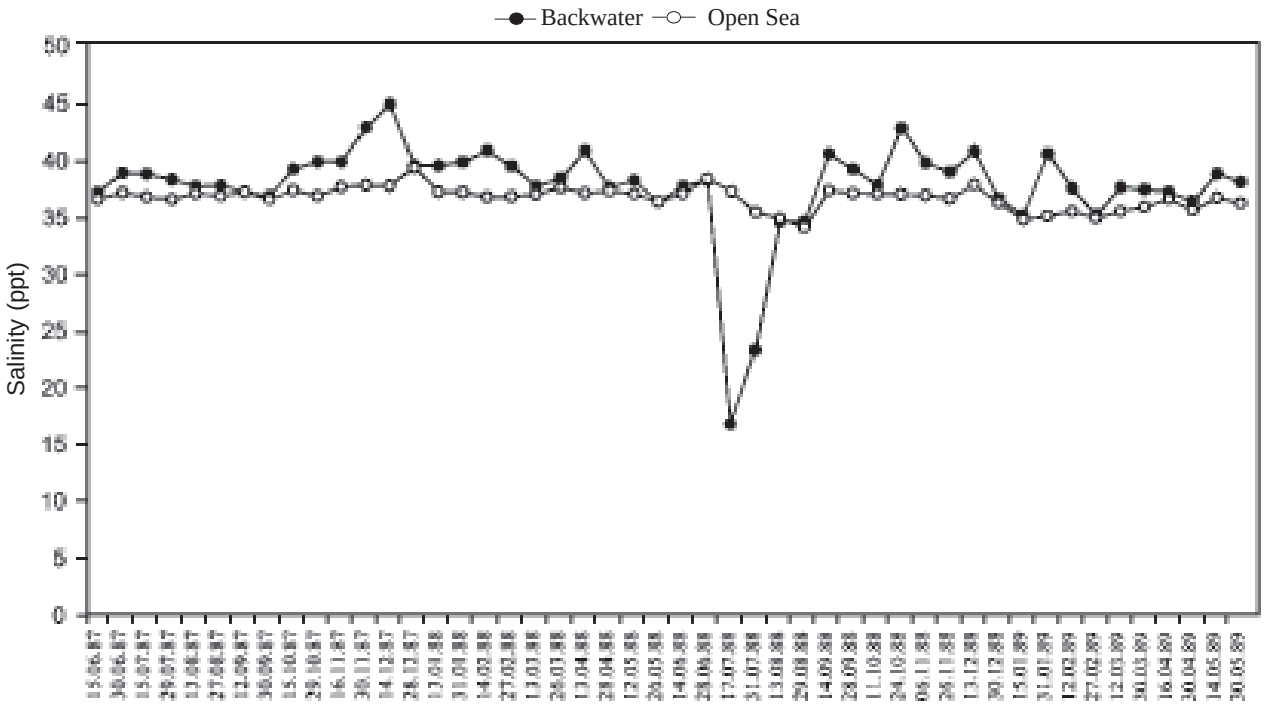


Fig 2. Salinity of the backwater and water from open sea during June 1987 - May 1989.

to be 37.98 ± 4.32 ppt (S.D.) (n = 48). During southwest monsoon period of 1987 (June to September), the average salinity of the backwater was 37.96 ± 0.75 ppt (S.D.) (n = 8)), whereas in the same monsoon period of 1988 (May to September), it was much lower i.e. 34.17 ± 7.73 ppt (S.D.) (n = 10). The average salinity of the backwater during the northeast monsoon period of 1987-1988 (November to March) was 40.44 ± 2.11 ppt (S.D.) (n = 10), while in the same monsoon period of 1988-1989 (November to March) the salinity was 38.22 ± 1.90 ppt (S.D.) (n = 10). The average salinity for the first year of study (June 1987 through May 1988) was 39.22 ± 1.92 ppt (S.D.) (n = 24), while for the second year of study (June 1988 through May 1989), it was 36.73 ± 5.55 ppt (S.D.) (n = 24).

The open sea salinity varied from 34.30 ppt on 29th August, 1988 to 39.48 ppt on 28th December, 1987. The average salinity for the study period was found to be 36.91 ± 0.97 ppt (S.D.) (n = 48). The average salinity during the southwest monsoon period of 1987 (June to September), was found to be 36.98 ± 0.27 ppt (S.D.) (n = 8), whereas, in 1988 southwest monsoon period it was 36.71 ± 1.30 ppt (S.D.) (n = 10). During the northeast monsoon period of 1987 - 1988 (November to March), the average salinity was 37.68 ± 0.73 ppt (S.D.) (n = 10). In the 1988-1989, northeast monsoon period (November to March), the average salinity was 36.20 ± 0.93 ppt (S.D.) (n = 10). During the first year of the study (June 1987 to May 1988), the average salinity was 37.33 ± 0.60 ppt (S.D.) (n = 24), whereas in the second year (June 1988 to May 1989), it was 36.51 ± 1.08 ppt (S.D.) (n = 24).

Seawater temperature. The variation in temperature of the backwater and of the open sea is presented in Fig 3. The values shown are only day temperatures recorded during low tides. The average temperature of the backwater for 24 months was found to be 28.9 ± 3.9˚C (S.D.) (n = 48). The lowest temperature 19.5˚C was recorded on 28th December, 1987, while the highest temperature 34.5˚C was recorded on 30th June, 1987. The average temperature for the southwest monsoon of 1987 (June to September) was found to be 32.50 ± 1.04˚C (S.D.) (n = 8), whereas in 1988 southwest monsoon (May to September) it was slightly lower, i.e. 30.90 ± 1.54˚C (S.D.) (n = 10). During northeast monsoon of 1987-1988 (November - March), the average temperature was 25.20 ± 4.14˚C (S.D.) (n = 10) and for the same monsoon period of 1988-1989 (November to March) it was slightly higher, i.e. 26.25 ± 2.62˚C (S.D.) (n = 10). On the other hand, the average temperature of the open sea for 24 months was found to be 27.2 ± 2.5˚C (S.D.) (n = 48) The lowest temperature 22.0˚C was recorded on 14th December, 1987 whereas the highest temperature 33.0˚C was recorded on 30th June, 1987. During the southwest monsoon of 1987 (June to September) and 1988 (May to September), the average values of seawater temperature was 29.88 ± 1.62˚C (S.D.) (n = 8) and 28.95 ± 1.79˚C (S.D.) (n = 10), respectively. Similarly during the Northeast monsoon of 1987-1988

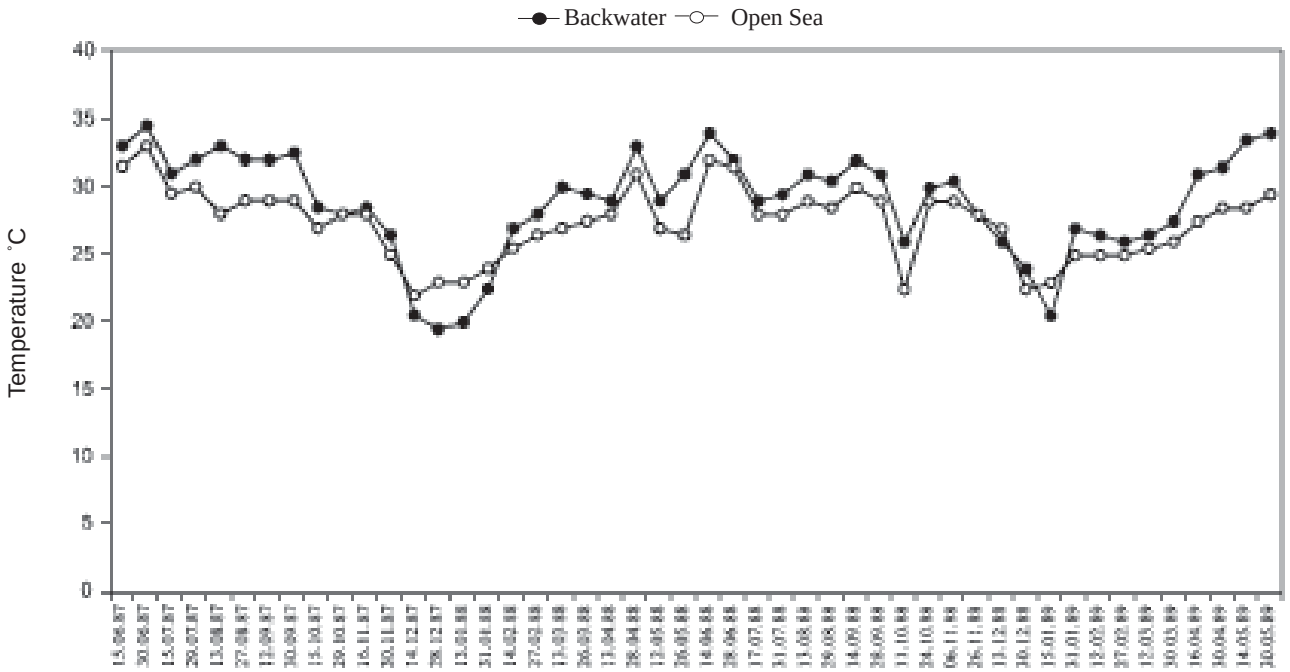


Fig 3. Temperature of the backwater and water from open sea during June 1987 - May 1989.

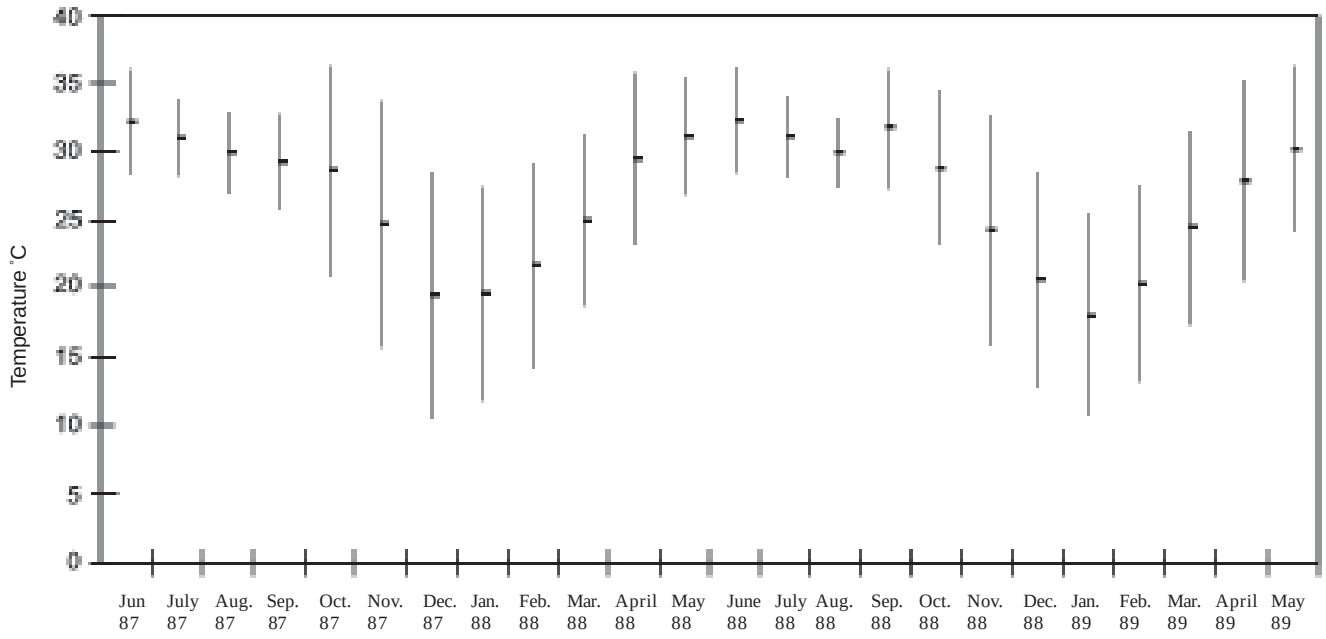


Fig 4. Air temperature at Karachi during the study period. Horizontal bar indicates mean monthly temperature and vertical iline shows range between monthly means of daily maximum and minimum temperature.

(November to March) and 1988-1989 (November to March), the average temperature was found to be 25.15 ± 2.10°C (S.D.) (n = 10) and 25.60 ± 2.02°C (S.D.) (n = 10), respectively.

Air temperature: Figure 4 shows the variation in air temperature of Karachi, from June 1987 through May 1989. The monthly mean of daily maximum temperature ranged between 25.6°C in January 1989 to 36.4°C in May 1989, while the monthly mean of daily minimum temperature ranged between 10.5°C in December 1987 to 28.4°C in June 1988. The mean monthly temperature ranged between 18.1°C in January 1989 to 32.3°C in June 1988. During the southwest monsoon of 1987 (May to September), the average air temperature was 30.58 ± 1.24°C

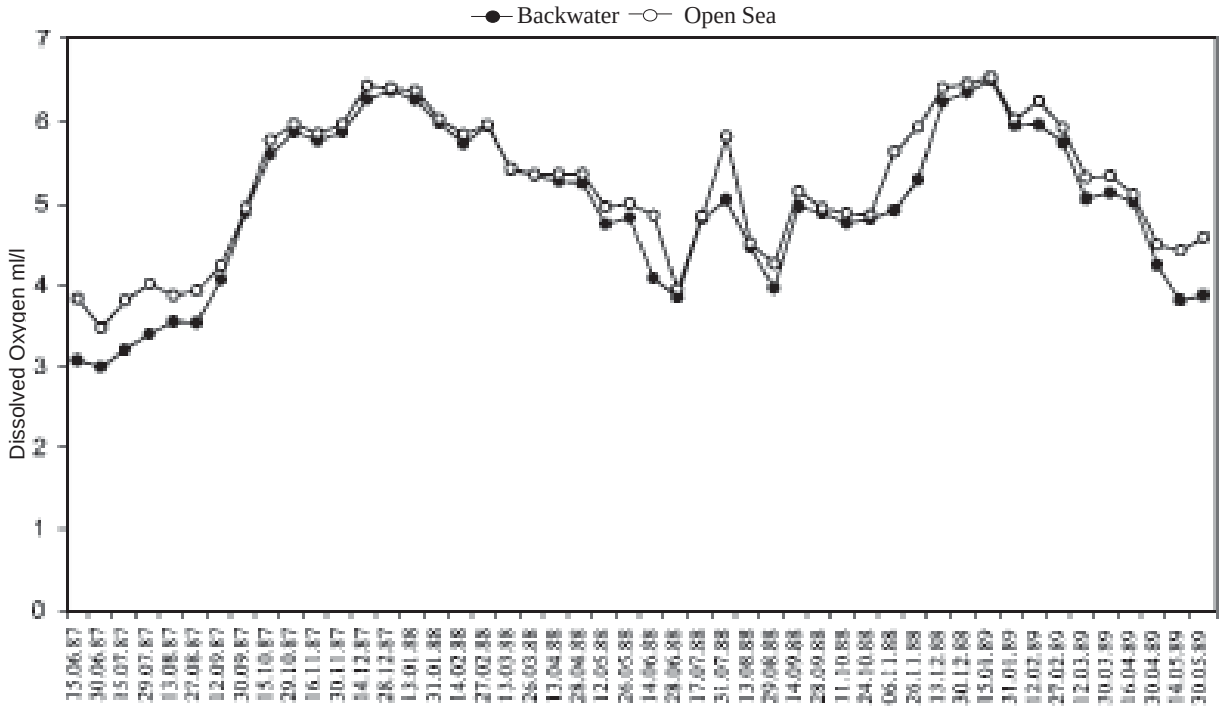


Fig 5. Dissolved oxygen in backwater and water from open sea during June 1987 - May 1989.

(S.E.) (n = 5), whereas in the same monsoon period of 1988 (May to September), it was 31.24 ± 0.90°C (S.E.) (n = 5). In the northeast monsoon period of 1987 - 1988 (November to March), the average air temperature was 22.10 ± 2.66°C (S.E.) (n = 5) and for the same monsoon period of 1988 - 1989 (November to March), the temperature was 23.22 ± 3.94°C (S.E.) (n = 5).

Dissolved oxygen. Figure 5 shows the amount of dissolved oxygen found in the backwater as well as in the water from the open sea during the study period. In the backwater, the mean value of dissolved oxygen was found to be 5.01 ± 0.97 ml/l (S.D.) (n = 48). The lowest value 3.00 ml/l was found on 30th June 1987, whereas the highest value 6.55 ml/l was recorded on 15th January, 1989. In the period of the southwest monsoon of 1987 (June to September), the average value of dissolved oxygen was 3.60 ± 0.63 ml/l (S.D.) (n = 8), while in the same monsoon period of 1988 (May to September) the average value was 4.60 ± 0.44 ml/l (S.D) (n = 10). During the northeast monsoon of 1987-1988 (November to March) the average dissolved oxygen was 5.91 ± 0.34 ml/l (S.D.) (n = 10) and 5.76 ± 0.58 ml/l (S.D.) (n = 10) was the average value for the same monsoon period of 1988-1989. During first year of the present investigation (June 1987 to May 1989), the average value of dissolved oxygen was 4.98 ± 1.13 ml/l (S.D.) (n = 24), while in the second year, it was found to be slightly higher i.e. 5.03 ± 0.81 ml/l (S.D.) (n = 24).

The average value of dissolved oxygen for the open sea was found to be 5.25 ± 0.85 ml/l (S.D.) (n = 48) during the study period. The lowest value 3.48 ml/l was found on 30th June, 1987, while the highest value 6.58 ml/l was recorded on 15th January, 1989. The average value of dissolved oxygen during the southwest monsoon period of 1987 (June to September) and 1988 (May to September) was found to be 4.02 ± 0.44 ml/l (S.D.) (n = 8) and 4.86 ± 0.51 ml/l (S.D.) (n = 10), respectively. Similarly, during the northeast monsoon of 1987-1988 and 1988 - 1989 (November to March), the average value was 5.98 ± 0.37 ml/l (S.D.) (n = 10) and 6.03 ± 0.44 ml/l (S.D.) (n = 10), respectively. During first year of the investigation, (June 1987 to May 1988), value of dissolved oxygen was 5.19 ± 0.95 ml/l (S.D.) (n = 24), while during second year (June 1988 to May 1989), it was 5.39 ± 0.72 ml/l (S.D.) (n = 24), slightly higher than the first year.

Rainfall. Out of 24 months, it rained only in six months, while the remaining 18 months were dry. The maximum rainfall (85mm) was recorded in August 1988, while the minimum rainfall (0.5mm) was recorded in January 1989. The other four months, which received rainfall were January 1988 (1mm), July 1988 (74mm), February 1989 (8.3mm) and March 1989 (1.5mm). The first year of the present investigation was dry, only 1mm rain fell in the month of January 1988 (northeast monsoon period), the other eleven months were dry. The southwest monsoon period, which is the main rainy season was dry and there was no rain at all. However, in the second year, 160.5 mm rain was recorded. Of this, 159.0 mm rain fell in the month of July and August (southwest monsoon period), the remaining

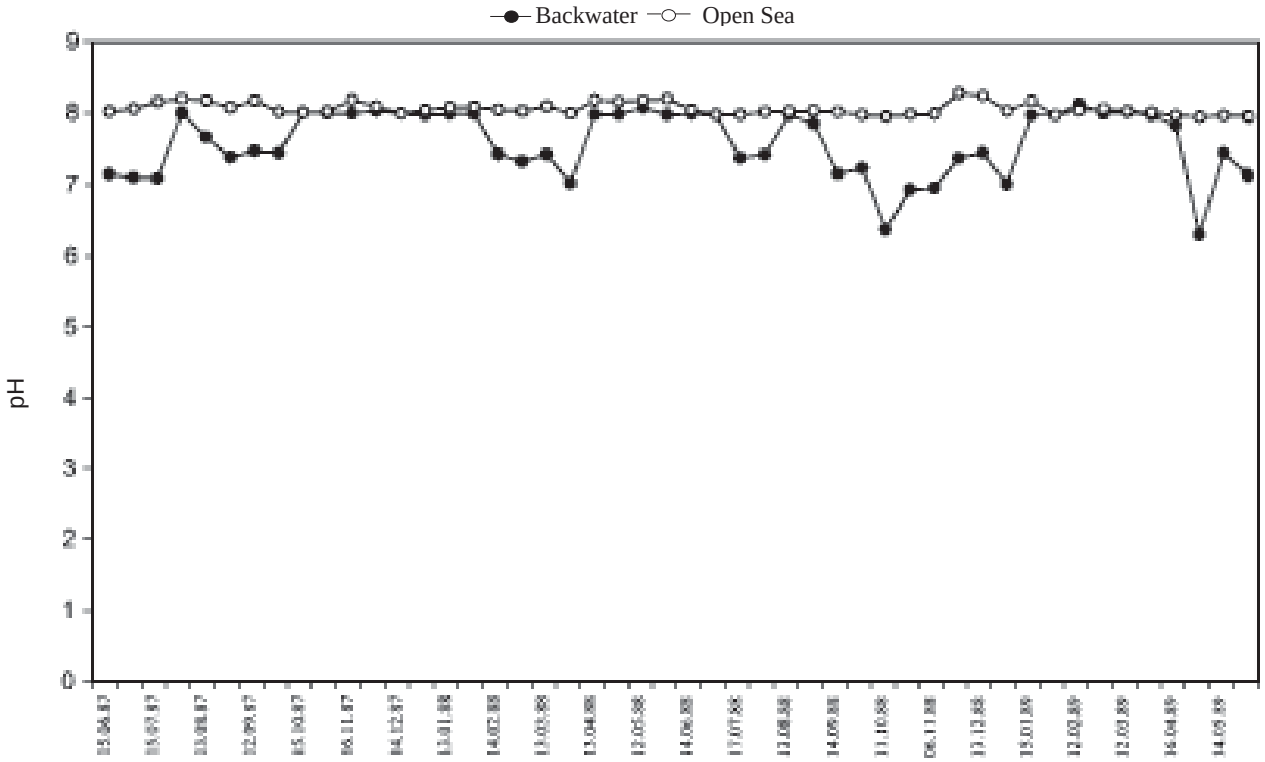


Fig 6. Variation in pH of the backwater and water from open Sea during June 1987 - May 1989.

1.5 mm rain fell in the month of March 1989 (northeast monsoon period).

Seawater pH. Figure 6 shows the fluctuation of pH values of the backwater and of the open sea, during the study period. The average pH was found to be 7.61 ± 0.46 (S.D.) (n = 48) for the period June 1987 to May 1989. The pH of the backwater ranged between 6.34 on 30th April, 1989 to 8.05 on 12th March, 1989. During the southwest monsoon of 1987 (June to September) and 1988 (May to September) the average value of pH was found to be 7.42 ± 0.31 (S.D.) (n = 8) and 7.73 ± 0.36 (S.D.) (n = 10), respectively. During the northeast monsoon of 1987-1988 and 1988-1989 (November to March), the average value of pH was 7.73 ± 0.37 (S.D.) (n = 10) and 7.71 ± 0.45 (S.D.) (n = 10), respectively.

The average value of pH was found to be 8.08 ± 0.08 (S.D.) (n = 48). The lowest pH was recorded on two occasions, first on 11th October, 1988 and then on 30th April, 1989. The highest pH 8.3 was recorded on 26th November, 1988. During the southwest monsoon period of 1987 (June to September) and 1988 (May to September) the average value of pH was 8.11 ± 0.08 (S.D.) (n = 8) and 8.07 ± 0.08 (S.D.) (n = 10), respectively. During the Northeast monsoon of 1987-1988 and 1988-1989 (November to March), the average value of pH was found to be 8.08 ± 0.06 (S.D.) (n = 10) and 8.11 ± 0.11 (S.D.) (n = 10), respectively. The average pH of the backwater was found to be 8.09 ± 0.52 (S.D) and of the open sea 8.08 ± 0.08 (S.D).

Grain-size analysis. Table 1 shows the grain size analysis of the sediment collected from the study area. The samples collected from sub-station 1-A (inter-tidal pool) had greater percentage of sand fraction (79.70-89.84%) as compared to those collected from sub-station 1-B (mudflat), which had 63.33 to 79.86% of sand fraction. During the post-monsoon (SW) period of 1987 and 1988, the sand fraction was 89.84% and 87.91% respectively for the samples collected from sub-station 1-A. From sub-station 1-B samples had 78.26% and 79.86% of the sand fraction for the post-monsoon (SW) period of 1987 and 1988, respectively. In the samples collected during the pre-monsoon (SW) period of 1988 and 1989, the sand fraction was found to be much lower. The two samples collected from station sub-station 1-A had 79.70 and 81.93% of sand in the pre-monsoon period of 1988 and 1989, respectively. Similarly in the same pre-monsoon periods, samples collected from sub-station 1-B had 66.80% and 63.33% of sand.

The highest percentage of silt (coarse and fine) i.e. 28.67% was found in the sample collected from sub-station 1-B on 24th October, 1988 (pre-monsoon), whereas, the lower percentage of silt (coarse and fine) 9.82 was found in the samples of sub station 1-A, collected on 29th October, 1987 (pre-monsoon). The

Table 2
Textural characteristics of the sediment samples collected from the Sandspit backwaters

Textural characteristis	29.10.1987		13.04.1987		24.10.1988		16.4.1989	
	Sub-station 1-A	Sub-station 1-B	Sub-station 1-A	Sub-station 1-B	Sub-station 1-A	Sub-station 1-B	Sub-station 1-A	Sub-station 1-B
M_Z (phi)	0.650	2.300	0.700	2.600	0.700	2.250	0.750	2.700
σ_1	1.280	2.464	1.950	2.560	1.540	2.290	2.290	2.800
Sk_1	0.609	0.423	0.789	1.000	0.690	0.440	0.780	0.390
K_G	2.950	3.684	1.410	0.860	1.440	1.800	1.770	0.760

Table 3
Organic carbon of the sediment collected from the Sandspit backwaters

	29.10.1987 post-monsoon (%)	13.04.1988 pre-monsoon (%)	24.10.1988 Post-monsoon (%)	16.04.1989 Pre-monsoon (%)
Sub-station 1-A Inter-tidal pool	6.06	3.19	5.87	4.05
Sub-station 1-B (Mud flat)	8.32	6.09	7.89	7.20

percentage of clay varied from 0.20 (substation 1-A) on 24th October, 1988 to 6.27 (sub-station 1-B) on 16th April, 1989.

The average grain size (Mz) of sub-station 1-A samples was found to vary from 0.65 to 0.75 ϕ (0.67500 to 0.61250 mm) and those of sub-station 1-B sample was found from 2.25 to 2.70 ϕ (0.21875 to 0.16250 mm) (Table 2). Mz values for the samples collected from sub-station 1-A during post-monsoon (SW) period, was found to be 0.650 ϕ (0.675 mm) to 0.700 ϕ (0.650 mm) for 1987 and 1988, respectively. Mz values for sub-station 1-B samples, was found to be 2.300 ϕ (0.21200 mm) and 2.250 ϕ (0.21875 mm), for post-monsoon period of 1987 and 1988, respectively. For pre-monsoon period of 1988 and 1989, the Mz values for samples collected from sub-station 1-B, was 2.600 ϕ (0.1750 mm) and 2.700 ϕ (0.1625 mm) respectively. The values of inclusive standard deviation, (σ_1) for sediment samples collected from sub-station 1-A were in the range of 1.280 to 2.290, while for the sample collected from sub-station 1-B, the values were found in the range of 2.290 to 2.800. Degree of graphic skewness (Sk_1) values were found from 0.609 to 0.789 for the samples of sub-station 1-A and 0.390 to 1.000 for the samples of sub-station 1-B. The value of graphic curtosis (K_G) varied from 1.410 to 2.950 for sub station 1-A samples; and from 0.760 to 3.684 for sub-station 1-B samples i.e. platykurtic to very leptokurtic (Table 2).

Organic carbon content. Table 3 shows the organic carbon content of the sediment samples collected from the study area on four different occasions. The organic carbon was found to range from 3.19% to 8.32%. The sample collected from sub-station 1-A (inter-tidal pool) had a low organic carbon as compared to those collected from sub-station 1-B (mud flat). In the post monsoon (SW) period of 1987 and 1988, the samples of sub-station 1-A had 6.06% and 5.87% organic carbon respectively, while samples from sub-station 1-B in the same period had 8.32% and 7.89% organic carbon, respectively. In the pre-monsoon (SW) period of 1988 and 1989, samples from sub-station 1-A contained 3.19% and 4.05% organic carbon, respectively. Samples from sub-station 1-B contained higher amount of organic carbon i.e. 6.09% and 7.20% in the pre-monsoon (SW) of 1988 and 1989, respectively.

The Karachi coast lies on the western margin of one of the major climatic regions of the world, the monsoon region. The southwest monsoon blows from May to September and brings rain, which is scant. The northeast monsoon lasts from November to March and the rain during this period is even less. October and April are transition periods.

Air temperature generally follows the typical pattern of the subtropical coastal zone. It reaches to its minimum in December and January, which gradually increases from February

onward and attains a maximum reading in June. Temperature declines during July and August mainly due to the rain. If there is no rain in July and August even then the temperature is comparatively low due to the strong wind and cloudy sky. A second but comparatively small rise in temperature may occur in September-October (this rise is more prominent in monthly means of maximum temperature as shown in Fig 4), which again decreases from October onward. During the summer months, air temperature has a narrow range, whereas in winter it has a wide range, which may affect the mean value of temperature to some extent. These findings are more or less similar to those reported by Niazi (1993).

The surface temperature of seawater exhibits a more or less similar pattern to that of air temperature. The surface seawater temperature of the backwater is generally higher (by 2 - 4˚C) than the open sea during summer months, but in winter the backwater temperature is lower (by 2 - 4 ˚C) than the open sea. The highest temperature was observed in May and June, while lowest temperature was found in December and January. The results of the present study, during which a range of 19.5˚C to 34.5˚C was observed for the backwater area, agrees well with the previous studies. In their study on the temperature and salinity of the Karachi coast (Keamari and Fish Harbour), Nooruddin and Khursheeduddin (1968) reported 30.4˚C as the highest average value of monthly surface seawater temperature in June and the lowest 19.13˚C in January. Similarly Javed and Mustaquim (1993) and Saeed *et al* (1995) reported a range of 20 to 28˚C surface seawater temperature from Manora channel during December 1992 to May 1993 and 21 to 35˚C from inter-tidal and near shore water of the Karachi coast during 1992-1993, respectively. According to Hussain and Samad (1995), seawater temperature from Manora channel (Baba and Chari Kund Channel) has a range of 21 to 32˚C.

Salinity of the Sandspit backwater was found to be highly variable as compared to that of the open sea. The difference between the minimum and maximum value of the seawater was 28.01 ppt, whereas, it was 5.18 ppt only for the open sea. The variation in salinity of the backwater was most pronounced in the second year of study, where there was heavy rain in the months of July and August 1988. The lowest salinity of 16.99 ppt was recorded on 17th July, 1988, when the sampling was done just after the rain had stopped. However the salinity of the open sea was not much affected by the rain and it remained 37.48 ppt. Although it rained more in August 1988 than in July 1988, the lowest salinity was observed in July 1988 because of the reason mentioned above. The sampling in August was done when there was no rain for a period of three or four days. This shows that the rain and flood of the Lyari river can lower the salinity of backwater to great extent but this low value of salinity does not remain for a longer period and may disappear within a period of two to three days or even less, depending on the magnitude of tidal water which flushes the backwater area.

The highest salinity (45 ppt) observed during the present investigation from the backwater is not unusual. Ahmed and Rizvi (1981) mentioned that the salinity might rise to as high as 41 - 42 ppt in the backwaters and tidal creeks of the Karachi area. Saifullah and Taj (1995) while studying the macro-algae of mangrove swamps of the Korangi Creek, Karachi, found a range of 40 to 44 ppt salinity during October and November 1991. However, salinity values of the present investigation have a wider range as compared to those reported by Nooruddin and Khursheeduddin (1968) and also by Hussain and Samad (1995). They reported a range of 27 to 37 ppt from the Keamari coast and 32-38 ppt from Manora (= Baba) channel, respectively. This could be due to the fact that they obtained seawater sample from sub-tidal region and that too during the high tide.

The amount of free oxygen dissolved in water is one of the environmental factors that is of basic importance in the study of any aquatic ecosystem (Reid and Wood 1976). The range of dissolved oxygen (3ml/l to 6.55 ml/l) observed during present investigation compare well with the data of earlier workers like Khan and Saleem (1988) who recorded average oxygen values from Karachi Harbour of 2.09 to 6.41 ml/l, whereas Hussain and Samad (1995) reported a range of 2.4 to 6.7 ml/l of dissolved oxygen from Manora (= Baba) channel. The lowest values of dissolved oxygen such as 2.09 or 2.4ml/l may occur towards the mouth of the Lyari River where sewage is discharged and is attacked by decomposers. These lower levels of oxygen was not observed during present investigation from the sampling sites, which is far away from the mouth of the Lyari River.

Ghamrawy (1982) while studying the ecology and biology of penaeid shrimp in the region of Jeddah, Saudi Arabia found the surface seawater dissolved oxygen inversely related to the temperature. Similarly Mahmood, (1990) who undertook a study on immigration of penaeid shrimp post-larvae in the estuarine area of Chakaria, Cox's Bazar, Bangladesh mentioned that the dissolved oxygen content of surface seawater was inversely related to the rise and fall of temperature. He concluded that the temperature mainly regulates the seasonal cycle of dissolved oxygen. He considered other factors like biological processes; flood discharge and chemical degradation of organic matter are less significant in the estuarine area of Chakaria. The amount of dissolved oxygen found during the present investigation also shows that it is inversely related to the temperature. The lower values of dissolved oxygen were

found during summer months or southwest monsoon, whereas higher values were observed during winter months or north-east monsoon.

It is well known that biological processes like respiration and decomposition producing carbon dioxide hardly affect the pH of seawater, nor does the removal of carbon dioxide in photo-synthesis. The carbonate buffer system maintains the pH of seawater in a state of weak alkalinity. However, in diluted seawater and in an isolated basin when H_2S is produced, the pH may approach 7.0 or even fall in the acidic range. The presence of "acid sulphate soil" in the coastal areas of tropical and sub-tropical countries is another factor, which brings down the pH of seawater in the acidic range (Simpson and Pedini1985). In their study on acid sulphate soil of the coastal aquaculture ponds of Bangladesh, Mahmood and Saikat (1995) found mangrove soil pH as low as 4.36. However, during present investigation the pH of open seawater was found to be slightly alkaline, whereas, pH of the backwater had a range of 6.34 to 8.05. This is comparable with the data of Hussain and Samad (1995), who observed a pH range of 6.5-8.2 in the Karachi Harbour area. The lower values of the pH may be attributed to the dilution of seawater or presence of H_2S but it is quite unlikely that the values of pH are due to "acid sulphate soil", a condition that has never been reported from the Karachi coast.

The sediment as a habitat for animals can be characterized on the basis of grain size and sorting. The coarse interstitial sediment drains fast and retains less water or organic matter. Fine sediment such as mud has poor water circulation and often, low oxygen retention. Medium and fine sands usually have abundant meio-fauna and macro-fauna. Muds have more organic matter per unit area and faunal densities are frequently highest here. During present investigation, it was observed that the intertidal area of the Sandspit backwater is mostly muddy with some sandy patches. This being the case, two substations were selected for sediment analysis, one was apparently sandy (sub station 1-A) and the other one is soft and muddy (sub station 1-B).

During southwest monsoon (*i.e*. May to September) the high energy wave normally changes the composition of the sediment in the areas close to the shore. Hence, more sand fraction is expected in the backwater sediments just after the monsoon period. When the monsoon is over, the wave action calms down a great deal and this enhance the process of siltation especially in the creek areas and in the backwaters. This was observed in the present study, when more sand fraction was found in the sediment samples collected in the post-monsoon period.

While grain size and sorting are probably the two most important characteristics that can be determined for sediment samples, other biologically important properties include the organic carbon content of the sediment. Although organic carbon was studied only on four occasions during the present investigation, it can be seen from the results obtained that the organic carbon content of the sediment increases with the fineness of the deposit.

It is evident that the Sandspit backwater area is not a typical estuary for most part of the year. It only acts as an estuary during monsoon seasons when there is rain. In the remaining months, the salinity is much higher than the open sea and the area becomes a negative estuary. It looses more water by evaporation than the Lyari river is able to deliver. Such estuaries are usually low in biological productivity. The biological productivity of the Sandspit backwater is not known. Hence, there is a need to investigate the primary and secondary productivity of the area.

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DISSOCIATION EXTRACTION PROCESS FOR THE SEPARATION OF ISOMERIC ORGANIC COMPOUNDS

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Separation of mixture of isomeric organic compound was achieved by exploiting the difference in their dissociation constants by employing dissociation extraction technique. Mixture of 3-picoline and 4-picoline was partially separated with sodium dihydrogen phosphate to demonstrate the phenomenon of dissociation extraction. A theoretical treatment of separation of isomers is given which can be utilized to predict the separation factor of dissociated and undissociated species of isomers. Increase in temperature had a positive influence on the separation of isomers and also on the phase disengagement between the aqueous and the organic phases. A process is described to develop a suitable chemical material bed in the column for gas-liquid chromatograph that reduces the retention time and gives high resolution of picolines.

Key words: Dissociation extraction, Separation factor, Dissociation constants.

Introduction

Dissociation extraction is a unique technique, capable of achieving the separation of isomeric mixture of organic acids and bases. Its research and applications have spread into the fields of extraction and separation of chemical substances (Wadekar & Sharma 1981; Lo & Baarid 1996; Hulton *et al* 2000; Ahmed *et al* 2002) from coal-tar oils, polyphenols, substituted phenols, amines alkaloids, herbicides and pharmaceutical products.

To achieve the separation, the dissociation extraction technique utilizes the difference in the strengths of the components as acids or bases, i.e. difference in their dissociation constants which are considerable due to different molecular arrangements in the isomers. Consider a mixture of components (e.g. two organic bases dissolved in an organic solvent) with a stoichiometric deficiency of an extracting agent in another phase (e.g. an aqueous acid). The term stoichiometric deficiency generally, implies that the amount of acid taken is just sufficient to neutralize the stronger base of the two. The organic bases enter the aqueous phase in their undissociated form and compete with each other to react with stoichiometrically deficient acid. The stronger base reacts preferentially with the acid. The aqueous phase becomes enriched with the stronger base while the organic phase is concentrated with the weaker base. The process is akin to the processes developed by authors (Ahmed *et al* 1989, Ahmed *et al* 1994) for solvent extraction of metals. According to the work of Anwar *et al* 1979 and Caira *et al* 1997 the picoline isomers possess different dissociation constants which provide a satisfactory basis for their separation. However, their attention was concentrated primarily on the use of dissociation extraction to effect separation of particular complex mixture in the laboratory. In the present paper, theoretical analysis of the technique is described to bring about the separation. Moreover, a Gas-Liquid Chromatography column bed is developed which gives 28 min retention time for separation as compared to 3 h by commercially available columns.

*Theory.*Consider the extraction of two organic bases A and B, dissolved in a water-immiscible orgnaic solvent by aqueous solution of weakly acid salt HX. The acid salt HX is taken in a stoichiometric deficiency as compared to base isomers, in order to have competition between the two isomers in the aqueous phase. The more strongly base A tends to react preferentially with hydrogen ions forming a dissociated salt in the aqueous phase. The base B does not react and remains in undissociated form increasing its concentrtion in the organic phase. The following reactions are expected in the aqueous phase:

$$A + HX \rightleftharpoons AH^+ + X^- \quad (1)$$

$$B + HX \rightleftharpoons BH^+ + X^- \quad (2)$$

Since, A is the stronger base; the overall reaction is an exchange reaction between bases A and B in the aqueous phases:

$$A + BH \rightleftharpoons AH^+ + B \quad (3)$$

The dissociation constants of the organic bases are defined as:

$$K_A = [AH^+]\,[X^-] / [A] \quad (4)$$

$$K_B = [BH^+]\,[X^-] / [B] \quad (5)$$

The distribution coefficients of the undissociated bases are:

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$$D_A = [A]_o / [A]_a \quad (6)$$
$$D_B = [B]_o / [B]_a \quad (7)$$

Where subscripts 'o' and 'a' denote organic and aqueous phases, respectively.

Overall distribution coeffecients are defined as:

$$D'_A = [A]_o / ([A]_a + [AH^+]) \quad (8)$$
$$D'_B = [B]_o / ([B]_a + [BH^+]) \quad (9)$$

The dissociation constants of the acidic reagent, K_x, and water, K_w, are defined as:

$$K_X = [H^+] / [X^-] / [HX] \quad (10)$$
$$K_W = [H^+] [OH^-] \quad (11)$$

The equilibrium constant for reaction (1), K_1, is given by:

$$K_1 = K_A K_X / K_W \quad (12)$$
$$K_2 = K_B K_X / K_W \quad (13)$$
$$K_3 = K_A / K_B \quad (14)$$

Showing that the equilibrium of the key exchange reaction is related to the relative strength of the two bases.

The equations presented above, contribute to the overall state of the system, the following two equations are derived from stoichiometry assuming that reactions (1) and (2) do not completely consume the acidic reagent, HX, for which the feed concentration is Z moles per litre.

For every mole of HX, one mole of AH^+ or BH^+ is formed:

$$[HX]_{feed} = Z = [HX]_{eq} + [AH^+] + [BH^+] \quad (15)$$

Where $[HX]_{eq}$ is the concentration of unreacted reagent in the aqueous phase at equilibrium.

$$[X^-] = [AH) + [BH^+] \quad (16)$$

The separation factor α_{AB} for the organic bases is:

$$\alpha_{AB} = D'_B / D'_A \quad (17)$$

From equations (17), (8) and (9):

$$\alpha_{AB} = D'_B / D'_A = \frac{[B]_o([A]_a + [AH^+])}{[A]_o([B]_a + [BH^+])} \quad (18)$$

From equations (6), (12), (15) and (16):

$$\frac{K_B K_X}{K_W} = K_1 = \frac{[BH^+][X^-]}{[B]_o[HX]} = \frac{([AH^+] + [BH^+])[BH^+]}{([B]_o / D_B)[Z - ([AH^+] + [BH^+])} \quad (19)$$

and similarly, from equation (7), (13) (15)and (16):

$$\frac{K_A K_X}{K_W} = \frac{([AH^+] + [BH^+])[AH^+]}{([A]_o / D_A)[Z - ([AH^+] + [BH^+])} \quad (20)$$

In equations (19) and (20), K_A, K_B, K_X and K_W are known as physical constants having literature values of 10.62×10^{-9}, 4.59×10^{-9}, 6.23×10^{-8} and 10^{-14}, respectively; Z, $[A]_o$ and $[B]_o$ are system variables which may be assigned an experimental values at equilibrium. Therefore, if D_A and D_B are known at the actual values of the system concentration variables, then we have two equations, (19)and (20) containing only two unknown: $[AH^+]$ and $[BH^+]$. The equation may be solved simultaneously, in order to yield the unknowns, However, if the equations are not linear and simple then the analytical solutions are not possible, whereas, a computer programme is the most convenient method of obtaining the real and positive solution values of $[AH^+]$ and $[BH^+]$.

On obtaining $[AH^+]$ and $[BH^+]$, $[HX]_{eq}$ and $[X^-]$ may be derived from relations (15) and (16) and $[A]_a$ and $[B]_a$ from equations (6) and (7). All the terms in equation (18) are now known and the required value of α_{AB} may be calculated. If desired, the values of the overall distribution coefficients may be calculated from equations (8) and (9).

Experimental

The system chosen for detailed studies was the separation of 3-picoline and 4-picoline which caused a separation problem in coal-tar industry. The extractant employed was sodium dihydrogen phosphate.

Preparation of GLC column. It is extremely important to have suitable material bed in the column that is capable of providing sufficient retention time to give high resolution of picolines. In order to achieve this objective, Matexil (the stationary phase material) 1.2g and potassium hydroxide 0.45g was dissolved in 35ml of methanol in a 150 ml round bottom flask. The low solubility of potassium hydroxide in methanol was overcome by the addition of a few drops of distilled water. Chromosorb G AW DMCS (the support material) 15.0 g of 80-100 mesh was added to the methanol solution with continuous stirring.

The flask was placed in a constant temperature bath at 65°C ± 1°C to evaporate methanol. The material was stirred with glass rod until the methanol was completely evaporated. The flask was then placed in an oven at 85°C ± 1°C for 20 h to obtain dried material with same flow characteristics as it had initially.

This process caused the chromosorb G AW DMCS to be coated uniformly on the Matexil. It is filled in the clean and dry glass coil-shaped column of 5 ft. length and 3 mm internal diameter to fit in Gas-Liquid Chromatograph.

To obtain the distribution coefficients of the undissociated species under various conditions, known volumes of bases in organic solvents and aqueous solution of sodium dihydrogen phosphate were shaken for 15 min at room temperature. The phases were separated and the organic phase injected in the

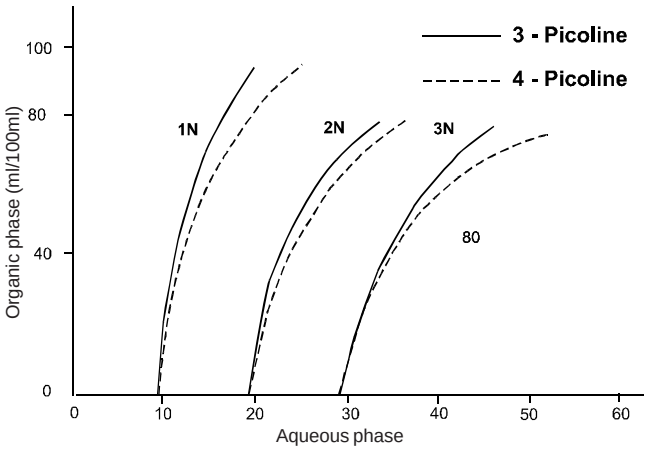


Fig 1. Distribution of bases between benzene and sodium dihydrogen phosphate.

Matexil bed for analysis by Gas-Liquid Chromatograph without any further treatment. The experimental and analytical procedure is the same as described in the previous paper (Ahmed *et al* 2002).

Experimetal work was also carried out to study separation factors directly and determine the effects of total isomer concentrations, isomer ratio and sodium hydrogen phosphate concentration.

The chemicals used were of Analar grade and obtained from BDH.

Results and Discussion

The equilibrium constant, K_e, for reaction between picoline and dihydrogen phosphate is given below in concentrations, neglecting activity coefficients:

$$K_e = \frac{[C_6H_7NH^+][HPO_4^{2-}]}{[C_6H_7N][H_2PO_4^-]}$$

Now, the dissociation constants for picolines, acid and water can be calculated by equations (4), (5), (10) and (11):

$K_A = 10.62 \times 10^{-9}$ 4-Picoline
$K_B = 4.59 \times 10^{-9}$ 3-Picoline
$K_X = 6.23 \times 10^{-8}$ Sodium dihydrogen phosphate
$K_W = 10^{-14}$ water

The dissociation constants for both picolines clearly show that a partial reaction takes place in the aqueous phase. Picoline is too weak a base to generate a high concentration of PO_3^{-3} ions, therefore, the presence of PO_4^{-3} ions in the aqueous phase is neglected. Furthermore, the dissociation constants are very small showing that any excess of base present in the aqueous phase after neutralization of the acid content will be almost entirely in the undissociated form. Analysis confirmed that the concentration of base in the organic phase is nearly zero, until sufficient base has been added to neutralize completely the aqueous acid. The rates of increase of base concentration in the organic and aqueous phases as shown in Fig. 1, virtually become constant representing distribution coefficients of the undissociated bases. The changes in gradient of the curves show that the distribution coefficients are inversely proportional to the increase in the base concentration. The effect of change of base concentration in the organic phase on the distribution coefficient is given in Table 1. It clearly shows that the presence of dissociated picoline affects the activity of undissociated picoline in the aqueous phase since the distribution coefficient decreases considerably with increase in the strength of acid in the aqueous phase. Similar observations were reported for the separation of phenolic derivatives (Hutton *et al* 2000), cresol isomers (Ahmed *et al* 2002) and picolines (Caira *et al* 1997).

The overall distribution coefficients of two isomers are determined individually, which usually donot give any guide whether the separation of a mixture of the two isomers is practicable by dissociation extraction. The isomers may have

Table 1
Distribution coefficients of undissociated 3- and 4-picoline between benzene and sodium dihydrogen phosphate

Concentration of base in organic phase (ml/100ml)	Distribution coefficient					
	1N		2N		3N	
	3-pic.	4-pic.	3-pic.	4-pic.	3-pic.	4-pic.
9.59	17.30	16.40	11.60	10.63	7.40	6.75
19.15	17.30	16.40	11.60	10.63	7.40	6.75
28.28	16.17	14.65	9.80	9.10	7.00	6.46
47.40	15.89	14.40	9.48	8.98	6.88	6.40
60.00	11.46	10.10	6.92	6.30	5.75	5.40
80.00	9.70	8.42	6.50	5.74	4.40	4.00

Table 2
Variation in separation factor of picolines at different temperatures

Temperature	Total conc. In the aqueous phase (ml/10ml)	Equilibrium concentration of the isomers in the conjugate phase				Separation factor
		Organic phase (Molar %)		Aqueous phase (Molar %)		
		3-pic.	4-pic.	3-pic.	4-pic.	
25°C	2.91	59.30	40.70	44.90	55.10	1.79
30°C	2.31	59.60	40.40	40.90	59.10	2.13
35°C	1.60	56.50	43.50	38.20	61.80	2.10

identical distribution coefficients when measured in this way, however, the dissociation constants are sufficiently different to influence the separation of these isomers by dissociation extraction.

Table 2 confirms that the separation factor increases with temperature due to an increase in the concentration of the solutes in the aqueous phase. The latter observation is of importance for a practical separation process, especially, in a multistage separation (Wadekar & Sharma 1981, Arif *et al* 1995) of isomers. Moreover, there was an improved phase separation under higher temperature and no traces of emulsion were noticed appearing in either phases.

The low concentration of the required products on the output phase is a feature adversed to the commercial application of this process. When 20% by weight sodium dihydrogen phosphate was equilibrated with 6.0 g picoline isomers in benzene then the phases were separated. The aqueous phase loaded with picoline was equilibrated with fresh feed of 6.0 g picoline mixture and again phases were separated after equilibration. This process was repeated which showed emulsification at 30% loading(Caira *et al* 1997) of picoline, because the specific gravities of the output phases became closer to each other. Nevertheless, the separation of 4-picoline from 3-picoline was improved, tremendously.

Conclusion

Dissociation extraction is a useful technique for separation of isomeric organic compounds of organic bases (or acids) which are difficult to separate by more common methods of separation such as extraction, crystallization, distillation, etc.

It can be seen from the results that the extent of reaction between the picoline isomers and sodium dihydrogen phosphate is modest. Thus, the competition between the isomers is limited. It would be desirable to find another reagent which would react more strongly and preferably whilst still allowing reversal of the reaction by the other physical separation processes. However, the theory and result are presented to establish the basic feasibility of achieving the separation by not so well known process of dissociation extraction.

Although, no attempt was made for the selection of solvent, the result shown gives a reasonable separation in the benzene media. Authors (Ahmed *et al* 1979; Wadekar & Sharma 1981) stress on the feasible choice for the selection of proper solvents.

Nomenclature

A,B	=	Organic bases
D'	=	Distribution coefficient of undissociated bases
D	=	Overall distribution coeffeicient
H^+	=	Concentration of cations
AH^+, BH^+	=	Concentration of dissociated base A and B, respectively
HX	=	Weak acid in the aqueous phsase
K	=	Dissociation constants
K_1	=	Equilibrium constants
OH^-	=	Activity coefficient of hydroxyl ions
X^-	=	Concentration of anions
Z	=	Total concentration of acid
$\propto$	=	Separation factor

Subscript

A	=	Refers to base A
a	=	Aqueous phase
B	=	Refers to base B
eq	=	Equilibrium
o	=	Organic phase
W	=	Refers to water
X	=	Refers to acid reagent

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THERMAL STABILISATION OF PVC WITH *JATROPHA* SEED, *KHAYA* SEED AND RUBBER SEED OILS. EFFECT OF BARIUM AND CADMIUM SOAPS OF THE SEED OILS ON THE THERMAL DEGRADATION OF PVC

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Polyvinyl chloride was mixed with barium and cadmium soaps of *Jatropha* seed, *Khaya* seed and rubber seed oils and mixtures of the metal soaps and degraded at 190°C under oxidative and nonoxidative conditions. The effectiveness of the additives in stabilizing PVC against thermal degradation was evaluated by comparing (a) the kinetic data measured at 1% conversion for the degradation of PVC in the presence of the additives with the corresponding values obtained in the absence of the additives (b) the intrinsic viscosity and level of unsaturation in the polymer samples degraded for the undergraded polymer and (c) the thermogravimetric data obtained for the degradation of PVC in the presence of the additives at a constant heating rate of 10°C min^{-1} up to 500°C. It was found that the additives retarded the rate of dehydrochlorination of PVC (by up to about 50% and 1% conversion) and reduced the extent of decomposition of the polymer by as much as 60%. The mixtures of the metal soaps containing more than 70%(wt) cadmium soap showed marked synergistic stabilising effect on the degradation of PVC.

***Key words*:** Thermal stabilisation, PVC synergistic effect, Metal soaps, Seed oils.

Introduction

Several workers (Bacaloglu and Fisch 1993, 1995; Geddes 1967; Iida and Goto 1980; Simen 1990; Simon and Valko 1990; Simon *et al* 1990; Varma and Sharma 1978; Verma *et al* 1987; Wypych 1986) have reported on the thermal degradation and stabilisation of PVC. At elevated temperatures well below its decomposition temperature, PVC loses HCl and becomes discoloured. Because these changes are accompanied by a deterioration in some of the useful properties of the polymer. It has become the practice to process PVC in the presence of heat stabilisers. Heat stabilising additives for PVC have been suggested to prevent or retard degradation reaction in a number of ways. Their proposed mechanism of action (Klemchuk 1968) include: (a) HCl scavenging; which is considered to preclude the autocatalytic effect of HCl on the degradation process, (b) modification of chain reactions leading to the inhibition of the elimination of HCl, (c) interrupting the formation of conjugated polyene sequence thus preventing the discolouration of the polymer, and (d) replacement of labile chlorine atoms within PVC structure with stabiliser moieties which are more stable to heat treatment. Heat stabilisers that have found commercial application in the processing of PVC include metal salts (soaps) of inorganic and organic acids, organometallic compounds and inhibitors of radical chain reactions. More recently interest has been shown in the use of poly epoxides (Hopff 1968) including epoxidised vegetable oils (May 1968; Okieimen 1999, 2000, 2001) and metal dicarboxylates (Ikhuoria *et al* 2001) as stabiliser for PVC. This report is part of on-going studies aimed at the development of industrially useful value-added products from local renewable resources and examines the stabilising effect of barium and cadmium soaps of *Jatropha multifida* seed, *Khaya senegalensis* seed and rubber seed oils on the thermal degradation of PVC. The physico-chemical characteristics and fatty acid composition of the seed oils (Aigbodion 1995; Eromosele 1998; Eromosele and Okieimen 1999; Okieimen and Okieimen 2001) have been presented in Table 1.

Experimental

Materials. PVC (BDH Ltd) ($\overline{Mn}$ 1.0 x 10^5, particle size of 100% passed B.S 60 mesh and 74% passed B.S 200) was purified by dissolution in THF/acetone and precipitation with constant stirring in a large excess of methanol. The precipitated polymer was filtered off after 24 h, washed with methanol and air-dried. *Jatropha* seed oil, JSO, was soxhlet - extracted with *n*-hexane from seeds collected in Benin City. *Khaya* seed oil, KSO was extracted with *n*-hexane from seeds collected from Michika, Adamawa; and rubber seed oil, RSO, was obtained from the Rubber Research Institute of Nigeria, Benin City.

Preparation of metal soaps from the seed oils. Metal soaps from the seed oils were prepared by metathesis in alcohol solution (Odilora 1989). Sodium soaps from the oils were first prepared by dissolving the oil sample (9.25g) in 50ml of hot ethanol followed by treatment with 20 ml of 20% (w/v)

Table 1
Physico-chemical characteristics and fatty acid composition of *Jatropha* seed, *Khaya* seed and Rubber seed oils

Parameter	Values		
	JSO	KSO	RSO
Physico-chemical characteristics			
Acid value (mg KOH/g)	5.61	24.00	23.00
Iodine value (gI2/100g)	157.37	68.00	155.56
Free fatty acid (%wt oleic acid)	2.70	7.64	11.26
Peroxide value (meq/kg)	5.11	26.00	0.40
Saponification value (mg KOH/g)	190.12	186.00	201.46
Specific gravity (at 30°C)	0.92	0.96	0.93
Fatty acid composition (%wt)			
Caprylic acid	4.46	3.86	-
Myristic acid	2.61	1.64	2.20
Palmitic acid	20.57	19.05	7.60
Stearic acid	15.64	10.42	10.70
Arachiolic acid	5.68	2.04	-
Behenic acid	1.61	3.26	-
Pamitoleic acid	3.59	-	-
Oleic acid	32.87	54.34	20.61
Linoleic acid	5.25	-	36.62
Linolenic acid	-	-	22.50

sodium hydroxide solution. To its mixture, 100 ml of 30% (w/v) solution of the metal salts were added slowly with continuous stirring. The precipitated metal soap was washed with hot water and air-dried. Soaps were prepared using, $BaCl_2.2H_2O$ and $Cd(NO_3)_2.4H_2O$.

Degradation. Thermal degradation was carried out using PVC powder, mixed with 3%(wt) of the metal soaps of the oils. In a typical experiment, the polymer sample was mixed thoroughly with appropriate amount of the additive and transferred into a degradation tube. The tube was connected to a source of nitrogen maintained at a flow rate of 60 ml/min. The degradation tube was immersed in a thermostat oil bath maintained at 190°C and controlled to better than ± 1°C. The HCl evolved was passed into a known volume of standard sodium hydroxide solution. The amount of HCl evolved was established by titration after various intervals of time. The extent of dehydrochlorination (%) was calculated from the ratio of HCl evolved to the amount available in the polymer.

Thermoxidative degradation was carried out in air as described for non-oxidative degradation except that dehydrochlorination rates were not monitored. Degradation was allowed to proceed for 30 min at the end of which the degradation tube was cooled down and the degraded polymer was recovered and stored in the dark.

Viscosity measurement. The degraded PVC samples were purified by dissolution in cyclohexane and precipitation in a large excess of methanol. The precipitated polymer was filtered off, washed several times with methanol and dried in vacuum at room temperature. The viscosities of the solutions of degraded polymer samples in cyclohexane were measured at 30°C. Intrinsic viscosities were determined from the measured viscosities using the relationship (Huggins 1942) is as under

$$\frac{\eta_{sp}}{C} = [\eta] + k_H [\eta]^{-2} C \quad \text{..... (1)}$$

Here, C is the concentration of the polymer solution in g/dl; η_{sp}/C is reduced viscosity calculated from the relationship $\eta_{sp}/C = (\eta_{rel} - 1)$ and $\eta_{rel} = \eta_{soln}/\eta_{solv} \cong t/t_o$; where 't' is the time of flow of polymer solution and 't_o' is the time of flow of solvent at the temperature of measurement, [η] is the intrinsic viscosity, measured at η_{sp}/C lim C→O and k_H is the Huggin's constant.

Determination of level of unsaturation in degraded PVC samples. The levels of unsaturation in the degraded PVC samples were determined by measuring the iodine values using the Wij's method (BSI 1976).

Thermogravimetric study of PVC degradation. A Perkin-Elmer TGA-7 Thermal Gravimetric Analyser (TGA) programmed at a heating rate of 10°C min^{-1} up to 500°C was used for studying, (a) barium and cadmium soaps of KSO, (b) unstabilised PVC, (c) PVC stabilised with barium and cadmium soaps of KSO and (d) PVC stabilised with admixtures of barium and cadmium soaps of KSO. About 150mg of PVC sample was mixed thoroughly with a known amount of the additive in a motar and an aliquot was carefully transferred into the platinum pan and placed in the TGA. The change in sample weight was recorded automatically as a function of temperature.

Results and Discussion

Nonoxidative degradation. The rates of dehydrochlorination of PVC at 190°C in the presence of barium soaps of the seed oils are shown in Fig 1. Similarly, plots were obtained for the degradation of PVC in the presence of cadmium soaps of the oils. The rates of dehydrochlorination at 1% conversion, R_{DH}, were determined and the time required for degradation to attain 1% conversion t_{DH} was noted. The effect of the metal soaps of the seed oils on the thermal degradation of PVC was evaluated by comparing the values of t_{DH} and R_{DH} with the corresponding values, t^o_{DH} and R^o_{DH}, obtained in the absence of the metal soaps of the seed oils (i.e. t_{DH}/t^o_{DH} and R_{DH}/R^o_{DH}) and have been shown in Table 2. For an additive to be considered an effective stabiliser for PVC, the value of $t_{DH}/$

Table 2
Non-oxidative thermal degradation of PVC at 190°C in the presence of barium and cadmium soaps of seed oils

	t_{DH}/t^{o}_{DH}			R_{DH}/R^{o}_{DH}			$[\eta]/[\eta]_o$			$(IV)/(IV)_o$		
Additive	JSO	KSO	RSO	JSO	KSO	RSO	JSO	KSO	RSO	JSO	KSO	RSO
Barium soap	1.90	1.13	1.69	0.70	0.73	0.83	0.60	-	0.73	-	-	12.00
Cadmium soap	2.39	2.00	1.80	0.65	0.58	0.67	0.87	-	0.63	-	-	11.00

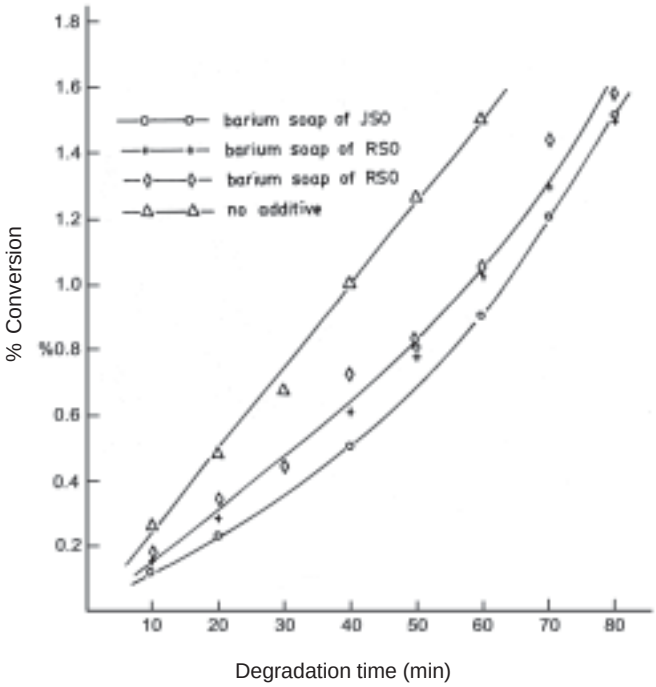


Fig 1. Non-oxidation dehydrochlorination of PVC in the presence of barium soaps of seed oils at 190°C.

t^{o}_{DH} should be greater than 1, and the values of R_{DH}/R^{o}_{DH} should be less than 1, the greater the divergence of the values from unit, the more effective the additive is considered in stabilising PVC against thermal dehydrochlorination. The result (Table 2) shows that the metal soaps of the seed oils are relatively effective in stabilising PVC against thermal degradation and that the cadmium soaps of the seed oils are more effective stabilisers than the barium soaps. The stabilising effect of the metal soaps is considered to be due to their facile reaction with HCl and substitution of labile chlorine atoms within PVC structure with additive moieties (Klemchuk 1968; Frye and Horst 1959). Cadmium soaps are generally considered more reactive than the corresponding barium compounds and consequently would be expected to be more effective stabi-lising PVC against thermal degradation. In a previous study (Okieimen 1999), it was reported that derivatives of rubber seed were more effective than the corresponding derivatives of unsaturated fatty acids (oleic and linoleic acids) in stabi-lising PVC against thermal degradation and explanations in terms of the contribution of the chemical identity (level of unsaturation) of the hydrocarbon moiety of the fatty acids to the overall stabilisation process were proffered. It has been suggested that the nature of the hydrocarbon portion of the fatty acid may influence PVC stability by altering the polymer matrix polarity (Starnes 1994). However, the result in Table 2 does not suggest any relationship between the levels of unsaturation of the seed oils and the stabilising effect of their metal soaps.

The intrinsic viscosity of PVC samples degraded at 190°C under nitrogen for 30 min in the presence of barium and cadmium soaps of the seed oils are presented in Table 2. Degraded PVC is semi-flexible in nature, with the conjugated polyene sequence in the polymer, following dehydrochlorination, constituting the rod-like part and the undegraded portion being flexible. It has been suggested (Okieimen 2000) that at low extents of dehydrochlorination (≤ 5% conversion) changes in the values of intrinsic viscosity correlate closely with the extent of degradation: the lower the value of intrinisic viscosity, the greater the extent of degradation. Therefore, the value of relative intrinsic viscosity, $[\eta]/[\eta]_o$, where $[\eta]_o$ is intrinsic viscosity of undegraded PVC can be used to evaluate the effect of additives on the degradation. The reactions that may accompany thermal dehydrochlorination of PVC include chain scission, which will be associated with reduction in the value of intrinsic viscosity and crosslinking which should lead to higher values on intrinsic viscosity. The intrinsic data in Table 2 show that the barium and cadmium soaps of the seed oils exert stabilising effect on the degradation of PVC and that chain scission may be the predominant reaction accompanying dehydrochlorination of PVC under the conditions of this study.

The elimination of HCl during thermal degradation of PVC leads to the formation of olefinic double bonds in the polymer molecule. Therefore, it is provided that the extent of degradation is low and dehydrochlorination is not accompanied by secondary reactions involving the double bonds, measurement of the level of unsaturation in the degraded polymer sample should provide satisfactory estimate of the relative

Table 3
Thermoxidative degradation of PVC at 190°C in the presence of metal soaps of seed oils

	$[\eta]/[\eta]_0$			$(IV)/(IV)_0$		
Additive	JSO	KSO	RSO	JSO	KSO	RSO
O	0.43	0.43	0.43	-	-	17.37
Barium soap	0.52	0.80	0.61	-	-	11.84
Cadmium soap	0.67	0.89	0.89	-	-	8.85

Degradation time = 30 min

stabilising ability of additives. The ratio of the iodine value of degraded PVC sample to the value of undegraded sample, $(IV)/(IV)_o$, can be used to assess the relative effect of the additives on the thermal degradation of PVC: the smaller the $(IV)/(IV)_o$ value, the greater the relative stabilising ability of the additive. The levels of unsaturation of PVC samples degraded at 180°C for 30 min in the presence of barium and cadmium soaps of the seed oils have been shown in Table 2. The results show a decrease of more than 40% in iodine value relative to the value obtained for unstabilised PVC. These results clearly show that the addition of the additives to PVC reduce the levels of unsaturation in the degraded polymer and indicate a stabilising effect of the additives on the thermal degradation of PVC.

Thermoxidative degradation. The intrinsic viscosity and levels of unsaturation of PVC samples degraded in air at 190°C for 30 min in the presence of barium and cadmium soaps of the seed oils are shown in Table 3. The results show that the values of intrinsic viscosity of the degraded PVC samples are lower than the value for the undegraded sample. The values of intrinsic viscosity of PVC samples degraded in the presence of barium and cadmium soaps of the seed oils are much higher (by between 30 and 60%) than the value obtained for the sample degraded in the absence of the additives. These results indicate that the extent of degradation of PVC in the presence of the additives is smaller than in the absence of the additives. The levels of unsaturation of PVC samples degraded in and at 190°C for 30 min have been shown in Table 3. The values of $(IV)/(IV)_o$ obtained for the polymer samples degraded in the presence of barium and cadmium soaps of the seed oils are 50% higher than the value obtained for PVC sample degraded in the absence of the additives. These results indicate that barium and cadmium soaps of the seed oils are effective in stabilising PVC against thermoxidative degradation.

Degradation in the presence of binary mixtures of barium and cadmium soaps of the seed oils. The effect of binary mixtures of barium and cadmium soaps of the seed oils on the thermal degradation of PVC was examined at 190°C under-oxidative and oxidative conditions. The variations of the values of R_{DH} and t_{DH} with composition of the soap mixtures are shown in Table 4. The results show that the effect of the mixtures of the metal soaps is not additive. The initial increase in the values of R_{DH} with increase in the cadmium soap content of the mixture attained maximum values at between 40-60%(wt) and thereafter, decreased with further increase in the cadmium soap content of the mixture. Minimum R_{DH} values of 1.62×10^{-2} % min^{-1} and 1.33×10^{-2} % min^{-1} were obtained at 90%(wt) cadmium soap content of the soaps mixtures of JSO and RSO, respectively. Similarly, maximum t_{DH} values of 110 min and 101 min were obtained at 90 % (wt) cadmium soap content of soap mixture of JSO and RSO, respectively. These results show that barium and cadmium soaps

Table 4
Effect of mixture of barium and cadmium soaps of seed oil on the thermal degradation of PVC at 190°C

Composition of soap mixture (%Cd soap)	$10^2 R_{DH}$(%min^{-1})		t_{DH} (min)		$[\eta]/[\eta]_o^*$		$(IV)/(IV)_o^*$	
	JSO	RSO	JSO	RSO	JSO	RSO	JSO	RSO
0	2.00	1.67	59.00	69.50	0.27	-	17.36	15.51
10	2.28	1.81	74.00	69.80	0.52	0.48	10.69	11.62
20	2.40	1.91	80.00	75.00	0.61	0.34	10.39	10.47
30	2.72	1.95	79.00	65.00	0.40	0.69	10.22	9.84
40	2.92	1.97	51.00	75.00	0.35	0.73	10.04	9.42
50	2.88	2.08	71.00	69.00	0.46	0.76	9.87	8.99
60	2.60	2.00	74.00	75.00	0.50	0.83	9.62	8.16
70	2.24	1.79	82.00	74.00	0.64	0.87	9.36	7.74
80	2.06	1.54	90.00	89.00	0.67	0.94	9.10	7.32
90	1.62	1.23	110.00	101.00	0.70	1.00	8.52	6.27
95	1.75	1.40	78.00	98.00	0.56	0.97	8.67	9.85
100	1.82	1.43	74.00	80.00	0.54	0.89	8.67	8.99

*Polymer samples degraded for 30 min in air.

Table 5
Thermogravimetric evaluation of relative stabilising effect of metal soaps of KSO on the thermal degradation of PVC

Additive	%Weight loss		Residue	t_{dmax}(°C)	Temperature at which degradation was attained (°C)	
	Stage I	Stage II			1%	5%
None	64.75	18.82	16.42	302.50	256.80	281.80
Barium soap of KSO	59.18	9.30	31.62	322.30	260.00	295.40
Cadmium soap of KSO	62.06	11.03	26.90	328.50	262.10	297.50
Mixture of Ba and Cd Soaps of KSO						
% Cd soap content						
10	61.23	10.02	28.75	324.40	268.30	304.70
50	60.59	10.59	28.84	328.40	274.60	301.60
90	60.49	9.99	29.90	329.50	276.60	303.70

of the seed oils exhibit synergistic stabilising effect on the thermal degradation of PVC. The combined effect of barium and cadmium soaps of the seed oils in stabilising PVC against thermal degradation can be described as monosynergism, i.e. synergism involving compounds of unequal activity that operate by the same mechanism. The reaction sequence include (David 1975; Onozuka & Asahina 1969) (a) facile reaction of the more reactive component (cadmium soap) with labile chlorine atom in PVC, (b) conversion of the cadmium chloride to the soap be reacted with barium soap; and (c) scavenging of the HCl formed during the early stages of dehydrochlorination. The reaction that is considered most critical for the synergistic stabilising effect of the soap mixture is the reaction between cadmium chloride and barium soap (David 1975; Onozuka & Asahina 1969). Although, the stiochiometry of the reactions, particularly the ester exchange reaction between cadmium chloride and barium soap taking place in the polymer matrix is not fully understood, the result (Table 4) indicates that barium and cadmium soap mixtures of the seed oils formulated to contain 90%(wt) cadmium soap would be effective in stabilising PVC against thermal degradation.

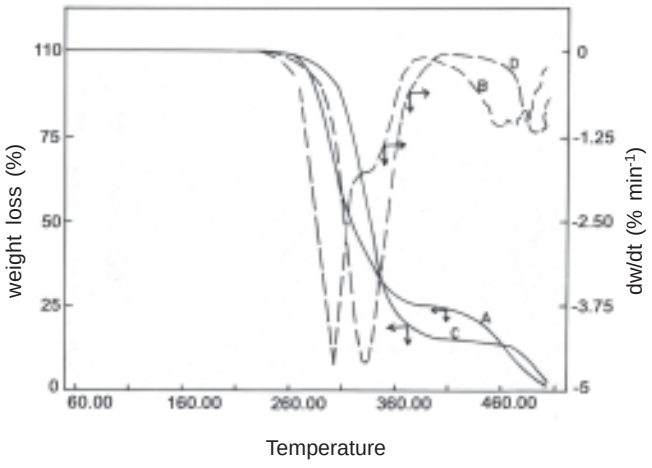


Fig 2. Thermograms of unstabilised PVC A-TG, B-DTG and PVC stabilised with cadmium soap of KSO C-TG, D-DTG.

The variations of the values of intrinsic viscosity and iodine value of PVC samples degraded in air at 190°C for 30 min have been shown in Table 4. The results show that mixture of barium and cadmium soaps of the seed oils exhibit antagonistic stabilising effect on the degradation of PVC at soap mixture composition up to about 60%(wt) cadmium soap and synergistic effect at higher cadmium soap content of the mixture.

Thermogravimetric studies on PVC degradation. The thermograms for unstabilised PVC stabilized with cadmium soap of KSO are shown in Fig 2. Similar thermograms were obtained for PVC stabilized with barium soap of KSO and PVC stabilized with mixtures of the soap. The values of weight loss of the polymer sample during degradation and the temperature at which maximum rates of degradation occurred, t_{dmax} were recorded automatically by the TG analyser and are shown in Table 5. The relative stability of PVC was also evaluated from the temperatures at which various extents of degradation (1 and 5%) occurred and shown in Table 5. The results show that the metal soaps of KSO reduce the extent of thermal degradation of PVC and that mixtures of barium and cadmium soaps of KSO exhibited synergistic stabilising effect on the degradation process.

Conclusion

This study examined the effect of barium and cadmium soaps of *Jatropha* seed, *Khaya* seed and rubber seed oils on the

degradation of PVC using rate, viscosity and iodine value measurements as well as thermogravimetric studies. The results show that the metal soaps of the seed oils retard dehydrochlorination rates and reduce the extent of degradation of PVC. Synergistic stabilising effect on thermal degradation was barium and cadmium soaps of the seed oils. These results demonstrate potential application of the derivatives of the seed oils as stabilizer in PVC formulations.

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Biological Sciences

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A Study on the Feeding Responses of a Filter - Feeding *Cyclops* sp. on Various Concentrations of *Chlorella vulgaris*

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The feeding responses of *Cyclops* sp. fed on *Chlorella vulgaris* at various concentrations were studied. The filtration rate of *Cyclops* ranged from 3.0 to 6.43 µl/h/individual. The range of food concentrations was 515 to 2039 cells/µl. The critical food concentration was 1435 cells/µl. The ingestion rate was recorded a maximum of 9971 cells/h/individual at the concentration of 771 cells/µl. This study also relates filtration rate with pH and dissolved oxygen.

Key words**:** *Cyclops* sp., *Chlorella vulgaris*, Feeding responses.

Introduction

The filter feeders especially rotifers are playing significant role as live food organisms in the food chain of fishes starting as starter food (Lubzens 1987; Tidwell *et al* 1997). These rotifers depend on the microalgae for their proper growth, development and reproduction (Maruyama 1993; Schwarz *et al* 1995; Habib 1998). Microalgae can biosynthesize these essential nutrients from the inorganic nutrient media or natural environment (Habib *et al* 1997; Vazhappilly and Chen 1998) which may be taken by rotifers after feeding them (Bennet and Boraas 1988; Hirayama *et al* 1989; Maruyama 1993; Habib 1998). Among microalgae,*Chlorella* sp., *Ankistrodesmus* sp., *Selenastrum* sp. etc. can successfully biosynthesize essential nutrients from the media (Duncan 1989) which are available in the ponds of Bangladesh Agricultural University Farm, Mymensingh. Among zooplankton, *Brachionus* sp., *Cyclops* sp., *Keratella* sp. etc. are found dominant in these farm ponds. These microalgae are smaller in size than others (Thompson *et al* 1988) and may be used to feed above zooplanktons after isolation. Therefore, the present work was undertaken to study the feeding responses of *Cyclops* sp. to different concentrations of *Chlorella* sp.

Materials and Methods

The samples of phytoplankton were collected from the ponds of Bangladesh Agricultural University (BAU) fish farm by plankton net of mesh size 30 µm. The cells of *Chlorella vulgaris* (isolate no. DAQ001) were isolated with the help of microcapillary pipette through different dilutions under microscope. Isolated microalgae was cultured in modified bold basal medium (MBBM) (Thompson *et al* 1988). Pure culture of isolated algae was done through repeated isolation and culture. Particle free medium (PFM), stock culture of *C. vulgaris*, design of experiment and mass culture were performed according to Miah (1993).

The samples of zooplankton were collected from the collected samples of BAU farm ponds with the help of plankton net of mesh size 55 µm. The dominant species of zooplankton as *Cyclops* sp. was isolated using pasteur soft micropipette under microscope. Pure culture of *Cyclops* sp. was performed through repeated isolation. It was fed with *C.vulgaris* cultured in the laboratory. Filtration and ingestion rates were calculated following the formula of Nimura (1980). In the evening before the day of experiment, zooplankton in stock culture was washed by sieving through a net of 50 µm mesh size by reverse filtration. This filtration was repeated 3 - 5 times for the experiment in higher food concentration while 4 - 8 times in lower food concentration. Washed zooplankton and algal suspension (both with aeration) were kept in the laboratory over night at 26 ± 2°C for acclimatization.

Just before the experiment, the algal suspension was diluted with PFM to have desired concentration. To the dilute algal suspension, the PFM was added in the ratio of 20:7 over control. The mixture was delivered to two control bottles, each containing 200 ml PFM. To the diluted algal suspension, the washed zooplankton were mixed in the ratio of 20:7. The mixture was delivered to four bottles each containing 200 ml water. Fifty individuals of zooplankton were released as initial density. All the bottles were kept in the laboratory at 26 ± 2°C under dim light condition. These bottles were slowly aerated to agitate the suspension according to Omori and Ikeda

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(1984). Sub sampling was performed at every 90 min for five times in each series including the initial one. The initial sampling was done from each bottle using a plastic syringe after gently mixing the medium. During sampling, dissolved oxygen and pH were measured with oxygen (Yellow Springs Instrument, Model 58) and pH meters (Jenway, Model 3020), respectively. The sample was used to count algae and zooplankton. All the samples were fixed with 5% particle free formalin after sampling immediately. The microalgae and zooplankton were counted using haemacytometer and Sedgewick Rafter counting chamber, respectively. Statistical analysis was done using excel of microsoft win 6.0.

The quantitative study of phytoplankton and zooplankton of samples was done by Sedgewick Rafter (SR) counting cell, which was 50 mm long, 20 mm wide and one mm deep. The volume of the chamber was one ml (1000 mm^3 or 1 cc). The counting chamber is equally divided into 1000 fields. Each field had a capacity of one 1µm. From the concentrated volume of the plankton samples, one ml was taken by a dropper and then put on the SR cell. The counting chamber was covered with a cover slip so as to eliminate the air bubbles and then it was placed under microscope for further analysis. Phytoplankton was counted from 30 random fields (units) out of the fields of the SR counting cell with the help of binocular microscope. The cell/l of cultured samples was calculated according to Ward and Whipple (1959). Identification of phytoplankton was done following the keys of Whitford and Schumachar (1973) and Pontin (1978).

Results and Discussion

A series of feeding experiments of *Cyclops* sp. was carried out with *Chlorella vulgaris* at 26 ± 2°C for getting filtration and ingestion rates. The filtration rate in *Cyclops* sp. ranged from 3.00 to 6.43 µl/h/individual. Fig 1 shows the filtration rate in *Cyclops* sp. relating to food concentrations. It was attained a plateau. The range of food concentration was 515 to 2039 cells/µl. The critical food concentration was 1435 cells/µl. The filtering rate was obtained scattered widely in respect to various food concentrations. One of the problem in measuring actual rate was the failure in keeping the same initial food concentrations in the control and the experimental, because food organisms were usually attached with zooplankton in spite of several washings especially in the case of low food concentrations. The contribution due to growth of food organisms and grazing was assumed as identical on the specific growth rate. The number of grazers in respect to time was little bid changed. The data for analysis were chosen from those which showed less than 33% mortality. Hirayama and Ogawa (1972) reported the effect of hunger on the filtration

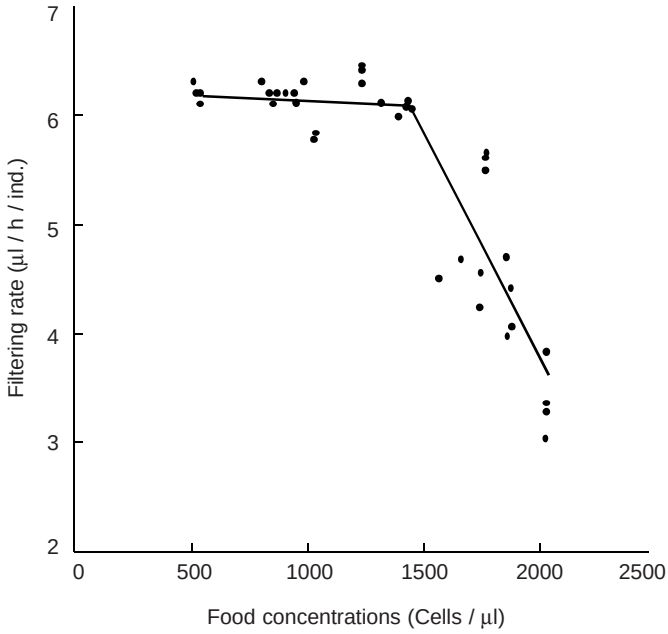


Fig 1. Filtering rate of *Cyclops* sp. in response to various food (*Chlorella* sp.) concentrations.

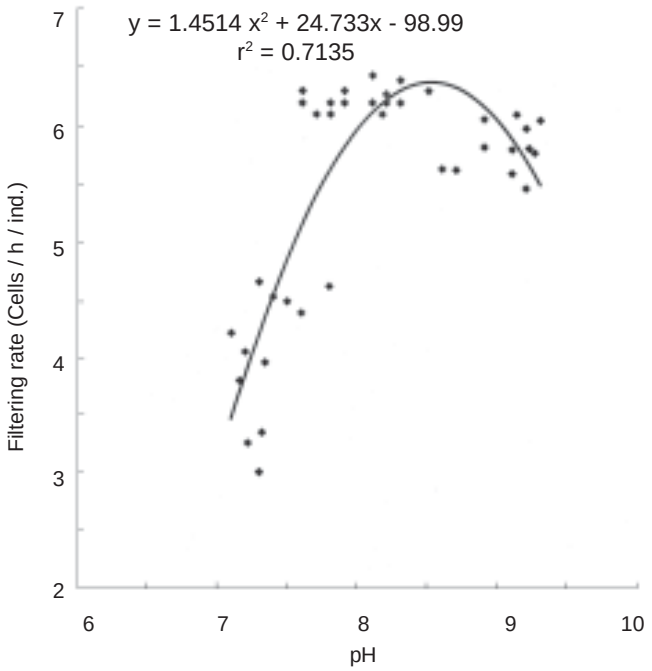


Fig 2. Filtering rate of *Cyclops* sp. in relation to pH.

rate in *Brachionus plicatilis*. A similar phenomenon was observed during the experiment. In low concentration, the initial data in filtering rate seemed to be larger than those obtained in later measurement. Hirayama and Ogawa (1972) obtained similar results during 60 min experiment with large food concentration. Rothhaupt (1990) considered more than 10 min for food to pass through the gut of *Brachionus*

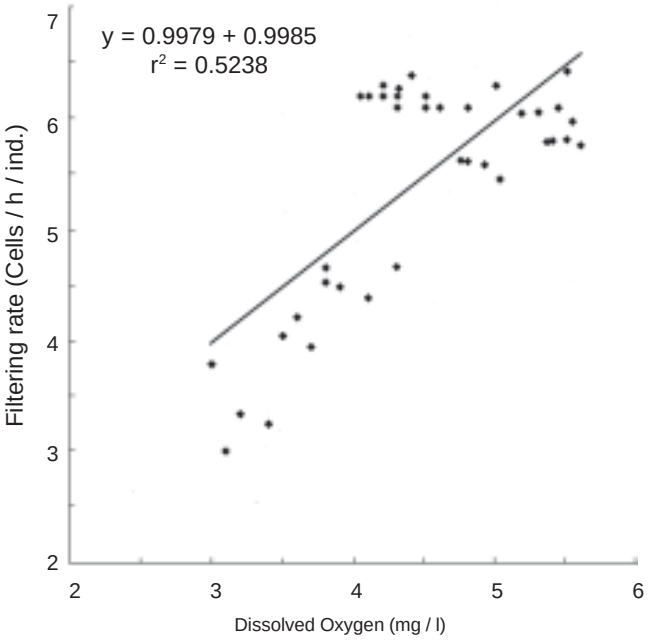


Fig 3. Filtering rate of *Cyclops* sp. in relation to dissolved oxygen.

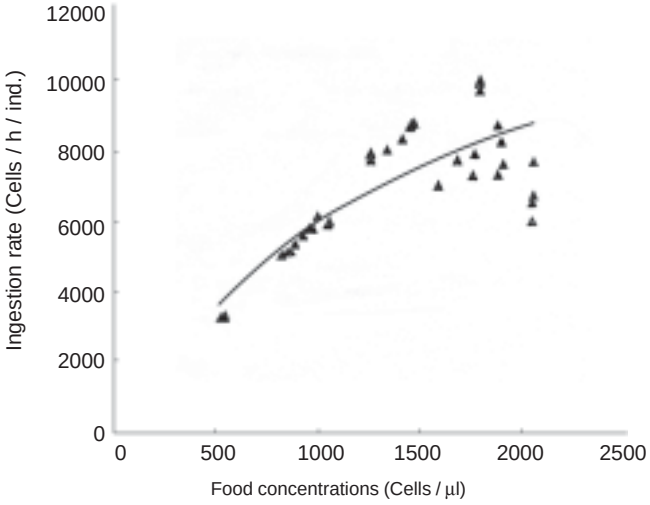


Fig 4. Ingestion rate of *Cyclops* sp. in response to various food (*Chlorella* sp.) concentrations.

calyciflorus. However, more or less similar results of present findings were reported by Hirayama and Ogawa (1972) and Chotiyaputta and Hirayama (1978).

The filtering rate of *Cyclops* sp. was positively related and highly significant ($p < 0.01$) with pH ($r^2 = 0.714$) (Fig 2). But it was observed from the graph that the filtering rate was decreased from pH 8.50. The filtering rate of *Cyclops* sp. was directly and significantly ($p < 0.01$, $df = 38$) related with dissolved oxygen ($r^2 = 0.524$) (Fig 3). There was no trend of decrease of filtering rate due to increase of dissolved oxygen. At present there is no comparable data regarding this aspect. The estimated critical food concentration in *Cyclops* sp. was 1435 cells/µl. This was very high in concentration with that of Chotiyaputta and Hirayama (1978) and Schlosser and Anger (1982) except in some cases of rotifers reported by Rothhaupt (1990). However, the slope obtained was usually larger than 1.0 which meant that the ingestion or food uptake rate increased above the critical food concentration. It was very difficult to know the reason for the difference in the cri-tical concentration from others at this moment. No comparable data on critical food concentration in *Cyclops* sp. was available.

Figure 4 shows that the food uptake rate (ingestion rate) of *Cyclops* sp. is related to the food concentration. The ingestion rate attained a maximum of 9971 cells / h / individual at the con-centration of 771 cells / µl. The ingestion rate increased with food concentration tested throughout. The minimal rate obtained was 3245 cells / h / individual at a concentration of 515 cells / µl. The maximal ingestion rate in *Brachionus plicatilis* reported by Doohan (1973) and Schlosser and Anger (1982) ranged from 0.04 to 0.29 µl / h / rotifer in the temperature ranged from 20 to 25°C. Rothhaupt (1990) reported that ingestion rate in *B. calyciflorus* and *B. rubens* ranged from 0.019 to 0.198 µl / h / rotifer at 20°C. The corresponding data of the expe-riment was 3245 - 9971 cells / h / *Cyclops* at 26 ± 2°C which was larger than the above reports. It was not formed a plateau. This may be due to the size and condition of food organisms.

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Effect of Cooking Method and Length of Cooking Time on Nutritive Value of Various Bean Broths

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Nutritive value of various bean broths was evaluated. Black grams, chick-peas, lentil, red and white kidney beans were subjected to different cooking methods for different time periods. Cooked broth was analyzed for total solids, °Brix, protein, minerals and total soluble sugars to assess the nutritive value. Variable amounts of these nutrients were obtained in these broths depending upon the cooking method and cooking time. These nutrients in broth showed a significant increase with cooking time. Total solids and °Brix, in these bean broths varied from 8.10 - 30.00 and 5.40 - 20.00 g/l, whereas the amount of protein, minerals and soluble sugars ranged from 4.10 - 17.00, 2.20 - 5.80 and 1.20 - 4.80 g/l, respectively. However, maximum amount of nutrients in broth was obtained by pressure cooking of beans.

Key words**:** Beans, Nutritive value, Cooking method.

Introduction

Beans, widely cultivated and consumed through out the world, are excellent sources of protein (20-25%) and carbohydrates (50-60%) and are fairly good sources of minerals and vitamins (Aykroyd and Doughty 1977). They are used as a substitute of relatively expensive animal protein in human diet. However, their wide acceptability is adversely affected by the presence of tannins, saponins and other anti-nutritional substances (Hentges *et al* 1991; Morrow 1991; Stanley 1992). They are usually cooked after soaking in water (De-Leon *et al* 1992; Wah *et al* 1997). Most of the macro and micronutrients are lost during cooking of beans (Rincon *et al* 1993; Addy *et al* 1995). Losses in proteins have also been ascribed to different cooking processes of beans (Phirke *et al* 1982). During bean preparation for consumption, the broth is often discarded. Chicken and vegetable soups are commonly used in many parts of the world including Pakistan due to their high nutritional value, whereas the use of bean broth as soup is not well-known. Broth obtained from various beans can also be used as soup as they may also be rich in various nutrients. Therefore, it is pertinent to ascertain the nutritive value of various bean broths. Further, these broths can also be sweetened with sugar and served as a dessert (Barroga *et al* 1985). Thus, present work was undertaken to investigate the effect of common bean variety, cooking method and cooking duration on the nutritional qualities of bean broth in order to make use of broth for nutritional purpose.

Materials and Methods

Black grams (*Vigna mungo*), chick-peas (*Cicer arietinum*), lentils (*Lens culinaris*), red kidney beans (*Vicia faba*) and white kidney beans (*Vigna sinensis*) were obtained from Ayub Agricultural Research Institute, Faisalabad (Pakistan). The beans were cleaned to remove broken seeds, dust and other foreign materials and cooked by three different methods after soaking in water (5 g/l) for 4 h.

Ordinary cooking. Presoaked beans were put in a round bottom flask fitted with a condenser. Tap water (5 g/l) was added and the samples were cooked on a hot plate for different time periods.

Pressure cooking. Presoaked beans were placed in one litre beakers containing tap water (5 g/l). Tops of beakers were covered with aluminum foil and then cooked in a pressure cooker at 15 lbs/inch^2 for different time periods.

Microwave cooking. Presoaked beans were placed in one litre beakers containing tap water (5 g/l). Tops of beakers were covered and then cooked in a domestic microwave oven for different time periods.

Chemical analysis. After cooking, excess water (broth) in each case was collected and then subjected to chemical analysis to evaluate their nutritive value.

Total solids of broth were determined by oven drying at 100°C for 24 h. Total minerals (ash) were estimated after ignition of dried material at 550 ± 5°C in a muffle furnace for a period of 4 h, while protein contents of the broth were estimated after digestion with concentrated sulphuric acid according to micro Kjeldahl method (AOAC 1984). Brix of the broth was determined by using a hand refractrometer, whereas total soluble

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Table 1
Total solids and °Brix of various bean broths (g/l)*

Type of cooking	Cooking time (min)	Total Solids					°Brix				
		Black gram	Chick-peas	Lentils	Red kidney beans	White kidney beans	Black gram	Chick-peas	Lentils	Red kidney beans	White kidney beans
Ordinary cooking	30	13.00 ± 0.73	12.30 ± 0.37	8.10 ± 0.44	21.30 ± 1.73	21.70 ± 1.34	6.00 ± 0.46	6.30 ± 0.41	6.90 ± 0.36	16.00 ± 0.77	16.30 ± 0.44
	60	16.40 ± 0.59	15.50 ± 0.56	10.03 ± 0.42	24.70 ± 1.63	25.70 ± 1.36	7.20 ± 0.73	7.70 ± 0.32	8.00 ± 0.40	17.50 ± 0.88	17.90 ± 0.73
	90	19.70 ± 0.73	18.80 ± 0.67	12.20 ± 0.39	27.50 ± 1.39	28.20 ± 1.44	9.30 ± 0.80	9.60 ± 0.28	9.80 ± 0.29	19.00 ± 0.90	19.10 ± 0.77
Pressure cooking	5	15.10 ± 0.82	14.60 ± 0.73	14.90 ± 1.00	24.40 ± 1.11	23.90 ± 1.74	6.40 ± 0.44	6.80 ± 0.22	7.20 ± 10.37	16.40 ± 0.67	16.80 ± 0.94
	10	18.60 ± 0.93	18.40 ± 0.82	16.00 ± 1.26	27.30 ± 1.84	27.60 ± 1.39	7.70 ± 0.60	8.40 ± 0.34	9.00 ± 0.44	17.70 ± 0.42	18.60 ± 0.46
	15	22.50 ± 1.20	20.70 ± 1.00	18.10 ± 0.83	30.00 ± 2.06	29.00 ± 1.09	9.80 ± 0.93	9.90 ± 0.33	10.70 ± 0.73	19.80 ± 0.49	20.00 ± 0.59
Microwave cooking	2	11.20 ± 1.11	10.70 ± 0.39	11.60 ± 0.33	20.70 ± 2.01	20.00 ± 2.22	5.70 ± 0.33	5.40 ± 0.81	6.00 ± 0.26	15.70 ± 0.53	16.00 ± 0.55
	4	14.10 ± 0.82	14.20 ± 1.02	14.90 ± 0.46	23.00 ± 1.77	23.90 ± 1.77	6.90 ± 0.40	7.00 ± 0.84	7.20 ± 0.22	16.70 ± 0.55	17.20 ± 0.77
	6	17.20 ± 1.02	17.20 ± 1.02	16.50 ± 0.49	25.90 ± 1.53	26.10 ± 1.40	8.60 ± 0.42	9.00 ± 0.49	8.80 ± 0.71	18.30 ± 0.85	18.80 ± 0.61

* Mean ± S.D. of Triplicate determinations.

sugar of the broth was determined by following the method as described by Yemm and Willis (1954). Total polyphenols in broth were determined by Vaillin-sulphuric acid method on spectrophotometer at 500 nm as described by Wilson and Blunden (1983). All determinations were carried out in triplicate and standard deviations (S.D.) were calculated according to the method of Steel and Torrie (1980). Duncan's multiple range test was applied to determine significant difference ($P < 0.05$).

Results and Discussion

Variable amounts of nutrients including protein, minerals and soluble sugars were present in the broth of black grams, chick-peas, lentils, red and white kidney beans. However, significant variation in total solids amongst varieties, cooking methods and cooking times was observed. Total solids in broth of red and white kidney beans ranged 20.70 - 30.00 and 20.00 - 29.00 g/l, whereas 8.10 - 18.10,11.20 - 22.50 and 10.70 - 20.70 g/l total solids were present in broths of lentils, black grams and chick-peas, respectively (Table 1). These results are in agreement with the observations of other workers who found significant variation in total solids of broth from bean seeds of three varieties (Gupta and Wagle 1987). Different cooking methods extracted total solids in broth to various extents. However, amount of total solids in broth was significantly ($P < 0.05$) higher after pressure - cooking compared to ordinary and microwave cooking. In fact, pressure - cooking caused greater destruction to tissue cells of beans, which consequently leached out greater amount of solids from bean seeds. It was also observed that total solids in broth also increased with increase in cooking time, suggesting that there was an increase in dispersion of solids from the beans with cooking time. The results for the °Brix are also reported in Table 1. There was a significant difference in soluble solids between varieties, cooking method and cooking time. °Brix in broth was also distinctly higher on cooking beans in a pressure cooker (6.40 - 20.00 g/l) compared to other cooking methods (5.40 - 19.10 g/l). On cooking in a domestic microwave oven least amount of °Brix (5.40 - 18.80 g/l) was obtained in broth. However, total soluble solids of the bean broth from these beans increased with increase in cooking time. It is apparent that longer the beans are cooked, the higher is the dispersion of solids and other soluble nutrients into the cooking medium. Similar observations had been made by Narasimha and Desikacher (1978) during cooking of split red beans.

Table 2 contains the protein contents in bean broth. Significant difference ($P < 0.05$) was observed between the varieties, cooking methods and cooking times. Highest protein content in broth was obtained from red kidney beans (8.10 - 17.00 g/l) and the lowest from lentils (4.10 - 9.20 g/l). Protein contents in broth were again comparatively higher in case of pressure-cooking compared to other cooking methods. Cooking time was also positively related to protein content in all the varieties of beans, probably as cooking continues, more bean seed

Table 2
Protein contents of various bean broths (g/l)*

Type of cooking	Cooking time (min)	Protein contents: Black gram	Chick-peas	Lentils	Red kidney beans	White kidney beans
Ordinary cooking	30	8.20 ± 0.44	16.00 ± 0.22	4.20 ± 0.21	9.00 ± 0.19	8.00 ± 0.26
	60	9.00 ± 0.56	7.00 ± 0.26	6.00 ± 10.19	12.02 ± 0.11	11.70 ± 0.43
	90	10.30 ± 10.63	9.00 ± 10.27	8.50 ± 0.28	14.00 ± 0.11	12.70 ± 0.44
Pressure cooking	5	9.00 ± 0.29	7.30 ± 0.29	6.00 ± 0.1	12.50 ± 0.21	12.00 ± 10.39
	10	12.00 ± 0.36	8.80 ± 0.22	7.80 ± 0.24	12.50 ± 0.36	14.60 ± 0.33
	15	14.00 ± 10.40	10.60 ± 3.33	9.20 ± 0.24	17.00 ± 0.16	16.60 ± 0.31
Microwave	2	6.00 ± 0.22	5.20 ± 0.21	4.10 ± 0.20	8.10 ± 0.19	7.40 ± 0.30
	4	8.80 ± 0.29	6.40 ± 0.20	5.50 ± 0.25	11.00 ± 0.26	10.60 ± 10.26
	6	10.20 ± 0.33	7.70 ± 0.19	8.00 ± 0.36	12.00 ± 0.32	12.00 ± 0.22

* Mean ± S.D. of Triplicate determinations.

materials disperse into the cooking medium carrying with them protein content, as reported by Ekpenyong and Borcheres (1980). Variation in protein content of bean broth with bean variety observed in this study is in agreement with findings of earlier workers (Okezie and Martin 1980; Kailasapathy and Nagalingam 1987). Protein contents in broth could be attributed to partial removal of certain essential as well as non-essential amino acids along with other nitrogenous compounds from the bean seeds during cooking process. Total soluble sugars of the bean broth from different varieties of beans are reported in Table 3. Significant variations in total soluble sugars between the varieties, cooking methods and cooking times were observed. Different cooking methods extracted total soluble sugars to various extents. However, maximum amount of total soluble sugars was present in broth, cooking in a pressure cooker. Cooking time was observed to be positively related to total soluble sugars. These observations are in agreement with Rao and Belavady (1978) who reported that cooking resulted in a distinct increase in soluble sugars. In fact, increase in the level of soluble sugars occurred mainly because of their increased solubility in water on cooking as it has already been reported by earlier workers (Sudesh *et al* 1986).

The results for total minerals in broth are also shown in Table 4. Maximum amount of minerals was present in broth, obtained after cooking beans in pressure cooker. This suggests that cooking under pressure extracted maximum amount of nutrients due to greater destruction of the tissue cells of beans. It is apparent that longer the bean seeds are cooked, higher is the dispersion of solids and other soluble nutrients including minerals into the cooking medium. This observation suggests that cooking was positively related to mineral contents. The findings of Kon (1979) also revealed that cooking caused some of the bean cells to separate rather than to break as a result of which cell contents including protein, sugars and minerals were released to the cooking medium and consequently, increased nutrients in bean broth.

Variable amounts of total polyphenols were present in broth of different beans (Table 5). General observation revealed that the amount of total polyphenols was comparatively higher in broth of coloured beans compared with white beans. Amount

Table 3
Total soluble sugar (g/l)* of various bean broths

Type of cooking	Cooking time (min)	Bean variety: Black gram	Chick-peas	Lentils	Red kidney beans	White kidney beans
Ordinary cooking	30	2.10 ± 0.11	1.70 ± 0.07	1.50 ± 0.06	2.40 ± 0.12	2.00 ± 0.13
	60	2.50 ± 0.14	2.00 ± 0.11	1.80 ± 0.07	3.00 ± 0.19	2.70 ± 0.16
	90	3.60 ± 0.14	3.10 ± 0.12	2.60 ± 0.07	3.70 ± 0.16	3.10 ± 0.18
Pressure cooking	5	3.30 ± 0.09	3.40 ± 0.17	2.70 ± 0.07	3.20 ± 0.17	2.80 ± 0.11
	10	4.00 ± 0.13	3.90 ± 0.11	3.40 ± 0.11	4.00 ± 0.22	3.50 ± 0.15
	15	4.80 ± 0.17	4.60 ± 0.14	4.10 ± 0.15	4.50 ± 0.21	4.00 ± 0.15
Microwave cooking	2	1.80 ± 0.09	1.50 ± 0.07	1.20 ± 0.11	2.00 ± 0.19	1.60 ± 0.19
	4	2.20 ± 0.08	2.00 ± 0.06	1.70 ± 0.12	2.40 ± 0.11	2.10 ± 0.11
	6	3.00 ± 0.06	3.20 ± 0.09	2.20 ± 0.14	3.00 ± 0.15	2.70 ± 0.13

* Mean ± S.D. of Triplicate determinations

Table 4
Total minerals (g/l)* of various bean broths

Type of cooking	Cooking time (min)	Bean variety				
		Black gram	Chick-peas	Lentils	Red kidney beans	White kidney beans
Ordinary cooking	30	2.80±0.16	2.50±0.11	2.70±0.15	2.60±0.14	2.90±0.11
	60	3.90±0.17	3.50±0.16	3.80±0.16	3.90±0.16	3.80±0.11
	90	4.40±0.19	4.00±0.17	4.90±0.11	4.20±0.11	4.00±0.15
Pressure cooking	5	3.30±0.11	3.00±0.13	3.20±0.12	3.60±0.19	3.50±0.16
	10	4.40±0.13	4.10±0.14	4.40±0.15	4.20±0.18	4.60±0.19
	15	5.70±0.17	5.00±0.12	5.70±0.11	5.00±0.16	5.80±0.18
Microwave cooking	2	2.20±0.16	2.20±0.16	2.40±0.17	2.00±0.15	2.40±0.18
	4	3.00±0.17	3.00±0.17	3.10±0.19	3.10±0.13	3.30±0.15
	6	4.00±0.13	3.70±0.16	4.00±0.13	3.60±0.13	4.10±0.16

* Mean ± S.D. of Triplicate determinations

Table 5
Total polyphenols (g/l)* in various bean broths

Type of cooking	Cooking time (min)	Bean variety				
		Black gram	Chick-peas	Lentils	Red kidney beans	White kidney beans
Ordinary cooking	30	0.25±0.05	0.14±0.01	0.18±0.05	0.21±0.04	0.10±0.02
	60	0.27±0.01	0.16±0.03	0.20±0.06	0.25±0.05	0.13±0.01
	90	0.28±0.02	0.19±0.02	0.22±0.02	0.28±0.03	0.16±0.03
Pressure cooking	5	0.28±0.04	0.17±0.03	0.20±0.01	0.36±0.03	0.17±0.04
	10	0.34±0.02	0.20±0.06	0.29±0.02	0.39±0.02	0.19±0.05
	15	0.35±0.03	0.21±0.02	0.24±0.01	0.42±0.01	0.21±0.04
Microwave cooking	2	0.24±0.04	0.11±0.01	0.13±0.04	0.18±0.02	0.12±0.02
	4	0.26±0.04	0.14±0.04	0.16±0.04	0.22±0.02	0.14±0.02
	6	0.29±0.05	0.15±0.04	0.19±0.03	0.24±0.04	0.18±0.01

* Mean ± S.D. of Triplicate determinations

of total polyphenols in black grams and red kidney beans was higher and ranged from 0.24 - 0.35 and 0.18 - 0.42 g/l, respectively. However, least amount of total polyphenols in white kidney beans and chick-peas were observed from 0.10 - 0.21 and 0.11 - 0.21g/l, respectively. These results are in accordance with our previous findings of other workers who found that the amount of polyphenols in red kidney beans was higher than white kidney beans (Rehman and Shah 1996). It is also apparent from these results, that cooking method and cooking time affected the extraction of the anti - nutrients from the beans to various extents. However, amount of the anti - nutrients in broth is so low that it would not adversely affect the nutritional quality of broth. Therefore, bean broth can safely be used for the preparation of soups and desserts for human consumption.

Conclusion

It is apparent from this study that the nutritive value of bean broth varied with cooking method, cooking time and bean varieties. Different cooking methods extracted different nutrients from the beans to various extents. However, pressure-cooking extracted comparatively more nutrients from beans than ordinary and microwave cooking methods. The concentration of proteins, soluble solids, soluble sugars and minerals increased in broth with increase in cooking time. Therefore, it may be concluded that broth of various beans con-

tains considerable amount of valuable nutrients, hence these may be used for soup and dessert preparations.

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MORPHOLOGICAL STUDIES OF THE SCHISTOSOMULUM OF *SCHISTOSOMA MANSONI* AND *SCHISTOSOMA MARGREBOWIEI* IN LUNGS OF MICE

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Morphological studies on the *Schistosoma mansoni* and *S. margrebowiei* schistosomulum development in the lung of definitive host mouse have been reported after 2 to 21 days post-infection. The greatest proportion of schistosomula were observed within the capillaries attached to the alveoli and few in the branches of the pulmonary arteries and veins. The length and shape of schistosomulum sections were highly variable due to the randomness of the plane of sections through the worm. The differences in the diameter of the body and cuticle of the *S. mansoni* and *S. margrebowiei* schistosomulum were non-significant ($P > 0.05$).

Key words: *Schistosoma mansoni, S. margrebowiei,* Schistosomulum, Lungs, Mice.

Introduction

The larval stage of *Schistosoma* (parasite) is called cercaria. These cercariae deposit a mucoid (PAS-positive) secretion from the postacetabular secretory glands as they loop over the skin of their mammal host during exploration at the site of penetration, enter into the horny and keratogenous zones during their passage through the skin and across the cellular epidermis. The mucoid post-acetabular secretion is adhesive, lubricative and serves in protective functions. The pre-acetabular secretion is primarily enzymatic (Stirewalt and Kruidenier 1961). Histolytic enzymes are secreted from the penetration glands and the cercariae burrow through the tissues. The tail of the cercaria is shed on penetration and the tail-less cercaria is then known as a schistosomulum. The schistosomulum is now no longer able to survive in fresh water (McLaren and Hockley 1977). The process of transformation takes less than one hour to complete *in vivo* (Cousin *et al* 1981).

Schistosomula leave the skin via the blood or lymphatic vessels and ultimately pass through the right side of the heart via the venous system to be distributed to the lungs via the pulmonary arterial system. The increase in numbers of schistosomula within the pulmonary capillaries from day 2 to day 7 post infection (p i) indicates their effectiveness as a physical barrier for further migration (Wheater and Wilson 1979). The arrival of schistosomula in the lungs via the pulmonary artery is a prelude to a sequence of developmental changes which are presumably necessary for further migration. Following arrival in the lungs of mice, schistosomula undergo a phase of elongation up to four times the length observed in the skin, with a concomitant reduction in diameter and no increase in mass (Wilson *et al* 1978). This process of elongation may be a necessary prelude to the passage of schistosomulum through the narrow lumina of capillaries in the lungs (Carbtree and Wilson 1986a). Schistosomula retain this capacity for elongation until they reach the hepatic portal system where it is believed to facilitate migration through capillary beds in the lungs and systemic organs (Miller and Wilson 1980). The aim of the present research paper is to describe the morphological changes in the development of the schistosomula in the lung of the mouse during schistosome infections.

Materials and Methods

Age-matched female mice of the Bantim and Kingman Tylers Original (BKTO) strain, weighed approximately 20 - 35g, each were infected with 200 cerariae of either *S. mansoni* [Puerto Rican strain maintained in albino *Biomphalaria glabrata* snails and random-bred TO mice following the methods of Taylor *et al* (1969) or *S. margrebowiei* (originally obtained from Lochinvar National Park, Zambia] and maintained in *Bulinus natalensis* intermediate host snails (the original stock was obtained from the Experimental Taxonomy Unit of the British Museum of Natural History, London, UK). Before administering the cercariae, the experimental animals were anaesthetized with sodium pentobarbitone (Nembutal) and the abdominal hair was clipped. The cercariae were applied to the abdominal skin by using ring. All mice were killed at day 2, 3, 4, 6, 8, 10, 16 and 21 and autopsies were performed immediately after the animals were killed by dislocation of neck region. The lungs from each animal were fixed in Heidenhain's Susa fixative, washed, dehydrated with ethanol, infiltrated and

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embedded in historesin. Selected 4 µm thick sections were stained in haematoxylin and eosin method. The sections were interpreted on Emst Leitz Wetzlar light microscope (Model No. 786554).

Results and Discussion

Morphological characteristics of *S. mansoni* and *S. margrebowiei* were studied in the lungs of mice.

Normal schistosomula were observed in both groups from day 2-16. During lung migration, the greatest proportion of schistosomula of both species were observed within the capillaries attached to alveoli and few were present in the branches of the pulmonary arteries (Fig 1-3). Some degenerated *S.mansoni* schistosomula were observed on day 21 in the branches of the veins. In *S.margrebowiei*, these were observed in the capillaries on day 8 and 21 pi. Few of the schistosomula were also observed in the pleural surface on days 2, 3, 6, 16 and 21 after infection.

Size of lung schistosomula. In the histological examined material, the length and shape of worm sections was highly variable due to the randomness of the plane of sections through the worm. In an attempt to compare sizes, the minimum diameter of transverse sections through the worms was determined. Observations on schistosomula in the mouse lungs were consistent with the organisms having an elongated thin shape. From 2-21 days pi, the diameter of the body and thickness of the cuticle of at least five healthy and five abnormal schistosomula were recorded in both species. The mean and standard deviations of the diameter of the body of the schistosomula of *S. mansoni* were 2.2560 ± 1.1280 µm and in *S. margrebowiei* they were 2.5790 + 0.9036 µm. The differ-

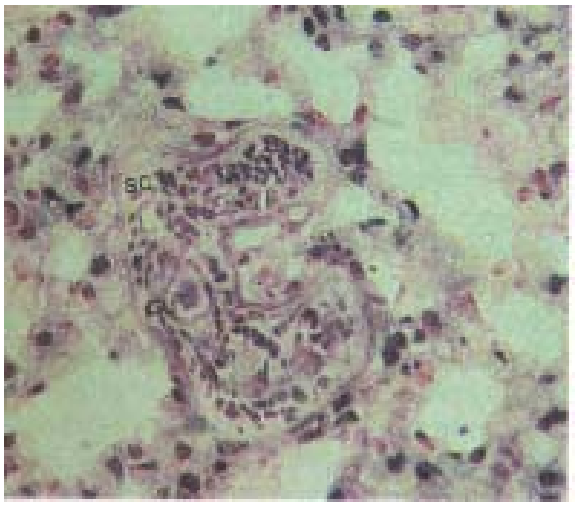


Fig 1. Mouse lung 3 days post-infection with *S.margrebowiei* cercariae. SC=Schistosomulum; BV=Blood vessel; N=Nuclei of differentiated cells. Stain; Haematoxylin and eosin.



Fig 2. Mouse lung 4 days post-infection with *S.Margrebowiei* cercariae. SC=Schistosomulum; GU=Gut caecum; N=Nuclei of differentiated cells. Stain: Haematoxylin and eosin.

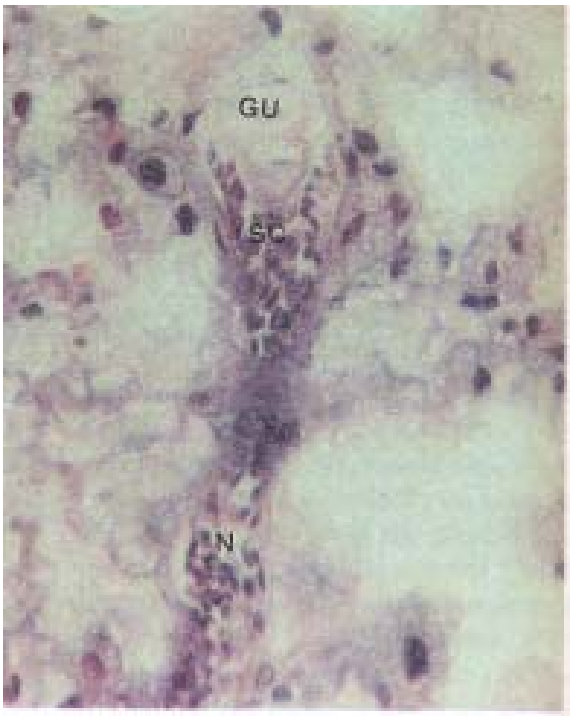


Fig 3. Mouse lung 4 days post-infection with *S.margrebowiei* cercariae. SC=Schistosomulum; GU=Gut caecum; N=Nuclei of differentiated cells. Stain: Haematoxylin and eosin.

Table 1
Mean diameter of the body and thickness of the cuticle of the *S. mansoni* and *S. margrebowiei* schistosomulum in the lungs of mice.

Days post infection	*S. mansoni* No.	Body µm	Cuticle µm	*S. margrebowiei* No.	Body µm	Cuticle µm
2	1	39.98	0.95	3	36.17	2.85
3	2	62.83	0.95	4	34.27	1.42
4	3	28.56	2.85	2	37.12	3.80
6	6	25.70	2.69	2	28.56	1.42
7	3	29.51	2.85	2	27.60	2.85
8	4	28.56	1.58	2	31.41	1.90
10	2	25.70	1.90	-	-	-
16	4	29.51	2.13	2	27.60	1.42
21	2	98.05	0.95	5	90.44	1.33

Abbrevation: dpi=Days post-infection, S=Schistosoma, µ=Micron.

ences in the diameter of the body of *S. mansoni* and *S. margrebowiei* schistosomulum were non significant, ($P > 0.05$). The mean and standard deviation of the thickness of the cuticle of *S. mansoni* larvae were 1.8720 + 0.8128 µm, whereas, in *S. margrebowiei* they were 2.1240 ± 0.9282 µm. The difference in the thickness of the cuticle of *S. mansoni* and *S. margrebowiei* schistosomulum was non significant, ($P > 0.05$). Hence there is no statistically significant difference in the diameter of the body and thickness of the cuticle of the two species of schistosomulum during their development in the lungs of mice between day 2 to 21 pi (Table 1).

Majority of the *S. mansoni* and *S. margrebowiei* schistosomula were presented in the capillaries of the lungs of mice from day 2 to 16 after infection, however a few were in the branches of arteries and veins of the lung. These observations are in close agreement with those of previously researched by Wheater and Wilson (1979) for *S.mansoni*, Ogbe (1983,1985) and Soomro (1996) for *S.margrebowiei*. Similar results were also reported by Yixun and Xinwu (1985) for *S.japonicum* schistosomula in the lungs of mice.

In the present study, the mean diameter of the body of the schistosomula of *S. mansoni* and *S. margrebowiei* was lower than reported by Carbtree and Wilson (1986a) where the minimum body diameter of the lung schistosomula was about 8 µm at day 7 pi Bloch (1980) reported that schistosomula had pigment in their caeca and the diameter varied between 40 - 175 µm. Clegg (1965) reported that average measurements of the 7 day old schistosomula (252 x 25 µm) are compatible with the view that the schistosomula elongates in the lung capillary but does not grow. Bruce *et al* (1974) reported that the mean cross section diameter of the intravascular organism was 20 µm. Wilson *et al* (1978) reported that maximum and minimum length of the 8 day schistosomulum was 365 µm and 197 µm respectively, giving maximum amplitude of 168 µm. The lung worm can constrict regions of its body to the diameter of less than 10 µm.

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NORTH CAROLINA DESIGN - 11 ANALYSIS FOR ESTIMATING GENETIC PARAMETERS IN COTTON *(GOSSYPIUM HIRSUTUM* L.*)*

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Genetic information is very important in formulating effective plant breeding programmes. North Carolina Design - 11 analysis was used to determine general combining ability (GCA), specific combining ability (SCA) and additive and dominant genetic variances for six quantitative traits in Upland cotton. In *per se* hybrid performance, parents CRIS - 241 and CRIS - 121 when crossed with other parents formed higher bolls per plant and produced more yield and lint %. It is usually predicted that *per se* hybrid performance is also reflected to SCA of the parents. This assumption did not always hold true, however, parents CRIS - 241 and CRIS - 121 that were good in *per se* hybrids and in GCA also formed good SCA when crossed with CRIS - 134 and CIM - 448, respectively. These results thus suggested that CRIS - 241 and CRIS - 121 are better parents simultaneously for *per se* hybrids, GCA and SCA. Genetic variation due to males (GCA), females (GCA) and males x females (SCA) were significant for all the traits except females for boll weight which indicated that both additive and dominant genes were important for characters under study. Similar to genetic variation due to GCA and SCA, estimates of additive and dominant genetic variances were also substantial suggesting that these characters can be improved either by hybridization and selection or suitable for hybrid cotton. The proportional contribution of GCA and SCA combining ability variances to total genetic variance further revealed that about equal proportions of additive and dominant genetic variances were available for all the traits except boll weight where additive variance was much higher than dominant variance.

Key words: General and specific combining abilities, Genetic parameters, Cotton.

Introduction

Improvement in metrical characters is usually based on progeny performance. In quantitative genetics, only additive genes determine progeny performance. Dominant genes, on the contrary, are specific to only genotypic value of an individual (Falconer 1989), thus do not contribute to progeny performance. Plant breeders make number of crosses among inbred parents to determine type of gene actions and also proportions of genetic variances attributable to additive and dominant genes for various plant characters. In earlier breeding performances, large number of crosses were also attempted and potential parents based on progeny performance were identified. These approaches of locating potential parents and genes functioning for various plant characters require huge resources in terms of manpower, space and time.

Information on genetic control of plant traits is also important before any breeding strategy is launched. Various mating designs such as diallels (Hayman 1954), Design-1, Design-11 (Comstock & Robinson 1948) and others are being used to estimate general combining ability (GCA) and specific combining ability (SCA) of parents and additive and non-additive genetic variances. The GCA effects are used to characterize the functions of additive genes whereas SCA effects and non-additive genetic variances are considered as the functions of dominant genes. North Carolina Design-11 analysis was used in the present study to estimate GCA and SCA effects and genetic components of variances for various cotton characters.

Materials and Methods

Four female parents that involved all advanced strains developed at the Central Cotton Research Institute, Sakrand, Sindh, Pakistan and four male parents of which two were cotton leaf curl virus (clcv) resistant from Punjab (CIM - 448 and CIM-443), one clcv resistant exotic (LRA - 5166) and one advance strain of CCRI-Sakrand (CRIS - 134) were crossed in North Carolina Design - 11 fashion. Thus, 4 x 4 parents were crossed and 16 F_1 hybrids were developed during 1998 crop season. Enough number of crosses were attempted to produce sufficient quantity of F_1 hybrid seed to be tested in replicated progeny trials. Sixteen F_1 hybrids were planted during 1999 in a Randomized Complete Block Design with four replications. The plot size was 10 x 35 feet, where row-to-row and plant-to-plant distances were kept at 2.5 feet and 9.0 inches, respectively. Thinning was exercised after 25 days of planting for maintaining proper spacing. In-organic fertilizers, 1 bag of DAP per hectare was applied at sowing time whereas 100 kg per hectare of nitrogen was applied in three split doses, 25 kg at sowing time + 25 kg with 2nd irrigation + 50 kg at peak flower-

ing. First irrigation was applied after 35 days of sowing whereas subsequent irrigations were given as and when required. The data on six quantitative traits such as yield per plant weighed in grams, average number of bolls counted on plant basis, average boll weight in grams, lint % calculated as the ratio of lint against cotton seed, fibre length measured in millimeters at 50% span length and seed index as the weight of 100 seeds in grams.

The general combining ability (GCA) and specific combining ability (SCA) effects were calculated by adopting Griffing's (1956) statistical procedures used by Singh and Choudhry (1979). The analysis of variance and genetic components of variances were determined as described by Comstock and Robinson (1948) adopted by Hallauer and Miranda (1986).

Results and Discussion

Genetic information to predict the relative contribution of desirable genes of parents to their offspring is very important in designing effective plant breeding programmes. Mating designs have been very useful in determining genetic control of various cotton characters. Diallel analysis has been used more than any mating designs in cotton breeding for determining general combining ability (GCA) and specific combining ability (SCA) of the parents.

The Design - 11 mating design as described by Comstock and Robinson (1948) is similar to diallel in obtaining genetic information. Two designs, however, are different in that the same parents are used as males and females in diallel, whereas, different sets of parents are used as males and females in design-11. Thus, design-11 uses more parents with small number of crosses as compared to that of diallel. Genetic information obtained from design-11, therefore, can be more reliable than diallel if same resources are to be used.

The *per se* performance of 16 F_1 hybrids for all six characters is shown in Table 1. In general, parents CRIS - 241 and CRIS - 121 when crossed with other parents gave more yield than the other parents in crosses. However, maximum yield of 155.0 gm was produced by the hybrid CRIS - 121 x CIM - 448. The next in the rank was CRIS - 241 x CIM - 443. Hybrids that gave higher yields generally set more bolls per plant but it did not always hold true because of variation in boll size. For instance, hybrids CRIS - 241 x CRIS - 134 which formed the highest number of bolls per plant (55.0) gave only 148.0 gm yield that actually ranked third in order. Boll weight, however, was found to be a contributing factor towards yield in that, as the boll weight increased, yields also increased with even less number of bolls set.

Though, boll weight varied from 2.3 to 3.8 gm, yet the best boll weight was 3.5 gm which gave maximum yield of (155.1 gm). These results thus suggested strong negative correlation between boll weight and number of bolls formed.

The *per se* hybrids' lint % varied from 34.6 to 40.0%, however, maximum lint was obtained from hybrid CRIS - 241 x CIM - 443. The next ginner hybrid was CRIS - 241 x CRIS - 134 that also

Table 1

Per se F_1 hybrid performance for yield, its components and fibre traits in cotton, *Gossypium hirsutum* L.

Hybrids	Yield per plant	Bolls per plant	Boll weight (gm)	Lint (%)	Staple length (mm)	Seed index (gm)
CRIS - 129 x CIM - 448	120.10	34.30	3.50	35.80	28.90	7.30
CRIS - 129 x CIM - 443	113.90	33.50	3.40	36.30	29.30	6.40
CRIS - 129 x LRA - 5166	139.90	37.80	3.70	34.60	30.60	6.90
CRIS - 129 x CRIS - 134	85.80	37.30	2.30	36.10	28.10	5.40
CRIS - 168 x CIM - 448	112.00	28.00	4.00	35.30	29.30	7.70
CRIS - 168 x CIM - 443	115.10	30.30	3.80	35.00	28.90	7.90
CRIS - 168 x LRA - 5166	134.60	40.80	3.30	35.10	26.90	6.10
CRIS - 168 x CRIS - 134	106.60	33.30	3.20	34.70	28.10	6.50
CRIS - 241 x CIM - 448	145.50	42.80	3.40	35.70	28.70	6.20
CRIS - 241 x CIM - 443	154.60	41.80	3.70	40.00	28.70	5.90
CRIS - 241 x LRA - 5166	107.20	33.50	3.20	36.10	27.80	5.80
CRIS - 241 x CRIS - 134	148.50	55.00	2.70	37.40	27.20	5.80
CRIS - 121 x CIM - 448	155.10	44.30	3.50	36.00	29.50	7.30
CRIS - 121 x CIM - 443	142.50	37.50	3.80	36.00	29.50	7.50
CRIS - 121 x LRA - 5166	113.40	37.80	3.00	35.70	29.10	6.10
CRIS - 121 x CRIS - 134	131.60	47.00	2.80	34.70	28.10	6.20
General mean	126.70	38.40	3.30	35.90	28.70	6.60

Table 2

North Carolina Design-11 analysis for yield, its components and fibre traits in cotton, *Gossypium hirsutum* L.

Source of variation	Degrees of freedom	Mean squares					
		Seed cotton yield	Bolls per plant	Boll weight (gm)	Lint (%)	Staple length (mm)	Seed index (gm)
Crosses	15	2066.20**	183.70**	0.77**	6.90**	3.58**	2.45**
Males (GCA)	3	423.40**	167.80**	2.69**	6.32**	5.41*	4.98**
Females (GCA)	3	4468.80**	372.10**	2.35	15.30**	4.87*	3.87**
Males x Females (SCA)	9	1817.60**	126.10**	0.28*	4.30**	2.55*	1.13**
Error	45	12.30	6.60	0.09	0.37	0.25	0.12
Genetic components of variances							
$^{i}d^{2}A$ (males)		347.90	10.408	0.604	0.505	0.716	0.963
$^{i}d^{2}A$ (females)		663.35	61.493	0.019	2.750	0.580	0.685
$^{i}d^{2}D$ (males x females)		1802.37	119.560	0.175	3.925	2.299	1.014
$^{i}d^{2}e$		12.25	6.580	0.099	0.373	0.248	0.116
Proportional contribution of males, females and their interaction to total variance							
Contribution of males (%)		4.10	18.27	69.87	18.32	30.22	40.65
Contribution of females (%)		43.25	40.50	9.09	44.35	27.21	31.59
Contribution of males x females (%)		52.69	41.19	21.82	37.39	42.73	27.67

**,*Significant at 1 and 5% probability levels, respectively.

ranked 3rd in yield (148.5 gm). For staple length, on an average, parents CRIS - 129 and CRIS - 121 when crossed with other parents gave longer fibre. The longest fibre of 30.6 mm however, was given by hybrid CRIS - 129 x LRA - 5166. The next in the rank were; CRIS - 121 x CIM - 448 and CRIS - 21 x CIM - 443, these produced 29.5 mm long fibre. The seed index of *per se* hybrids varied from 5.4 to 7.9 gm where maximum (7.9 gm) index was given by hybrid CRIS - 168 x CIM - 443. The three high yielding hybrids gave index value in the range of 5.8 to 7.9 gm. These results, therefore, indicate that for obtaining higher yields, index value should not exceed to 7.3 gm. Generally, seed index was positively correlated with boll weight, that means, as the boll weight increased, the index value also increased but the number of bolls reduced and as a consequence, yields decreased. The highest seed index (7.9 gm) giving hybrid CRIS - 168 x CIM - 443, however, gave less yield of only 115.1 gm that is even less than the general mean.

The mean squares of crosses (Table 2) for all the six characters were significant that allowed to further partition this factor into variation due to males (GCA), females (GCA) and males x females (SCA) components. The variation due to males, females and males x females were also significant for all the traits except only due to females for boll weight. For yield and number of bolls, the proportion of variance due to females was higher than males suggesting that female parents had more additive genes as compared to male parents. Males x females source of variation for both traits were also siginificant implying the importance of dominant genes for yield and bolls also. But small variation due to males x females compared to their respective males and females further indicated that both additive and dominant genes are functioning for these traits. Nevertheless, additive genes were more important than dominant genes in controlling yield and bolls per plant. Mean squares for boll weight due to females were non-significant but they were significant due to males suggesting that males retained more additive genes against females. Males x females factor for boll weight was also significant indicating the importance of non-additive genes controlling boll weight. Regarding lint %, the mean squares due to males, females and males x females were significant but the proportions of variations due to GCA were higher than SCA implying greater importance of additive genes than non-additive genes in expressing this trait. For staple length, variation due to males, females and males x females were all significant connoting the importance of additive as well as dominant genes for this trait. Baloch *et al* (2002) from line x tester analysis which is analogous to our Design - 11 analysis reported that mean squares due to GCA and SCA were significant implied that both additive and dominant genes were functioning for seed cotton

yield, bolls per plant, boll weight, lint percent, and fibre length Table 2.

Baloch *et al* (2000) from diallel analysis also noted the importance of both additive and non-additive genes for seed cotton yield, bolls per plant, lint%, and staple length. In previous studies, Baloch *et al* (1999), however, observed that number of bolls, lint % and staple length were controlled by only additive genes. Additive genes predominantly controlled seed index, nevertheless, dominant genes were also important as reported by Tunio *et al* (2002). Bhutto *et al* (2001), nonetheless, observed that seed index was predominantly controlled by dominant genes.

The estimates of additive and dominant genetic variances were higher than environmental variances (Table 3) suggesting that inferences regarding genetic control of cotton traits in the present study are quite reliable. Similar to mean squares, predictions of additive and dominant genetic components for all the traits were substantial connotating that both additive and dominant genes are responsible for these traits. Results further suggested that all the characters under study could be improved by the selection in segregating generations or suitable for hybrid vigor, consequently, for hybrid crop development.

The proportional contribution of males (GCA), females (GCA) and males x females (SCA) variances to total genetic variance (Table 2) further revealed that about equal proportion of additive and dominant genetic variances is available for all the traits except boll weight. By using line x tester analysis, Baloch *et al* (1999), recorded controversial results where additive genetic variance for lint % was significantly higher than non-additive variance. Ubaidullah and Hussain (1997) however, did not find any significant genetic variance for cotton traits they studied. Genetic models, mating designs and material used in their studies, could explain these controversies.

Plant breeders generally prognosticate that parents, which perform well in hybrids *per se*, also perform similar for GCA and SCA as well. However, this divination did not always hold true (Srinivasan & Gururajan 1973; Baloch *et al* 1993,1995 & 1997). Our results for GCA estimates (Table 3) indicated that parents CRIS - 241 and CRIS - 121, which generally were based in hybrids *per se*, were also good general combiner for seed cotton yield and number of bolls. Surprisingly, both of these parents exhibited negative GCA estimates for boll weight, suggesting that boll weight was negatively correlated with yield and boll numbers. In other words, as the boll weight in-creases above certain point, the number of total bolls per plant drops-off, consequently yield per plant also reduces. In respect to lint %, parent CRIS - 121 formed the maximum GCA (1.400) and the next in the rank was CIM - 443 (0.913). The GCA estimates for staple length and seed index were not prominent, however, parents CIM - 443 and CIM - 448 were good general combiners for these traits and parent CRIS - 129 was good combiner for seed index only. Our GCA estimates, therefore, suggested that parents CRIS - 241 and CRIS - 121 are good general combiner simultaneously for yield, number of bolls and lint % and to improve these characters, the above three parents are good choices for hybridization and selection programmes. On the other hand, for staple length and seed index, parents CIM - 443 and CIM - 448 are desirable parents.

Specific combining ability estimates are presented in Table 4. Though good general combining ability parents, CRIS - 241 and CRIS - 121, were not always good specific combiners for

Table 3

General combining ability estimates for yield, its components and fibre traits in cotton, *Gossypium hirsutum* L.

Males	Yield per plant (gm)	Bolls per plant	Boll weight (gm)	Lint (%)	Staple length (mm)	Seed index (gm)
CIM - 443	- 0.940	- 2.660	0.344	0.913	0.441	0.369
CIM - 448	6.440	- 1.090	0.250	- 0.219	0.434	0.569
LRA - 5166	0.560	- 0.970	- 0.025	- 0.525	- 0.084	- 0.338
CRIS - 134	- 6.060	4.720	- 0.569	- 0.169	- 0.791	- 0.600
General mean	126.700	38.400	3.300	35.900	28.700	6.600
S.E (gi)	0.935	0.641	0.079	0.153	0.124	0.085
Females						
CRIS - 129	- 8.440	- 2.720	- 0.106	- 0.213	0.547	- 0.063
CRIS - 168	- 19.440	- 5.340	0.219	- 0.875	- 0.366	0.506
CRIS - 241	13.500	4.840	- 0.056	1.400	- 0.572	- 0.650
CRIS - 121	14.380	3.220	- 0.056	- 0.313	0.391	0.206
General mean	126.700	38.400	3.300	35.900	28.700	6.600
S.E. (gi)	0.940	0.640	0.079	0.153	0.123	0.085

Table 4

Specific combining ability estimates of yield, its components and fibre traits in cotton, *Gossypium hirsutum* L.

Hybrids	Yield per plant	Bolls per plant	Boll weight (gm)	Lint (%)	Staple length (mm)	Seed index (gm)
CRIS - 129 x CIM - 448	- 10.560	- 0.340	0.019	0.299	- 0.747	0.257
CRIS - 129 x CIM - 443	- 0.690	0.470	- 0.149	- 0.232	- 0.403	- 0.493
CRIS - 129 x LRA - 5166	30.560	3.030	0.469	- 0.569	1.497	0.763
CRIS - 129 x CRIS - 134	- 19.310	- 3.160	- 0.337	0.599	- 0.347	- 0.525
CRIS - 168 x CIM - 448	- 4.310	- 3.970	0.194	0.487	0.566	0.063
CRIS - 168 x CIM - 443	- 8.190	- 0.160	- 0.199	- 0.944	0.185	0.488
CRIS - 168 x LRA - 5166	12.560	- 8.660	- 0.231	0.619	- 1.340	- 0.606
CRIS - 168 x CRIS - 134	- 0.060	- 4.530	0.238	- 0.163	0.591	0.056
CRIS - 241 x CIM - 448	- 9.490	0.590	- 0.106	- 1.388	0.147	- 0.281
CRIS - 241 x CIM - 443	7.380	1.160	0.101	1.806	0.191	- 0.381
CRIS - 241 x LRA - 5166	- 24.130	- 8.780	- 0.031	- 0.681	- 0.234	0.199
CRIS - 241 x CRIS - 134	26.250	7.030	0.038	0.262	- 0.105	0.462
CRIS - 121 x CIM - 448	24.000	3.720	- 0.006	0.599	0.035	- 0.037
CRIS - 121 x CIM - 443	1.500	1.470	0.151	- 0.532	0.029	0.388
CRIS - 121 x LRA - 5166	- 18.990	- 2.910	- 0.206	0.631	0.079	- 0.376
CRIS - 121 x CRIS - 134	- 6.870	0.660	0.063	- 0.701	- 0.140	0.006
General mean	126.700	38.400	3.300	35.900	28.700	6.600
S.E. (Si)	1.750	1.280	0.158	0.305	0.249	0.170

yield with every other parents in crosses, yet both the parents, CRIS - 241 with CRIS - 134 and CRIS - 121 with CIM - 448 formed good SCA estimates. However, hybrid CRIS - 129 x LRA - 5166 formed the best SCA effect of 30.56. In respect of bolls per plant, the above-mentioned hybrids also ranked 2nd and 3rd for SCA estimates. These results thus concluded that some of the parents are simultaneously good in *per se* hybrid performance, GCA and SCA as in our case CRIS - 241 and CRIS - 121 are parents of better choice for hybridization and selection and also for hybrid cotton development.

Baloch *et al* (1997) also noted that some of the parents performed equally well either in *per se* hybrids, GCA or SCA. The high GCA and SCA estimates of some parents for yield, bolls per plant and lint % indicated that both additive and dominant genes are function for these characters.

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Performances of Newly Developed Cotton Strains For Economic and Fibre Traits in National Coordinated Varietal Trials

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To find out the responses of new cotton, strains were conducted at Central Cotton Research Institute, Multan along with a commercial variety NIAB - 78. The highest seed cotton yield was noted in CIM - 443 followed by CIM - 435. The longest staple length was noted in strain CIM - 435 followed by CIM - 443. The strain CIM - 443 was significantly better in ginning outturn percentage and number of bolls/plant followed by CIM - 435. The boll weight was the highest in CIM - 435. The newly developed strain CIM - 443 showed the best characteristics, among all the tested strains.

Key words: Genotypes, Response, *Gossypium hirsutum* L. NCVT.

Introduction

Cotton, which is called as 6F plant, i.e. utilized as food, feed, fibre, fuel, fodder and fertilizer, worldwidely. In Pakistan, cotton adds to our economy with annual production of worth 136739.60 millions rupees by exporting various products and byproducts of cotton, (Agricultural Statistics of Pakistan 1999 - 2000) along with the labour opportunities for the rural and urban population of our country.

Cotton is a very sensitive crop, and a strain, which is high yielder in one location or year, may become poor yielder at other location or year. Therefore, it is a challenge for breeders to evolve such strains which could retain stability in different environment/years for seed cotton yield, number of bolls, boll weight, ginning outturn percentage and staple length.

A lot of research works were conducted on the above parameters, from which the works of some prominent scientists are shown below. Afzal *et al* (2002) searched out significant differences in years for plant height and seedcotton yield, while non-significant differences in number of bolls per plant and boll weight. For genotypes, the variations were significant in all the characters studied i.e. number of bolls per plant, boll weight, plant height and seedcotton yield. The environment x genotype interactions were also significant for boll weight and seedcotton yield. Afzal *et al* (2001) concluded highly significant variations among years, genotypes and year x genotypes interaction for plant height, number of bolls/plant, and seedcotton yield. Hanif *et al* (2001) searched out significant differences among years (environment), varieties (genotypes) and their interaction in four newly developed genotypes and one commercial variety i.e. Karishma. Baluch (2001) studied that there are significant differences among various genotypes/strains for boll weight, ginning outturn percentage, staple length and seed cotton yield. Shad *et al* (1996) noted highly significant differences for staple length among the strains. Ahmad *et al* (1993) studied that seed cotton yield and staple length are mostly affected by environmental factors (years). Geng *et al* (1987) and Soomro (1994) studied that staple length was mostly influenced by strains than years. Soomro *et al* (1986) reported significant differences in yield, ginning outturn percentage (GOT) and staple length for varieties, locations and years. Meredith (1984) reported highly significant seasonal effects on staple length, strains and years interaction. Ahmad *et al* (1982) obtained highly significant differences in yield of upland cotton due to varieties, locations and yield components. Gupta and Katiyar (1980) reported significant differences in yield of upland cotton due to strains, environment and their interactions. Soomro and Memon (1979) reported that the effect of site and season (year) on the yield and GOT percentage in desi cotton were due to varieties only. Sharif and Ahmad (1978) studied that there are two major factors for determining the yield of any crop species, (a) genetic makeup (b) environmental factors and their interactions. Singh *et al* (1973) reported that significant differences in raw cotton yield for American cotton was due to varieties.

Thus the present research is conducted to find out the strains having desirable fibre characters among the tested strains.

Materials and Methods

The experiments were conducted for two years at Central Cotton Research Institute Multan, during the cotton growing season, 1997 - 1998 and 1998 - 1999. In order to find out the strains, having desirable yield and fibre characters in five newly

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Table 1

Mean squares from combined analysis of variances for various characters in cotton strains tested over two years at CCRI, Multan during 1997-1998 to 1998-1999

Source of variation	D.F.	Mean squares				
		No. of bolls	Boll weight (g)	GOT (%)	Staple length (mm)	Seed cotton yield (kg/ha)
year	1	1.67	3.22**	0.17	44.55**	148603.00
Factor A (variety)	4	308.43**	3.78**	41.76**	33.19**	2677377.00**
LA	4	36.55**	0.24**	6.53**	0.67**	650727.00**
Error	24	3.48	0.02	0.19	0.17	22001.00

**, * Significant at 1 % and 5 % probability levels respectively.

Table 2

Average economic and fibre traits performance of newly evolved cotton strains tested over two years at Central Cotton Research Institute Multan, during 1997 - 1998 to 1998 - 1999

S. No.	Strain/ variety	Characters				
		No. of bolls	Boll weight (g)	GOT (%)	Staple length (mm)	Seed cotton yield (kg/ha)
1	CIM - 435	26.70	4.34	37.55	28.13	2896
2	CIM - 443	36.00	3.28	37.98	26.43	3036
3	BH - 95	26.10	3.85	36.43	25.72	2491
4	CRIS - 19	23.10	2.98	32.84	24.05	2144
5	CRIS - 82	21.80	2.70	33.92	22.94	1912
6	NIAB - 78	20.70	3.04	35.06	25.08	1784
	LSD. (0.05%)	1.69	0.13	0.39	0.37	134

developed cotton strains, i. e. CIM - 435 and CIM - 443 of Central Cotton Research Institute (CCRI) Multan, BH - 95 of Cotton Research Station Bahawalpure, CRIS - 19 and CRIS - 82 of Cotton Research Institute Sakrand against a commercial variety NIAB - 78 of National Institute for Agriculture and Biology Faisalabad. The crops were sown on 1st June, during 1997 - 1998 (Y_1), and 31st May during 1998 -1999 (Y_2), with five replications, in randomized complete block design, having row to row and plant to plant distances of 75 and 30cm, respectively. Normal agronomic practices, i.e. fertilizer application, weeding, irrigation, and plant protection measures were adopted as and when required. Ten randomly selected plants from each replication of each strain were selected for recording the data. Data were recorded on the following parameters. Number of bolls/plant, boll weights (g), ginning outturn (%), staple length (mm), and seed cotton yield (kg/ha).

The data obtained were statistically analyzed by using MSTATC, a computer software package Bricker (1991).

Results and Discussion

Significant variations were observed in years (Table 1) for boll weight, and staple length, which showed that different years affected boll weight and staple length of the cotton crop differently. Thus the present research findings are in agreement with the research work of Ahmad *et al* (1993), Hanif *et al* (2001), Meredith (1984), Soomro *et al* (1986) and in contrast with the results of Afzal *et al* (2002), Geng *et al* (1987) and Soomro (1994). While non-significant differences were noted for number of bolls per plant, ginning outturn percentage and seed cotton yield, which are in conformity with the result of Afzal *et al* (2001), Singh *et al* (1973), Soomro and Memon (1979).

There also existed significant variations among the strains (Table 1) for all the characters i.e. number of bolls per plant, boll weight, ginning outturn percentage, staple length and seed cotton yield (Table 2). The research work is in agreement with the research finding of Afzal *et al* (2001), Afzal *et al* (2002), Ahmad *et al* (1982), Baluch *et al* (2001), Geng *et al* (1987), Hanif *et al* (2001), Shad *et al* (1996), Sharif and Ahmad (1978), Singh *et al* (1973), Soomro and Memon (1979), Soomro *et al* (1986) and Soomro (1994).

Significant strain x year interactions were observed (Table 1) in all the characteristics i.e. number of bolls/plant, boll weight, ginning outturn percentage, staple length and seed cotton

yield. The significance of strains x years interaction indicated that the strains performed differently over the years. The present research work is in agreement with the experimental research of Afzal *et al* (2001), Afzal *et al* (2002), Hanif *et al* (2001), Sharif and Ahmad (1978) and Soomro *et al* (1984).

The average performance of strains are presented in Table 2, suggesting that the strain CIM - 443 gave significantly higher seed cotton yield (3036 kg/ha) against the other testing strains. Where strain CIM - 435 ranked next in order by giving 2896 kg/ha and the lowest yielder was NIAB - 78 (1784 kg/ha). The longest staple length was recorded for strain CIM - 435 (28.1mm) followed by CIM - 443 (26.4mm) and the shortest was noted for strain CRIS - 82 (22.9mm). While in case of ginning outturn percentage, the highest ginning outturn percentage was recorded for strain CIM - 443 (37.98%), followed by CIM - 435 (37.55%) and the bottom position was again retained by CRIS - 19 (32.84%). In respect of boll weight, the heaviest boll weight was noted for strain CIM - 435 (4.3g) and the lesser boll weight, were recorded for CRIS - 82 (2.7g). The highest number of bolls/plant were found in strain CIM-443 i.e. 36.0 followed by CIM - 435 (26.7) and the lowest number of bolls/plant were recorded in strain NIAB - 78 (20.7).

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NUTRITIONAL QUALITIES OF SMOKED SHRIMP FROM THE SUNDARBANS MANGROVE AREA, BANGLADESH

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Quality aspects of a traditional smoked product from the shrimp *Metapenaeus brevicornis* in the Sundarbans mangrove area have been discussed. Studies on the physical properties, proximate composition, mineral content and amino acid value show that the quality of traditional smoked product is quite good. During 90 days of storage period the product was found to lose its characteristic properties and overall acceptability. However, smoked shrimp retained its overall acceptability up to 60 days of storage.

Key words: Smoking, Shrimp, Proximate composition, Micronutrient, Amino acid

Introduction

In artisanal fisheries of coastal Bangladesh, the common methods of preservation are sun drying and smoking with the exception of salted hilsa (*Tenualosa ilisha*). In the Sundarbans area, artisanal fishing dominates in both inshore and offshore water, about 60% of the catch is used for sun drying. Of the rest, small-sized shrimps, which represent 20-30% of catch, are smoke-dried. These shrimps, which are found in vast quantity, the fisherfolk heat and smoke over a fire using forest wood, and the semi-dried, characteristic brown coloured product can be stored for substantial period (Hossain 1984). This product is economically high-priced and is used as food by large numbers of coastal people.

In tropical countries, particularly in Africa, smoking is the most important method of fish preservation. Smoking is also practiced as a means of fish preservation in many south-east Asian countries, e.g. fish and prawn in India (Salagrama 1990), tuna in Maldives and Sri Lanka (BOBP 1988).

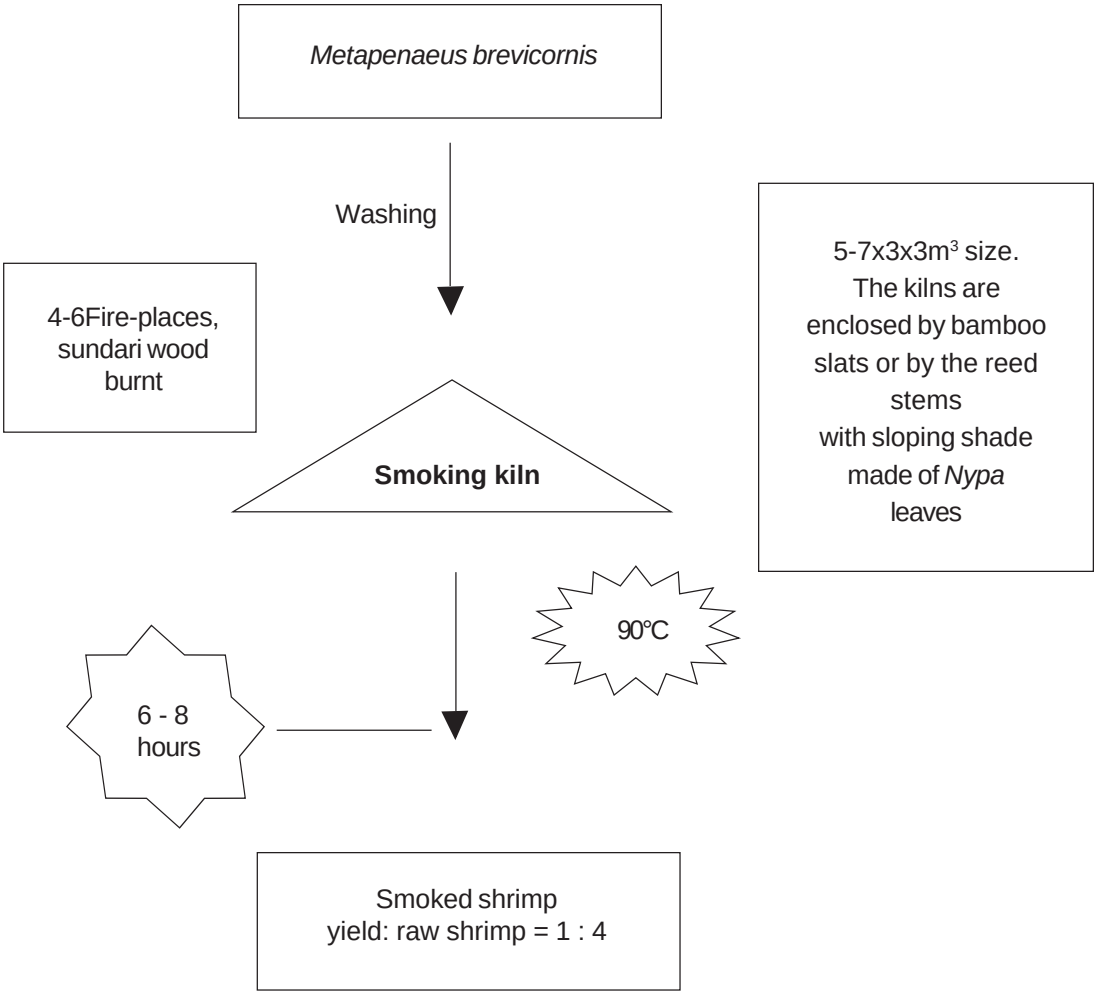
The importance of fisheries products is related to its nutritional quality, market value and acceptability to consumers. Smoking of shrimp in Sundarbans area has been considered as a technique for preservation since time immemorial due to availability of shrimp and smoking wood. This "hot smoking" process is traditional and the acceptable storage duration of the product is comparatively short. In this paper attempts have been made to evaluate the smoking process and the product of *Metapenaeus brevicornis* from nutritional point of view, and also storage quality.

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Materials and Methods

Smoking process. The traditionally smoked product is prepared near the coastal rivers. The smoking kilns are bamboo structures with a sloping roof of mangrove origin and thatched golpata (*Nypa fruticans* leaves). Sometimes a one meter high muddy wall surrounds the kiln. Usually, the size of the kiln is about 5-7x3x3 meters which can be opened along one side like a flap. A platform made of bamboo slats and woven reeds is made about 1.5 m high from the floor. At least 4 fire-place are operated inside kiln and the wood like "sundri" (*Heritiera fomes*) is used as fuel. The characteristic nature of sundri wood is that it burns very slowly and produces smoke. Burning inside the kiln is controlled by a long bamboo handle, which is operated through the holes of kiln. The kiln has enough ventilation to exit the smoke produced.

Smoking kilns normally run during the rainy season (mid June to mid September) when plenty of mixed species of shrimp/ prawns are available in the rivers of the Sundarbans mangrove forest. Usually, shrimp/prawns of commercial importance *Penaeus monodon*, *P.indicus*, *Metapenaeus monocerous*, *Macrobrachium rosenbergii* are marketed fresh or frozen for export. Other species like *Metapenaeus brevicornis, M. dobsoni, Palaemon styliferus, Macrobrachium* spp. are used for smoking. In the present study, *M. brevicornis* was used as raw material and the smoking operation was carried out in a kiln located near the Sundarbans (Scheme 1). Whole operation was slightly improved over that of the traditional method of smoking. After collection from local fishermen, the *Metapenaeus brevicornis* locally known as "kucho chingri" were sorted out from other species groups and those above



Scheme 1. Schematic diagram of smoking process.

4 cm total length were used as raw material. At the time of sorting, other foreign particles like leaf-fragments, molluscan shell, other fish and shrimp species were removed. Then the shrimps were placed in bamboo baskets and washed several times with tube-well water. Generally, during traditional method mixed shrimp species are used in smoking process and proper handling and washing is not done, hence, there is ample scope for quality deterioration.

After washing and removal of excess water, the shrimps were weighed (130 kg) and placed on the bamboo platform and the wood in the fire-place is lit. The quantity of fire-wood used not measured, but it was enough to produce adequate smoke. After 2 h, when the shrimps turned reddish colour, they were turned upside down with the help of a long arm wooden handle. The fire-place was operated for 6 h and the burning charcoal extinguished with water, and smoked shrimp were kept inside the kiln for another 2 h to be cooled. After that the folded door was opened and the end products were taken out and weighed for yield estimation.

The inside temperature of the kiln was recorded at 15 to 30 min interval using a Celsius thermometer. Temperature was measured on the layer of shrimps placed on bamboo platform, and recorded at 3 different places inside the kiln. For a better understanding of temperature distribution inside a traditional two other smoking kilns, nearby, were monitored and the temperature during smoking operation was recorded on consecutive days.

Immediately after smoking, ready to be marketed, smoked shrimps were placed in air tight clear polythene bags and divided into 3 groups to be analyzed after 30, 60 and 90 days of storage study. While some of the shrimps, were analyzed for various parameters and this was taken as zero-time sample. Polythene bags containing smoked shrimps, were then placed in a cardboard box and kept in the laboratory at room

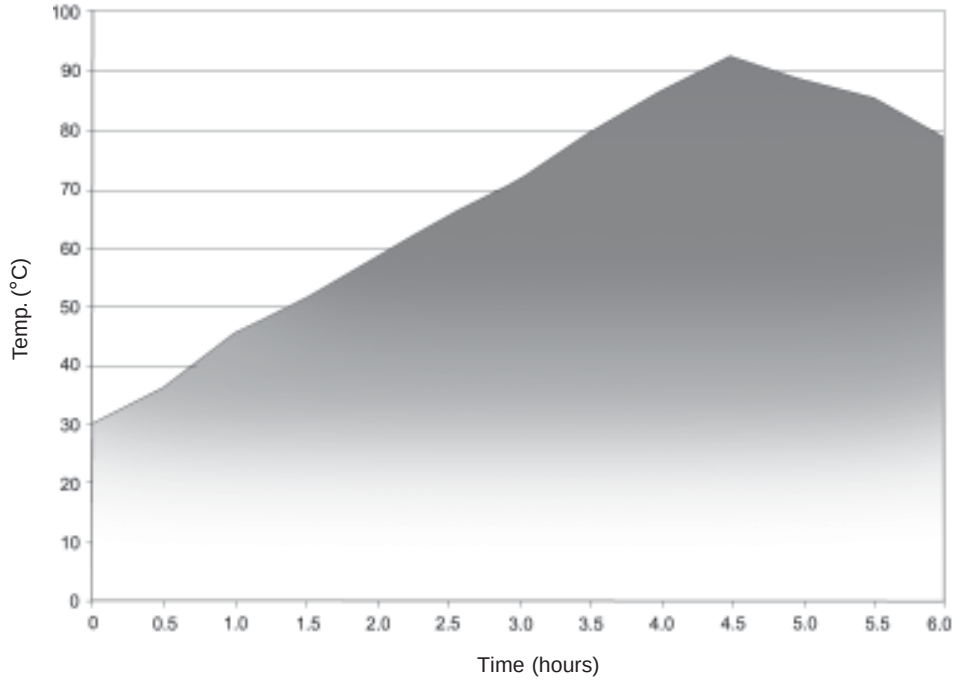


Fig 1. Mean inside temperature of smoking kiln during 6 h time.

temperature. The smoked product is normally stored in bamboo baskets or reed stem bags and marketed during the lean season.

Market samples of smoked shrimp were collected from the city of Chittagong to compare with the above.

Ambient temperature, rainfall and relative humidity data were collected from the local Meteorological Station.

Analytical procedure. Proximate composition of the raw shrimp and smoked shrimp at different storage periods was conducted by following the methods of AOAC (1980).

For pH determination, 2 g of ground sample was diluted in 10 ml of distilled water and pH was measured using a direct reading digital pH meter (pH 523, WTW, Germany).

Reconstitution property was assessed as percentage of water imbibed by 100 g of sample soaked in 400 ml of water, both at room temperature and 80˚C for a period of 2 h by following the method of Valsan (1975).

Total Volatile Base Nitrogen (TVB-N) of the smoked samples were determined accordingly to the method described by Commission of the European Communities (1995).

Peroxide Value (POV) of samples was determined by following the methods described by Egan *et al* (1981).

Total bacterial count expressed as colony forming units (CFU/g) of smoked products was estimated by standard plate count method on plate count agar by following the dilution technique.

For mineral content, pre-digested sample composition was determined using Perkin-Elmer 2380 Atomic Absorption Spectrophotometer by following modified method of Cresser and Parson (1979).

Amino acid composition of smoked product was determined at Stirling University, UK using an LKB 4151 Alpha-Plus Amino acid analyzer (LKB Bichrom Ltd., Cambridge).

Results and Discussion

Mean inside temperature of the 3 smoking kilns is presented in Fig 1. The initial temperature recorded was 30˚C.During 1 h period the temperature increased to 45˚C, after 2 h when the shrimp on bamboo platform turned upside down the temperature remained at 60˚C (66˚C in experimental kiln) then, it further increased to 92-94˚C in 4.5 h period. The temperature then gradually decreased to78˚C (75˚C in experimental kiln) when smoking operation was closed down. As the kiln was made of bamboo splits structure, it had enough ventilation through which some heat loss is expected. As 6 fire-places were placed uniformly inside the kiln, the temperature generated inside the kiln varies within a 4-7˚C range.

Table 1 represents the physical properties of smoked product. During 90 days storage, the smoky odour of the product was greatly reduced, pH of the product was initially 6.44, after 90 days, the pH value remained at 7.65. The pH of raw shrimp was 7.41. Water reconstitution property gradually increased with the storage at room temperature from 98.75 to 157.18% and was higher at 80˚C (130.43-163.44%) during 90 days of storage.

Table 1
pH, Reconstitution properties and sensory evaluation of smoked shrimp (*Metapenaeus brevicornis*)

Days		pH	Reconstitution (%)		Organoleptic properties		
			Room temperature	80°C	Smoky odour	Colour	Texture
Fresh shrimp		7.41	-	-	-	-	-
Smoked shrimp	0 day	6.44	98.75	130.43	Strong	Reddish brown	Springy
	30 days	7.32	121.78	139.94	Medium	Yellowish red	Less springy
	60 days	7.57	135.64	148.15	Less	Brownish yellow	Medium soft
	90 days	7.65	157.18	163.44	Poor	Reddish yellow	Soft

Table 2
Biochemical composition and bacterial load of smoked shrimp

Days	Moisture (g/100g)	Protein (g/100g)	Lipid (g/100g)	Ash (g/100g)	TVBN (mg/100g)	POV (meq/kg)	Bacterial load (cfu/g)
Fresh shrimp	79.95	72.37	11.62	15.46	-	-	-
Smoked - 0 day	12.70	77.98	7.88	13.08	22.75	17.40	2.46×10^4
30 days	16.89	77.58	9.54	11.71	25.93	31.50	2.70×10^4
60 days	21.42	72.75	12.59	13.97	27.16	46.05	1.65×10^6
90 days	24.19	68.74	15.38	14.72	29.33	55.30	1.41×10^7
Market sample	19.51	75.57	9.83	12.87	-	-	6.50×10^4

During the smoking operation, the ambient temperature was recorded as 29.3°C (September). After 30 days, the temperature decreased to 21.2°C with moderately heavy rainfall (82.5 mm). The day-time temperature decreased from 15.2 - 10.8°C in the next 60 days. The humidity (RH) during storage was recorded as 96 to 71%.

Storage quality of the smoked product was also studied for 90 days, the results of which is presented in Table 2. Moisture level at 90 days interval increased to 24% from initial 13%. The protein content of raw shrimp was 14.51%, after smoking the protein content found to be 68.08% due to loss of about 60% moisture. After one month storage protein content reduced to 64.48%. The protein percentage further reduced to 57.17% after 60 days and at 90 day it stands at 52% due to gradual increase of moisture content. Lipid content gradually increased to 11.66% from 6.88%. Proximate composition and bacterial load study in market sample revealed increased moisture level (19.51%) and decreased protein content (60.83%) with a microbial load of 6.5×10^4 cfu/g.

Total volatile base nitrogen (TVBN) value increased with storage time from 22.75% at ' 0' day to 29.33% at 90 day. Peroxide value (POV) increased largely from initial 17.4 to final 55.3 meq/kg of oil. Total bacterial counts of the smoked product increased to 1.41×10^7 cfu/g from an initial 2.46×10^4 cfu/g over 90 days (Table 2).

In an attempt to evaluate the biological and nutritive value of smoked shrimp, nutrient and amino acid composition of the product are given in Tables 3 and 4. Smoked shrimp was found to be rich in calcium, phosphorus, potassium, magnesium, iron, and essential amino acids.

There are more than 100 shrimp smoking units now operating in and around the Sundarbans, although the total production could not be estimated. Abundant shrimp catch and easily available fuel woods enable fishermen to operate these traditional smoking kilns during a particular season. Although collection of wood specially sundri from Sundarbans is prohibited, unauthorized collection of wood not only for fuel but for many other purposes is a common practice within the Sundarbans boundaries. Shrimps are smoked mainly to give it a flavour rather than to preserve it. Moreover, people from eastern boundaries of the country traditionally like to eat smoked shrimp.

The good smoky odour of the product lasts up to 30 days. Reconstitution property gradually increased with time due to changes in texture. Water reconstitution properties of 123-144% were observed in a market sample of smoked snakehead fish (*Channa* spp.) (Lilabati *et al* 1997). The pH value after 60 days indicated slightly alkaline nature of the smoked product. A good quality product that keeps well must lose 12-14% of its original weight during traditional smoking.

Table 3
Nutrient composition (mg/100g) of smoked shrimp

Mineral	Content	Mineral	(% Dry matter basis) Content
Phosphorus	1070	Copper	4.99
Potassium	500	Zinc	6.99
Magnesium	180	Manganese	2.76
Calcium	100	Iron	36.08

products of Bangladesh (Khan *et al* 1997). Moisture content increased rapidly during initial 30 days then slightly with the lapse of storage time. Smoked shrimps were stored in polythene bags, but due to tiny pores created by the pointed rostrum and telson of shrimps, air-tight conditions for the product could not be maintained. Moreover, moist weather due to rainfall may have caused increased moisture level in smoked product. For the storage and packaging of *Sardinella* spp. best results were obtained by misty and clear polythene rather than aluminium foil, brown paper or jute bags at ambient

Table 4
Amino acid composition (g/100g) of smoked shrimp

Amino acid	Content	Amino acid	Content	Amino acid	Content
Aspartic acid	4.13	Alanine	2.57	Tyrosine	1.43
Threonine	1.89	Cystine	0.45	Phenylanine	1.91
Serine	1.70	Valine	1.99	Histidine	1.11
Glutamic acid	5.85	Methionine	0.88	Lysine	3.24
Proline	2.20	Isoleucine	1.83	Arginine	3.35
Glycine	2.49	Leucine	3.11		

Traditional kilns often give the operator little or no control over the smoking process, although shrimps are turned upside down on bamboo split platform after 2 h when the inside temperature rises to 66˚C in experimental kiln and final smoking was completed in 4.5h at 92˚C. After that the folded door was partially opened to reduce the inside temperature to 75˚C at closing of smoking process (extinguishing of fuel woods). The way in which shrimps are placed on the bamboo platform in a kiln, in most cases too thickly, coupled with uneven heat distribution results in varying and poor quality of products. Although brining is recommended in hot smoking for flavour development but it was not done in the smoking process as the raw shrimps originated from moderately salt water sources (river salinity10-20 ppt) (Bannerman 1976). Fish that are hot smoked are cooked during the process, the kiln temperature may be as high as 80˚C and the fish temperature may reach 60˚C (Burgess and Bannerman 1963). The wood normally used for fuel is often freshly fallen from mangrove forests and the moisture it contains will affect its burning and drying qualities, and also smoking time. Fuel efficiency is not easy to measure accurately, but 0.5-1 kg of fire wood will be needed to process 1 kg of fresh fish of approximately 30-50% moisture (Ward 1995).

Although, previous data on smoked shrimp is not available, the composition and nutritive quality of the smoked shrimp was found to be comparable with exportable dried/salted dried

temperature (Eyabi and Ningo 1985). The quality of smoked fish products can be deteriorated due to mold growth if the moisture content is above 15% (Kaneko 1976). Attempts should be made to reduce the rate of moisture absorption of the product by using appropriate packaging material or storage facilities. Proximate composition of the present product is also comparable with the smoked dried products of Nigeria (Ikeme 1991), but much higher protein content was found in the present product than smoked prawn (protein content of 31.15%) (Lobao *et al* 1989). Total bacterial load increased near to double after 90 days of storage. Bacterial spoilage in smoked fish is mostly due to presence of nonspore forming rods which are most likely to be introduced during handling of the product (Graikoski 1973).

Mineral and amino acid content of the smoked product is satisfactory. Mineral content of smoked shrimp is much higher than smoked *Barbus* spp. (Qudrat-i-khuda *et al* 1962) and comparable to local sun dried shrimp (INFS 1977). A smoking temperature of about 150˚C will affect the availability of lysine, one of the essential amino acids found in fish protein (Virulhakul 1995) but this is higher than the temperature used to smoke prawns. It is well known that high temperature can damage food proteins (Hoffman *et al* 1977). During smoking of Atlantic mackerel, it was observed that 25% of lysine and 7% of other amino acids were lost (Bhuiyan *et al* 1986). Available lysine in smoked shrimp was 3.24%, which is comparable

with smoked *Sardinella* spp. from Nigeria (Egwele *et al* 1985). In the tropics, loss of available lysine on drying of fish is small and not significant from nutritional point of view (Blake 1990). From a nutritional point of view smoked shrimp can be considered as a product with good nutritive value. Economically, the product is comparatively high priced. One kg of smoked shrimp costs about 4 US$ at production site and during marketing the price increased to 5 US$.

Smoked shrimp produced in Sundarbans located in south-west corner of the country is mainly marketed in south-east part and requires about 15 h road journey. For transportation smoked shrimp is generally packed in special cylindrical basket made of reed stems and marketed in bamboo basket. Smoked products are vulnerable to damage and loss after processing (Poulter *et al* 1988). The mode of transportation and storage supports microbial spoilage which may deteriorate the quality of the product. Proper storage and keeping moisture at low level will help to improve product's quality. The smoked product available in local market only before the winter season as fish drying in coastal belt generally, starts with the beginning of winter. So, most of the product is consumed within 2-3 months of production and present study revealed that smoked shrimp is acceptable in good condition at 60 days of storage.

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EFFECT OF RADIATION ON THE PHYSICO - CHEMICAL CHARACTERISTICS OF TOMATO DURING STORAGE

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Tomato samples, collected from Peshawar market, were irradiated with different doses of gamma radiation, 0.2, 0.3, 0.4, 0.5, 1.0, 2.0, 3.0 and 4.0 kGy and a control sample was kept for comparison. The irradiated samples were analysed every day for physico-chemical analysis i.e. pH, acidity, optical density, ascorbic acid (vitamin - C) and % ripening. Ascorbic acid and acidity were determined titrimetrically while optical density was measured with the spectrophotometer. Physical separation, ripening and spoilage were determined by visual examinations. The results showed that the ascorbic acid contents were higher in control as well as in samples irradiated with lower doses while it decreased to a level of 12.3 mg/100 g for high doses. The acidity and optical density also decreased during storage. The ripening process was delayed at higher doses.

Key words*:* Tomato, Irradiation, Physico - chemical analysis.

Introduction

Tomato is one of the most popular vegetable grown throughout the world. This edible fruit belongs to the genus *Lycopersicon esculentum*. It can be consumed either fresh or processed into many different products. The common tomato plays a vital role in maintaining health and vigour and helpful in healing wounds because of antibiotic properties found in the ripe fruit. It is also a good source of vitamins (Anon 1982). Tomato is a perishable fruit and its average life may not exceed more than a few days under normal circumstances. It is claimed as vegetable as well as a fruit in the literature (Kader and Morris 1976; Luh and Woodroof 1982).

The use of neutrons, X-rays, and beta and gamma rays is described and interpreted, with some new data on levels of radiation required to preserve different foods (Vidal 1960). Irradiation can be applied for ripening delay, preservation and extending storage life of various crops, fruits and vegetables (Ahmad *et al* 1972; Khan *et al* 1985; Badshah *et al* 1992). Some physico-chemical changes may occur after radiation treatment of fresh fruits and vegetables (Poole 1956; Burns and Desrosier 1957; El-Sayed 1978; Assi *et al* 1997). The chemical changes brought about by irradiation treatment in foods have been studied very thoroughly owing to the fears that some of these changes may produce toxic compounds (Vidal 1960; Maxie and Sommer 1968; Tencheva 1976). Gamma irradiation is also used for decontamination of food items (Akhtar *et al* 1995).

Because of various preservation methods, tomato is now available throughout the year. Techniques like freezing, canning, dehydration and salting are applied to extend the shelf-life of the commodity. Irradiation is a new technique enabling the extension of tomato shelf-life upto 13 days (Burns and Desrosier 1957; Kader *et al* 1965; Assi *et al* 1977; El-Sayed 1978). Effect of gamma irradiation on the post-harvest behaviour of tomatoes (Bhatti *et al* 1967; Sattar *et al* 1970) and cooking methods on the nutritional and organoleptic properties of two vegetables are reported (Zeb *et al* 1995). The research reported here was carried out to see the quality changes in tomato fruits after postharvest treatment with gamma radiation used for extending the shelf-life.

Materials and Methods

A) *Sampling*. Tomato samples were purchased from the local market of Peshawar. Fruits of equal size and uniform maturity were selected and washed with distilled water.

B) *Instrumentation*. Following radiation source and instruments were applied in the present study:

i. Co-60 gamma irradiator (ISSLEDOVATEL)
ii. Shimadzu UV-Visible Recording Spectrophotometer UV-160
iii. pH Meter 215 Corning Incorporated USA

C) *Procedure*. About 1 kg (10-15 tomatoes) were grouped for each radiation dose. Different radiation doses i.e. 0.2, 0.3, 0.4, 0.5, 1.0, 2.0, 3.0 and 4.0 kGy were given. A control sample was taken without irradiation. As tomato perishes rapidly, fruits showing sign of deterioration were separated from the lots. Physico-chemical analyses were conducted in triplicate after every 24 h. The standard procedures for selective parameters (Williams 1984) are outlined below:

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Table 1
Doses for treatment of fresh fruits and vegetables

Class	Description	Purpose of Irradiation	Dose(kGy) max
1	Bulbs, roots and tubers	To inhibit sprouting during storage	0.2
2	Fresh fruit and vegetables (other than class 1).	a) To improve keeping quality by delaying ripening	1.0
		b) To control insect infestation.	1.0
		c) To prolong storage life by partial elimination of spoilage organisms.	2.0
		d) To satisfy quarantine requirements.	1.0
3	Cereals/pulses, their products dried vegetables/nuts/fruits.	To control insect infestation.	1.0
4	Raw fish and seafood and their products (fresh and frozen).	a) To ensure hygienic quality by reducing the number of pathogenic microorganisms.	5.0
		b) To prolong shelflife by partial elimination of spoilage organisms.	3.0
5	Raw poultry and meat and their products (fresh and frozen).	a) To ensure hygienic quality by reducing the number of pathogenic microorganisms.	5.0
		b) To prolong shelflife by partial elimination of spoilage organisms.	3.0
6	Dried herbs, spices, condiments.	a) To ensure hygienic quality by reducing the number of pathogenic microorganisms.	10.0
		b) To control insect infestation.	1.0
7	Dried food of animal origin.	To control insect infestation.	1.0

i. pH. The pH was measured in slurry of sample using pH meter.
ii. Acidity. An aliquot of 10 ml from sample solution (10 g/ 100 ml) was titrated against 0.1N NaOH using phenol-phthalene indicator.
iii. Vitamin - C. A 10 ml aliquot of sample solution (1 g sample in 10 ml 0.4% oxalic acid) was titrated against a standard dye (2,6-dichlorophenolindophenol). Standard solution of ascorbic acid (1 mg/ml) was used for calibration.
iv. Optical density. Samples were filtered and optical density was determined with the help of spectrophotometer at 520 nm.
v. % Ripening. The % ripening of tomato sample was noted subjectively.

Results and Discussion

The moisture content of tomato is 93% and hence, it is normally spoiled due to chemical reactions, autolysis and microbial activities. Storage of tomato under low temperature using refrigerator, may prolong their shelf-life. But in very rare cases such food lasts for upto 4 weeks. Tomatoes are normally grown throughout the year, therefore it is necessary to reduce their production losses, extend their shelf-life and reduces the chances of spoilage. To achieve these goals, food processor commercially used some food preservation methods based on the principle that is removal of chemical and physical reactions, microbial activity and losses caused by attack of insects.

Application of ionising radiation is carried out for preservation and disinfestation of food. The absorption of electromagnetic radiations by food depends upon several factors including their nature, source and intensity (Balla and Kiszel 1963). Previous studies have revealed that irradiation has the ability to inhibit sprouting of root crops and tubers, delay ripening in fruits, prevent reproduction in insects, kill insects and parasites, and destroy or inactivate moulds, yeasts and bacteria, including spores (Burns and Desrosier 1957; Balla and Kiszel 1963). Detailed report on irradiation of food in Pakistan are available in the literature (Muhammed and Sattar 1971; Sattar 1992).

The technique of food preservation by ionising radiations has become established among other technological methods. The main advantage of these radiations lies in its strong

Table 2
Effect of different radiation doses on change in pH of tomatoes during one week storage

Dose	Storage time (days)							
(kGy)	0	1	2	3	4	5	6	7
0.00	4.60	4.00	4.30	4.00	4.20	4.20	4.00	3.80
0.20	4.60	3.80	4.30	4.60	4.10	4.10	4.10	3.70
0.30	4.60	4.00	4.60	4.30	4.20	4.20	4.20	3.90
0.40	4.60	3.80	4.60	4.30	4.20	4.20	4.20	4.30
0.50	4.60	3.80	4.70	4.40	4.20	4.20	4.20	4.20
1.00	4.60	3.70	4.40	4.60	4.20	4.30	4.20	4.20
2.00	4.60	3.60	4.50	4.60	4.10	4.50	4.20	4.60
3.00	4.60	3.60	5.00	4.60	4.40	4.60	4.50	4.20
4.00	4.60	3.50	4.60	4.60	4.60	4.60	4.40	4.50

Table 3
Effect of different radiation doses on change in % acidity of tomatoes during one week storage

Dose	Storage time (days)						
(kGy)	0	1	2	3	4	5	6
0.00	0.54	0.52	0.52	0.43	0.41	0.39	0.38
0.20	0.54	0.53	0.53	0.42	0.40	0.38	0.38
0.30	0.54	0.52	0.52	0.45	0.46	0.43	0.40
0.40	0.54	0.54	0.54	0.48	0.48	0.44	0.43
0.50	0.54	0.49	0.49	0.44	0.42	0.40	0.39
1.00	0.54	0.54	0.54	0.47	0.45	0.43	0.40
2.00	0.54	0.51	0.51	0.44	0.43	0.39	0.35
3.00	0.54	0.51	0.51	0.47	0.45	0.35	0.33
4.00	0.54	0.50	0.49	0.44	0.41	0.38	0.37

penetrating power. Gamma and X-rays have germicidal properties and are being exploited in food industry for preservation. The X-rays are produced by the X-ray tubes, while gamma rays are emitted from radioactive substances such as cobalt-60 and caesium-137. (Balla and Kiszel 1963). Still the most commonly employed unit for measurement of radiation energy absorbed has been the 'rad' (Awan 1995). A rad is equivalent to 10^{-5} Joules (J) of energy absorbed per gram of material receiving ionising radiation. Kilorad (krad) is used when heavy doses are employed. In accordance with the recommendations of the International Organisation for Standardisation (ISO), the rad has been replaced by Gray (Gy) which corresponds to 100 rad. The Gy is the new unit of System International (SI) system (IAEA 1994).

A dose range of 70-220 krad was used for tomato irradiation in a study (Assi *et al* 1997). Low levels of radiation appear to have the most promising immediate application in the food field (Burns and Desroiser 1957). Pre-irradiation was effective in controlling rot decay during storage of irradiated fruits. A dose of 1.0 kGy extended the tomato shelf-life for 13 days without effect on fruit quality measured in terms of their content of vitamin - C, sugars and amino acids (El-Sayed 1978). But the use of ionising irradiations in food preservation is slow to gain the desired acceptability, owing to fears that consumption of foods may prove toxic to the consumer (Awan 1995). It is claimed that radiations bring changes in food materials that are not fully understood and that products of radiolysis could prove toxic (Tencheva 1976). The effects of ionising radiations on the different storage properties of fruits are given (Clarke 1968). Recent studies (Sattar *et al* 1992; Sattar 1993) have removed all fears, the radiolytic products have been detected and FAO/IAEA/WHO (IAEA 1997; IAEA 1998) expert committee has permitted irradiation of food materials to any dose level whereby the technological properties are not affected. Initially, the FAO/IAEA/WHO experts committee had allowed irradiation of foods upto a maximum of 10 kGy. Keeping this in view, present study was conducted to evaluate the effect of radiation on the physico-chemical characteristics of tomato during storage. Doses in the range of 20-400 krad were used for irradiation. The prescribed doses for treatment of fresh fruits and vegetables are given in Table 1 (Anon 1996).

The results for pH, acidity, ascorbic acid, optical density and % ripening are given in Tables 2 - 6. The data show that the pH (Table 2) decreased from 4.6 to 3.7 at lower doses while at higher doses very slight decrease was observed. As is clear from Table 3, the % acidity also decreased from 0.54 to 0.43, nevertheless, no proper pattern appeared. Brecht *et al* (1976) reported that table ripe tomatoes were higher in titratable acidity and lower in pH of the mature-green fruits. Kader *et al* (1976) found significant differences in the levels of pH and titratable acidity. The pH was lowest in fruits picked at the breaker (B) and typical mature green (TMG) stages while fruits picked at or near the table-ripe (TR) stage had highest pH values. The differences for titratable acidity were not significant but generally inversely related to pH.

The results on ascorbic acid (vitamin - C) are given in Table 4. The levels of vitamin - C increased at lower doses during storage, while remained almost stable at doses of 2.0 and 3.0 kGy. At higher dose i.e. 4.0 kGy, the vitamin - C contents decreased. Ionizing radiations decreased ascorbic acid in peprika puree and increased dehydroascorbic acid (Balla and Kiszel 1963). No prominent differences in total ascorbic acid were noted (Kader and Morris 1976). There were large differences in ascorbic acid content, fruits analyzed soon after picking (AR stage) had the highest level. The earlier the picking stage, the lower the ascorbic acid concentration, fruit harvested green and ripened at 20°C contained about 55 - 65 %

Table 4
Effect of different radiation doses on change in vitamin - C contents (mg/100g) of tomatoes during one week storage

Dose	Storage time (days)						
(kGy)	0	1	2	3	4	5	6
0.0	16.00	15.35	15.87	16.42	16.92	18.99	19.50
0.2	15.00	15.26	15.58	15.90	16.44	17.65	18.54
0.3	15.00	15.52	15.80	18.40	19.28	19.50	20.36
0.4	15.00	15.50	15.66	16.00	16.40	17.34	18.55
0.5	15.00	15.14	15.45	17.14	17.46	17.85	18.75
1.0	15.00	15.56	15.64	17.41	18.76	19.99	18.62
2.0	15.00	15.18	15.65	17.14	16.36	16.16	15.24
3.0	15.00	15.20	15.68	16.74	16.48	16.36	15.47
4.0	15.00	14.04	13.55	12.85	12.72	12.45	12.31

Table 5
Effect of different radiation doses on change in optical density of tomatoes during one week storage

Dose	Storage time (days)							
(kGy)	0	1	2	3	4	5	6	7
0.0	0.381	0.296	0.126	0.106	0.048	0.043	0.043	0.047
0.2	0.381	0.292	0.205	0.120	0.430	0.041	0.041	0.042
0.3	0.381	0.302	0.170	0.133	0.061	0.098	0.074	0.052
0.4	0.381	0.342	0.222	0.074	0.049	0.041	0.042	0.040
0.5	0.381	0.187	0.199	0.087	0.046	0.051	0.051	0.047
1.0	0.381	0.283	0.169	0.159	0.051	0.470	0.052	0.040
2.0	0.381	0.163	0.106	0.122	0.065	0.061	0.046	0.042
3.0	0.381	0.165	0.152	0.131	0.065	0.052	0.058	0.047
4.0	0.381	0.252	0.196	0.081	0.051	0.046	0.043	0.043

ascorbic acid relative to those picked at TR stage. The importance of physiological state as it may affect vitamins content is reported earlier (Maxie and Soommer 1968). Mature green fruits radiated to 4.0 kGy, then subsequently ripened showed 8-6 % loss in ascorbic acid, while table ripe fruits subjected to 3.0 kGy lost 20.4%. However, in the latter fruit there was initially almost twice as much as ascorbic acid, so that even with higher percentage of destruction by irradiation, the fruits still contain 5 mg/100 g more than the fully ripened unirradiated fruits harvested as mature green. As reported earlier (Enachescu 1956), ascorbic acid contents of riped tomatoes kept for 15-17 days at room temperature, do not show large variations. The riped tomatoes contain more ascorbic acid than unripened ones.

The optical density determined during the studies is given in Table 5. The levels decreased in all cases by a factor of about

Table 6
Effect of different radiation doses on change in ripening percentage of tomatoes during one week storage

Dose	Storage time (days)							
(kGy)	0	1	2	3	4	5	6	7
0.0	0	0	5	7	12	15	13	15
0.2	0	0	2	3	4	8	9	9
0.3	0	0	3	3	3	5	7	7
0.4	0	0	1	1	1	9	8	8
0.5	0	0	4	4	6	9	9	10
1.0	0	0	1	2	3	6	4	5
2.0	0	0	1	1	2	3	1	2
3.0	0	0	0	0	1	8	4	5
4.0	0	0	0	0	1	4	3	4

10 during one-week storage. Physical separation, ripening and spoilage were carried out with the help of naked eye. The results on % ripening are given in Table 6. Ripening percentage in the samples irradiated at lower doses was relatively stable during storage while higher doses showed comprehensive delayed ripening scores. Irradiation induced softening which was evident in fruits. Fruit irradiated at mature green stage softened during post-irradiation (Assi *et al* 1997). Van Kooy (1968) studied the effect of gamma irradiation. Dose levels are 0.5, 1.0, 2.0 and 3.0 kGy on tomatoes at different stages of maturity. The largest increases are found at the low dose level, indicating the existence of an optimum at the 2.0 kGy dose level.

Irradiation induced softening was evident in mature green and pink fruit within hours following irradiation and differences between irradiated and control fruit persisted through post-irradiation storage. Trends of firmness loss were much more consistent and showed much greater dose dependency in pericarp tissue than in whole fruit (Assi *et al* 1997).

Conclusion

Irradiation is of particular advantage in developing countries where the standards of hygiene are low and it is usually difficult to maintain strict hygiene in food processing areas. However, the commercial application of irradiation has been slow mainly due to high economic risks involved in the initial investment and reluctant attitude of consumer towards the consumption of such foods.The effects of irradiation on some physico-chemical parameters were determined in the present study. Overall, the data showed that values of acidity, vitamin C and optical density were almost stable at doses less than 4.0 kGy. Our results are in line with reported data.

Keeping in view, the importance of tomato consumption in Pakistan more work on irradiation as well as higher storage is desired for complete assessment in order to see the effect of irradiation on physico-chemical qualities of tomatoes. In general, limited data are available on the specific changes induced by irradiation in fruit constituents. Hence, more knowledge is required on the effect of irradiation on the changes occurring during the ripening process i.e. the extension of shelf life by understanding the basic mechanism. It is concluded that a great deal of research needs to be completed in this new field.

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Retention of Nutritional Quality of Soybean during Extrusion Cooking

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Trypsin inhibitor (TI) is one of the major anti-nutritional components of soybean and must be inactivated before its protein content can be safely and efficiently utilized for food and feed purposes. However, retention of the protein quality is also a prime consideration while inactivating TI. This research was conducted to study the effect of extrusion process conditions (temperature, screw speed and moisture content) on trypsin inhibitor activity (TIA) and nitrogen solubility index (NSI) and to develop a model for prediction of TI inactivation during extrusion cooking based on its reaction kinetics. A laboratory size single screw extruder was used for extrusion cooking of full-fat soybean implementing a (4x4x4)x2 full factorial design. TIA was measured using a standard procedure and NSI by AACC procedure. The reaction rate constant for loss of TIA was calculated based on its activation energy from literature and experimental TIA data. The statistical models correlating product temperature with operating conditions and activation energy were combined with mathematical equations for predicting TIA during the cooking process. TIA and NSI of the soybean (William 82 variety) were found to be 47.0 TIU mg^{-1} and 78% respectively. Trypsin inhibitor inactivation ranged from 90% of that of raw soybean at low screw speed (75 rpm) and high barrel temperature (170˚C) (LSHT) to 50% for higher screw speed (150 rpm) and low barrel temperature (140˚C) (HSLT). Reduction in NSI for similar extrusion conditions ranged from 95% at LSHT to 50% at HSLT of that of raw soybeans. Variations between predicted and measured TIA values were less than 1% for the given conditions. Results indicated that reduction in TIA and NSI occurred mainly in the compression and metering sections of the extruder and that they paralleled each other, thereby making it difficult to retain high NSI while inactivating TI. However, the efficiency of extrusion cooking for TI inactivation has been proved. The model can be used for determining optimum conditions for extrusion cooking of soybean for food and feed purposes.

Key words: Soybean, Trypsin inhibition, Extrusion cooking, Nitrogen solubility index.

Introduction

Soybean, by virtue of its large fraction of high quality protein, is appearing as the most promising source for satisfying the world's protein requirements. In raw form, it contains approximately 35-50% protein, 12-25% fat, 33-35% carbohydrate and 5% ash (Smith 1989). However, soybean also contains anti-nutritional factors such as trypsin-inhibitor (TI) that limit protein digestibility, inhibit growth, and cause pancreatic hypetrophy and flatulence (Reddy and Pierson 1994). TI, a protein by itself, reacts with the trypsin (protein digesting enzyme) and reduces its protein digesting capability besides causing other problems. TI, therefore, must be inactivated before the protein content can be safely utilized for food and feed purposes. Macrae *et al* (1993) suggested that inactivation of TI in different legumes is a function of temperature, heating duration, particle size and moisture content. The capability of the extrusion cooking process in providing a lively control over these variables has resulted in its evolution as a low-cost and effective method for cooking soybean.

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The high temperatures encountered by soybean during extrusion cooking, although for a short time, not only denatures TI but also other proteins resulting in reduced nutritional and functional properties. Very limited studies are available on modeling of TI inactivation during extrusion cooking. The present study aimed at optimizing the process conditions for extrusion cooking of soybeans on one hand and development of a model for prediction of TI inactivation during the process is based on TI reaction kinetics and temperature history, on the other. Development of such a model will help a great deal in predicting quality of processed soybean as well as in design of extruders for this purpose.

Materials and Methods

The extruder. The laboratory size single screw extruder (C.W. Brabender Instrument, S. Hackensack, NJ) used in these experiments had a length to diameter (L/D) ratio of 25:1 and compression ratio of 5:1. The extruder had four heating zones (three for the barrel and one for the die) that could be independently heated to set temperatures. Cooling and temperature control was accomplished by compressed air flow

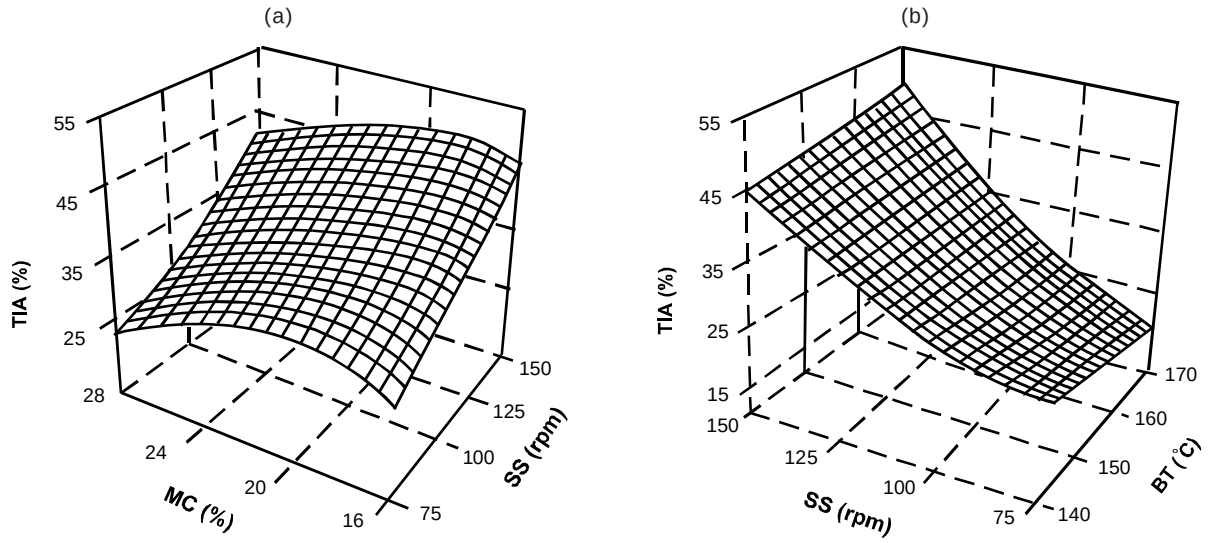


Fig 1. Effect of **(a)** Moisture Content (MC) and Screw Speed (SS) and **(b)** Screw Speed (SS) and Barrel Temperature (BT), on Trypsin-Inhibitor Activity (TIA).

Table 1
Experimental plan

Parameters	A	B	C	D
Moisture content (%db)	16	20	24	28
Temperature (°C)	140	150	160	170
Screw speed (rpm)	75	100	125	150

through jackets surrounding the barrel. Barrel temperatures in the feed and compression sections were set at 60°C and 100°C respectively and maintained constant throughout these experiments. Temperatures of the metering section and die varied according to the experimental plan (Table 1). A long (11cm) tubular tapering die with 1.10 cm and 0.48cm entrance and exit diameters respectively, was used on the extruder.

Product (Soybean) temperature inside the extruder was measured with thermocouples attached at the end of feed and compression sections and at the die. Pressure at the die was measured with a pressure transducer (Dynasco, Model TPT 432A-3M-6/18) in the same port along with thermocouple. The temperature and pressure data were recorded through an IMB personal computer in every six seconds during each extrusion run.

Experimental design. A 4x4x4 full factorial design (Table 1) was employed in duplicate with screw speed, moisture content and barrel temperature (metering zone & die) as the control variables. As mentioned earlier, barrel temperature in the feed and compression sections were kept constant for all the experiments. Statistical models for prediction of TIA and NSI were developed using non-linear regression. TI inactivation along the screw channel based on temperature history was computed by a program developed for this purpose (Khan 1996).

Soybean preparation for extrusion cooking. The soybean (William 82 1994 Missouri crop), purchased from Missouri Seed Foundation (Columbia MO) were cracked and de-hulled using a Fitz Mill (Model No.DASO6, The Fitzpatrick Co. Elmhurst, IL) such that each seed was broken into 3 or 4 pieces. Moisture content of the cracked soybean was measured using a moisture balance (CSC Scientific Co. Inc.). The quantity of water needed for adjusting moisture content to the desired level (Table 1) was calculated using the following moisture balance equation:

$$Wm = \frac{Ws.(DMC-OMC)}{1 - DMC}$$

Where

Wm: Quantity of Additional Water Required (kg)
Ws: Total Weight of Soybean (22.73 kg per Batch)
DMC: Desired Moisture Content (Table 1)
OMC: Original or Measured Moisture Content

Moisture content of each batch was adjusted to the desired level of 24 h before extrusion cooking and stored in moisture proof plastic containers. The material was thoroughly mixed (Leland Food "Double Action Mixer", Model L-100DA) immediately before the extrusion run. Samples collected during these runs were stored at - 4°C for analysis.

Chemical analysis. The frozen extrudate samples were freeze-dried (Labconco Corporation, Kansas City, MO),

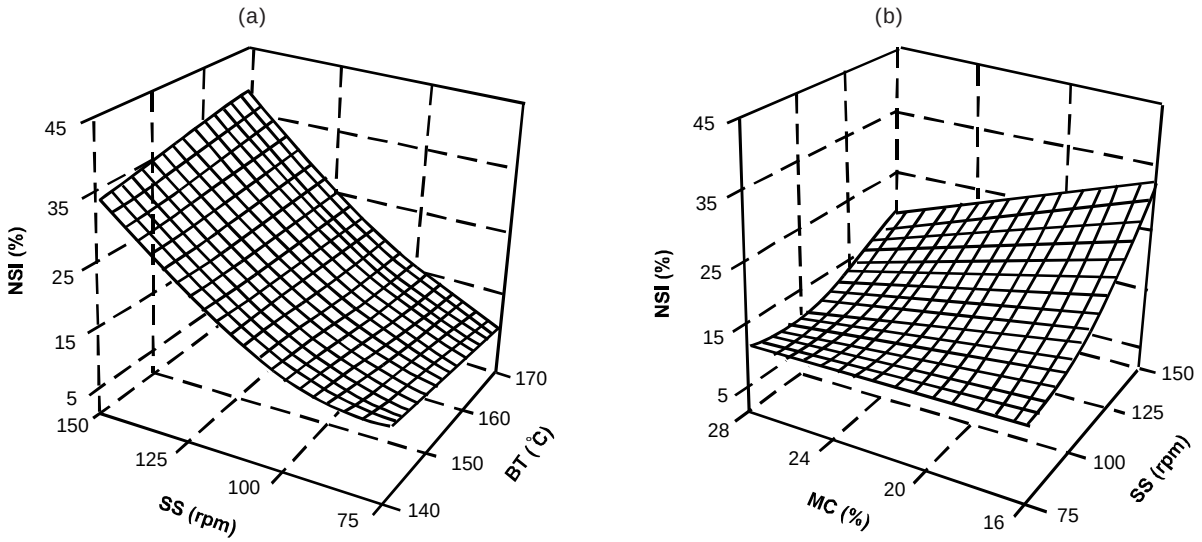


Fig 2. Effect of **(a)** Screw Speed (SS) and Barrel Temperature (BT), and **(b)** Moisture Content (MC) and Screw Speed (SS), on Trypsin-Inhibitor Activity (TIA).

ground and de-fatted by solvent extraction. TIA of soybean samples was determined using a slight modification of the standard procedure (AACC 1995a) suggested by Hamerstrand *et al* (1981). NSI was determined by using a standard procedure (AACC 1995b).

Results and Discussion

TIA and NSI of raw soybean were 47.0 TIU mg^{-1} and 78% respectively. Maximum (85%) and minimum (45%) TI inactivation were observed at screw speeds of 75 and 150 rpm respectively (Fig 1a) indicating an inverse relationship. The lower screw speed allowed for a longer residence time at high temperatures and proved more effective for TI inactivation. The effect of barrel temperature and moisture content, although statistically significant (Table 2) was unexpectedly less pronounced. The small changes in TIA with barrel temperature (Fig 1b) occured because of the small changes in product's temperature history inside the extruder (Khan 1996). TIA varied nonlinearly over a small range with moisture content. Although it has been shown previously (Macrae *et al* 1993) that increasing moisture content caused increased reduction in TIA. It seems that moisture content was too high and therefore, did not show any difference in effectiveness in the given range. A study of lower range of moisture content would be valuable. These results indicated that screw speed or in other words residence time and shear had the most important effect on TIA for a given temperature history.

Nitrogen solubility index (NSI) was more sensitive to extrusion operating conditions than TIA (Fig 2a). However, the trend for changes in NSI was similar to that of TIA except for moisture content (Fig 2b). Maximum reduction in NSI (95%) was observed at 28% moisture content, 170˚C barrel temperature and 75 rpm screw speed while minimum reduction (60%) was observed at 16% moisture content, 170˚C and 150 rpm (Table 3). The effect of moisture is more significant at 150 rpm as compared to 75 rpm. For example, at 170˚C and 150 rpm, NSI decreased from 38% at 16% moisture content to 18% at 28% moisture content. While for the same barrel temperature and 75 rpm, NSI remained constant with increasing moisture content, thus suggesting that the effect of moisture is enhanced by shearing at high screw speed.

Selection of extrusion conditions for cooking of soybean will mainly depend on the purpose of the end product. If the soybean or soy protein is intended for direct consumption as food or feed without any further heat treatment, conditions giving lowest TIA should be used. However, if the soybean protein is intended for imparting functional properties, conditions giving high NSI should be used.

Table 2
Model for trypsin inhibitor activity, $R^2 = 0.54$

Parameter	Estimate	Pr > F
Intercept	87.719	0.0349
MC	4.845	0.0045
BT	-7.76×10^{-1}	0.0008
SS	-1.05	0.0056
BT x SS	6.47×10^{-3}	0.0011
MC^2	-1.16×10^{-1}	0.0026
SS^2	-1.78×10^{-3}	0.0703

MC: Moisture Content (%), BT: Barrel Temperature (˚C), SS: Screw Speed (rpm).

Table 3
Model for nitrogen solubility index, $R^2 = 0.75$

Parameter	Estimate	Pr > F
Intercept	87.474	0.0001
BT	-6.13×10^{-1}	0.0001
SS	-8.99×10^{-1}	0.0001
MC x BT	-8.61×10^{-3}	0.0001
BT x SS	6.14×10^{-3}	0.0001
MC x BT x SS	-1.18×10^{-4}	0.0001
SS^2	2.59×10^{-3}	0.0001

MC: Moisture Content (%), BT: Barrel Temperature (˚C), SS: Screw Speed (rpm).

Reaction kinetics of loss of trypsin inhibitor (TI). The data for reaction rate constant (k) for inactivation of TI (Table 4) was taken from literature (Savage *et al* 1995, Rouhana *et al* 1996). The two data sets covered consecutive ranges of temperature and were combined to cover a broader temperature range from 90 to 150˚C. Although this data was not obtained under extrusion conditions, it covers the temperature range usually encountered during extrusion cooking and can therefore be used for estimating the reaction rates in this temperature range.

Rouhana *et al* (1996) showed that 60% of the TIA was caused by Kunitz trypsin inhibitor (KTI) and 40% by the Bowman-Birk inhibitor (BBI). Reaction rate constant (k) in Table 4 is the sum of k_{KTI} times 0.6 and k_{BBI} times 0.4. The data was fitted to the Arrhenius equation:

$$k = k_O e^{\frac{-E_a}{RT}}$$

E_a is activation energy, R is gas constant (8.314 Joule.g^{-1} mol^1. K^{-1}) and T is absolute temperature (˚K). A linear regression of 1n(k) vs T^{-1} had an R^2 of 0.98 and resulted in the following equation:

$$k = 1924160.e^{-7513.53\frac{1}{T}}$$

Table 4
Reaction rate constant data

Temperature (˚C)	k_{KTI} (S^{-1})	K_{BBI} (S^{-1})	K (S^{-1})	
90			0.00185	Savage *et al* 1995
95			0.00255	″
100			0.00283	″
110	0.01156	0.00237	0.00788	Rouhana *et al* 1996
120	0.01428	0.00569	0.01084	″
130	0.01803	0.01158	0.01545	″
140	0.02049	0.02204	0.02111	″
150	0.02372	0.05386	0.03578	″

The above mentioned relationship covered the temperature range from 90 to 150˚C, while inactivation of TI starts at 30˚C (Kunitz 1948). The reaction rate was constant for the entire range of temperature inside the extruder was derived by using the given activation energy.

Residence time for given extrusion conditions was determined using the equation of continuity. The mass flow rate through the extruder is given by:

$$Q_m\left(\frac{kg}{s}\right) = \rho\left(\frac{kg}{m^3}\right).A\,(m^2).V\left(\frac{m}{s}\right)$$

or

$$V\left(\frac{m}{s}\right) = \frac{Q_m\left(\frac{kg}{s}\right)}{\rho\left(\frac{kg}{m^3}\right).A(m^2)}$$

Q_m: mass flow rate (kg s^{-1}) measured experimentally
ρ: 1300 kg m^{-3} (Density of Soybean), measured experimentally
A1: 5.43×10^{-5} m^2 (Cross-Sectional Area of the Screw Channel in the Feed Section)
A2: 3.72×10^{-5} m^2 (cross-Sectional Area of the Screw Channel in the Compression Section)
A3: 1.86×10^{-5} m^2 (Cross-Sectional Area of the Screw Channel in the Metering Section)

Table 5
Predicted TIA (TIU/mg) values using the kinetics and experimental data for 20% moisture content

Screw speed	k_o		Pred.TIA		Actual TIA	
(rpm)	(140˚C)	(150˚C)	(140˚C)	(150˚C)	(140˚C)	(150˚C)
75	11941590	10641590	12.56	9.59	12.45	9.51
100	12641590	9341590	17.36	18.05	17.33	18.02
125	15841590	13941590	17.78	16.33	17.67	16.24
150	16841590	12341590	21.04	22.67	20.95	22.61

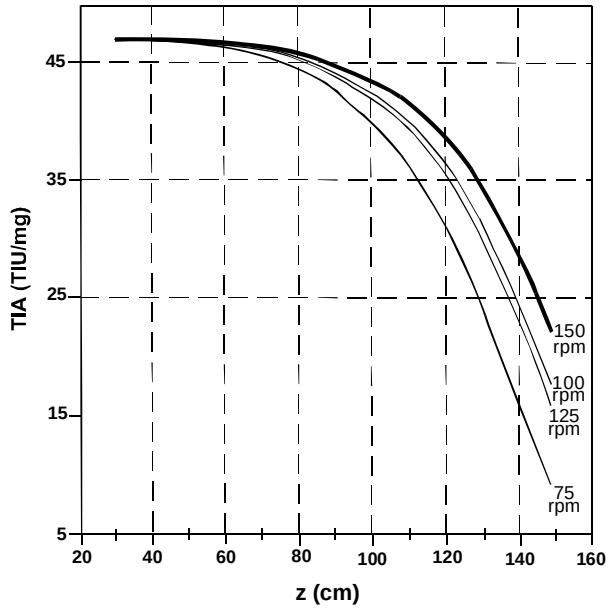


Fig 3. Inactivation of Trypsin Inhibitor (TI) along the Screw Channel (z) at 20% Moisture Content and 150˚C Barrel Temperature and 75,100, 125 and 150 rpm Screw speeds.

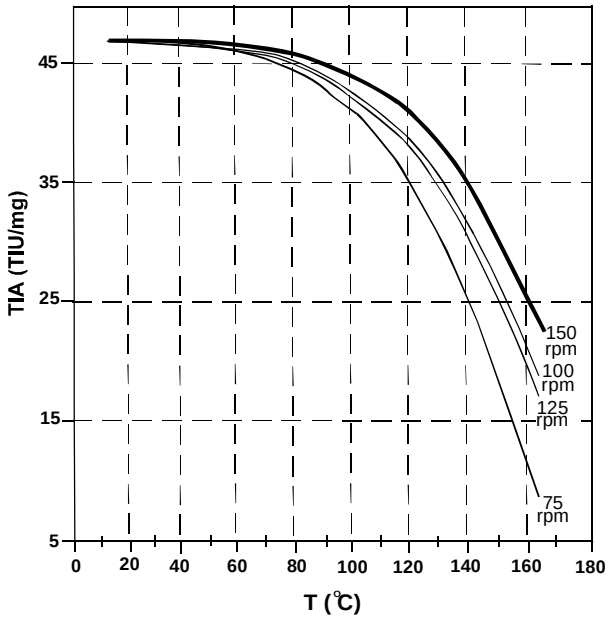


Fig 4. Inactivation of Trypsin Inhibitor (TI) with increasing Soybean Temperature (T) at 20% Moisture content, 150˚C Barrel Temperature and 75,100,125 and 150 rpm Screw speeds.

The velocity V is assumed constant in a given section of the extruder, although it changed from the feed section to the compression section and from compression section to the metering section because of changes in the cross-sectional area. The velocity in a given section is also:

$$V\left(\frac{m}{s}\right) = \frac{Dz(m)}{t(s)}$$

The entire screw channel (164 cm) (Khan 1996) was divided into small steps (Dz) 0.1 cm each. Since Dz was fixed and V already known, the residence time t over a small distance could be calculated.

Beginning at Z1 (distance from feed hopper in down channel direction) equal to zero, product temperature was calculated using the experimental temperature profile. If the temperature was less than 30˚C, Z1 was incremented by 0.1 until the product temperature reached to 30˚C. If Z1 was less than 95 cm (feed section) between 95 and 127 cm (compression section) or between 127 and 164 cm (metering section), corresponding cross sectional area of the screw channel was used to determine the residence time over the 0.1 cm length in the section. The activation energy was assumed constant and a value for K_o was initially used as a guess. The reduction in TIA was calculated step by step incrementing Z1 by 0.1 until Z1 reached 164 cm. Then the residual TIA was compared with experimental TIA for that condition. If the two values were not close, K_o was incremented or decremented depending on whether calculated TIA was larger or smaller than the experimental value.

The K_o and experimental and predicted values of TIA for 20% moisture content and 140 and 150˚C barrel temperatures and 75, 100, 125 and 150 rpm screw speeds are shown in Table 5. A computer program was written for these computations by using Matlab (1992).

The k value for any given condition can then be predicted using the Arrhenius equation and covers the range from 30 to 140 or 150˚C depending on the product exit temperature.

It can be seen from Table 5 that the k_o and k for 140˚C barrel temperature increased with increasing screw speed. Since k is independent of residence time, this data suggested that k increased with increasing shear rate that results in smaller particles and thus instant heating. TI inactivation also depends on particle size (Macrae *et al* 1993). Thus it can be concluded that TIA is also affected by shear rate along with moisture content, temperature and residence time.

The reduction in TIA of soybeans as they pass along the screw channel for 20% moisture content, 140˚C barrel temperature and 75, 100, 125 and 150 rpm screw speeds is shown in Fig 3. As shown TI inactivation started in the feed section at a lower rate and most of the inactivation occurred in the compression and metering sections at higher temperatures.

Reduction in TIA with rising temperature during extrusion cooking has the same trend as it had with channel length (Fig 4). This relationship comes from the fact that soybean temperature increased linearly with distance along the screw

channel. Figs. 3 and 4 suggested that TI inactivation started in the feed section. However, most of the inactivation occurred in the compression and metering sections. It can be concluded that the chemical changes in soybean occurs in the compression and metering sections and that any model describing the process only for metering section will not be adequate. Also, it is noteworthy, that the temperature history was not affected so much by the barrel temperature as would be expected.

Conclusion

TIA and NSI of the soybean were mainly affected by screw speed. Lower screw speeds resulted in higher TI inactivation and larger reduction in NSI. Since TI inactivation and reduction in NSI paralleled each other, selection of suitable parameters for extrusion cooking of soybean will depend on whether complete TI inactivation or higher NSI is more desirable. The reaction rate constant for TI inactivation sharply increased in the compression and metering sections, thereby showing that most of the inactivation occurred in these sections. These studies also revealed that TIA is affected by shear rate along with the other variables. The degree of TI inactivation and NSI reduction can be estimated using the given models within the range of the experimental conditions.

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