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Editorial Note

The year 2007 marks completion of 50 years of publication of ***Pakistan Journal of Scientific and Industrial Research (Pak. J. Sci. Ind. Res.)***, the prime Journal of Pakistan Council of Scientific and Industrial Research (PCSIR) which was founded by Prof. Dr. Salimuzzaman Siddiqui (late), way back in 1953 with a small nucleus of scientists, for performing research on projects of national importance. As soon as quite enough research results accumulated, the journal was launched in the year 1958 as a quarterly; presently, it is a bimonthly research Journal. Contributions to the Journal are received from the scientists all over the world and the published papers are pre-reviewed by the eminent scientists and subject experts of international stature.

It would be pertinent here to acknowledge the commendable part played by the distinguished members of the Editorial Board of the Journal in evaluating the manuscripts submitted for publication. The present issue includes the review of an eminent scientist Dr. Muhammad Anwar Waqar and Co-workers on the interesting subject of "The Protective Action of Prostaglandins on the Fetus and the Neonate and its Modulation". Such reviews of our elite members in the area of their expertise would appear in *Pak. J. Sci. Ind. Res.* regularly during the current year.

This year also boasts of the activation of the website of the Journal with a number of built-in features, designed to facilitate on-line users, authors and referees of the Journal, (http://www.pjsir.org), in an effort to further improve the scholarly quality of the Journal. By logging-in to the website, they shall have free access to the contents of the Journal. Through creation of a log-in account, on one hand, the author will find it easier to submit the manuscripts and correspond with the Editor, and will be able to know the updated status of all his submissions as well. On the other hand, it shall assist the referee in book keeping of the papers assessed or awaiting assessment.

Work on creation of archives of the Journal is in full swing and presently, the users can view abstracts of 7 volumes of the Journal, for which quick search option is available on the website.

The Editor would welcome suggestions for improving the Website.

Dr. Kaniz Fizza Azhar
Executive Editor

Physical Sciences

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Geotechnical Study of Sub-Surface Sediments in Sirajgonj Town and its Adjoining Areas, Bangladesh

Md. Sultan-Ul-Islam*, Md. Shamsuzzaman and Md. Badrul Islam
Department of Geology and Mining, University of Rajshahi, Rajshahi-6205, Bangladesh

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Abstract. Engineering bore logs of 86 holes, installed in the Sirajgonj town of Bangladesh and adjoining areas, were used to identify the geotechnical characteristics of the sub-surface sediments. The study area was divided into four major zones on the basis of surface and sub-surface geological characters, engineering properties of soil, such as lithology, atterberg limit, consistency, SPT, bearing capacity, plasticity, compaction, consolidation and compressibility etc., and geotechnical map. Each zone can be used for different types of construction purposes, characterized by specific engineering properties. Detailed engineering geological information should be used for sustainable land-use planning and development to reduce environmental hazards.

Keywords: geotechnical study, land-use planning, Sirajgonj, Bangladesh

Introduction

Sirajgonj is one of the first growing towns in Bangladesh with respect to population and development. People started to fill up nearby low-lying areas to construct civil structures without any plan. As a result they have been facing disastrous environmental problems, like foundation failure and structural subsidence etc. Similar problems have been recently recorded in and around many towns such as Pabna, Chandpur, Shibgonj, Dhaka, Keranigonj in Bangladesh and abroad (Shamsuzzaman *et al.*,2005; 2004a & b, 2003; BWDB, 2002; Asaduzzaman *et al.*, 1999, 1996; Mulder *et al.*, 1996; Natani, 1996; Prame, 1996; Suhari, 1996; Khan *et al.*, 1991). The most important geological factors that have to be considered in urban development are the suitability of the soils and rocks for foundations, sources of hazards, sub-surface as well as surface geological data etc. For more safe and sustainable urbanization, the planners and developers have to consider engineering geological factor before planning, designing and constructing or developing the area.

The engineering geological investigations of the area in detail are non-existent though several papers have been published on geomorphology, urban geology, sedimentation and hydrology of the surrounding areas (Shamsuzzaman *et al.*, 2005, 2004a & b, 2003; Rahman, 2001a & b; Islam, 2000; BWDB, 1985; Chowdhury, 1973; Coleman, 1969). The main aim of the present study is to determine the geotechnical status of the study area and prepare a geotechnical map, which will be helpful in urban planning and development of the area.

The study area occupies the Jamuna and Karatoya floodplain deposits measuring 301 sq.km. It extends between 23° 22′ and 24°32′N latitudes and 89°34′ and 89°45′E longitudes (Fig. 1) and is located about 100 km, northwest from Dhaka. The principal drainage systems of the area are the Jamuna, Karatoya, Katakhal and Ichhamoti Rivers. The town is connected with the surrounding districts by metal roads, railways and rivers.

Materials and Methods

The following materials and data from different sources as well as direct fieldwork were used in the present study:

a. Overall geological information (Geological Survey of Bangladesh).
b. Topographic sheets (Survey of Bangladesh, 1991, 1967, 1954, 1952, 1921).
c. Satellite imagery (hard and soft copies of SPOT satellite image of 1989. (SPARRSO, 1989).
d. Climate, population, culture, historical, geographical and other relevant information (Anon,1978).
e. Sub-soil investigation reports (LGED, 1990-2005; NBSI, 1990-2005; CPSI, Bogra, 1990-2005; Soil Associates Engineering, Dhaka, 1998-2000).
f. Deep tubewell borelog data and soil-type map (Bangladesh Agricultural Development Corporation, 1990-2001).
g. Town development planning and land-use map (Sirajgonj Pourashava).
h. Field data from direct field surveying and local people. Sub-soil samples were also collected by hand augers to determine their engineering properties.

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*Author for correspondence; E-mail: sultan_ul_islam@yahoo.com

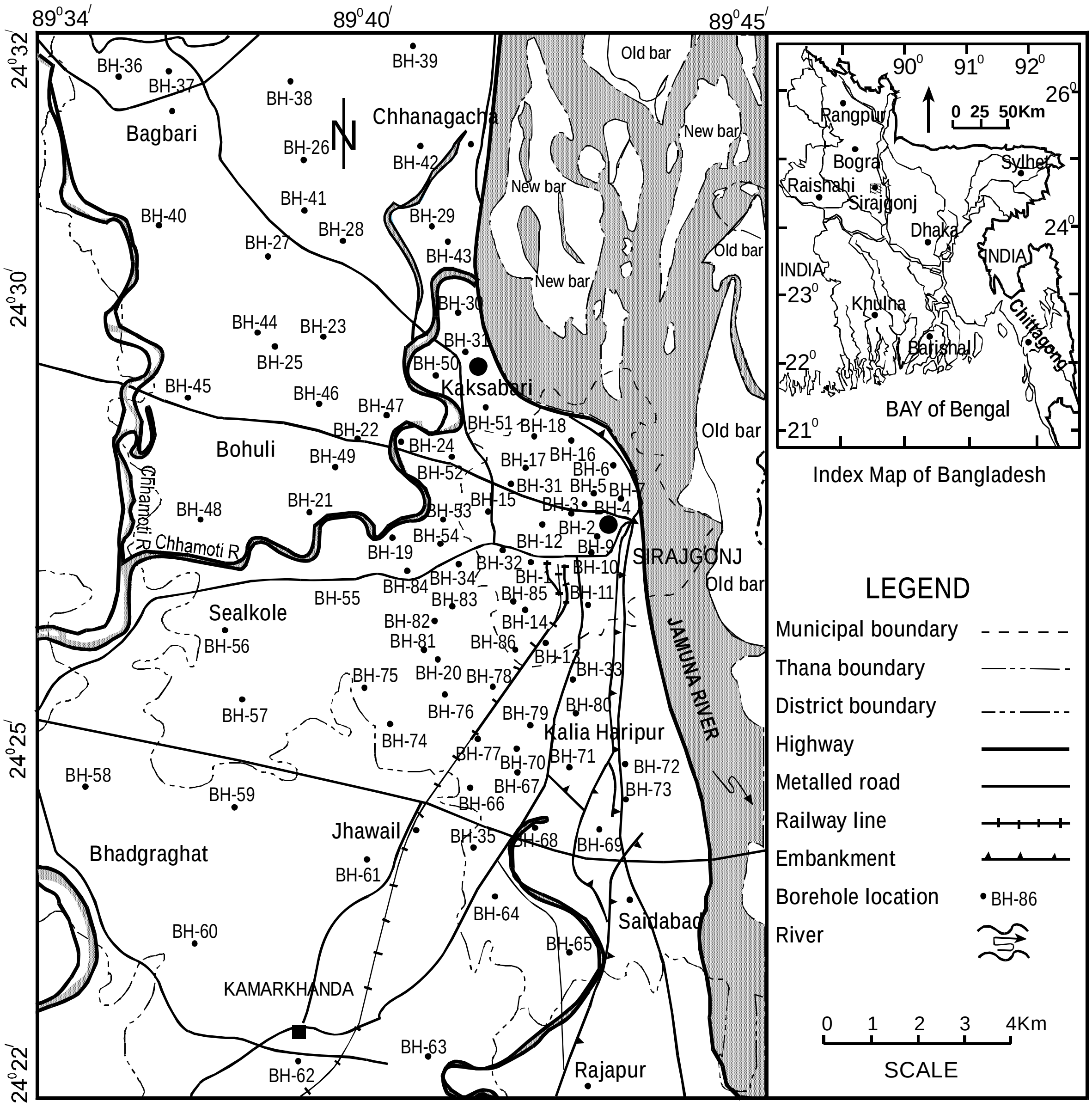

Fig. 1. Location map of the study area.

Besides the use of the existing data, various types of maps, published and unpublished reports and documents relevant to the field of study and laboratory analysis were also consulted. At first a preliminary morphogeological map was prepared with the aid of SPOT satellite image, topographic maps, thana map (LGED, 2000) and visual and digital image interpretation techniques. The sub-surface undisturbed soil samples of different morphologic units were studied in shallow cores through auguring and coring to identify the geotechnical characters of soil. The final morphogeological map was then prepared, where major morphogeological units have been delineated by integrating morphologic and lithologic data. The SPT-zonation map were prepared on the basis of SPT value and sub-surface stratigraphic cross-section. Finally, the geotechnical map has been prepared considering the morphogeological features, engineering properties of the soil and the sub-surface stratigraphy.

Index properties, mechanical properties and bearing capacity of soil were calculated after determining individual parameter using standard methods.

Results and Discussion

It is essential to know the engineering properties of the soil for heavy construction. For it, the index properties of soil (moisture content, void ratio, unit weight, liquid limit, plastic limit, shrinkage limit, plasticity index and liquidity index), mechanical properties of soil (compaction, cons olidation, shear strength, unconfined compression test and permeability) and bearing capacity of the soil were calculated (Table 1).

For measurement of the bearing capacity of the sub-surface soils, standard penetration test (SPT) was done. The bearing capacity is the ability of the foundation sediments and soils to support superimposed load without causing failure or rupture of the supporting soil (Sehgal, 1988). 86 Engineering boreholes were used to calculate the SPT curve (Fig. 2). The SPT zonation maps (Fig. 3.) have been prepared on the basis of SPT value of different engineering boreholes according to which each zone has been divided into high, moderate and low strength zones.The high SPT strength indicates high suitability of soil for heavy construction and vice versa.

Plasticity is defined as the property of soil, which allows it to be deformed rapidly, without rupture, elastic rebound and volume change (Punmia, 1994). During construction of an engineering structure, it is pre-requisite to identify the plasticity behaviour of the soil of that area, because plasticity indicates the bearing capacity. Plasticity index is the first condition to determine the plasticity of soils. For the preparation of geotechnical map (Fig. 4), the geological map, subsurface lithology and geotechnical characteristics of soils were taken into consideration. The mapped area has been divided into the following 4 major zones:

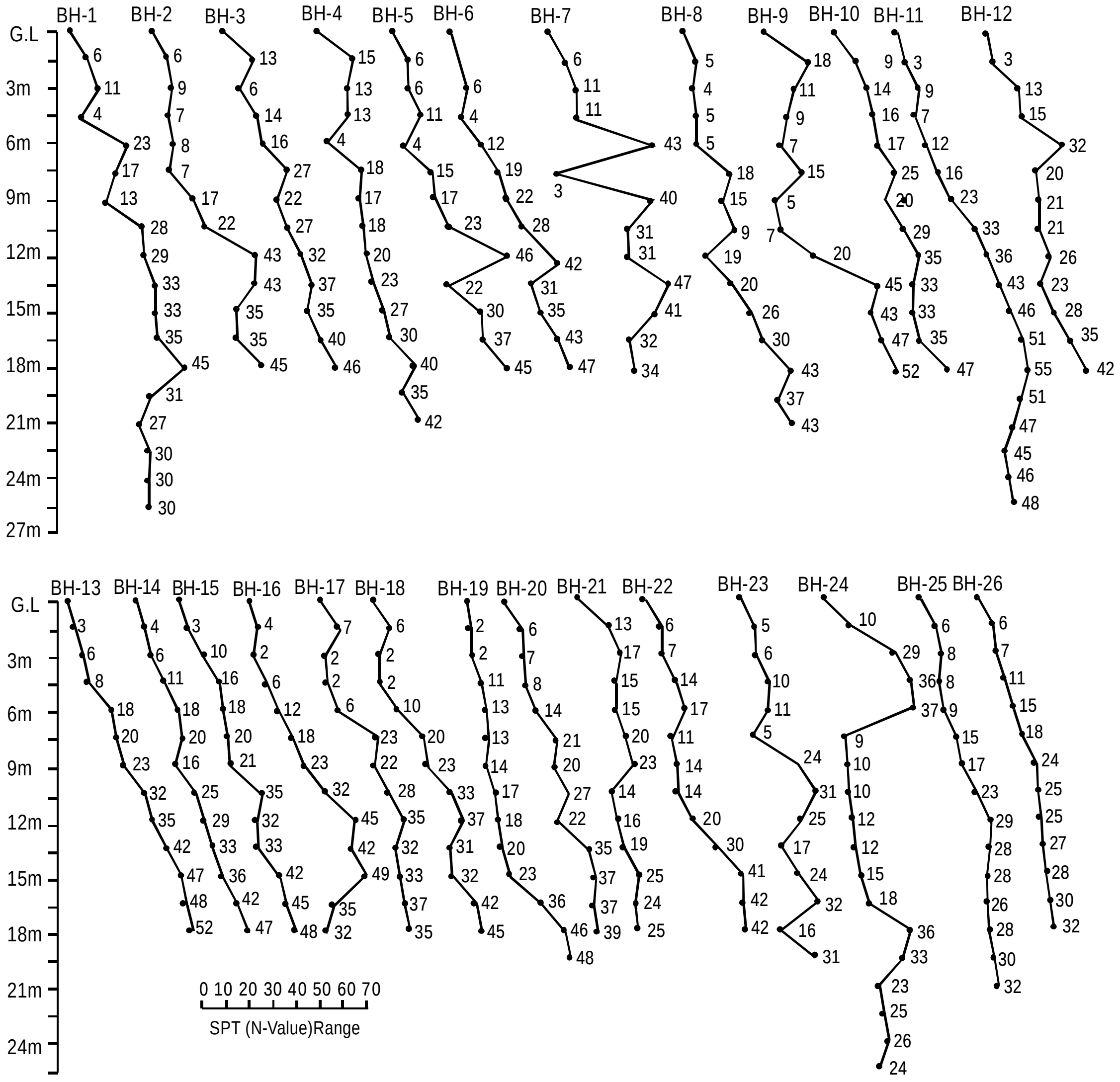

Fig. 2. SPT Curve of different engineering boreholes of Sirajgonj town and its adjoining areas upto the depth of 18 m (LGED, NBSI, RHD, SAE; 1990-2005).

Table 1. Physical and geotechnical properties of near surface soils in the Sirajgonj town and its adjoining areas (from NBSI, CPSI, SAE, LGED, RHD, 1990-2001 and laboratory tests)

Borehole No.	Engineering geologic unit	Geologic units	Particle size						Atterberg limit			Specific gravity	Natural moisture content (%)
			Sand %	Silt %	Clay %	D_{10} in mm	D_{30} in mm	D_{60} in mm	Liquid limit (LL)%	Plasticity limit (PL)%	Plasticity index (PI)%		
BH-1	III	Natural levee, Channel, Meander Scar, Crevasse splay	94-75	06-25	-	0.09-0.04	0.15-0.08	0.18-0.12	28-30	25-26	3-4	2.66-2.67	21.28-22.63
BH-2	III	Natural levee, Channel, Meander Scar, Crevasse splay	85-82	15-18	-	0.06-0.01	0.09-0.01	0.15-0.02	25-26	22-23	3-4	2.75-2.73	20.60-43.10
BH-3	III	Natural levee, Channel, Meander Scar, Crevasse splay	84-75	16-25	-	0.06-0.02	0.15-0.04	0.15-0.03	27-29	22-23	5-6	2.65-2.68	28.64-31.66
BH-4	III	Natural levee, Channel, Meander Scar, Crevasse splay	91-70	9-30	-	-	-	-	30-32	27-28	3-4	2.66-2.65	30.96-36.55
BH-5	III	Natural levee, Channel, Meander Scar, Crevasse splay	80-70	15-23	5-7	-	-	-	38-42	33-36	5-6	2.64-2.69	25.56-31.23
BH-6	III	Natural levee, Channel, Meander Scar, Crevasse splay	67-43	40-52	3-5	-	-	-	37-39	33-34	4-5	2.67-2.66	25.02-31.3
BH-7	III	Natural levee, Channel, Meander Scar, Crevasse splay	82-75	15-20	3-5	-	-	-	36-38	32-33	4-5	2.65-2.61	21.25-23.64
BH-8	III	Natural levee, Channel, Meander Scar, Crevasse splay	95-90	5-10	-	0.15-0.043	0.28-0.07	0.1-0.045	22-24	21-22	1-2	2.65-2.66	20.10-35.99
BH-9	III	Natural levee, Channel, Meander Scar, Crevasse splay	96-88	4-12	-	-	-	-	23-27	22-25	1-2	2.65-2.66	16.45-28.04
BH-10	III	Natural levee, Channel, Meander Scar, Crevasse splay	95-85	5-15	-	0.1-0.03	0.22-0.05	0.32-0.08	26-25	24-29	2-3	2.66-2.68	21.5-33.70
BH-11	III	Natural levee, Channel, Meander Scar, Crevasse splay	95-90	5-10	-	0.15-0.043	0.27-0.06	0.03-0.9	26-25	25-23	1-2	2.66-2.70	20.57-26.18
BH-12	III	Natural levee, Channel, Meander Scar, Crevasse splay	95-92	5-8	-	0.15-0.05	0.4-0.1	0.64-0.14	26-25	25-23	1-2	2.63-2.61	24.87-38.99
BH-13	III	Natural levee, Channel, Meander Scar, Crevasse splay	95-90	3-7	2-3	-	-	-	20-25	18-22	2-3	2.67-2.70	21.04-30.12
BH-14	II	Floodplain	90-85	8-12	2-3	-	-	-	43-46	24-26	19-20	2.66-2.68	21.5-33.70
BH-15	II	Floodplain	85-80	13-17	2-3	-	-	-	42-45	29-31	13-14	2.65-2.66	16.45-28.04
BH-16	I	Flood basin, Abandoned Channel, Ox-bow lake	75-65	10-15	15-20	-	-	-	48-56	20-22	25-34	2.69-2.68	26.44-32.02
BH-17	I	Flood basin, Abandoned Channel, Ox-bow lake	75-65	10-15	15-20	-	-	-	42-44	25-26	17-18	2.69-2.68	26.44-32.08
BH-18	I	Flood basin, Abandoned Channel, Ox-bow lake	75-65	15-20	10-15	-	-	-	46-62	19-30	27-32	2.70-2.68	31.04-36.12
BH-19	III	Natural levee, Channel, Meander scar, Crevasse splay	98-95	2-5	-	-	-	-	25-27	24-25	1-2	2.63-2.60	23.86-37.10
BH-20	II	Floodplain	90-74	10-26	-	-	-	-	40-38	25-20	15-18	2.65-2.69	20.86-24.93
BH-21	III	Natural levee, Channel, Meander scar, Crevasse splay	90-80	10-20	-	-	-	-	24-27	19-20	5-7	2.67-2.69	21.63-25.13
BH-22	III	Natural levee, Channel, Meander scar, Crevasse splay	95-80	5-20	-	-	-	-	26-30	22-25	4-5	2.66-2.68	20.96-24.14
BH-23	II	Floodplain	92-70	8-30	-	0.08-0.03	0.17-0.08	0.25-0.13	47-52	30-32	17-20	2.66-2.67	24.55-34.20
BH-24	III	Natural levee, Channel, Meander scar, Crevasse splay	90-94	10-6	-	0.075-0.09	0.2-0.15	0.4-0.2	23-25	22-23	2-3	2.64-2.66	18.65-24.57
BH-25	I	Flood basin, Abandoned Channel, Ox-bow lake	45-50	15-20	40-30	-	-	-	56-67	26-27	30-35	2.59-2.71	18.43-25.37
BH-26	II	Floodplain	60-65	10-15	30-20	-	-	-	48-42	30-22	18-20	2.65-2.72	22.13-26.49
BH-27	II	Floodplain	60-70	28-20	12-10	-	-	-	44-42	32-25	12-17	2.69-2.67	25.44-30.12
BH-28	II	Floodplain	75-80	13-10	12-10	-	-	-	46-49	29-30	17-19	2.64-2.69	28.56-32.15
BH-29	II	Floodplain	80-85	13-10	7-5	-	-	-	43-42	33-27	10-15	2.69-2.67	30.12-36.56
BH-30	IV	Active Channel	98-99	2-1	-	-	-	-	-	-	-	2.66-2.64	15.26-20.96
BH-31	IV	Active Channel	97-98	3-2	-	-	-	-	-	-	-	2.64-2.62	14.26-18.21
BH-32	II	Floodplain	68-75	20-15	12-10	-	-	-	43-45	31-28	12-17	2.69-2.67	25.02-31.3
BH-33	II	Floodplain	70-75	15-20	15-5	-	-	-	47-51	34-33	13-18	2.67-2.66	25.03-30.2
BH-34	I	Flood basin, Abandoned Channel, Ox-bow lake	50-60	15-20	35-20	-	-	-	56-67	26-29	30-38	2.67-2.66	33.6-34.3
BH-35	I	Flood basin, Abandoned Channel, Ox-bow lake	-	10-12	90-88	-	-	-	45-55	10-13	35-42	2.59-2.67	30.84-34-47

Table 1. (Continued from previous page)

Borehole No.	Engineering Geologic Unit	Unit weight (g/cc)		Soil classification USC	Porosity	Void ratio	Permeability	Consistency	SPT	Direct shear test		Plasticity
		Wet weight	Dry weight							Angle of Internal friction (degree)	Cohesion (TSF)	
BH-1	III	1.85-1.82	1.53-1.48	ML-MH	Low to Medium	0.9-1.3	Moderate to Low	Medium to Hard	6-30	35°-30°	0.0-0.045	Low
BH-2	III	1.85-1.82	1.52-1.30	ML-MH	Low to Medium	1.1-1.2	Mederate to Low	Medium to Hard	6-45	31°-29°	0.04-0.05	Low
BH-3	III	1.85-1.82	1.44-1.32	ML-MH	Low to Medium	0.9-1.4	Moderate to Low	Stiff to Hard	13.46	38°-30°	0.09-0.20	Low
BH-4	III	1.88-1.86	1.44-1.36	ML-MH	Low to Medium	1.1-0.9	Moderate to Low	Stiff to Hard	13.42	31°-32°	0.11-0.09	Low
BH-5	III	1.70-1.78	1.36-1.35	ML-MH	Low to Medium	0.9-1.0	Moderate to Low	Mediumj to Hard	4-45	32°-27°	0.02-0.01	Low
BH-6	III	1.92-1.87	1.54-1.42	SL-MH	Low to Medium	0.8-0.9	Moderate to Low	Medium to Hard	4-47	29°-33°	0.14-0.06	Low
BH-7	III	1.80-1.79	1.48-1.46	ML-MH	Low to Medium	0.9-1.0	Moderate to Low	Medium to Hard	6-47	33°-30°	0.05-0.06	Low
BH-8	III	1.94-1.83	1.62-1.35	ML-MH	Low to Medium	1.2-1.3	Moderate to Low	Medium to Hard	4-43	36°-29°	0.04-0.11	Low
BH-9	III	1.84-1.76	1.56-1.38	ML-MH	Low to Medium	1.1-1.3	Moderate to Low	Medium to Hard	5-52	31°-28°	0.00-0.11	Low
BH-10	III	1.83-1.78	1.50-1.42	ML-MH	Low to Medium	0.9-1.1	Moderate to Low	Medium to Hard	9-47	32°-33°	0.06-0.16	Low
BH-11	III	1.85-1.81	1.53-1.43	ML-MH	Low to Medium	1.1-1.2	Moderate to Low	Medium to Hard	9-48	29°-30°	0.05-0.04	Low
BH-12	III	1.79-1.73	1.43-1.24	ML-MH	Low to Medium	1.1-1.4	Moderate to Low	Stiff to Hard	13-42	31°-32°	0.18-0.02	Low
BH-13	III	1.86-1.81	1.54-1.46	ML-MH	Low to Medium	1.1-1.3	Moderate to Low	Soft to Hard	3-52	32°-30°	0.01-0.05	Low
BH-14	II	1.87-1.84	1.52-1.47	CL-CH	Low	1.2-1.5	Low	Medium to Hard	4-47	32°-31°	0.01-0.04	Medium
BH-15	II	1.89-1.83	1.59-1.47	CL-CH	Low	1.1-1.4	Low	Soft to Hard	3-48	31-32	0.00-0.06	Medium
BH-16	I	1.75-1.74	1.38-1.35	CL-MH	Low	0.8	Low to very Low	Soft to Hard	2-49	32-30	0.06-0.05	High
BH-17	I	1.75-1.76	1.38-1.36	CL-MH	Low	0.9	Low to very Low	Soft to Hard	2-37	32-30	0.01-0.06	High
BH-18	I	1.74-1.75	1.37-1.34	CL-MH	Low	0.6	Low to very Low	Soft to Hard	2-45	32-30	0.06-0.05	High
BH-19	III	1.87-1.82	1.52-1.46	SP-MH	Low to Medium	0.80-1.0	Moderate to Low	Soft to Hard	2-48	31-28	0.00-0.11	Low
BH-20	II	1.86-1.75	1.53-1.40	SP-ML	Low	-	Low	Medium to Hard	6-39	31-27	0.04-0.08	Medium
BH-21	III	1.84-1.80	1.51-1.43	SP-MH	Low to Medium	-	Moderate to Low	Stiff to very Stiff	13-25	32-31	0.41-0.05	Low
BH-22	III	1.85-1.79	1.53-1.44	SP-MH	Low to Medium	-	Moderate to Low	Medium to Hard	6-42	28-29	0.03-0.05	Low
BH-23	II	1.84-1.76	1.48-1.31	SM-MH	Low	-	Low	Medium to Hard	5-13	34-33	0.05-0.07	Medium
BH-24	III	1.89-1.82	1.59-1.46	SP-MH	Low to Medium	-	Moderate to Low	Stiff to Hard	10-37	33-34	0.06-0.08	Low
BH-25	I	1.76-1.74	1.46-1.39	CH-ML	Low	0.7	Low to very Low	Medium to very Stiff	6-32	23-24	0.06-0.04	High
BH-26	II	1.88-1.85	1.51-1.48	CL-ML	Low	1.0-1.3	Low	Medium to very Stiff	6-32	34-32	0.00-0.04	Medium
BH-27	II	1.78-1.80	1.45-1.43	CL-ML	Low	-	Low	-	-	-	-	Medium
BH-28	II	1.76-1.75	1.31-1.30	CL-OL	Low	-	Low	-	-	-	-	Medium
BH-29	II	1.68-1.72	1.44-1.40	CL-OL	Low	-	Low	-	-	-	-	Medium
BH-30	IV	1.85-1.83	1.48-1.43	SP	High	-	High	-	-	-	-	Non-plastic
BH-31	IV	1.89-1.83	1.53-1.48	SP	High	-	High	-	-	-	-	Non-plastic
BH-32	II	1.82-1.80	1.47-1.43	ML-OL	Low to Medium	-	Low	-	-	-	-	Medium
BH-33	II	1.79-1.80	1.45-1.43	ML-OL	Low to Medium	-	Low	-	-	-	-	Medium
BH-34	I	1.64-1.75	1.30-1.35	OH-OL	Low	-	Low	-	-	-	-	High
BH-35	I	1.70-1.77	1.44-1.42	OH-OL	Low	-	Low	-	-	-	-	High

Table 1. (Continued from previous page)

Borehole No.	Engineering geologic unit	Compressibility	Suitability of the material		Remarks
			Value for the foundation characteristics	Workability as construction materials	
BH-1	III	Low to very Low	Fair to good bearing value	Fair	Shallow foundation is possible (R. C. C. Square footing)
BH-2	III	Low to very Low	Fair to good bearing value	Fair	Shallow foundation is possible (R. C. C. Square footing)
BH-3	III	Low to very Low	Fair to good bearing value	Fair	Shallow foundation is possible (R. C. C. Square footing)
BH-4	III	Low to very Low	Fair to good bearing value	Fair	Shallow foundation is possible (R. C. C. Square footing)
BH-5	III	Low to very Low	Fair to good bearing value	Fair	Shallow foundation is possible (R. C. C. Square footing)
BH-6	III	Low to very Low	Fair to good bearing value	Fair	Shallow foundation is possible (R. C. C. Square footing)
BH-7	III	Low to very Low	Fair to good bearing value	Fair	Shallow foundation is possible (Caste in situ pile)
BH-8	III	Low to very Low	Fair to good bearing value	Fair	Shallow foundation is possible (R. C. C. Square footing)
BH-9	III	Low to very Low	Fair to good bearing value	Fair	Shallow foundation is possible (R. C. C. Square footing)
BH-10	III	Low to very Low	Fair to good bearing value	Fair	Shallow foundation is possible (R. C. C. column footing)
BH-11	III	Low to very Low	Fair to good bearing value	Fair	Shallow foundation is possible (R. C. C. Square footing)
BH-12	III	Low to very Low	Fair to good bearing value	Fair	Shallow foundation is possible (R. C. C. Square footing)
BH-13	III	Low to very Low	Fair to good bearing value	Fair	Shallow foundation is possible (R. C. C. Square footing)
BH-14	II	Medium	Fair to good bearing value	Fair	Shallow to medium foundation possible with care
BH-15	II	Medium	Fair to good bearing value	Fair	Shallow to medium foundation possible with care
BH-16	I	High	Poor bearing value	Poor	Very deep foundation required and bad for civil structure
BH-17	I	High	Poor bearing value	Fair	Very deep foundation required and bad for civil structure
BH-18	I	High	Poor bearing value	Poor	Very deep foundation required and bad for civil structure
BH-19	III	Low to very Low	Fair to good bearing value	Fair	Shallow foundation is possible (R. C. C. Square footing)
BH-20	II	Medium	Fair to good bearing value	Fair	Shallow to medium foundation possible with care
BH-21	III	Low to very Low	Fair to good bearing value	Fair	Shallow foundation is possible (R. C. C. Square footing)
BH-22	III	Low to very Low	Fair to good bearing value	Fair	Shallow foundation is possible (Caste in situ pile)
BH-23	II	Medium	Fair to good bearing value	Fair	Shallow foundation is possible (R. C. C. Square footing)
BH-24	III	Low to very Low	Fair to good bearing value	Fair	Shallow foundation is possible (R. C. C. Square footing)
BH-25	I	High	Poor bearing value	Poor	Very deep foundation required and bad for civil structure
BH-26	II	Medium	Fair to poor bearing value	Fair	Shallow to medium foundation possible with care
BH-27	II	Medium	Fair to poor bearing value	Fair	Shallow to medium foundation possible with care
BH-28	II	Medium	Fair to poor bearing value	Fair	Shallow to medium foundation possible with care
BH-29	II	Medium	Fair to poor bearing value	Fair	Shallow to medium foundation possible with care
BH-30	IV	Very Low	Good bearing value	Good	Very suitable for civil structure but it is affected by bank erosion
BH-31	IV	Very Low	Good bearing value	Good	Very suitable for civil structure but it is affected by bank erosion
BH-32	II	Medium	Fair to poor bearing value	Fair	Shallow to medium foundation possible with care
BH-33	II	Medium	Fair to poor bearing value	Fair	Shallow to medium foundation possible with care
BH-34	I	High	Poor bearing value	Poor	Very deep foundation required and bad for civil structure
BH-35	I	High	Poor bearing value	Poor	Very deep foundation required and bad for civil structure

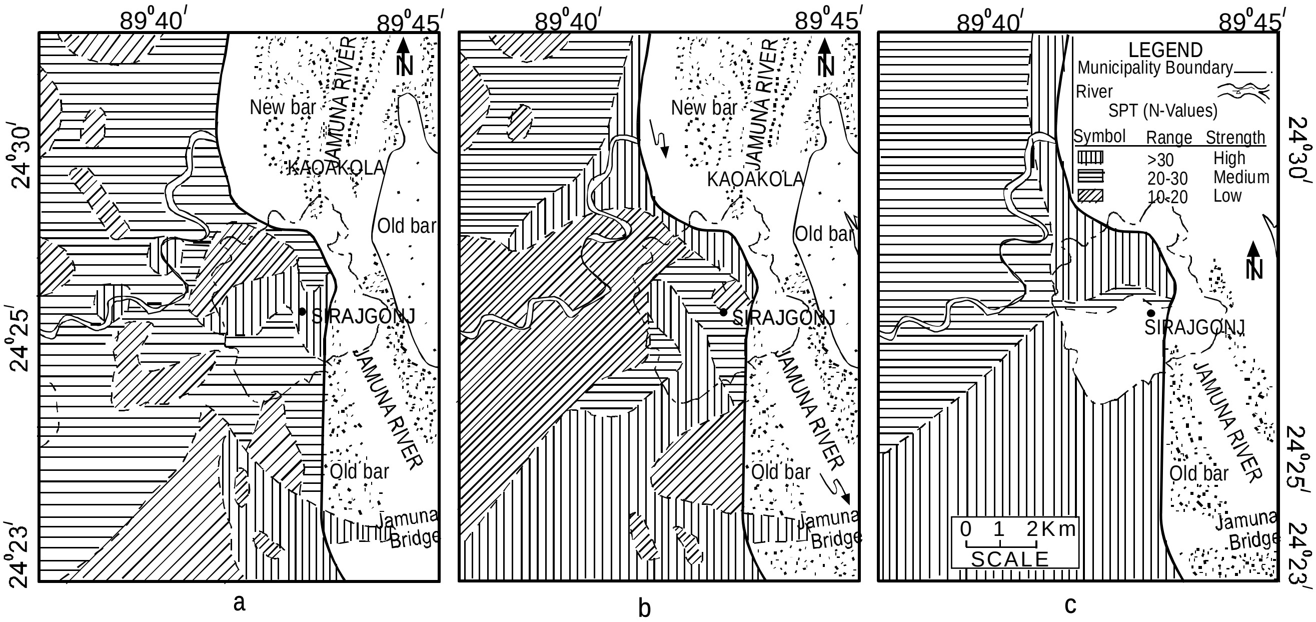

Fig. 3. SPT (N values) zonation map of Sirajgonj town and its adjoining areas, (**a.** upto depth of 6 m, **b.** upto depth of 6 to 12 m and **c.** upto depth of 12 to 18 m).

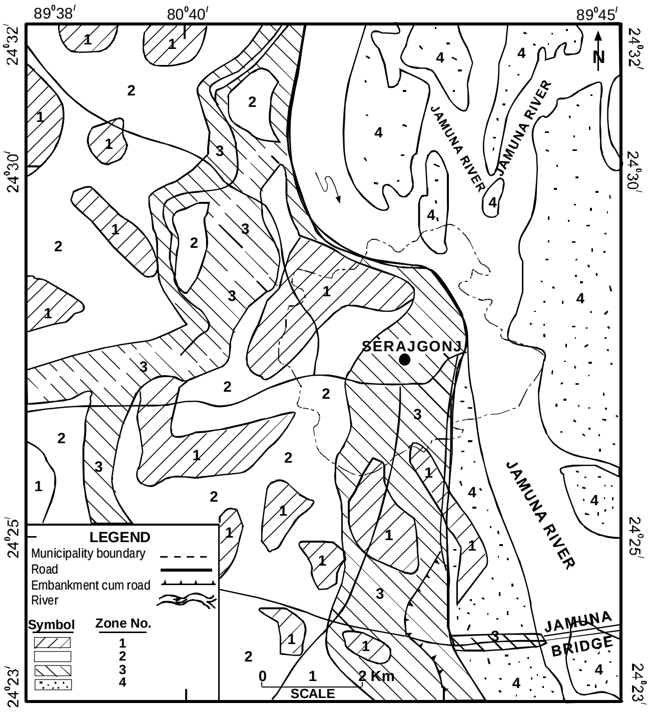

Fig. 4. Geotechnical map of Sirajgonj town and its adjoining areas.

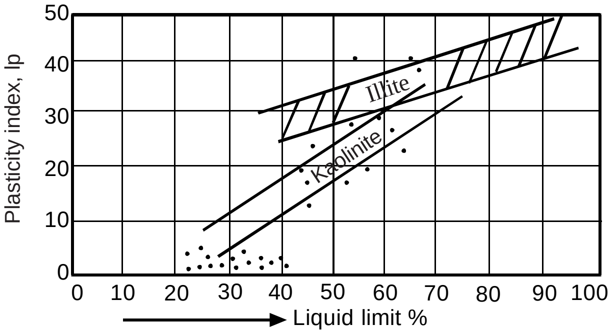

Fig. 5. Liquid limit versus plastic index for the common clay minerals (Modified after Grim, 1962).

Zone 1: This zone is mainly composed of light gray to gray kaolinitic clay (Fig. 5), decomposed and partially decomposed vegetable matter and light gray silty clay, which are found in flood basin, ox-bow lake and abandoned channel. The sediments of this zone are poorly compacted, highly plastic, cohesive and compressive. The plasticity index and liquid limit of Casagrande (1976) have been plotted in the plasticity chart (Fig. 5), the soil type of this unit is mainly CH-OH (llay-organic clay). The consistency of soil of this zone is very soft to hard when dry and SPT-value varies from 1 to 26. It is a very weak foundation layer and very deep foundation will be required for any civil construction with normal foundation.

Zone 2: This zone comprises mainly of grayish to brownish yellow silty clay, often black to gray organic rich kaolinitic clay (Fig. 5), decomposed and partially decomposed grass roots, animal burrows and silty sands of flood plain sediments. These sediments are low compacted, medium plastic, cohesive and medium compressible. The plasticity index and liquid limit of Casagrande (1976) have been plotted in the plasticity chart (Fig. 6). The soil type of this unit is mainly CL-ML (clay-silt), which is low to medium plastic. The consistency of soils of this unit is soft to hard and SPT-values vary from 3 to 48. It is a good foundation layer and is suitable for construction of low to moderately high-rising buildings with normal foundation.

Zone 3: This zone comprises mainly yellowish gray to gray silty clay, silty sand and fine to very fine and medium-grained sands of natural levee, crevasse splay and meander scar sediments. The sediments of this unit are moderate to high compact, low plastic and low to very low compressible. Plotting of the plasticity index and liquid limit in the Casagrande (1976) plasticity chart (Fig. 6) indicates that the soil type of this zone is SP-MH (medium to coarse sand-silty sand micaceous) to ML-MH (silt-silty sand). The consistency of soil is stiff to hard and SPT-values vary from 10 to 55. This zone is generally situated above flood level and shallow foundation is required for construction of low to moderately high-rising buildings.

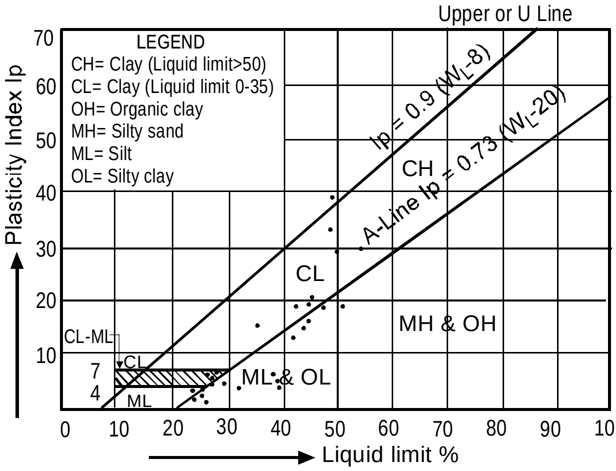

Fig. 6. Position of cohesive sub-soil of Sirajgonj town and adjoining areas on the Casagrande Plasticity Chart (Eden, 1963).

Zone 4: This zone consists of active channel deposits, which are mainly light brown to gray, fine to medium-grained sand with little sandy silt and silty sand. These sediments are moderately compact, non-plastic and low to very low compressible. According to Bowels (1984), the soil type of this unit is SP (poorly graded sand). Although this zone bears good foundation layer for construction, it has a major problem of being situated in high bank erosion of the Jamuna River each year. So, permanent urbanization is not suitable in this area. The sediments of this unit may be used for filling land and construction materials.

Conclusion

From the study of overall geotechnical aspects, it is suggested that the soil test must be done carefully before construction of any civil engineering structure. With reference to SPT zonation map and geotechnical map, zone-1 does not seem suitable for heavy construction as very deep foundation (mat or pile) is required for construction of buildings. Zone-2 is moderately suitable for heavy construction with shallow to medium foundation (R.C.C square or column footing). Zone-3 is the most suitable area for heavy construction, with normal foundation depth of 3 to 6 m below the surface. Zone-4 is unsuitable for heavy construction and permanent urbanization in this area is not suggested.

Acknowledgement

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Synthesis of 3′, 4′, 5, 6, 7-Pentamethoxy-8-C-Prenylflavone

M. Amzad Hossain[a*] and Zhari Ismail[b]
[a]Chemistry Division, Atomic Energy Centre, GPO Box No. 164, Ramna, Dhaka - 1000, Bangladesh
[b]School of Pharmaceutical Sciences, University Sains Malaysia, 11800 Pulau Pinang, Malaysia

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Abstract. 3′, 4′, 5, 6, 7-Pentamethoxy-8-C-prenylflavone, isolated from the leaves of Malaysian O*rthosiphon stamineus*, was synthesized starting with treating 2, 4, 5, 6-tetrahydroxyacetophenone with dimethyl sulphate which yielded several other minor compounds as well. The synthesized title compound is identical in all respects with the natural sample.

Keywords: flavones, medicinal plants, *Orthosiphon staminens*, methoxy-prenylflavone

Introduction

Orthosiphon stamineus Benth (Lamiaceae), is one of the popular traditional medicinal plants and is extensively used in southeast Asia for the treatment of a wide range of diseases. In Indonesia, it is used for rheumatism, diabetes, urinary lithiasis, edema, eruptive fever, influenza, hepatitis, jaundice, biliary lithiasis and hypertension etc. (Hossain and Ismail, 2005a; Tezuka *et al*., 2000), in Vietnam, for urinary lithiasis, edema, eruptive fever, influenza, hepatitis, jaundice, biliary lithiasis (Hossain and Ismail, 2005a; Tezuka *et al*., 2000) and in Malaysia, to alleviate diabetes and kidney stone diseaes. Owing to its pharmaceutical utility, it is under systematic cultivation in Malaysia and is locally known as ‘Misai kucing’ meaning ‘Cat’s whisker’ and consumed as healthy Java tea to facilitate body detoxification. In particular, extracts of *O. stamineus* are now widely used in Malaysia as drugs for the treatment of diabetes and kidney stone diseases. The recent surge of interest in chemistry of this plant has led to the isolation of its more than 60 components with different biological acitivies (Tezuka *et al*., 2000). Prenylated flavones have been found to display a variety of biological activities such as behavioural depression and muscle relaxation; they are also known to be antihypertensive and to have β_1-adrenergic inhibition and antimicrobial activities (Hossain *et al*., 2004). The title prenylated flavonoid, isolated from *O. stamineus*, was recently found to exhibit some important bioactivities including antioxidant and aldose reductase inhibitory effects (Tezuka *et al*., 2000). Such bioactivities, as well as the lack of synthesis routes, attracted our interest in this compound.

Materials and Methods

Melting points of the compounds were determined using an electrothermal melting point apparatus (Gallenkamp) and are uncorrected. IR spectra (ν_{max} per centimeter) were recorded (KBr discs) on FT-IR spectrophotometer, ^{1}HNMR and ^{13}CNMR spectra on Bruker R-32 (300 MHz) instrument in $CDCl_3$ with TMS as an internal standard (chemical shifts in δ, ppm) and UV spectra on a HATACHI, U-2000 spectrophotometer Ultrospeck in methanol (λ_{max} in nm). TLC was performed with silica gel GF_{254}. All solvents used were of analytical reagent grade.

2-Hydroxy-4, 5, 6-trimethoxyacetophenone (2). To 10 g a solution of 2, 4, 5, 6-tetrahydroxyacetophenone (**1**) in dry acetone (125 ml), dimethyl sulphate (9.03 g) and anhydrous K_2CO_3 (40 g) were added. The mixture was refluxed for 3 h. Acetone was removed by distillation and water was added to the residue. It was extracted with ether, dried over anhyd. Na_2SO_4 and evaporated to dryness. The ether extract, on column chromatography using petroleum spirit (40-60 °C), petroleum spirit-hexane (3:1, 3:2) and increasing quantities of hexane as eluent gave the major compound 2-hydroxy-4, 5, 6-trimethoxyacetophenon (**2**) and several other minor compounds. The compound **2** was obtained as white crystals (2.54 g), mp: 76 °C; (M^+, 226); R_f: 0.69 (choroform-methanol; 95:5); UV: 230, 245, 278 nm; IR: 3455, 2976, 2855, 1645, 1605, 1595, 1543, 1470, 1410, 1378, 1340, 1236, 1212, 1190, 1153, 1132, 1050, 1043, 1005, 945, 885, 765, 664 cm^{-1}; ^{1}HNMR (δ, DMSO-d_6): 2.48 (s, 3H, $-COCH_3$), 3.98, 3.99 and 4.00 (3s, 9H, $-OCH_3$x3), 6.41 (s, 1H, H-3), 12.71 (s, 1H, -OH); ^{13}CNMR (δ, DMSO-d_6): 158.06 (C-6), 89.06 (C-5), 156.43 (C-4), 102.62 (C-3), 131.47 (C-2), 98.04 (C-1), 182.07 (C=O), 60.76 (6- OCH_3), 55.69 (4- OCH_3), 54.40 (5- OCH_3), 19.43 ($-CH_3$). (Found: C, 58.4; H, 6.19. $C_{11}H_{14}O_5$ requires C, 58.4; H, 5.5%).

2-Hydroxy-4, 5, 6-trimethoxy-3-C-prenylacetophenone (3). Compound **2** (2.26 g) was added to cool solution of KOH (5 g) and absolute methanol (20 ml). The mixture was treated with prenyl bromide (0.98 g) slowly while shaking. After keeping

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*Author for correspondence; E-mail: dramzadh@yahoo.com the reaction mixture for 20 h at room temperature, it was diluted with ice-cold water, acidified and extracted with ether. The ethereal solution was successively extracted with 7% sodium carbonate and 1% KOH solution. The sodium carbonate fraction gave unreacted 2-hydroxy-4, 5, 6-trimethoxy-acetophenone (0.38 g), mp: 116 °C; (M^+, 294). The KOH fraction after acidification gave a solid product which after crystallization from petrol formed white shining plates (1.08 g), mp: 112 °C; (M^+, 294); UV: 234, 256, 348 nm; IR: 3510, 1640, 1605, 1593, 1376, 1363 cm^{-1}; ^{1}HNMR (δ, DMSO-d_6): 1.75 [s, 6H, $>C(CH_3)_2$], 2.43 (s, 3H, -$COCH_3$), 3.53 (d, 2H, *J*=7Hz, -CH_2-CH=), 3.95, 3.98 and 3.99 (3s, 9H, -OCH_3x3), 5.49 (t, 1H, *J*=7Hz, -CH_2-CH=), 12.78 (s, 1H, -OH); ^{13}CNMR (δ, DMSO-d_6): 158.26 (C-6), 88.36 (C-5), 156.49 (C-4), 101.68 (C-3), 131.56 (C-2), 94.44 (C-1), 181.17 (C=O), 60.26 (6-OCH_3), 55.12 (4-OCH_3), 54.49 (5-OCH_3), 19.43 (-CH_3), 76.10 (C-1′), 76.28 (C-2′), 76.68 (C-3′), 75.99 (C-4′), 77.88 (C-5′); (Found: C, 65.30; H, 7.48. $C_{16}H_{22}O_5$ requires C, 65.44; H, 7.5%).

2′-Hydroxy-3, 4, 4′, 5′, 6′-pentamethoxy-8-C-prenylchalcone (4). A mixture of **3** (1 g) and 3,4-dimethoxybenzaldehyde (0.43 g) in ethanolic solution of KOH (50%, 15 ml) was kept at room temperature for 3 days. The reaction mixture was diluted with ice-cold water, acidified with dil. HCl and extracted with ether. The ether layer was washed with water, dried over anhydrous sodium sulphate and evaporated to dryness. The residue was purified by preparative TLC over silica gel 60 using hexane-acetone (15:1) as developing solvent. The product was crystallized from dil. methanol to give yellow crystals (0.35 g), mp: 101 °C, (M^+, 442); R_f 0.76 (chloroform-methanol; 9.5:0.5); UV: 228, 254, 370 nm; IR: 3424, 2987, 2855, 2345, 1642, 1605, 1595, 1375, 1365 cm^{-1}; ^{1}HNMR (δ, DMSO-d_6): 1.73 [s, 6H, $> C(CH_3)_2$], 3.53 (d, 2H, *J*=7Hz, -CH_2-CH=), 3.94, 3.96, 3.97, 3.99 and 4.00 (5s, 15H, -OCH_3x5), 5.49 (t, 1H, *J*=7Hz, -CH_2-CH=), 6.94 (s, 1H, H-2), 7.32 (d, 2H, *J*=9Hz, H-5 and 6), 7.44 (d, 1H, *J*=9Hz, H-α), 8.01 (d, 1H, *J*=9Hz, H-β), 13.01 (s, 1H, -OH); ^{13}CNMR (δ, DMSO-d_6): 98.44 (C-1′), 131.86 (C-2′), 102.43 (C-3′), 156.88 (C-4′), 89.26 (C-5′), 158.66 (C-6′), 181.92 (C=O), 60.99(6′- OCH_3), 55.87 (4′ -OCH_3), 53.93 (5′- OCH_3), 55.64 (3-OCH_3), 47.22 (4-OCH_3), 151.41 (C-β), 125.79 (C-α), 118.51 (C-1), 124.78 (C-2), 111.23 (C-3), 144.43 (C-4), 111.77 (C-5), 118.55 (C-6), 77.45 (C-1″), 77.12 (C-2″), 77.62 (C-3″), 76.88 (C-4″), 77.95 (C-5″); (Found : C, 67.87; H, 6.78. $C_{25}H_{30}O_7$ requires C, 67.77; H, 6.63%).

5, 6, 7, 3′, 4′ -Pentamethoxy-8-C-prenylflavone (5). To a solution of compound **4** (1 g) in dry dioxan (25 ml), 2, 3-dichloro-5, 6-dicyano-1, 4-benzoquinone (DDQ) (85 mg) was added. The mixture was refluxed for 3 h washed with water, dried over anhydrous sodium sulphate and evaporated to dryness. It was purified by preparative TLC over silica gel GF_{254} using hexane-acetone-ethyl acetate (7:5:1) as developing solvent. It was crystallized from methanol as orange needles (0.543 g); mp: 89 °C; (Hossain and Ismail, 2005b), mp: 134 °C); R_f 0.67 (hexane-methanol; 95:5), (M^+, 440); UV: 318, 288, 213 nm; IR: 3435, 2964, 2936, 2832, 2361, 1649, 1610, 1595, 1452, 1376, 1364, 1271, 1200, 1128, 1046, 1030, 879, 832, 800 cm^{-1}; ^{1}HNMR (δ, DMSO-d_6): 1.71 (s, 6H, - > $C(CH_3)_2$, 3.53 (d, 2H, *J*=7Hz, -CH_3-CH=), 3.93, 3.94, 3.97, 3.99 and 4.00 (5s, 15H, -OCH_3 x 5), 5.57 (t, 1H, *J*=7Hz, -CH_3-CH=), 6.99 (d, 1H, *J*=9Hz, H-2′), 7.35 (d, 2H, *J*=9Hz, H-5′ and H-6′); ^{13}CNMR (δ, DMSO-d_6): 153.47 (C-2), 126.27 (C-3), 183.07 (C-4), 159.16 (C-5), 90.97 (C-6), 159.16 (C-7), 104.95 (C-8), 153.47 (C-9), 111.10 (C-10), 119.50 (C-1′), 126.27 (C-2′), 112.75 (C-3′), 146.45 (C-4′), 112.75 (C-5′), 119.50 (C-6′), 77.80 (C-1″), 77.58 (C-2″), 77.38 (C-3″), 76.95 (C-4″), 77.38 (C-5″), 61.23 (5-OCH_3), 56.69 (7-OCH_3), 56.54 (6-OCH_3), 55.64 (3′-OCH_3), 47.38 (4′-OCH_3); (Found: C, 68.18; H, 6.36. $C_{25}H_{28}O_7$ requires C, 68.44; H, 6.30%).

Results and Discussion

In this paper, synthesis of 3′, 4′, 5, 6, 7-pentamethoxy-8-C-prenylflavone is described, starting with 2, 4, 5, 6-tetrahydroxy-acetophenone (**1**) which on treatment with dimethyl sulphate afforded 2-hydroxy-4, 5, 6-trimethoxyacetophenone (**2**) and several other minor compounds. Compound **2** on prenylation yielded 2-hydroxy-4, 5, 6-trimethoxy-3-C-prenylaceto-phenone (**3**) (Hossain, 1999). Alkaline condensation of **3** and 3, 4-dimethoxybenzaldehyde furnished 2′-hydroxy-3, 4, 4′, 5′, 6′-pentamethoxy-8-C-prenylchalcone (**4**) (Hossain *et al.*, 1993). DDQ treatment of **4** gave the title compound 3′, 4′, 5, 6, 7-pentamethoxy-8-C-prenylflavone (**5**) (Fig. 1) (Hossain and Tarafdar, 1998). Its melting point and spectral characteristics agreed with those reported by Hossain and Ismail (2005b) for the natural sample.

The compound **1** was subjected to methylation to give compound **2** and several other minor compounds. The formation of **2** was ascertained by spectral studies and elemental analysis. Compound **2** had the molecular formula $C_{11}H_{14}O_5$ as evidenced by HR-EIMS. IR spectrum of compound **2** showed absorption frequencies at 3455 and 1645 cm^{-1} indicating the presence of hydroxyl and ketonic groups, respectively. In ^{1}HNMR spectrum, a singlet at δ 2.48 indicated the presence of one methyl group on the aromatic ring and a sharp singlet at δ 12.71 indicated the presence of one hydroxyl group on the aromatic ring. Other three singlets at δ 3.98, 3.99 and 4.00 indicated the presence of three -OCH_3 protons, which confirmed that the methylation has taken place. Compound **3** was obtained by the nuclear prenylation (methanolic KOH/

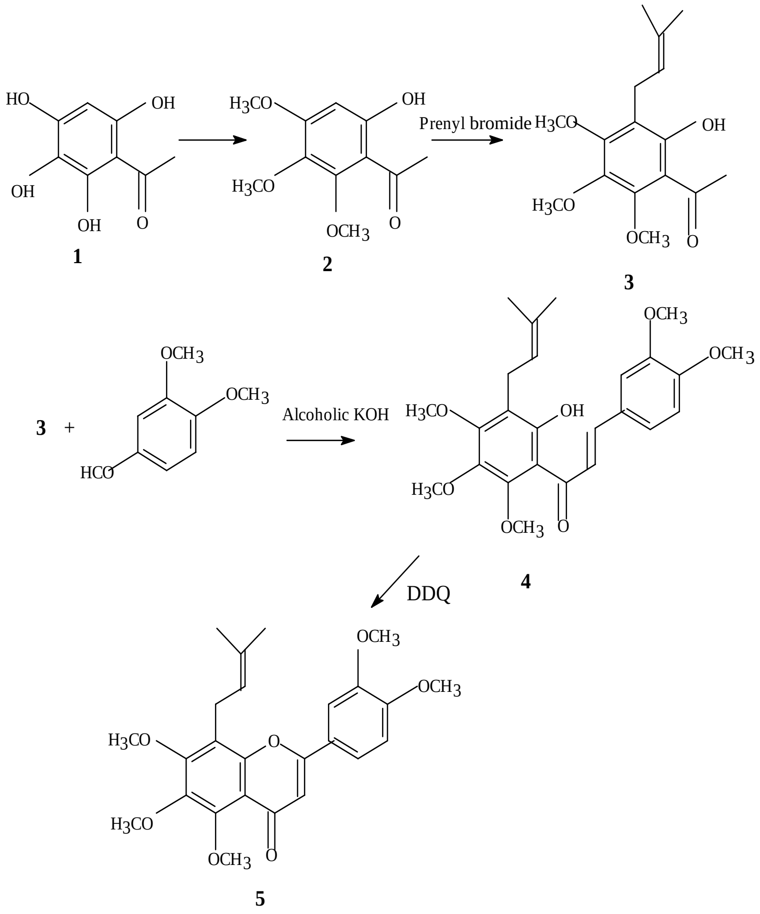

Fig. 1. Synthesis route of 3′, 4′, 5, 6, 7-pentamethoxy-8-C-prenylflavone

prenyl bromide) of **2**; its formation agrees with the data of spectral and elemental analysis. The ^{1}HNMR spectrum of the prenylated compound **3** indicated the presence of C-prenyl unit. A sharp singlet at δ 1.75 revealed the presence of gem-dimethyl group and the presence of -CH2- and -CH= protons attached to the aromatic ring was indicated by the doublet at δ 3.53 and a triplet at δ 5.49, respectively. Compound **3** on a cross-aldol condensation with 3, 4-dimethoxybenzal-dehyde in the presence of 50% ethanolic KOH afforded compound **4** after dehydration of the initial aldol product. The charcteristic IR absorption frequencies at 1642 cm^{-1} showed the presence of conjugated ketonic group and the absorption peaks at 1605 and 1595 cm^{-1} indicated the presence of unsymmetric ethylenic double bond and aromatic rings, respectively. The singlet for methyl protons of acetyl group disappeared while two new doublets at δ 7.44 and 8.01 appeared showing the presence of two vinylic protons (α and β protons). The elemental analysis for C and H showed satisfactory results (within ± 0.4%). DDQ treatment of **4** gave the corresponding title compound **5**. The formation of the title compound (**5)** was confirmed by comparing its spectral data and elemental analysis with that of the reported values of the natural sample.

In conclusion, a new and efficient synthesis of 3′, 4′, 5, 6, 7-pentamethoxy -8-C-prenylflavone has been described through the condensation of 2-hydroxy-4, 5, 6-trimethoxy-3-C-prenyl-acetophenone with 3, 4-dimethoxybenzaldehyde and finally cyclocondensation catalyzed by 2, 3-dichloro-5, 6-dicyano-1,4-benzoquinone (DDQ). The procedure is simple, rapid and high yielding, and will find many applications in organic synthesis. In addition, the CD data should usefully contribute towards the assessment of the absolute configuration of this class of flavonoids.

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Effect of Microwave and Sand Roasting on Physicochemical Values and Fatty Acids of *Cicer arietinum* (White Gram) and *Vigna mungo* (Black Gram) Oils

Lubna Liaquat, Shahnaz Hamid*, Bushra Khalid and Javed Iqbal Khan
[a]Applied Chemistry Research Centre, PCSIR Laboratories Complex, Lahore-54600, Pakistan

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Abstract. The oils extracted from raw seeds of *Cicer arietinum* and *Vigna mungo* of different areas of Punjab province of Pakistan were found to possess, more or less, the same physicochemical properties and fatty acid composition. The oils of both the grains showed an increase in the peroxide and free fatty acids values after both, sand and microwave roasting. Other physicochemical values did not show any significant change in the roasted seed oils. The oils of *Vigna mungo* showed a higher concentration of unsaturated fatty acids. The oils of both the species had a significant amount of linoleic acid in raw, microwave and sand roasted seeds.

Keywords: *Cicer arietinum, Vigna mungo*, fatty acids, microwave roasting, sand roasting

Introduction

Cicer arietinum (chickpea, locally known as Kabuli channa or white gram) and *Vigna mungo* (chickpea, locally known as Kala channa) are known as the poor man's meat owing to the rich amount of good quality protein content. These are the most popular legumes and are eaten traditionally by the local people. These legumes contain about 25.3% of protein in comparison to their oil content (Kritikar and Basu, 2000).

Both the species are considered valuable for human and animal consumption in the south east Asia from nutritional point of view due to their high protein and lysine content as well as energy components such as starch and lipid (Saini and Knights, 1984). In Pakistan, chickpea is eaten in sand roasted as well as in boiled/softened form. Traditionally roasting in sand is a very common method as it imparts appetizing flavour to the fresh warm product.

In the present work, effect of sand and microwave roasting on the lipids of *C. arietinum* and *V. mungo* has been studied. Microwaves are very short waves of electromagnet energy which interact with the food molecules changing their polarity with each cycle. This agitation heats up the food and may also change the composition of food (Maga *et al*., 1977).

Although a number of studies have been undertaken on chickpea as a rich protein source, little work is available on its fatty acid profile versus traditional and modern roasting practices. The earlier investigations indicate high percentage of unsaturated acids in the lipids of chickpea. The present study was undertaken in view of the importance of leguminous seeds as food items and the lack of earlier systematic investigations on lipids of these seeds, particularly in Pakistan. The study was focussed on comparing the traditional sand roasting with modern microwave roasting with reference to fatty acid changes.

Materials and Methods

The seeds of three varieties of *C. arietinum* and *V. mungo*, each, from Mianwali, Bakhar and Layea districts of Punjab were purchased from the local market. After grinding, 100 g powder of each seed was extracted with hexane in soxhlet apparatus. The solvent was removed under nitrogen atmosphere using rotary evaporator. Pale yellow oils were obtained from all the three samples of *C. arietinum*, whereas the oil obtained from *V. mungo* of the above mentioned areas was of darker shade (brown). All the chemicals used in this study were of AR-grade.

Physicochemical values of the oils. The raw seed oils of *C. arietinum* and *V. mungo* samples were subjected to physicochemical investigations. Standard methods (AOAC, 2002) were used to determine refractive index, free fatty acids, saponification value, peroxide value and iodine value.

Microwave and sand roasting. Seeds of each sample (100 g) were roasted by microwave roasting at high microwave radiation intensity and sand roasting at 220 °C for 5 min, each. Oil were extracted from roasted seeds and their physicochemical values and fatty acid profile were determined.

Gas chromatographic analysis. The oils extracted from the samples were treated with borontrifluoride-methanol reagent

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*Author for correspondence

to prepare methyl esters of fatty acids for their identification by GC (AOAC, 2002). These esters were analysed on Shimadzu GC-14A gas chromatograph equipped with hydrogen flame ionization detector and data processor. A PEG capillary column (25 m x 0.2 mm i.d) was used and the column oven temperature was maintained at 180 °C for 5 min, then raised to 220 °C at a rate of 2 °C/min. The temperature of the injector and the detector was set at 250 °C and 300 °C, respectively. The peaks were recorded on Shimadzu C-R4A Chromatopac and identified by comparing their retention time with those of the standard methyl esters, analyzed under the same conditions. All the experimental work was done in triplicate to authenticate the results.

Results and Discussion

The percentage of oil extracted from *C. arietinum* of Mianwali, Bakhar and Layea were 3.72, 3.69 and 3.48% in raw seeds, 3.50, 3.70 and 3.65% in microwave roasted seeds and 3.71, 3.63 and 3.46% in sand roasted seeds, respectively. In case of *V. mungo* of the same localities, it was 3.34, 3.30 and 3.23% in raw, 3.33, 3.29 and 3.2% in microwave roasted seeds and 3.3, 3.27 and 3.22% in sand roasted seeds, respectively. These values are considered as low concentrations of oil. Some earlier studies in this regard have indicated 4-6% oil content in different cultivars of chickpea of different areas (Saini and Knights, 1984). The variation in oil content may be due to the soil composition and environmental effects of different geo graphical areas. The present study indicates that the oil contents in two varieties of chickpea of the same area, are almost equal. The colour of the oils of *C. arietinum* raw seeds is slightly lighter than that of *V. mungo* raw seeds. In all the varieties, the colour of seeds became darker after microwave and sand roasting both, irrespective of the place of origin. The colour change may be due to change in composition of pigments or decomposition of pigments present in the seeds, on heating.

In respect of the physicochemical properties of the oil extracted from the three samples of *C. arietinum* of Mianwali, Bakhar and Layea, it was observed that the iodione value of raw seeds ranged from 126.8-128.4. The values decreased to 127.0-127.9 in microwave roasted seeds and 123.9-124.7 in the sand roasted seeds of the three localities. Similarly a slight change was observed in the saponification value with a slight decrease in the oil of microwave roasted as well as sand roasted seeds. On the other hand an appreciable change was observed in the free fatty acid (FFA) and peroxide values (PV) of the oils after micowave roasting and sand roasting of the seeds of the *C. arietinum* (Table 1).

This change in FFA and PV during heating process may be confined to oxidation and hydrolysis at high temperature. Further more, the increase in FFA shows that high molecular weight fatty acids break into lower molecular weight fatty acids during heating which in turn increased the FFA. Such a change in PV and FFA has already been observed in the study of Vieira and Regitano D'Arce (1999). *V. mungo* of the three areas also showed the same trend of change (Table 2) in iodine value, saponification value and refractive index in raw, microwave roasted and sand roasted seed oils. In this case also, FFA and peroxide values of oils significantly changed after microwave and sand roasting. All the recorded physicochemical values recorded catagorise the oils as edible oils (Tables 1-2).

The extracted oils were further analysed for their fatty acid composition. It was found that Mianwali variety of *C. arietinum* contained 75.0% unsaturated fatty acids in the raw seed oil; the amount decreased to 74.48% and 74.5% when roasted in microwave and sand, respectively. Similar trend was observed in the Bakhar and Layea varieties of *C. arietinum*. The minor decrease in unsaturation in all the cases may be due to the oxidation and hydrolysis of the unsaturated acids on heating.

Table 1. Physicochemical values of raw, microwave and sand roasted seed oils of *C. arietinum* of different areas

Parameter	Raw seeds			Microwave roasted seeds			Sand roasted seeds		
	Mianwali	Bakhar	Layea	Mianwali	Bakhar	Layea	Mianwali	Bakhar	Layea
Oil percentage (DW)	3.72	3.69	3.48	3.50	3.70	3.65	3.71	3.63	3.46
Free fatty acid	1.22	1.25	1.30	1.29	1.30	1.40	1.28	1.34	1.40
Refractive index	1.4673	1.4675	1.4670	1.4688	1.4687	1.4683	1.4679	1.4680	1.4677
Iodine value	126.8	128.4	127.9	127.0	127.9	127.5	123.9	124.7	124.3
Saponification value	196.7	196.5	196.2	193.8	194.4	193.9	192.1	191.2	191.6
Peroxide value	5.25	5.27	5.26	14.23	14.29	14.30	19.12	19.25	19.43

DW = dry weight

The unsaturated acids contained a significant amount of linoleic acid which was about 45.5% in the raw seed oils of all the three samples of *C. arietinum*. A minor decrease was observed on heating in microwave and sand (1.3-1.5%). Amount of oleic acid was almost the same in all the three samples (23.69-26.35%) alongwith lesser amount of linolenic acid (4.0-5.59%) (Tables 3-4). The high amount of linoleic acid, which is an essential fatty acid, has beneficial effect on the health. It acts as carrier of and assists in the absoption of fat - soluble vitamins (A, D, E, K). It also provides a convenient source of stored energy to the body and seves as a precursor to the biologically important prostaglandins. Generally linolenic acid level in excess of 1% is considered to have an adverse effect on flavour stability (MacLeod and Coppock, 1976). Comparison of the two varieties of different areas is presented in Tables 3 and 4.

The range of saturated fatty acids was 24.07-25.27 in all the samples (Table 3). The predominant acids were myristic, palmitic and stearic acids which showed a significant increase after roasting in different ways (Table 4).

Table 2. Physicochemical values of raw, microwave and sand roasted seed oils *of V. mungo* of different areas

Parameter	Raw seeds			Microwave roasted seeds			Sand roasted seeds		
	Mianwali	Bakhar	Layea	Mianwali	Bakhar	Layea	Mianwali	Bakhar	Layea
Oil percentage (DW)	3.34	3.30	3.23	3.33	3.29	3.20	3.30	3.27	3.22
Free fatty acid	1.120	1.12	1.12	1.20	1.23	1.25	1.22	1.26	1.24
Refractive Index	1.4690	1.4675	1.4670	1.4690	1.4689	1.4682	1.4675	1.4685	1.4686
Iodine value	131.40	130.40	130.59	126.25	126.75	125.95	128.98	129.12	129.52
Saponification value	191.07	192.06	192.53	190.73	191.23	191.68	193.72	192.89	193.30
Peroxide value	6.76	6.27	5.72	19.32	19.12	19.52	19.65	19.75	19.53

DW = dry weight

Table 3. Fatty acid composition of raw, microwave and sand roasted seed oils of *C. arietinum* of different areas

Fatty acid	Raw seeds			Microwave roasted seeds			Sand roasted seeds		
	Mianwali	Bakhar	Layea	Mianwali	Bakhar	Layea	Mianwali	Bakhar	Layea
$C_{14:0}$	8.85	7.98	7.35	6.73	7.32	8.1	6.94	6.90	7.52
$C_{16:0}$	10.42	10.56	11.62	12.0	12.69	11.46	12.63	11.95	11.85
$C_{18:0}$	5.35	5.53	6.3	6.0	6.13	6.0	5.65	6.2	6.4
$C_{18:1}$	24.6	25.0	26.35	25.75	23.69	24.15	25.0	25.35	24.83
$C_{18:2}$	45.00	44.5	43.15	44.0	45.29	44.69	44.2	43.85	43.54
$C_{18:3}$	5.40	5.8	5.0	4.73	4.3	4.9	5.3	5.20	5.01
Unidentified	0.38	0.58	0.32	0.79	0.58	0.7	0.98	0.55	0.85

Table 4. Fatty acid composition of raw, microwave and sand roasted seed oils of *V. mungo* of different areas

Fatty acid	Raw seeds			Microwave roasted seeds			Sand roasted seeds		
	Mianwali	Bakhar	Layea	Mianwali	Bakhar	Layea	Mianwali	Bakhar	Layea
$C_{14:0}$	0.65	0.69	0.65	0.86	0.95	0.75	0.85	0.95	0.74
$C_{16:0}$	18.97	17.8	18.49	18.79	18.10	19.37	18.07	17.10	18.0
$C_{18:0}$	3.60	3.75	3.54	3.40	3.72	3.15	3.96	3.95	3.76
$C_{18:1}$	28.39	28.53	27.95	28.30	28.59	28.50	29.99	29.69	29.15
$C_{18:2}$	42.95	44.29	43.85	44.56	43.01	43.05	42.91	42.39	43.30
$C_{18:3}$	5.59	4.19	5.5	4.0	4.7	4.35	4.4	4.5	4.2
Unidentified	0.95	0.75	0.95	0.75	0.99	0.83	0.89	0.81	0.85

The oils extracted from *V. mungo* showed more unsaturation than *C. arietinum* oils as shown in Table 4. This high level of unsaturation may have marked influence on the functional properties and average stability of processed legume flours (Vieira and Regitano D'Arce, 1999). The fact that linoleic acid was again the prominent fatty acid among the unsaturated acids of *V. mungo* showed close relationship of the samples of different areas.

Generally the fatty acid composition of the oils, extracted after microwave roasting and sand roasting of seeds, was almost similar to that of raw oils which showed that both types of roasting did not have any significant effect on the fatty acid composition.

Comparison of fatty acids of the oils from seeds of the three localities is shown in Tables 3 and 4.

Earlier studies of Caponio *et al.* (2003) and Mai *et al.* (1980), indicated that microwave cooking did not change the fatty acid composition of many food lipids nor cause isomerization of the unsaturated fatty acids. Hassanein *et al.* (2003) had reported that microwave baked potatoes showed relatively lower percentage of total unsaturated fatty acids as compared to that of conventionally baked potatoes and in addition it increased the *trans* fatty acids. The present study in which *C. arietinum* and *V. mungo* were roasted by microwave and conventional sand roasting method did not show any significant change in the fatty acid pattern of both the legumes from three different areas, which corresponds to the study of Mai *et al.* (1980). However, the results are in contrast to the earlier study of Maga *et al.*(1977).

In conclusion the oils extracted from the raw seeds of *C. arietinum* and *V. mungo* of different areas have more or less the same properties but the fatty acid composition of *V. mungo* showed higher degree of unsaturation in comparison to *C. arietinum*; furthermore, the effect of microwave roasting and sand roasting did not produce any significant change in their fatty acid composition thus eliminating the presumption that such treatments lead to the formation of toxic compound.

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Peanut Hull as Biosorbent for Removal of Reactive, Acid and Disperse Dyes from Aqueous Solutions

S. Rehman Khan*, Asma Inayat and H. Rehman
Leather Research Section, Applied Chemistry Research Center, PCSIR Laboratories Complex,
Ferozpur Road, Lahore-54600, Pakistan

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Abstract. In the investigations on peanut hull as a low cost locally available biosorbent for the potential to remove reactive, acid and disperse dyes from aqueous solutions, the acid treated peanut hulls exhibited maximum adsorption efficiency as biosorbent.

Keywords: peanut hull, biosorbent, dye removal, textile dyes

Introduction

Textile factories use various types of dyes. Presently there are more than 10,000 dyes available commercially (Nigam *et al.*, 2000), most of which are difficult to biodegrade due to their complex aromatic molecular structure and synthetic origin (Seshadri *et al.*, 1994). The extensive use of dyes often poses pollution problems in the form of coloured wastewater discharged into the surrounding water. This wastewater contains a variety of organic compounds and toxic substances, which are harmful to fish and other aquatic organisms (Ramakrishna and Viraraghavan, 1996). Such wastewater when get mixed with normal water makes it unacceptable for human consumption. Even small quantity of dye can colour large water bodies, which not only affects esthetics but also reduces light penetration and photosynthesis. Some dyes are even toxic or mutagenic or carcinogenic (Chen *et al.*, 2003). Thus it is desirable to eliminate dyes from textile wastewater before its disposal.

There are many methods of colour removal from textile wastewater which include coagulation and floccation (Panswed and Wongchaisuwan, 1986), oxidation or ozonation (Malik and Saha, 2003; Koch *et al.*, 2002) membrane separation (Ciarderlli *et al.*, 2000) and active carbon sorption (Venkata and Sastray, 1987). Although these methods are highly efficient, they are uneconomical. Thus there is a growing need for some low cost and renewable, locally available materials that can remove dye colours. Some of the low cost agricultural by-products, that have been investigated, include orange peel (Namasivayam *et al.*, 1996), cassava peel (Rajeshwarisivaraj *et al.*, 2001), banana pith (Namasivayam *et al.*, 1998), plum kernels (Juang *et al.*, 2000) apple pomace, wheat straw (Robinson *et al.*, 2002), cotton waste, rice husk, teakwood bark (Mckay *et al.*,1986), sawdust (Garg *et al.*, 2003), bagasse pith, maize cob (Nassar and El-Geundi, 1991) palm fruit bunch (Nassar *et al.*, 1995) etc.

Peanut hull is a low cost agricultural waste residue that is easily available in large quantities. The purpose of this work is to investigate the possibility of using peanut hulls as biosorbent for the removal from aqueous solutions of acid, reactive and disperse dyes which are extensively used by textile units for dyeing cotton, rayon, wool, silk and jute fibres.

Materials and Methods

Dyes. The dyes selected for the study were reactive yellow 1 (RYI), acid orange 2 (AO2) and disperse orange 25 (DO25). The Society of Dyers and Colourists (SDC) has given specific numbers to the dyes. For each dye the number is unique and is called Colour Index Number (C.I.No.). Properties of the dyes used in the present study with their C.I. Nos. and structures are given in Table 1, Fig. 1.

Preparation of peanut hull sorbent. The peanut hull was obtained from local market. The collected biomaterial was extensively washed with tap water to remove soil and dust, sprayed with distilled water and then dried at 80 °C in a hot air convection oven (Memmert-Germany) to a constant weight. Dry biomass was crushed into powder, sieved and preserved in a desiccator for use.

Treatment of crushed peanut hulls. The sieved powder was divided into three parts and given one of the following three different treatments:

1. Powder + distilled water (PH_W)
2. Powder + 37% formaldehyde + 0.2 N sulphuric acid (ratio 1:20) (PH_A)
3. Powder + 0.1 sodium hydroxide (PH_B)

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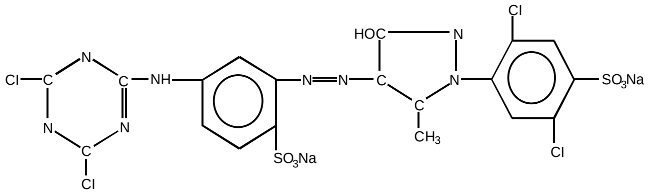
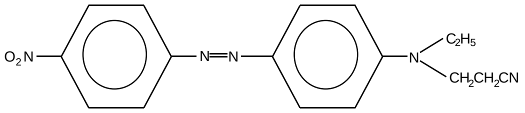
*Author for correspondence; E-mail: shahid_pcsir@hotmail.com

Table 1. Description of dyes used in the study

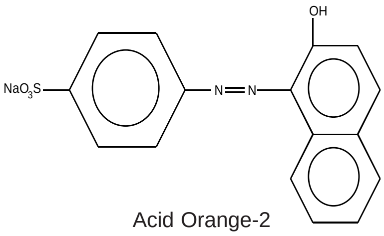
Type of dyes	C. I. No.	Formula weight	λ_{max}(nm)
Reactive yellow 1 (anionic azo dye)	18971	744	415
Acid orange 2 (anionic azo dye)	15510	328	486
Disperse orange 25	11227	323	492



Reactive Yellow-1



Disperse Orange-25



Acid Orange-2

Fig. 1. Structures of dyes used in the study.

The treated samples were filtered and rinsed with distilled water until the pH remained constant at 6-8. The treated material was dried again at 80 °C for 12 h, sealed in plastic bags and stored in desiccator.

Preparation of dye solutions and standard curve. Different initial concentrations of each dye i.e. reactive yellow I (RY-1), acid orange 2 (AO-2) and disperse orange 25 (DO-25) (from 5 ppm-30 ppm) were prepared and their absorbance were noted at their respective lambda max (λ_{max}), (415 nm for RY-1, 486 nm for AO-2 and 492 nm for DO-25) using Unicam Helios Alpha Spectrophotometer (Model Helios α 9423 Spectronic Unicam). The absorbance vs concentration were plotted at the corresponding λ_{max} of each dye. Absorbance obtained against different concentrations of dyes is shown in Table 2.

Table 2. Concentration of dyes and absorbance

Concentration (ppm)	Absorbance		
	RY-1 415 nm	AO-2 486 nm	DO-25 492 nm
5	0.117	0.15	0.044
10	0.234	0.312	0.083
15	0.339	0.455	0.113
20	0.461	0.612	0.144
25	0.571	0.742	0.170
30	0.688	0.902	0.209

Treatment of dyes with peanut hull. Three types of treated peanut hull were used to absorb different dyes. For each type of treated peanut hull and each dye tested, four flasks were used: three contained treated peanut hull and 150 ml of 5 ppm dye solution, and the fourth was control which contained only dye solution. All flasks were shaken at the room temperature at 140 rpm on orbital shaker for 12 h. Six ml of the dye solutions were collected from each flask for assay using a 10 ml syringe. All samples were centrifuged (3000 Centurion, Scientific Ltd.) and the absorbance was measured. Similar process was applied to dye concentrations of 10, 15, 20, 25 and 30 ppm.

Determination of sorption. The concentration of each dye was determined before and after each treatment by measuring absorbance at its respective λ_{max} using spectrophotometer, with the help of calibration curve. The amount of each dye sorbed by the biomass was calculated by the difference in the initial dye concentration (Ci) and the final dye concentration (Ce) in the solution after treatment. The percentage sorption by each type of biomass was calculated as follows:

$$\text{Sorption (\%)} = \frac{\text{Ci} - \text{Ce}}{\text{Ci}} \times 100$$

Results and Discussion

On treating, three types of treated peanut hulls (PH_A, PH_B and PH_W) with the dye reactive yellow 1, it was revealed that among the three biosorbents, the acid treated peanut hull (PH_A) had the highest dye removal efficiency for reactive yellow 1 in dilute solutions, (Table 3, Fig. 2).

The results for the other two dyes i.e. AO-2 and DO-25 are shown in Table 4 and 5 and corresponding Fig. 3 and 4, respectively The results reveal that among the three types of treated biosorbents ($PH_{A,}$ PH_B and PH_W), the acid treated

Table 3. Sorption of reactive yellow by the treated peanut hull

Ci (ppm)	Absorb before treatment	Absorb. after treatment			Ce (ppm)			% sorption: $\frac{Ci - Ce}{Ci}$ x 100		
		PH_A	PH_B	PH_W	PH_A	PH_B	PH_W	PH_A	PH_B	PH_W
5	0.117	0.098	0.117	0.117	4.19	4.99	4.99	16.19	0.23	0.18
10	0.234	0.192	0.232	0.233	8.20	9.92	9.95	18.11	0.85	0.51
15	0.339	0.271	0.335	0.335	12.01	14.82	14.86	19.92	1.2	0.92
20	0.461	0.368	0.454	0.453	15.98	19.7	19.69	20.11	1.51	1.81
25	0.571	0.450	0.450	0.562	19.7	24.7	24.6	21.29	1.29	1.62
30	0.688	0.521	0.679	0.604	22.7	29.6	29.3	24.22	1.34	2.26

Ci = initial dye concentration (ppm); Ce = equilibrium dye concentration (ppm) in liquid phase

Table 4. Sorption of acid orange dy by peanut hull

Ci (ppm)	Absorb. before treatment	Absorb. after treatment			Ce (ppm)			% sorption: $\frac{Ci - Ce}{Ci}$ x 100		
		PH_A	PH_B	PH_W	PH_A	PH_B	PH_W	PH_A	PH_B	PH_W
5	0.15	0.047	0.146	0.124	1.56	4.85	4.14	68.81	3.00	17.10
210	0.312	0.096	0.302	0.258	3.07	9.68	8.27	69.23	3.24	17.21
15	0.455	0.139	0.437	0.373	4.48	14.40	12.29	70.11	3.88	18.11
20	0.612	0.166	0.581	0.610	5.43	19.00	16.15	72.83	4.53	19.21
25	0.742	0.172	0.696	0.592	5.79	23.44	19.94	76.81	6.23	20.21
30	0.902	0.198	0.828	0.770	6.57	27.54	23.15	78.11	8.19	22.82

Ci = initial dye concentration (ppm); Ce = equilibrium dye concentration (ppm) in liquid phase

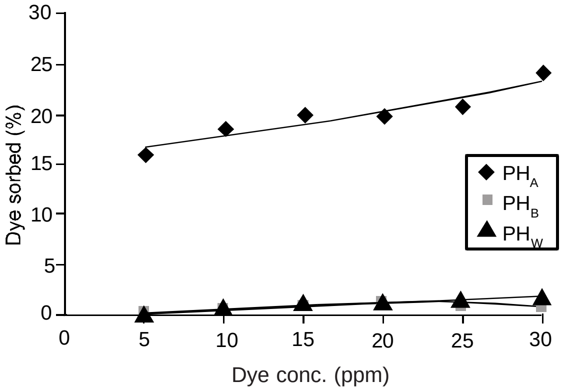


Fig. 2. Sorption of reactive yellow on treated peanut hull.

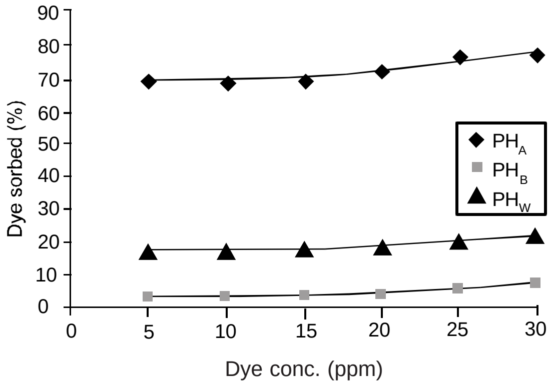


Fig. 3. Sorption of acid orange on treated peanut hull.

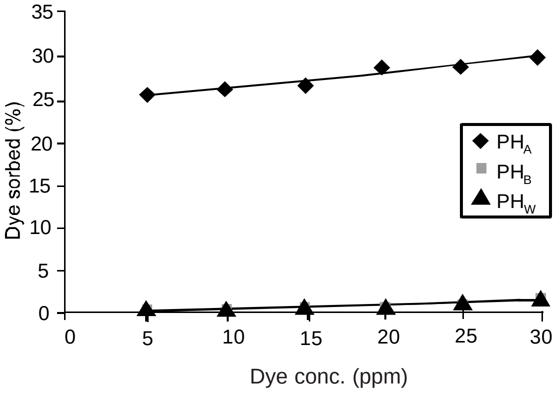


Fig. 4. Sorption of disperse orange 25 on treated peanut hull.

peanut hull (PH_A) had the highest dye removal efficiency for the other two dyes used in this study, as well.

Conclusion

In the study on determination of the potential of low-cost biosorbents for removing dyes from aqueous solutions, the sorption efficiency of peanut hull was increased by three types of treatment i.e., acid, base and water. The acid treated peanut hulls showed maximum dye removal efficiency. Peanut hull is an economicaly and easily available biosorbent which has

Table 5. Sorption of disperse orange 25 by peanut hull

Ci (ppm)	Absorb. before treatment	Absorb. after treatment			Ce (ppm)			% sorption: $\frac{Ci - Ce}{Ci}$ x 100		
		PH_A	PH_B	PH_W	PH_A	PH_B	PH_W	PH_A	PH_B	PH_W
5	0.044	0.034	0.044	0.044	3.88	4.98	4.99	22.41	0.28	0.11
10	0.083	0.064	0.083	0.083	7.75	9.97	9.98	22.49	0.29	0.13
15	0.113	0.087	0.113	0.113	11.62	14.95	14.98	22.51	0.30	0.13
20	0.144	0.111	0.193	0.143	15.46	19.94	19.97	22.67	0.30	0.14
25	0.170	0.131	0.169	0.169	19.28	24.92	24.97	22.88	0.30	0.15
30	0.209	0.157	0.208	0.208	22.85	29.82	29.91	24.82	0.61	0.31

Ci = initial dye concentration (ppm); Ce = equilibrium dye concentration (ppm) in liquid phase

been found to be the most effective adsorbent for the removal of dyes and phenols from their aqueous solutions.

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Short Communication

Steroid Estimation in Different Dental Applications

Lubna Iqbal[a]*, Kauser Siddique[a], Nighat Afza[a], Salma Rehman[b], Afsheen Arshad[a] and Mohammad Saleem[b]

[a]PCSIR Laboratories Complex, Shahrah-e-Dr. Salimuzzaman Siddiqui, Karachi-75280, Pakistan
[b]PCSIR Laboratories Complex, Ferozepur Road, Lahore-54600, Pakistan

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Abstract. Study of various brands of regular and medicated toothpastes of the local markets of Lahore and Karachi for the presence of steroids revealed the presence of synthetic steroids in medicated toothpastes only while all common, popular brands were devoid of steroids with a few exceptions.

Keywords: steroids, cortisone, toothpastes

Introduction

Toothpaste are generally used by people for cleaning of teeth as well as for other oral health benefits such as for control of caries and plaque and gum health (Fachin and Zaki, 1991). Mouthwashes are also used for the same purpose besides for mouth freshness. Being an excellent vehicle for delivering miscellaneous oral health benefits, in addition to necessary ingredients, many therapeutic agents are generally added to the toothpastes, some of which are stated to be steroids. The use of mild topical steroids can start spontaneous healing of mouth lesions and consequent decrease in the pain. Kenalog is a commonly used topical corticosteroid and dexamethasone is used as rinse for multiple ulcerated areas but usage of both of them requires doctor's prescription (Lehner and Lyne, 1999).

Steroids constitute a class of compounds which are widely distributed in nature and have diverse biological activities such as the development and control of reproductive system and act as cardiotonics (digitoxin), vitamin-D precursors (ergosterols), anti-inflammatory agents (corticosteroids) and anabolic agents (androgens) (Tayler *et al.*,1988).

The potential benefits and risks of steroids vary with the nature and severity of disease being treated, presence or absence of other alternative treatments and of other significant medical problems. Occurrence of side effects depends upon dose, type of steroid and length of the treatment. Some side effects are more severe than others. (Fields, 2005).

Some common side effects of steroids include weight gain, increase in appetite, sudden mood swings, muscle weakness, blurred vision, low resistance to infections, osteoporosis, insomnia, nervousness, restlessness, acne, growth of body hair, swollen face, cataract, glaucoma and water retention in the body (Porter and Scully, 2000).

Presently the use of corticosteroids has become a common practice, especially in herbal products, topical applications, beauty creams, soaps and toothpastes etc. to enhance the effect of the product. Use of corticosteroids in toothpastes is gaining attention as these agents have been found to be highly effective in the management of large number of acute and chronic lesions of oral mucosa. (Rosenberg *et al.*, 1997). The present survey of toothpastes was undertaken in response to reports in media about use of steroids in commercial toothpastes and associated risks and side effects.

For the survey, eighteen samples of toothpastes and toothpowders of various brands were purchased from the local markets of Lahore and Karachi. These brands were tested at PCSIR Laboratoroes at Karachi during 2002 and 2004 using standard methods of AOAC (2000); afterwards, the same brands of toothpaste were obtained from commercial outlets of Lahore and were evaluated for steroids at PCSIR Laboratories at Lahore during June 2004, using standard methods of United States Pharmacopoeia (USP, 2004). Evaluation of commercial samples was carried out using chemical tests, thin layer chromatography, UV spectroscopy and high performance liquid chromatography. All the samples were also tested for tetrazolium blue test according to the British Pharmacopoeia (2003).

In the analysis carried out at PCSIR Laboratories Complex (KLC), presence of synthetic steroids was indicated in the medicated toothpastes, whereas no such compounds were detected in the regular brands. However, only two of the medi-

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*Author for correspondence

Table 1. Synthetic steroids in toothpastes tested at Karachi Labs. Complex (KLC) and Lahore Labs. Complex (LLC) in 2002 and 2004

Toothpaste/ toothpowder code	Synthetic steroids (%)		
	KLC (2002)	KLC (2004)	LLC (2004)
K-A/R	-	-	-
K-B/R	-	-	-
K-C/R	-	-	-
K-D/R	-	0.055	-
K-E/R	-	0.510	-
K-F/R	-	-	-
K-G/R	-	-	-
K-H/R	-	-	0.210
K-I/R -	-	-	0.334
K-J/M	-	0.085	0.452
K-K/M	0.160	0.054	0.225
K-L/M	0.186	0.280	0.560
K-M/M	-	-	0.304
K-N/M	0.126	0.155	-
K-O/M	-	-	-
K-P/MW	0.346	0.825	0.280
K-Q/M	-	-	-
K-R/M	-	-	0.450

cated toothpaste samples contained steroidal components according to the 2nd evaluation carried out at KLC in 2004.

Steroids were found mainly in medicated toothpaste brands of Lahore markets; only one of the regular brands contained steroids. Thus the results of LLC are in general conformity with those of KLC.

For inflammatory conditions of mouth, use of aqueous anti-inflammatory steroids in dentifrices may be recommended for patients. In acute and chronic oral mucosal diseases, such gels are primarily ameliorative or suppressant in their action (Martin *et al.*, 1998). Systematically administered corticosteroids are however, relatively seldom prescribed by dentists, exceptions being in severe allergic reactions or conditions which are more commonly thought of as associated with systemic diseases (Remington, 1970). According to Balch and Balch (1998), 1-2 mg/day of steroids can be used, e. g. orally in pathological conditions. On prolonged use e. g. for months, steroids can cause few or all side effects (Merck, 1999). Some preparations containing corticosteroid dexamethasone are typically applied for 1-2 days prior to mounting the crown in the mouth, for reducing inflammation (Shannon and Fields, 2007).

It is, therefore, suggested that incorporation of steroids in medicated toothpastes must be mentioned on the label and addition of any type of steroid in regular toothpastes, toothpowders or mouthwashes may be banned and made liable to punishment.

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Biological Sciences

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Different Agroresidues Used in Solid Substrate Fermentation for α-Amylase Production by *Bacillus subtilis*-239

M. Yousaf*, M.B. Bhatty, S. Baig, M. Nadeem and Z. Nasreen
Food and Biotechnology Research Centre, PCSIR Laboratories Complex, Lahore - 54600, Pakistan

(received April 5, 2007; revised February 8, 2008; accepted February 12, 2008)

Abstract. The best mass ratio for agroresidue fermentation for α-amylase production by locally isolated *Bacillus subtilis*-239 was found to be wheat bran to rice bran 2:1 with 70% initial moisture content for 60 h incubation time. Among different inorganic nitrogen sources supplemented, sodium nitrate and ammonium chloride (0.5% w/w) increased the enzyme yield upto 178 U/ml and 176 U/ml, respectively, whereas all the organic nitrogen sources decreased the enzyme production. Addition of glucose (1% w/w) as a carbon source enhanced α-amylase synthesis to 185 U/ml as compared to the control (134 U/ml).

Keywords: α-amylase production, fermentation, *Bacillus subtilis*, agroresidues

Introduction

Enzymes are among the most important products obtained for human needs through microbial sources. A large number of industrial processes in the area of industrial environment and food technology utilize enzymes, at some stage or the other. Solid substrate fermentation holds tremendous potential for the production of various enzymes such as proteases, lipases, pectinases and amylases. It can be of special interest in those processes where crude fermented products may be used directly as enzyme source (Pandey *et al*., 2000; Benjamin and Pandey, 1998). Amylases are among the most important and widely used enzymes whose spectrum of applications has widened in many sectors such as baking, brewing, detergent, textile, paper and distillery industries (Ramachandran *et al*., 2004).

Amylases are reported to occur in microorganisms, although they are also found in plants and animals. Two major classes of amylases have been identified in microorganisms, namely α-amylase and glucoamylase. α-amylase (endo-1,4-α-D-glucan glucohydrolase E.C. 3.2.1.1) is extracellular enzyme that randomly cleaves the 1,4-α-D-glucosidic linkages between adjacent glucose units in the linear amylose chain. Glucoamylase (exo-1,4-α-D-glucan glucanohydrolase E.C. 3.2.1.3) hydrolyzes single glucose units from the non-reducing ends of amylose and amylopectin in stepwise manner (Pandey *et al*., 2000). The cost of enzyme production in submerged fermentation is high which can be reduced by adopting alternate method i.e. solid substrate fermentation. The contents of synthetic media are very expensive and might be replaced with more economically available agricultural by-products to reduce the cost of production medium. The use of agricultural wastes makes solid substrate fermentation an attractive alternate method for enzyme production (Ellaiah *et al*., 2002).

In the present study, α-amylase production through fermentation by locally isolatedby *Bacillus subtilis*-239 has been investigated, using various agricultural wastes like wheat bran, rice bran, soybean meal, cassava bagasse, rice husk and their combinations in different mass ratios.

Materials and Methods

Organism and cultivation conditions. The organism used in this study was *B. subtilis*-239, earlier isolated in our laboratory (Yousaf *et al*., 2007). The culture was maintained on nutrient agar medium, containing (g/l) peptone 6.0, casein hydrolyzate 4.0, yeast extract 3.0, glucose 2.0, beef extract 1.5 and agar 15.0. After inoculation the culture was incubated at 37 °C for 48 h.

Inoculum preparation. A 24 h old vegetative inoculum was employed in the present work. Nutrient broth, (50 ml) was sterilized in 250 ml conical flask at 121 °C for 15 min. After cooling, the medium was inoculated with culture of *B. subtilis*-239 and incubated at 37 °C for 24 h on a rotary shaker with 150 rpm.

Fermentation procedure. In order to choose the best substrate for fermentation, 40 g of each of various locally purchased defatted agricultural wastes like rice bran (RB), soybean meal (SBM), cassava bagasse (CB), wheat bran (WB), rice husk (RH), cotton seed oil cake (CSOC) and mustard oil cake (MOC) were taken separately, in 1:1 and in different mass ratios in 1 litre cotton plugged conical flask and moistened with 20 ml of phosphate buffer (pH 7.0). The flasks were autoclaved at

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*Author for correspondence; E-mail: usaf_374@hotmail.com

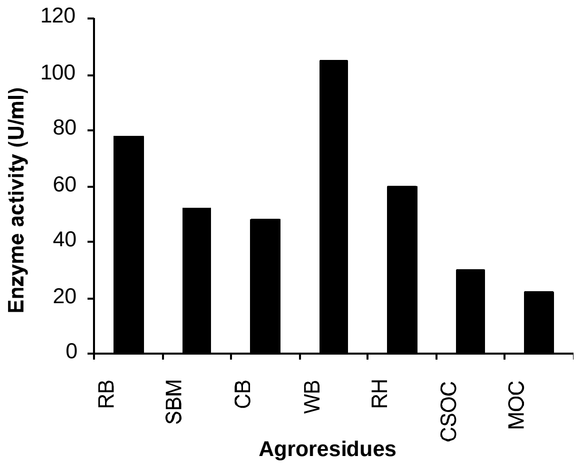
121 °C for 15 min and the sterilized solid substrates were inoculated with 8 ml of 24 h old inoculum and incubated at 37 °C for 48 h.

Preparation of enzyme extract. 400 ml of phosphate buffer (pH 7.0) was added to the fermented substrate for the extraction of crude enzyme. The flask was placed on a rotary shaker for 10 min (150 rpm). The substrate suspension was filtered using ordinary filter paper and the filtrate was used for the enzyme assay.

Analytical method. Enzyme activity was assayed by adding 1 ml of diluted enzyme solution to 1 ml (1% w/v) starch solution and incubated at 25 °C for 3 min. The reaction was terminated by adding 2 ml of 1% DNS (3,5-dinitro salicylic acid) reagent. Colour due to the liberated reducing sugars was developed by placing the reactants in boiling water for 5 min, then rapidly cooling them to room temperature. The extinction values were determined at 550 nm on spectrophotometer (Stein and Fisher, 1961). One amylase unit liberates reducing sugar equivalent to 1 mg maltose hydrate under the assay conditions. Triplicate samples were assayed for the enzyme activity and the confirmation of the result, whereas average of the values are shown in tables and figures.

Results and Discussion

Screening of agroresidues as substrate. Various defatted agroresidues were used for the production of α-amylase by *B. subtilis*-239 (Fig. 1). Among these, wheat bran gave the highest enzyme activity (108 U/ml). Wheat bran has also been reported to be the best substrate in solid substrate fermentation by *Bacillus* species because the produced metabolites are concentrated and purification procedure is less costly (Malimani and Ramalingam, 2000). Most of the mixtures of agroresidues containing wheat bran caused sufficient increase in enzyme production but the highest activity of the enzyme was at 156 U/ml, when the mixture of wheat bran and rice bran was used as substrate in mass ratio 1:1 (Table 1). The maximum activity of enzyme in different ratios of wheat bran and rice bran achieved was (178 U/ml) in a mass ratio 2:1 (Table 2). Agroindustrial wastes are rich sources of carbon, protein, minerals and other growth factors for microorganisms to produce amylases and in the present study a mixture of wheat bran and rice bran in mass ratio 2:1 has proved to be the best substrate for α-amylase production, whereas in the past, a mixture of wheat bran and coconut oil cake was found to be the best substrate for α-amylase production by fungal culture with optimum yield of 160 U/ml (Ramachandran *et al.*, 2004).



RB = rice bran; SBM = soybean meal; CB = cassava bagasse; WB = wheat bran; RH = rice husk; CSOC = cotton seed oil cake; MOC = mustard oil cake

Fig. 1. Screening of agroresidues for the production of α-amylase by *B. subtilis*-239

Effect of initial moisture content of the medium on production of α-amylase. Solid substrate fermentation is the microbial growth on solid particles without presence of free water. The

Table 1. Screening of substrate mixture (1:1) for the production of α-amylase by *B. subtilis*-239

Substrate mixture (1:1)	Enzyme activity (U/ml)
RB + SBM	84
SBM + CB	50
CB + WB	122
WB + RB	156
WB + RH	118
WB + CSOC	63
WB + MOC	52
RH + CSOC	42
CSOC + MOC	26

RB = rice bran; SBM = soybean meal; CB = cassava bagasse; WB = wheat bran; RH = rice husk; CSOC = cotton seed oil cake; MOC = mustard oil cake

Table 2. Production of α-amylase by *B. subtilis*-239 in different ratios of wheat bran (WB) and rice bran (RB)

WB:RB	Enzyme activity (U/ml)
1:1	151
2:1	178
3:1	122
1:2	135
1:3	89

water remains in complex form within the solid matrix or as a thin layer either absorbed to the surface of the particles or less tightly bound within the capillary region of the solid substrate. Moisture level is a critical factor for the production of enzyme in solid substrate fermentation and generally bacterial growth requires higher water activity. The necessary moisture in solid substrate fermentation is likely to be more advantageous for growth because of the possible efficient oxygen transfer (Raghavarao *et al.*, 2003). Enzyme synthesis gradually increased with increase in moisture content and maximum activity (182 U/ml) was achieved when substrate moisture was 70% (v/w) (Fig. 2). At insufficient moisture content, enzyme yield is found to be less as it does not allow good diffusion of solutes and gases due to which cell metabolism slows down or stops completely (Gervais and Molin, 2003). Higher moisture level also decreases the enzyme production because it decreases porosity, changes particle structure, increases stickiness and lowers the oxygen transfer (Ramesh and Lonsane, 1991). Optimal moisture level of 60-75% has been recommended by Lonsane *et al.* (1985) for α-amylase production by *B. licheniformis* in solid state fermentation, whereas in our study, the moisture level is within this range.

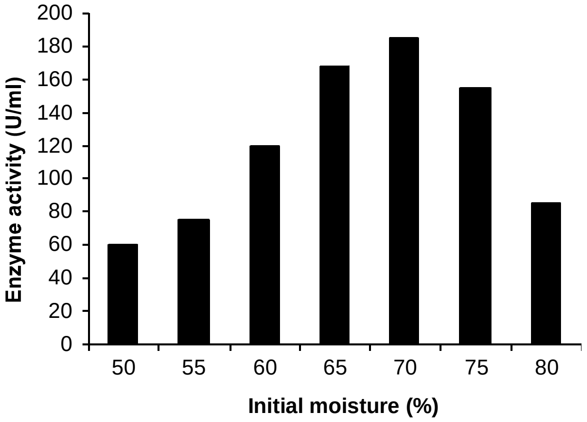


Fig. 2. Effect of initial moisture content of medium on the production of α-amylase by *B. subtilis*-239.

Effect of incubation period on α-amylase production. In solid substrate fermentation process, incubation period is very critical which depends upon the characteristics of the culture, growth rate and production of enzyme. There is a regular increase in enzyme formation and it becomes maximum i.e. 184 U/ml after 60 h incubation, after which a gradual decrease in enzyme production is observed with further increase in fermentation time (Fig. 3); this is caused by denaturization or decomposition of α-amylase due to interaction with other components of the medium (Ramesh and Lonsane, 1987).

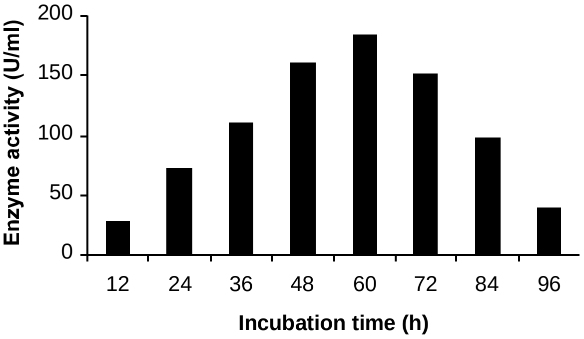


Fig. 3. Effect of incubation period on α-amylase production.

Effect of inorganic nitrogen sources on α-amylase production. Studies on the supplementation of different inorganic nitrogen sources at 0.5% (w/w) concentration to the fermentation medium showed a mixed trend on enzyme production (Table 3). Among the added inorganic nitrogen sources, sodium nitrate (178 U/ml) and ammonium chloride (176 U/ml) were found to increase enzyme yield as compared to the control (135 U/ml) whereas other sources inhibited the enzyme synthesis by *B. subtilis*-239. The increase in the enzyme activity on addition of ammonium chloride and sodium nitrate shows that any one of these two can be used alternatively as nitrogen source to enhance the enzyme yield; this is in accordance with the results reported earlier by Ikram *et al.* (2002). The inhibitory effects of some inorganic nitrogen sources on α-amylase production by *Aspergillus niger* using wheat bran in submerged and solid-state fermentation were also observed by Kocher *et al.* (2003).

Effect of complex nitrogen source on α-amylase production. The nutritional requirements of *Bacillus* sp. are reported to be complex for the growth and the enzyme synthesis as the structural macromolecules of agroresidues provide an inert matrix within which various organic acids are already present to provide complex nitrogen in solid substrate fermentation.

Table 3. Effect of inorganic nitrogen sources on α-amylase production

Nitrogen source	Enzyme activity (U/ml)
Sodium nitrate	178
Potassium nitrate	85
Ammonium sulphate	92
Ammonium chloride	176
Ammonium bicarbonate	72
Ammonium oxalate	60
Ammonium acetate	53
Control	135

Different organic nitrogen sources in complex form such as casein, yeast extract, urea, peptone, tryptone and beef extract were supplemented at the rate of 1% w/w to the medium for biosynthesis of α-amylase (Fig. 4). Addition of all the organic nitrogen sources resulted in considerable decrease in α-amylase production by *B. subtilis*-239. This result is in agreement with the findings in which repression in production of α-amylase was observed during the solid-substrate fermentation when organic nitrogen sources like casein, gelatin and soy meal were added (Malimani and Ramalingam, 2000). However these results differ with the investigations in which organic nitrogen sources along with wheat bran in solid substrate fermentation by *Aspergillus niger* enhanced α-amylase production (Kocher *et al.*, 2003). On addition of complex nitrogen sources, the reduction in α-amylase yield may be due to the production of protease or other unfavourable catabolites.

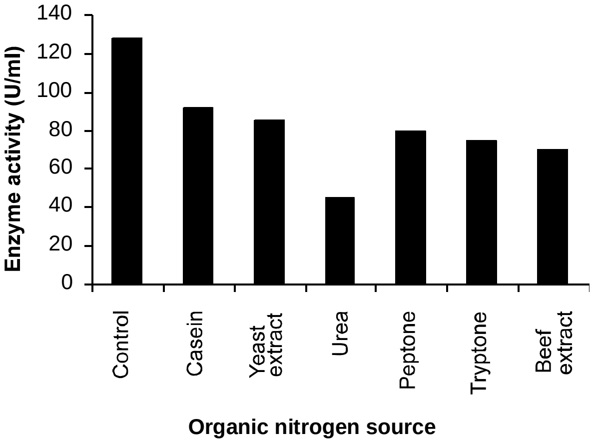


Fig. 4. Effect of complex nitrogen sources on α-amylase production.

Effect of carbon sources on α-amylase production. To investigate the effects of various carbon sources on α-amylase production, *B. subtilis*-239 was grown in the medium containing different monosaccharides, disaccharides and polysaccharides such as glucose, galactose, maltose, lactose, sucrose and starch at the rate of 1% (w/w). Maximum amylase yield (185 U/ml) was obtained in the medium containing glucose (Fig. 5). The glucose has been reported to be the best carbon source for the production of α-amylase by *B. theroolevorans* (Narang and Satyanarayana, 2001). It was also observed that lactose and starch enhanced the enzyme production to marginal limits whereas galactose, maltose and sucrose inhibited α-amylase synthesis. It has also been reported that formation of carbohydrate degrading enzymes in most species of the genus *Bacillus* were subjected to catabolic repression by some of the readily used carbon sources (Lin *et al.*, 1998).

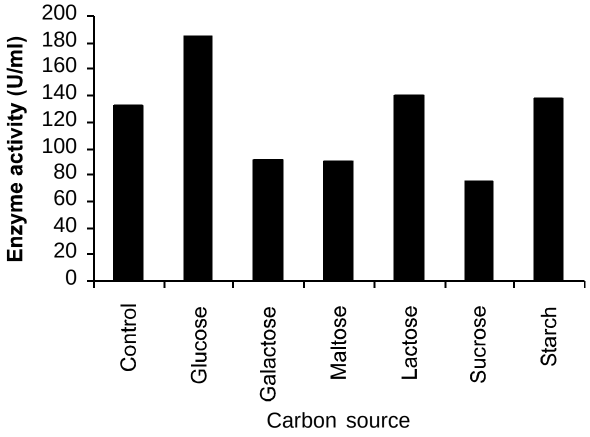


Fig. 5. Effect of carbon sources on α-amylase production.

Conclusion

The production of α-amylase by *Bacillus subtilis*-239 using a combination of wheat bran and rice bran in the ratio 2:1, under the optimized conditions is an economical method for the production of enzyme for industrial use as both of the agroresidues are cheap and easily available.

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Growth Response and Ionic Relation in Two *Brassica* Species under Water Stress Conditions

Badr-uz-Zaman[a]*, M. Salim[a], Rehana Asghar[b], B.H. Niazi[a], Imdad Ali Mahmood[a] and Arshad Ali[a]
[a]Land Resources Research Programme, Institute of Natural Resources and Environmental Sciences,
National Agricultural Research Centre, Islamabad, Pakistan
[b]Botany Department, University of Arid Agriculture, Rawalpindi, Pakistan

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Abstract. A glasshouse study of *Brassica campestris* and *Brassica juncea* showed that the growth and the ionic parameters of both the species were significantly ($p < 0.01$) affected due to water stress. Shoot length of both the species decreased consistently with decrease in solute potential (ψ_s) in the root medium. Relative growth rate and dry mass was higher in *B. juncea* than *B. campestris*, but leaf area was less. Concentrations of K^+, Ca^{2+}, P and S generally decreased with gradual increase in water stress. *B. campestris* was more susceptible to water stress than *B. juncea*.

Keywords: *Brassica* sp., water stress, growth, ionic parameters

Introduction

Plant growth is influenced by physical, chemical and biological components in the environment (Westwood, 1978). Water is essential at every stage of plant growth, from seed germination to plant maturation and yield (Reisdorph and Koster, 1999; Turner, 1991). World wide crop losses due to drought probably exceed the losses caused by all the other stresses (McWilliam, 1986). Physical process of water uptake leads to the activation of metabolic processes (Katembe *et al.*, 1998; Begum and Paul, 1993; Mondal and Paul, 1992).

Among the oil seed crops, *Brassica campestris* and *Brassica juncea* are grown in wide ecological niche. Both the crops are harvested at vegetative stage for the production of vegetables or at maturity for that of oilseeds. Owing to the capacity to thrive well under poor moisture conditions, mustard is seldom irrigated and is generally raised as a rainfed crop in Indo-Pak-Bangladesh subcontinent (Mondal and Paul, 1995). Water stress affects cell division and cell expansion process and, therefore, affects leaf area (Turner and Begg, 1981). Polyethylene glycol (PEG) is commonly used to induce water stress in laboratory experiments (Livingston and de Jong, 1990; Smith *et al.*, 1989). *Brassica* is a major oilseed crop and its drought resistance potential needs to be further studied. The present study was conducted to observe the effect of water stress on important macronutrients and growth of *B. juncea* and *B. campestris*.

Materials and Methods

Seeds of *B. campestris* (cv. BSA) and *B. juncea* (cv. BARD-1) were germinated using moist quartz sand with distilled water. Nutrient solution (Hoagland and Arnon, 1950) along with 5, 10, 15 and 20% polyethylene glycol $(PEG)_{6000}$, having osmotic concentrations of -0.21, -0.24, -0.56 and -0.75 MPa were prepared. The osmotic potential of the nutrient solution was measured by vapour pressure osmometer, VAPRO, Wescor. One week old seedlings were foam-plugged in the lids of plastic pots containing 2.5 litre of nutrient solution that was continuously aerated and fresh nutrient solution was replaced weekly. The study was conducted in the glass house under sun light. The pH of the solution was adjusted to 6.0 ± 0.2 with HCl or KOH and was monitored regularly. The treatments were applied in quadruplicates. Two harvests were taken with three weeks interval after transplantation of seedlings to pots. Leaf area was measured on CI-202, CID Inc., USA. After recording fresh mass (FM), the plants were rinsed with deionized water, and were separated into shoot and root portions. Plant samples were dried at 65 °C. Dry mass (DM) of each sample was recorded and was grindid to pass a 40-mesh Wiley Mill. The ground samples of root and shoot were separately digested in 2:1 perchloric-nitric di-acid mixture (Rayan *et al.*, 2001). Relative water contents (RWC) were calculated according to Misra and Dwivedi (2004); relative growth rate (RGR) and leaf area ratio (LAR) were calculated using the formula given by Franklin *et al.* (1985). Potassium and calcium ions in the digested material were determined by atomic absorption spectroscopy and sulphur and phosphorus, by the method given by Verma *et al.* (1977) and Chapman and Pratt (1961), respectively. The data were statistically analyzed according to two factors randomized complete block design (RCBD) and the treatment means were compared using least significant difference (LSD) test (Gomez and Gomez, 1984).

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*Author for correspondence; E-mail: badruaar@yahoo.com

Results and Discussion

B. campestris and *B. juncea* were grown for 6 weeks after transplanting the seedlings under water stress at ψ_s -0.21, -0.24, -0.56 and -0.76 MPa using PEG_{6000}. Significant ($p < 0.01$) difference was observed in the growth and the ionic concentrations of both the species.

Shoot length of *B. campestris* decreased 27, 35, 51 and 78% than the control at ψ_s -0.21, -0.24, -0.56 and -0.76 MPa, respectively (Table 1), whereas that of *B. juncea* also decreased by 25, 32, 45 and 60% than the control in the same increasing order of ψ_s, respectively. The root length of *B. campestris* decreased 17, 31, 45 and 75% than the control at ψ_s -0.21, -0.24, -0.56 and -0.76 MPa, respectively and that of *B. juncea* also decreased 14, 17, 33 and 64% in the same order of water potential, respectively.

In *B. campestris*, shoot fresh mass (SFM) decreased 47, 72, 98 and 99% than the control at ψ_s -0.21, -0.24, -0.56 and -0.76 MPa, respectively. In *B. juncea*, SFM increased 4% as compared to the control at ψ_s -0.21 MPa but it decreased 72, 95 and 98% than the control at ψ_s -0.24, -0.56 and -0.76 MPa, respectively. In *B. campestris*, root fresh mass (RFM) decreased 48, 75, 98 and 99% than the control at ψ_s -0.21, -0.24, -0.56 and -0.76 MPa, respectively. In *B. juncea*, RFM also decreased 22, 66, 98 and 99% than the control at ψ_s -0.21, -0.24, -0.56 and -0.76 MPa, respectively. In *B. campestris* shoot dry mass (SDM) decreased 35, 55, 90 and 94% than the control at ψ_s -0.21, -0.24, -0.56 and -0.76 MPa, respectively; in *B. juncea* as well, SDM decreased 20, 60, 80 and 87% in the same sequence of ψ_s. In *B. campestris* root dry mass (RDM) decreased 35, 57, 90 and 95% than the control at ψ_s -0.21, -0.24, -0.56 and -0.76 MPa, respectively. Also in *B. juncea*, RDM decreased 17, 47, 91 and 96% than the control in the same order of ψ_s, respectively.

In *B. campestris*, shoot and root length, SFM, RFM, SDM and RDM decreased with an increase in water stress. In *B. juncea*, shoot and root length, RFM, SDM and RDM also decreased with increasing water stress. In *B. juncea* SFM increased at low ψ_s (-0.21 MPa). According to Katembe *et al.* (1998), imbibition supports elongation of tissue and cell expansion; *B. juncea*, therefore, might have been able to manage low level of water stress to accumulate water in the shoots. No significant effect of moisture regime on roots length was found in mustard. In the present study, root and shoot length decreased with an increase in water stress in both the species. *B. campestris* was less susceptible to water stress for growth than *B. juncea*. According to Theodore and Xu (2000), under water deficiency, growth is readily inhibited and osmotic adjustment occurs slowly and wall loosening ability either does not increase substantially or actually decreases, leading to marked growth inhibition.

In *B. campestris* shoot relative growth rate (SRGR) decreased 9, 15, 36 and 46% than the control at ψ_s -0.21, -0.24, -0.56 and -0.76 MPa, respectively. In *B. juncea*, SRGR increased 5% than the control at ψ_s -0.21 MPa but SRGR decreased 9, 30 and 36% at ψ_s -0.24, -0.56 and -0.76 MPa, respectively. In *B. campestris*, root relative growth rate (RRGR) decreased 9, 19, 39 and 51% than the control at ψ_s -0.21, -0.24, -0.56 and -0.76 MPa, respectively. In *B. juncea* RRGR was less affected at ψ_s -0.21 MPa and it decreased 13, 32 and 43% than the control at ψ_s -0.24, -0.56

Table 1. Growth of *B. campestris* and *B. juncea* under water stress (means of 4 replications)

ψ_s (MPa)	Shoot length (cm)	Root length (cm)	Fresh mass (g/plant)		Dry mass (mg/plant)		Relative growth rate (µg/g/d)		Relative water content (%)	
			Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
B. campestris										
Control	14.4 a	11.3 b	2.89 b	1.40 c	272.8 b	132.8 c	101.4 ab	98.9 a	90.6 ab	90.5 a
- 0.21	10.5 c	9.4 c	1.53 c	0.73 d	176.8 c	85.7 e	92.2 bc	89.7 b	88.5 ab	88.3 ab
- 0.24	9.3 d	7.8 e	0.80 e	0.35 e	123.2 e	57.4 f	86.4 c	80.3 c	84.6 b	83.7 b
- 0.56	7.1 f	6.2 g	0.05 f	0.03 f	26.3 h	12.8 gh	64.8 de	60.7 de	47.8 d	50.9 c
- 0.76	3.2 h	2.8 h	0.02 f	0.01 f	15.4 h	6.4 h	54.9 e	48.6 f	30.3 f	47.5 c
B. juncea										
Control	15.1 a	12.6 a	3.49 a	2.17 a	348.6 a	212.3 a	100.6 ab	99.1 a	90.0 ab	90.2 a
- 0.21	11.4 b	10.8 b	3.62 a	1.70 b	280.4 b	176.1 b	106.0 a	100.1 a	92.3 a	89.6 ab
- 0.24	10.2 c	8.4 d	0.99 d	0.74 d	138.1d	111.2 d	91.2 bc	86.5 bc	86.1 b	85.1 ab
- 0.56	8.3 e	6.9 f	0.16 f	0.04 f	70.5 f	18.1 g	70.2 d	66.9 d	55.5 c	53.4 c
- 0.76	6.1 g	3.3 h	0.08 f	0.02 f	46.3 g	9.5 h	64.6 de	56.7 e	39.6 e	48.1 c

Means sharing similar letter(s) in a column do not differ significantly at $p < 0.01$

and -0.76 MPa, respectively. In *B. campestris*, shoot relative water content (SRWC) decreased 2, 7, 47 and 67% than the control at ψ_s -0.21, -0.24, -0.56 and -0.76 MPa, respectively. In *B. juncea*, SRWC increased 3% than the control at ψ_s -0.21 MPa but it decreased 4, 38 and 56% than the control at ψ_s -0.24, -0.56 and -0.76 MPa, respectively. In *B. campestris* root relative water content (RRWC) decreased 2, 8, 44 and 48% than the control at ψ_s -0.21, -0.24, -0.56 and -0.76 MPa, respectively; in *B. juncea* as well, RRWC decreased 1, 6, 41 and 47% than the control in the same sequence of ψ_s, respectively.

In *B. campestris* SRGR, RRGR, SRWC and RRWC decreased with an increase in ψ_s while in *B. juncea* SRGR, RRGR and RRWC increased at lower ψ_s (-0.21 MPa). Variations in relative growth rates in both the species are in conformity with the findings of Poorter (1989), that wide variation in relative growth rate was observed when the plant species were grown under stress conditions.

In *B. campestris*, leaf area ratio (LAR) decreased by 4, 12, 24 and 88% than the control at ψ_s -0.21, -0.24, -0.56 and -0.76 MPa, respectively (Fig. 1). In *B. juncea*, LAR decreased by 5, 9, 25 and 40% than the control in the same sequence of ψ_s, respectively. Leaf area is a valuable index in identifying plant growth and development. It is also related to light interception, transpiration and photosynthesis and thus it is considered the most important single determinant of dry matter accumulation and yield (Chan *et al.*, 1998; Noggle and Fritz, 1983). Water stress caused a drastic decrease in leaf area and shoot length, probably due to the decrease in cell enlargement (Hsiao, 1973). In *B. juncea*, relatively more leaf area contributed towards an increase in dry mass as compared to *B. campestris*. Leaf is a site of metabolic processes involved in photosynthesis and Janick (1979) found that growth occurs only in living cells by metabolic processes involved in the synthesis of proteins, nucleic acids, lipids, and carbohydrates at the expense of metabolic energy provided by photosynthesis and respiration. Therefore, the difference in LAR of both the species may be due to difference in the genetic make up.

In *B. campestris* and *B. juncea*, K^+, Ca^{2+}, P and S had antagonistic relations with the increase in water stress (Fig. 2a-d). However, in shoots and roots of *B. juncea*, K^+ increased 4 and

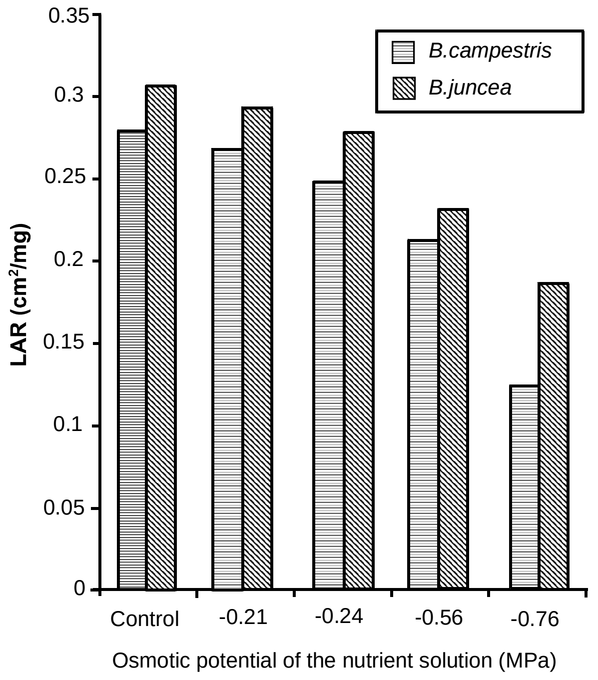
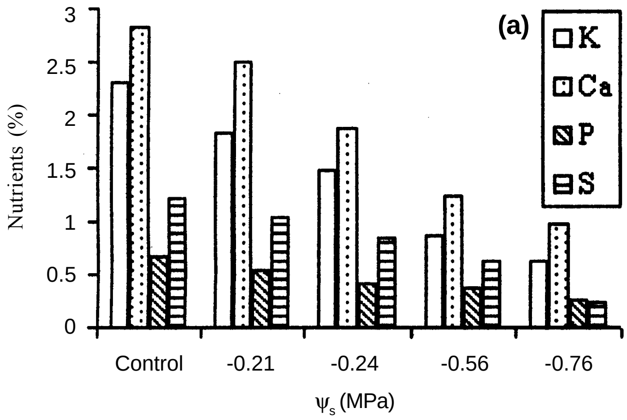
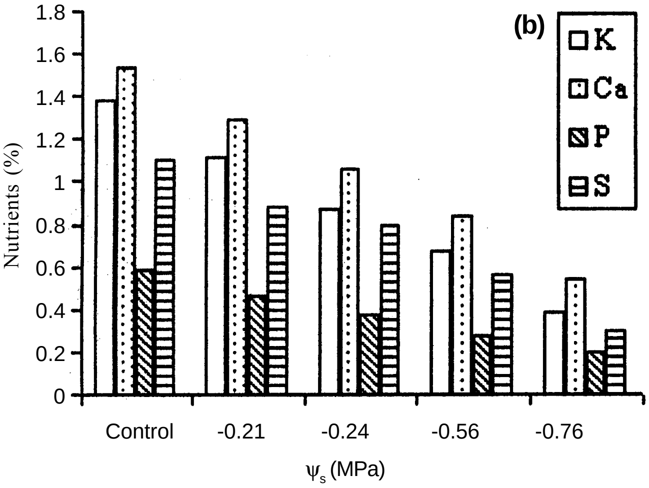


Fig. 1. Effect of water stress on leaf area ratio (LAR) in *B. campestris* and *B. juncea*.

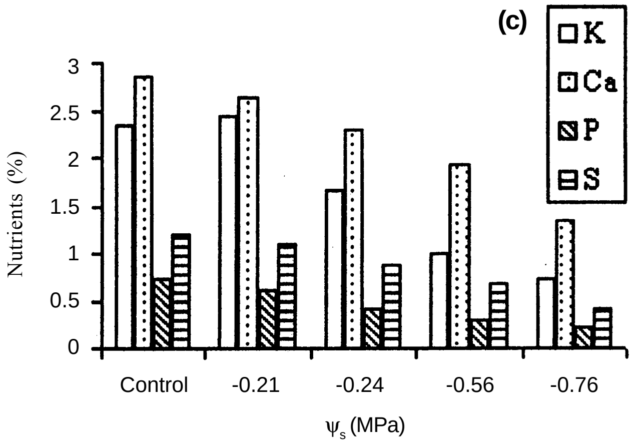


Shoots of *B. campestris*

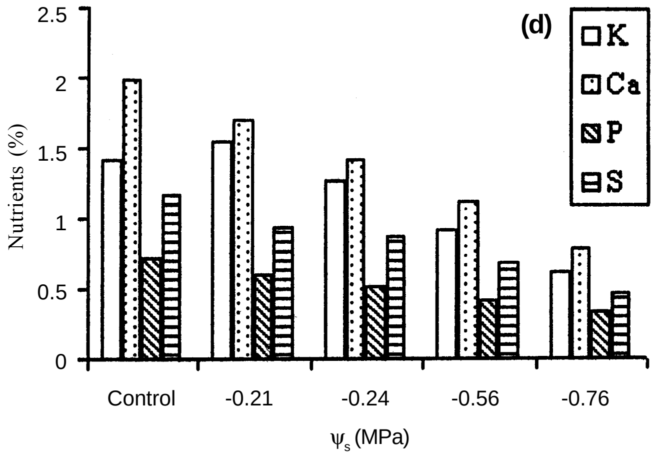


Roots of *B. campestris*

Fig. 2a-b. Nutrient relations in (a) shoots and (b) roots of *B. campestris* under water stress conditions.



Shoots of *B. juncea*



Roots of *B. juncea*

Fig. 2c-d. Nutrient relations in (c) shoots and (d) roots of *B. juncea* under water stress conditions.

8%, respectively than the control at ψ_s -0.21 MPa. The entry of water molecules is initially a passive transport. Active transport of water correlates with metabolic processes of growth. Reduced entry of water molecules may become a cause of low concentration of Ca^{2+}, K^+, S and P in the shoot and root system. *B. juncea* may have the ability to accumulate K^+ ions in shoots and roots at a low water stress. Fricke *et al.* (1994) found that epidermal cells are virtually unable to produce organic solutes and rely heavily on inorganic ions (mainly K^+) for osmotic adjustment. Talbott and Zeiger (1996) also observed that potassium uptake occurred during stomatal opening but this ability was lost at higher water stress.

By generating external osmotic stress, with the application of PEG_{6000} on the growth of *B. campestris* and *B. juncea*, it can be assumed that lesser availability of water caused lesser flow of nutrients from the roots to the shoots. However, in *B. juncea*, compared to *B. campestris*, at low ψ_s growth of shoots and roots improved in the form of dry mass and K^+ concentration.

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Estimates of Genetic Parameters from Line x Tester Mating Design for Some Quantitative Traits in Upland Cotton, *Gossypium hirsutum* L.

Muhammad Jurial Baloch*, Moula Bux Kumbher, Wajid Ali Jatoi and Nasreen Fatima
Department of Plant Breeding & Genetics, Sindh Agriculture University, Tando Jam, Sindh, Pakistan

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Abstract. Combining abilities of cotton varieties were evaluated using a line x tester design with eight lines and 4 testers. Good performance combination was found between the varieties CRIS-134 and BH-147. The former was a good candidate for fibre length improvement and the latter, a good parent for yield improvement. The specific combining ability suggested that both additive and dominant genes controlled the characters. Hybrid performance *per se* may be used to predict the parental performance for specific combining ability and thus for hybrid crop development.

Keyword: cross breeding, combining ability estimates, fibre length, *Gossypium hirsutum*

Introduction

In cotton breeding, some crosses make better combinations than the others in forwarding desirable genes to their offsprings. Richy and Mayer (1925) thus emphasized that due to lack of any definite association in the performance between the parents and their offspring, selection for combining ability must be based on the performance of the inbred parents in cross combinations rather than the inbred parents *per se*. Thus it is essential that those inbred parents may be identified which produce good individuals in specific combinations, commonly referred to as specific combining ability (SCA) of the inbred parents, and those parents may be marked which form good combinations with other parents in a series of crosses, referred to as general combining ability (GCA) of the parents (Sprague and Tatum, 1942). The former types of inbreds are useful for hybrid crop development whereas the latter are desirable to generate segregating populations for selection.

In quantitative genetics, genotypic value of an individual is determined by various types of gene actions such as additive, dominance and their interactions (Falconer, 1989). Theoretically, inbreds which show higher estimates of SCA are those which predominantly possess dominant genes; such genes are not transferable to their progenies. Whereas inbreds which perform well in general terms are referred to as general combiners; they posses predominantly additive genes. Baloch *et al*. (2002, 2000, 1997, 1995 and 1993) and Bhutto *et al*. (2001) observed that inbred parents were very inconsistent in their *per se* hybrid performance and GCA and SCA estimates. They pointedout that generally, parents which formed best GCA did not perform similarly for SCA estimates. However, Baloch (2004) and Baloch and Bhutto (2003) reported that not all but some parents perform equally well in hybrids *per se*, for GCA and also for SCA estimates. It was, hence, believed that combining ability is the property of an individual parent in hybrid combination rather than inbred parents, *per se*. It is, therefore, very essential for plant breeders that every inbred parent be specified for its potential in terms of GCA or SCA. Ever since cotton leaf curl virus (clcv) disease erupted in Pakistan during 1991, cotton breeders had no choice but to include one of the clcv resistant parents with other high yielding commercial varieties in their hybridization programme. Breeders are also aware of the phenomenon that making a large number of crosses between clcv resistant and high yielding parents and thereby separating potential inbreds based on progeny performance seems a chance game. This method of identifying desirable inbreds is eventually very laborious and expensive in terms of inbred selection and also requires vigorous selection in segregating populations. At present, though numerous clcv resistant inbreds have been evolved which do not require screening of segregated population in the clcv hot spots if both parents in crosses are resistant, yet not all the clcv resistant parents yield good harvest or other fibre traits. It is, thus, important for cotton breeders to determine the GCA and SCA estimates of the parents to be used in hybridization programme for producing potential progenies. In this way, more dependable hybridization programme could be launched. The essential objectives of conducting line x tester analysis is therefore to determine or predict the combining ability of the clcv resistant inbred parents, for knowing the types of gene actions controlling different traits. The general and specific combining ability effects of individual

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*Author for correspondence; E-mail: dr_mjr@hotmail.com parents further demonstrate the saturation of additive or dominant genes in the parents. So far, controversial results have been presented by many researchers on the type of genes controlling various traits in cotton and the types of genes present in individual parents. Baloch (2004), Baloch and Bhutto (2003) and Tang *et al*. (1993) reported that both additive as well as dominant genes were involved in the expression of seed cotton yield, bolls per plant, boll weight, lint percentage and the fibre length. They, however, observed that parents were still quite different in exhibiting general and specific combining ability effects for these traits. The results of above researchers are quite justifying in that F_1 hybrids do show heterosis or vigour due to dominant gene action but at the same time, much improvement in such characters have also been made by selection from segregating populations. Such results suggest that additive genes are also important for the above mentioned traits. It appears that types of gene action vary a little depending upon the mating design used; nevertheless, the general and specific combining ability are solely dependent on the individual parents. For this purpose, diallel analysis has been extensively used but line x tester mating design is still superior as it uses more number of crosses, thus providing more reliable estimates of genetic components of variance as compared to diallel analysis. Actually, it gives two estimates of GCA from both female lines and male testers. The objectives of the present research were to determine the GCA and SCA effects of clcv resistant parents and also genetic variations due to GCA and SCA, thereby knowing the importance of additive and dominant genes attributable to various quantitative traits in intra-hirsutum F_1 hybrids.

Materials and Methods

Eight commercially grown cotton leaf curl virus (clcv) resistant varieties from Punjab (CIM-707, CIM-499, CIM-506, FH-1000, FH-925, VH-142, NIAB-111/S and BH-147) were used as female lines and crossed with four pollinators (testers) from Sindh (CRIS-134, CRIS-342, CRIS-191 and CRIS-377) in a line x tester mating design during 2003-2004. Thus a total of 32 cross combinations were developed. The crossed bolls were grown as F_1 during crop year 2004-2005. The experiment was conducted in a randomized complete block design with four replications in a plot size of 35.0×7.5 feet (3 rows, each 35 feet long). The seeds were hand dibbled by putting only 3 seeds per hole. After 25 days of planting, the extra two plants per dibble were thinned leaving only one vigorously growing plant per dibble. The plant-to-plant and row-to-row distances were maintained at 9.0 inches and 2.5 feet, respectively. All the inputs like fertilizer, irrigation and pesticides were applied as and when required in the doses recommended for the local conditions. For recording the observations, 20 plants from each genotype per replication were randomly tagged and kept as index plants. The data was recorded on seed cotton yield per plant in g, number of bolls per plant, lint percentage (taken as the ratio of lint from seed cotton sample), fibre length (in mm measured with digital fibrograph at 2.5% span length) and earliness percentage (as the number of open bolls after 120 days of planting). The analysis of variance and general and specific combining ability estimates were determined according to the procedures of Singh and Choudhry (1979).

Results and Discussion

The hybrids' performance *per se*, the general combining ability (GCA), specific combining ability (SCA) estimates and mean squares from line x tester analysis are presented in Tables 1, 2, 3 and 4, respectively.

The mean performance of hybrids *per se* (Table 1) indicated that pollinator CRIS-342 when crossed with female lines BH-147, CIM-707 and CIM-499, pollinator CRIS-374 crossed with lines FH-1000 and CIM-506 and pollinator CRIS-134 crossed with lines VH-142 and NIAB-111/S gave higher yields. Yield results thus generally connoted that majority of pollinators formed good combinations for yield with at least one of the eight female lines used. Nevertheless, very interestingly, all of the pollinators produced higher yields when crossed with a female line BH-147. It means that female line BH-147 is the choice parent to be crossed with any of the pollinators to obtain maximum yields. It was also interesting to note that hybrids which gave higher yields *per se* also correspondingly produced more number of bolls per plant; this suggested that number of bolls and yield are parallel attributes and are highly correlated traits in cotton. As regards to lint percentage, pollinator CRIS-134, with a few exceptions, always ranked first when crossed with female lines. These results, therefore, indicated that if lint percentage is to be improved via selection from segregating populations, CRIS-134 is one of the best pollinator parents. Pollinator CRIS-134 always produced fibres of longer length when crossed with any of the female parents and thus may be considered as one of the best parents for the hybridization and selection programme.

In respect of earliness percentage, again pollinator CRIS-134 when crossed with any of the female lines, opened maximum number of bolls (Table 1) after 120 days of planting except female BH-147; with the latter, pollinator CRIS-342 opened maximum number of bolls (81.0%). Results regarding hybrids' performance *per se* suggested that in general pollinato CRIS-134 and line BH-147 may be given priority in hybridization programme with the

Table 1. Hybrid performance *per se* of cotton leaf curl virus resistant intra-hirsutum F_1 hybrids

F_1 hybrids	Seed cotton yield (g)	Number of bolls per plant	Lint (%)	Fibre length (mm)	Earliness (%) at 120 DAP*
CIM-707 x CRIS-134	194.50	52.38	37.80	29.30	82.13
CIM-707 x CRIS-342	195.50	54.00	33.90	28.20	71.80
CIM-707 x CRIS-191	126.50	35.68	34.80	28.70	74.87
CIM-707 x CRIS-377	115.75	31.00	35.50	27.70	82.50
CIM-499 x CRIS-134	151.50	40.18	38.10	28.70	86.30
CIM-499 x CRIS-342	177.00	44.75	35.80	27.70	65.70
CIM-499 x CRIS-191	134.25	36.13	37.60	27.10	82.30
CIM-499 x CRIS-377	116.50	30.88	35.90	26.70	79.70
CIM-506 x CRIS-134	145.00	38.13	36.10	28.50	93.40
CIM-506 x CRIS-342	132.00	34.75	34.70	27.20	87.50
CIM-506 x CRIS-191	152.25	39.50	34.60	27.03	76.00
CIM-506 x CRIS-377	160.13	42.75	35.90	26.50	69.00
FH-1000 x CRIS-134	158.50	41.75	37.90	28.20	75.50
FH-1000 x CRIS-342	119.75	32.63	39.70	26.40	75.00
FH-1000 x CRIS-191	164.50	43.00	36.30	26.30	60.70
FH-1000 x CRIS-377	216.50	57.88	35.80	25.70	67.50
FH-925 x CRIS-134	119.00	31.63	37.80	28.40	67.20
FH-925 x CRIS-342	122.25	33.25	36.40	27.20	46.50
FH-925 x CRIS-191	132.50	35.58	38.20	26.80	58.00
FH-925 x CRIS-377	131.25	35.75	35.30	26.90	53.70
VH-142 x CRIS-134	145.00	38.68	38.50	28.50	91.50
VH-142 x CRIS-342	116.50	30.88	36.50	27.50	87.13
VH-142 x CRIS-191	120.50	32.25	37.90	27.00	82.05
VH-142 x CRIS-377	132.00	35.05	36.30	26.50	83.90
NB-111/S x CRIS-134	132.25	35.42	36.70	27.80	83.50
NB-111/S x CRIS-342	121.25	40.00	35.50	27.20	67.00
NB-111/S x CRIS-191	108.00	29.00	36.10	26.40	61.00
NB-111/S x CRIS-377	121.25	32.00	34.50	27.70	77.20
BH-147 x CRIS-134	221.50	58.38	36.70	27.00	79.70
BH-147 x CRIS-342	236.00	62.63	35.80	26.50	81.00
BH-147 x CRIS191	204.00	53.83	36.40	26.00	72.70
BH-147 x CRIS-377	221.00	58.38	36.70	25.50	70.70
Avg.	152.32	40.56	36.43	27.80	74.78
LSD (5%)	1.17	0.74	0.33	0.28	1.57

DAP = days after planting.

prediction of improvement in yield and number of bolls of cotton.

Cotton breeders are aware that inbred parents are quite different in transferring the breeding value to their offsprings. Thus, Sprague and Tatum (1942) used the term "combining ability" to designate the ability of the parents to contribute favourable genes to their progenies.

It is generally predicted that parents which perform well in hybrids' *per se* similarly exhibit good general combining ability as well as specific combining ability. (Baloch and Baloch, 2005; Baloch, 2004; Baloch and Bhutto, 2003; Baloch *et al*., 1997, 1995, 1993; Srinivasan and Gururajan 1973). However, in the present study, this assumption was not always found correct except some parents which performed well in hybrids *per se* also similarly exhibited good GCA and SCA. For example, tester CRIS-134 which formed good combinations in hybrids, also expressed maximum GCA estimates for all the traits studied whereas tester CRIS-342 ranked next for only yield and bolls but expressed negative estimates for the other three traits (Table 2). Among the female lines, BH-147, which gave maximum yields in hybrids'*per se*, performed similarly by giving the highest GCA values for yield (68.3g) and bolls (17.69). These

Table 2. General combining ability (GCA) estimates of lines and testers inbred parents for some quantitative traits in upland cotton

Variety	Seed cotton yield (g)	Bolls per plant	Lint (%)	Fibre length (mm)	Earliness (%)
Lines (females):					
CIM-707	5.738	2.70	-0.91	1.23	3.08
CIM-499	-7.512	-3.28	0.42	0.28	3.73
CIM-506	-4.980	-1.78	-1.13	0.01	6.67
FH-1000	12.488	3.25	0.99	-0.62	-5.10
FH-925	-26.074	-6.51	0.47	0.06	-18.41
VH-142	-23.820	-6.35	0.86	0.10	11.37
NB-111/S	-24.136	-6.46	-0.72	0.08	-2.60
BH-147	68.300	17.69	-0.02	-1.03	1.28
General mean	152.320	40.56	36.43	27.28	74.78
SE (gi)	0.209	0.132	0.058	0.05	0.279
SE (gi-gj)	0.295	0.187	0.082	0.07	0.395
Testers (pollinators/males):					
CRIS-134	6.080	1.50	1.02	1.02	7.62
CRIS-342	3.960	1.04	-0.39	-0.02	-2.07
CRIS-191	-9.510	-2.45	0.05	-0.37	-3.82
CRIS-377	-0.530	-0.11	-0.67	-0.61	-1.73
General mean	152.320	40.56	36.43	27.28	74.78
SE (gi)	0.147	0.093	0.041	0.035	0.198
SE (gi-gj)	0.209	0.132	0.058	0.050	0.279

SE = Standard error

results are coinciding with the results of Baloch and Baloch (2005) and Baloch (2004) who noted that not all but some parents exhibit equally good performance in hybrids' *per se* and in GCA, both. The GCA results thus suggested that CRIS-134 and CRIS-342 among the pollinators and BH-147, FH-1000 and CIM-707 among the female lines could be used in hybridization programme for significant improvement in all the traits through selection from segregating populations.

It is generally believed by the plant breeders that *per se* hybrid's performance is also reflected in specific combining ability estimates. This prediction does not hold true exactly; however, in the present study, some hybrids FH-1000 x CRIS-377, CIM-707 x CRIS-342, CIM-707 x CRIS-134 expressed maximum hybrid performance *per se* for yield per plant and correspondingly gave maximum SCA estimates (Table 3). These three high scoring specific combiners displayed similar SCA estimates for bolls per plant also.

The SCA results for lint percentage indicated that parents which performed better as *per se* hybrids, performed almost similar for SCA estimates except little change in rank orders. The SCA estimates of staple length in present research are in accordance with those of Baloch and Baloch (2005) and Baloch (2004) who noted that SCA estimates are not so prominent in cotton as compared to other parameters. Regarding SCA estimates for earliness; many hybrids gave fairly higher estimates, however the top three high scorers were CIM-506 x CRIS-342 (8.11%), CIM-499 x CRIS-191 (7.58%) and FH-1000 x CRIS-342 (7.39%). These results clearly indicated that inbred CRIS-342 is one of the best specific combiner among the pollinator parents.

The GCA variances for the traits like yield, bolls per plant and earliness were higher in lines (females) whereas among the testers/pollinators (males), the GCA variances were larger for lint percentage and fibre length, suggesting that these traits are controlled by additive genes and could successfully be improved through selection from segregating populations. The variances for SCA (line x tester) were also significant for all the traits (Table 3) indicating that dominant genes are also responsible for the expression of these traits hence insinuating the feasibility of hybrid crop development in the present material.

The analysis of variance presented in Table 4 reveal that mean squars of F_1 hybrids for all the traits were significant for further partitioning of this source of variation into other components such as line (estimates the GCA variances of

Table 3. Specific combining ability (SCA) estimates of lines and testers inbred parents for some quantitative traits in upland cotton

F_1 hybrids	Seed cotton yield (g)	Bolls per plant	Lint (%)	Fibre length (mm)	Earliness (%)
CIM-707 x CRIS-134	30.36	7.61	1.25	-0.23	-3.34
CIM-707 x CRIS-342	33.46	9.70	-1.19	-0.24	-3.91
CIM-707 x CRIS-191	-22.55	-5.14	-0.73	0.16	0.86
CIM-707 x CRIS-377	-41.78	-12.14	0.64	-0.15	6.40
CIM-499 x CRIS-134	0.61	0.69	0.19	0.12	0.16
CIM-499 x CRIS-342	28.23	5.73	-0.62	0.16	-10.69
CIM-499 x CRIS-191	-1.05	0.59	0.66	-0.09	7.58
CIM-499 x CRIS-377	-32.79	-6.92	-0.22	-0.20	2.97
CIM-506 x CRIS-134	-8.43	-0.55	-0.23	0.16	4.28
CIM-506 x CRIS-342	-20.44	-5.07	2.80	-0.10	8.11
CIM-506 x CRIS-191	14.41	3.17	-0.79	0.10	-1.64
CIM-506 x CRIS-377	13.30	4.08	1.23	-0.18	-10.73
FH-1000 x CRIS-134	-12.40	-3.56	-0.53	0.54	-1.81
FH-1000 x CRIS-342	-44.52	-12.23	2.71	-0.19	7.39
FH-1000 x CRIS-191	9.19	1.64	-1.21	-0.04	-5.11
FH-1000 x CRIS-377	52.21	14.17	-0.99	-0.30	-0.45
FH-925 x CRIS-134	-13.34	-3.93	-0.13	0.06	3.25
FH-925 x CRIS-342	-7.96	-1.84	-0.15	-0.07	-7.80
FH-925 x CRIS-191	15.76	3.97	1.26	-0.15	5.45
FH-925 x CRIS-377	5.52	1.81	-0.97	0.14	-0.89
VH-142 x CRIS-134	10.41	2.96	0.18	0.10	-2.28
VH-142 x CRIS-342	-20.96	-4.38	-0.39	0.16	3.04
VH-142 x CRIS-191	1.51	0.49	0.52	-0.01	-0.28
VH-142 x CRIS-377	4.02	0.95	-0.33	-0.27	4.53
NB-111/S x CRIS-134	-2.02	-0.18	0.02	-0.54	3.69
NB-111/S x CRIS-342	19.10	4.86	0.20	-0.03	-3.11
NB-111/S x CRIS-191	-10.68	-4.65	0.26	-0.56	-7.36
NB-111/S x CRIS-377	-6.41	-1.99	-0.49	1.06	6.80
BH-147 x CRIS-134	-5.20	-1.43	-0.73	-0.27	-4.48
BH-147 x CRIS-342	11.42	3.28	-0.35	0.27	7.01
BH-147 x CRIS191	-7.11	-2.03	-0.01	0.12	0.51
BH-147 x CRIS-377	0.92	0.19	1.08	-0.14	-4.12
General mean	152.32	40.56	36.43	27.28	74.78
SE (si)	0.417	0.264	0.116	0.100	0.559

SE = Standard error

Table 4. Line x tester analysis: Mean squares for some quantitative traits in upland cotton

Source of variation	Degree of freedom	Mean squares				
		Seed cotton yield (g)	Bolls per plant	Lint percentage	Fibre length (mm)	Earliness (%)
Replication	3	35.413	2.073	0.126	0.102	2.209
F_1 hybrids	31	5404.350*	373.109*	7.043*	3.581*	487.179*
Lines (females)	7	15463.205*	1066.801*	10.827*	6.966*	1304.064*
Testers (males)	3	1529.601*	99.604*	17.534*	16.595*	853.985*
Line x tester	21	2604.934*	180.950*	4.284*	0.593*	162.483*
Pooled error	93	0.696	0.279	0.054	0.040	1.249

* = significant at 1% probability level.

females), testers/pollinators (estimates the GCA variances of males) and lines x testers (estimates SCA variances of males x females) The importance of GCA and SCA variances, hence reflects the importance of additive and dominant gene actions, respectively.

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Short Communication

Formulation and *in vitro* Evaluation of a Cosmetic Multiple Emulsion from Olive Oil

Naveed Akhtar[a]*, Mahmood Ahmed[a], Marina Akram[a] and Ahmad Mahmood Mumtaz[b]
[a]Faculty of Pharmacy and Alternative Medicine, Department of Pharmacy, The Islamia University of Bahawalpur, Bahawalpur, Pakistan
[b]University College of Pharmacy, Punjab University, Lahore, Pakistan

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Abstract. Stability of multiple emulsion prepared using olive oil was evaluated at different storage temperatures. The formulation remained stable at lower temperatures for 28 days without any phase separation. Significant changes in the pH were observed in the formulation, kept at 8 °C from the 5th day onwards, in the electrical conductivity at 40 °C from 14th day onwards and in the globule size, at 40 °C and 40° C + 75% RH condition from 24 h onwards.

Keywords: Multiple emulsion, olive oil, globule size, stability.

Multiple emulsion systems are novel complex systems (Vyas and Khar, 2004) finding potential biopharmaceutical applications, including prolonged drug delivery systems (Nakhare and Vyas, 1997), protection of active substances (Silva-Cunha *et al.*, 1997), as sorbent reservoir in drug over-dosage treatment, as adjuvant vaccines etc. (Antimisiaris *et al.*, 1993) as well as in cosmetics (Gallarate *et al.*, 1994) and house hold products.

However, multiple emulsions are inherently unstable especially if natural oil is used. Olive oil contains a number of fatty acids and sterols. In this study, stability of an emulsion containing olive oil was observed; the latter was used because of its cosmetic benefits (British Pharmacopoeia, 2004). Abil-EM90 was used as lipophilic emulsifier and Tween-80, as hydrophilic emulsifier.

Accelerated stability studies were performed on the formulation for 28 days at 8 °C, 25 °C, 40 °C as well as at 40 °C + 75% relative humidity (RH) at different time intervals. The data so obtained was evaluated by applying the statistical tests.

After heating of the oil phase (olive oil + lipophilic emulsifier) and aqueous phase (water + magnesium sulphate) at 72 °C ± 1 °C, the two were mixed and stirred and cooled to room temperature (primary emulsion). Next, Tween 80 and water were added and homogenized (formulation).

The possible mechanisms for deterioration of multiple emulsions include swelling of internal drops, rupture of the oil layer or coalescence of the oil globules or internal water droplets (Florence and Whitehill, 1982). Electrolytes promote an osmolar balance between the internal and external aqueous phases and also increase the stability of multiple emulsions by formation of a rigid interfacial layer between oil and aqueous phase (Grossiord and Seiler, 1998).

The freshly prepared primary emulsion and the formulation were white in colour. There was no change in colour of primary emulsion and the formulation or major liquefaction or phase separation at 8 °C, 25 °C, 40 °C and at 40 °C + 75% relative humidity (RH), till 28 days. The formulation at 40 °C showed slight liquefaction after 14 days and the sample at 40 °C + 75% RH, after 21 days.

There was a tendency of phase separation in the samples of formulation at 40 °C after a period of 14 days which increased with time but in sample at 40 ºC + 75% RH condition, phase separation was observed after a period of 21 days which persisted till the end. This indicated that the formulation was stable up to a period of 28 days at lower temperature.

No phase separation after centrifugation was seen in any of the samples of primary emulsion, kept at different storage conditions upto 21st day but slight phase separation on centrifugation was seen on 28th day of observation in the samples kept at 40 °C and 40 °C + 75% RH condition.

In the case of formulation, no phase separation after centrifugation was seen in any of the samples kept at 8 °C and 25 °C till the end of 28th day. But slight phase separation on centrifugation was seen in the sample kept at 40 °C on 21st day and more phase separation on centrifugation was observed on 28th day. While in the sample kept at 40 °C + 75% RH condition, slight phase separation on centrifugation was seen on 21st day of observation, which persisted up to 28th day.

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*Author for correspondence; E-mail: nakhtar5@mul.paknet.com.pk; nakhtar567@hotmail.com

The pH of the sample of formulation kept at 8 °C, 25 °C, 40°C + 75% RH remained constant for 24 h and then increased continuously (Fig. 1). The increase in pH might be due to the production of basic by-products by any of the ingredients of the formulation or release of hydroxyl ions from glass containers.

ANOVA at 5% level of significance, showed that the change in pH of the formulation was significant at different levels of time and temperature. By using LSD technique it was found that most significant change in pH was observed in the formulation kept at 8 °C from 5th day up to the end of the study.

Increase in the electrical conductivity of multiple emulsions is due to the transfer of electrolytes entrapped in the inner aqueous phase to external aqueous phase and decrease in electrical conductivity is due to the transfer of electrolytes which are lost into external aqueous phase during the process of manufacture towards internal aqueous phase.

In this study, electrical conductivity of freshly prepared formulation was 0.0 µS/cm which increased with the passage of time and no conductivity difference was seen in any of the samples up to 72 h. The electrical conductivity of sample of formulation kept at 8 °C, 25 °C and 40 °C + 75% RH condition, increased continuously from the 7th day onwards and that kept at 40 °C increased continuously from 5th day (Fig. 2). Increase in conductivity might be due to the diffusion of electrolytes from internal aqueous phase to external aqueous phase.

ANOVA at 5% level of significance, revealed that the change in electrical conductivity of formulation was significant at different levels of time and temperature and by using LSD technique, it was found that the most significant change in pH was observed in the formulation kept at 40 °C and from14th day till the end of the study.

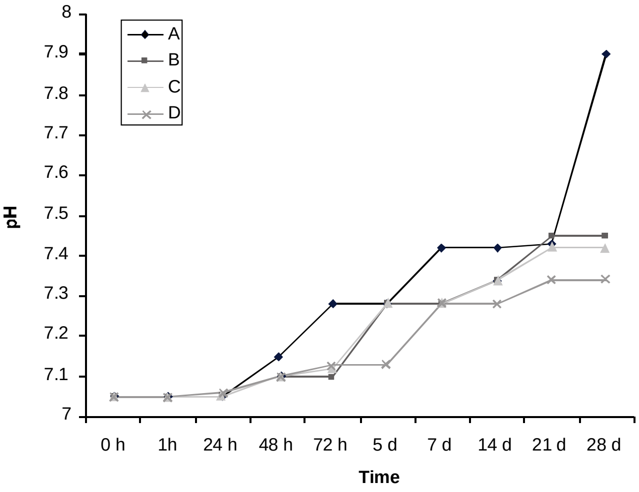

Fig. 1. pH values of formulation kept at different storage conditions, A = 8 °C; B = 25 °C; C = 40 °C; D = 40 °C + 75% RH.

The increase or decrease in the globule sizes indicates the process of instability (Florence and Whitehill, 1982). Globule sizes of all the samples of formulation kept at different storage conditions increased continuously from 21.83 µm, which was in the range of average globule size of multiple emulsions (Florence and whitehill, 1982), from 1st h after preparation till the end of observation period i.e., 28 day (Fig. 3).

By using two-way analysis of variance (ANOVA) techniques at 5% level of significance, it was found that the change in globule size of formulation was significant at different levels of time and temperature. Insignificant changes were observed in the sample of formulation kept at 8 °C.

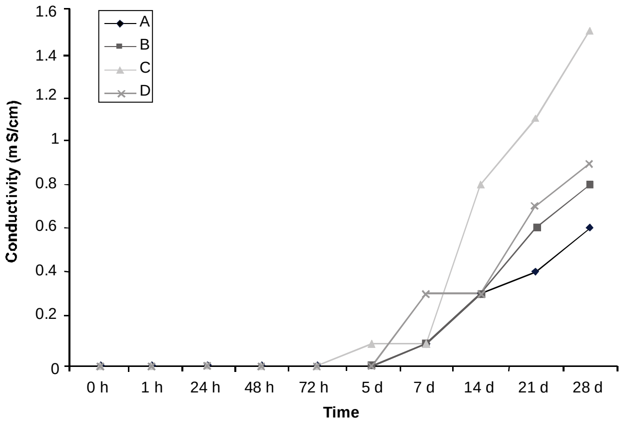

Fig. 2. Conductivity values of formulation kept at different storage conditions, A = 8 °C; B = 25 °C; C = 40 °C; D = 40 °C + 75% RH.

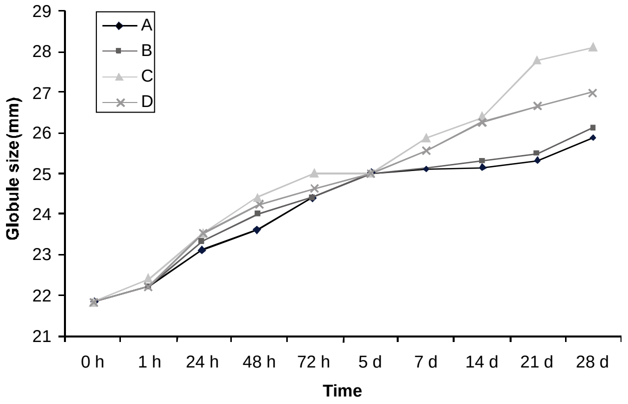

Fig. 3. Globule sizes of formulation kept at different storage conditions, A = 8 °C; B = 25 °C; C = 40 °C; D = 40 °C + 75% RH.

Conclusion

A stable w/o/w emulsion can be formulated by using olive oil in a concentration of 22% with 4% Abil.

Acknowledgement

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Technology

Beneficiation Studies on Low-Grade Stibnite Ore of Chitral, NWFP, Pakistan

M. Arif Bhatti*, K. R. Kazmi, M. Sharif Nizami, M. Shafique Anwar and M. Habib
PCSIR Laboratories Complex, Ferozepur Road, Lahore-54600, Pakistan

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Abstract. Antimony ore of Chitral in North West Frontier Province (NWFP) of Pakistan, containing stibnite as an economical mineral, was upgraded by froth flotation technique. Through optimizing the flotation parameters, the grade of the final antimony concentrate produced was raised to 62% Sb with 95 % recovery.

Keywords: stibnite, beneficiation, flotation, antimony, Chitral, Pakistan

Introduction

Stibnite (Sb_2S_3), being the main source of antimony metal and its compounds, is the principal mineral ore. It is found in veins with quartz and occasionally with various antimony minerals resulting from the decomposition of stibnite (Blackburn and Dennen, 1988). Of less commercial importance are the senarmontite (Sb_2O_3 cubic) and valentinite (Sb_2O_3 rhombic), kermesite (Sb_2S_2O) and stibiconite ($H_2Sb_2O_5$). Antimony ores and their concentrates are used as sources of antimony metal and its salts. Antimony metal is used in the manufacture of various alloys such as type metal (Pb,Sb,Sn), bearing metal (Pb,Sb,Sn,Cu) and white metal (Cu,Sb,Sn,Zn). Crude antimony is used on the striking surface of safety match boxes and in detonating caps for ammunition. It is also used in lead storage batteries while its compounds are used in medicine, as mordant in dying, as opacifier in vitreous enamels and glazes, as refining agent in optical glasses, as flame retardant in polymers like P.V.C, for vulcanizing and colouring rubber and also as paint pigments (Johnstone and Johnstone, 1961).

Stibnite deposits are found at various places in Pakistan. However, economically important and sizeable reserves are found at Krinj area of Chitral which contain significant amount of antimony content (Ahmad and Siddiqi, 1993). Although, the ore containing 5-10% Sb content is considered feasible for the recovery of metal but it is better to beneficiate such an ore as it improves the production economics to a great extent. Generally, low-grade ores are beneficiated into concentrates containing 40-65% Sb before the production of chemicals and extraction of metal (Wang *et al.*, 2001). Depending upon the nature of ores, various gravity and flotation techniques are used on lean antimony ores. Gravity concentration technique is mostly used for coarse-grained ores. While on the other hand, fine-grained and disseminated ores require more grinding for the liberation of minerals and can only be beneficiated economically by froth flotation technique (Jain, 1986).

The undertaken beneficiation work is so important that several researchers have been engaged in it under different parameters. Liu (1988) separated antimonite and boulangerite by flotation from lead containing antimony ores. Lead was leached from boulangerite with aqueous $AlCl_3$ for its recovery by crystallization. Peng *et al.* (1991) studied the beneficiation of 81% oxidized antimony ore from Yunnan province, China and obtained antimony sulphide concentrate by flotation and antimony oxide concentrate by gravity separation with an overall 74% recovery. Kursun *et al.* (1994) performed bulk and selective flotation experiments for the beneficiation of Etibank Halikoy antimonite ore and were able to produce an antimony concentrate containing 61%Sb with 95% recovery, using Aero 3477 as collector and NaCN as depressant. While, Solozhenkin *et al.* (1994) improved the grade and recovery of antimony ore containing antimonite, senarmontite, hydromardite, kermesite, stibiconite and other antimony containing minerals by using a combined flotation agent, consisting of dialkyldithiocarbamate and butyl xanthate. Later on, Wang and Lei (2000) treated low-grade antimony ore (15-35%) by bath smelting continuous fuming process, in a furnace using coal, limestone and iron ore and produced antimony oxide containing 76.4% Sb with 90% recovery. Wang *et al.* (2001) applied another method for the separation of antimony from ore, involving smashing, milling, classifying, flotation and dewatering. They used lead nitrate as activating agent, xanthate as collecting agent and terpineol as frothing agent. Yin and Long (2003) attempted the direct leaching of sodium antimonate from antimony ore, using sodium sulphide and a mixture of sodium sulphide and sodium hydroxide, followed by solid liquid separation, washing and drying. Zhang (2004) studied three different methods

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*Author for correspondence

to recover antimony from the ore of Lu'erba Mine. Their experimental results indicated that the process of flotation and acid leaching was a better method. They first beneficiated the ore by flotation and then recovered antimony from flotation concentrate by acid leaching.

The objective of the present study was selection and development of a suitable process for concentration of indigenous antimony ore. For this purpose, the stibnite ore of Chitral, NWFP, Pakistan, was beneficiated, on bench scale, to produce antimony concentrate by froth flotation technique. Following is the comprehensive account of the experimentation and the results in this regard.

Materials and Methods

The flow sheet developed for preparing antimony concentrate has been shown in Fig.1.

Sample preparation. A bulk sample of the ore, weighing about 10 tonnes was supplied by Sarhad Development Authority (SDA). The bulk sample consisted of lumps of 100-250 mm in size from which about 100 kg of the ore was collected by random sampling. Some of the ore was kept for mineralogical study while the remaining ore was comminuted by primary crushing using laboratory jaw crusher set at 20-25 mm and by secondary crushing using roll crusher set at 5-6 mm. A representative sample for chemical analysis and further processing was obtained by coning-quartering and riffling of the crushed ore.

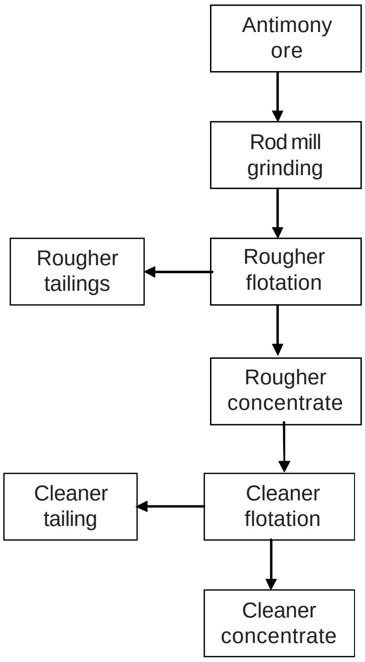

Fig. 1. Flow-sheet for the beneficiation of stibnite ore of Chitral.

Chemical analysis. Representative sample was pulverized to 100% minus 200 mesh size (74 µm) and its chemical analysis was performed by gravimetric and volumetric methods (Jeffery, 1989). Silica, alumina, lead and sulphur were determined gravimetrically, antimony by iodometric titration, iron by oxidation reduction titration, calcium and magnesium by complexometric titration, while sodium and potassium were estimated using flame photometer (PFP7, Jenway Ltd., England).

Mesh of liberation. The product of roll crusher was subjected to sieve shaker (International Combustion, England) for size analysis using different sieves. The fraction retained on each sieve, was analyzed for the liberation of stibnite grains using microscope particles counting method. The percentage liberation at various size fractions was calculated by the following relation (Atiq *et al.*, 2005):

Stibnite liberation (%) = Free stibnite (%) ×100/ [Free stibnite (%) + locked stibnite (%)]

Flotation tests. Flotation tests were carried out in a Denver flotation machine (Model: D-12), using stainless steel cells of 1, 2 and 4 l capacity. The flotation parameters such as grain size, pulp density, pH of the pulp, type of reagents, their consumption and the conditioning time were optimized. The ore was ground from 85% to 100% passing 100 mesh to study the effect of grinding. Flotation feeds were prepared by dry grinding of roll crusher product in rod mill (Denver, USA) and grinding time was adjusted according to the required size of feed. The effect of pulp density was studied by varying the solid to liquid concentration from 20% to 35% and similarly the effect of pH was studied by varying it with soda ash from 7.0 to 8.5. Sodium silicate was added to depress the silicate gangue minerals and its quantity was varied from 100-400 g/ton. Impeller speed was increased from 900-1200 rpm to study the effect of aeration. Potassium amyl xanthate was used as anionic collector and its dosage was varied from 100-200 g/ton while, polypropylene glycol was utilized as frother and its dosage was varied from 30-60 g/ton. Lead acetate was employed to activate stibnite and its amount was varied from 500-2000 g/ton. Optimum conditions and metallurgical balance of the typical tests are recorded in Table 3 and 4 separately.

Results and Discussion

The results of the chemical analysis of the representative sample are given in Table 1. The percentage of antimony in head sample was found to be 19.05. The obtained grade is sufficient to exploit the ore. However, the presence of silica appeared to be the main impurity. It was decided, in this perspective, to involve the flotation technique which has the potential to reduce it effectively.

Percentage liberation of various sizes of stibnite particles is given in Table 2. As flotation technique is very selective and the success of the froth flotation depends upon the nature and size of mineral particles distributed in the ore, therefore, ore is ground to such an extent that all of the mineral grains are fairly liberated from the matrix. The average size of stibnite grains in Krinj, Chitral ore was observed to be around 150 µm. It is evident from the Table 2 that about 98 % mineral grains (phases) are liberated around 100 mesh (150 µm). Therefore, the ore under study was ground in rod mill to 100 mesh sieve so that it may have a fair proportion of free valuable mineral particles in subsequent flotation operation.

The results of flotation tests are presented in Table 3 and Table 4. Our findings in this respect are in good agreement with the above referred researchers. Beneficiation of the antimony ore, on the bench scale, showed very encouraging results, particularly with reference to the percentage recovery. The flotation tests showed that this ore can be easily enriched to a high-grade concentrate containing over 62% Sb by a very simple flotation operation. It is clear from the results that the flotation operation proved to be successful in reducing the silica content of the ore significantly. The production of chemical and metallurgical grade antimony concentrate in two step flotation with 95% recovery of antimony from an ore containing around 19% metal content is quite reasonable. It is evident from the Table 4 that better grade antimony concentrate was produced after cleaning stage of flotation.

Table 1. Chemical composition of ore

Constituents	Percentage
SiO_2	64.00
Pb	1.55
Sb	19.05
Al_2O_3	0.50
Fe_2O_3	4.5
CaO	1.40
Na_2O	0.18
K_2O	0.15
S	8.21

Table 2. Mesh of liberation of stibnite

Mesh No. (BSS)	Particle Size of ore (µm)	Liberation of mineral (%)
60	250	75
70	212	80
85	180	92
100	150	98

It is obvious from the chemical analysis of the final concentrate (Table 5) that the antimony content of the investigated ore was raised from 19.05% to 62.02%.

The data generated regarding particle size versus grade and recovery has been shown graphically in Fig.2. It is clear from the Fig. that the grade and recovery of antimony was improved by decreasing the particle size and maximum recovery was obtained at feed size of 95% passing 100 mesh (150 µm). This,

Table 3. Antimony flotation parameters/conditions

Parameters	Conditions	
	Rougher	Cleaner
Particle size	95%-100#	95%-100#
Impeller speed	1100 rpm	900 rpm
Pulp density (%solids)	30%	20%
pH of the pulp	~8.0	~8.0
Gangue depressant (sodium silicate)	300 g/t	50 g/t
Stibnite activator (lead acetate)	1500 g/t	Nil
Collector (potassium amyl xanthate)	150 g/t	Nil
Frother (polypropylene glycol)	50 g/t	Nil
Conditioning time	20 min	10 min

Table 4. Metallurgical balance for antimony flotation

Product	Weight (%)	Grade (%) Sb	Recovery (%) Sb
Cleaner concentrate	29.12	62.02	95.05
Cleaner tailings	2.40	16.30	2.06
Rougher concentrate	31.52	58.54	97.11
Rougher tailings	68.48	0.82	2.89
Head sample	100	19.00	100

Table 5. Chemical analysis of final concentrate

Constituents	Percentage
SiO_2	2.15
Pb	2.68
Sb	62.02
Al_2O_3	0.12
Fe_2O_3	2.55
CaO	0.54
Na_2O	0.06
K_2O	0.02
S	27.88

being the maximum liberation limit of stibnite, was considered as optimum feed size and selected for further investigation. It is interesting to note that the grade and recovery of antimony sharply decreases on further grinding of ore. On excessive grinding, the generation of slimes might have lowered the grade and recovery; Parsonage (1985) pointed out that the particles in the size range of 20 μm and below have a deleterious effect on the process of flotation.

The percentage of solid feed in the pulp is another very important factor in recovery. The data for solid percentage versus grade and recovery is given in Fig. 3 from which it is clear that the increase in pulp density decreases the grade slightly but recovery is increased. Maximum recovery was achieved at 30% solids beyond which it decreased because coating by collector is not proper due to excess amount of solids. Therefore, this value was selected for the remaining study. It was observed that lower pulp density at rougher stage although produced higher grade but recovery decreased. While at cleaning stage of flotation, a pulp density of 20% produced a concentrate with good grade and recovery.

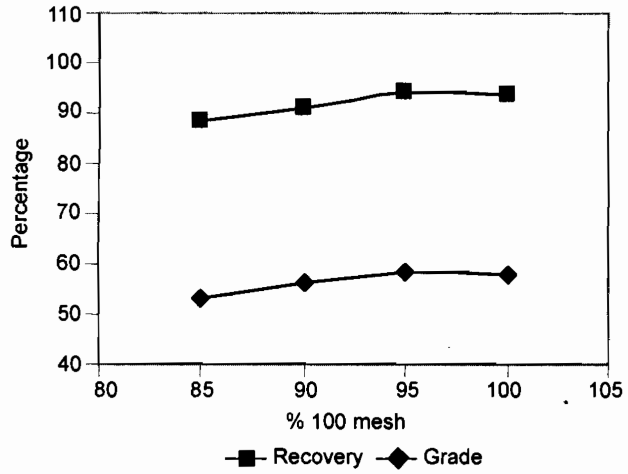

Fig. 2. Effect of particle size.

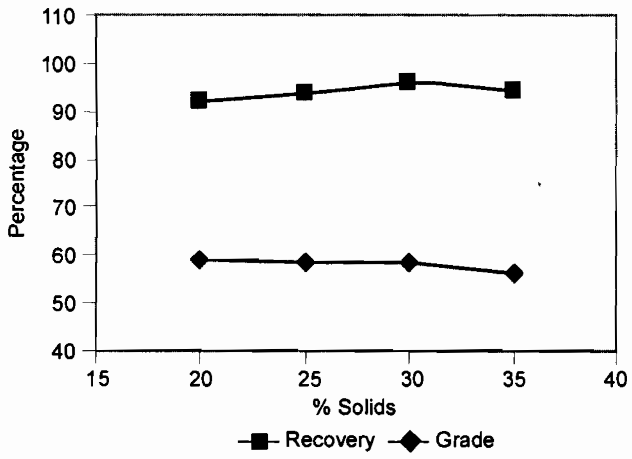

Fig. 3. Effect of pulp density.

The pH of pulp is very critical factor during the flotation of sulphide minerals. The results obtained, at various pH values, are presented in Fig. 4. It is clear that under constant condition of particle size and solid to liquid ratio, the grade and recovery increase with increase in pH from neutral to slightly alkaline medium and then decrease. It was observed that the maximum recovery of antimony is obtained at a pH 8.0, at rougher and cleaner stages. It was also noted that practically a small variation in pH showed a significant change in the grade and the recovery. This effect may be attributed to the maximum stability of xanthates in specific alkaline pH (Weiss, 1985).

Another important factor affecting the purity and recovery of antimony is the impeller speed. Data collected regarding the effect of impeller speed is shown in Fig. 5. It is obvious that an aeration speed of around 1100 rpm gave better results at rougher stage. It is due to the fact that this rpm makes the collector-coated mineral particles containing liberated as well as some partially exposed mineral particles float by attaching onto air bubbles and thus increasing the recovery. While on the other hand, at cleaning stage, a lower aeration speed of 900 rpm improved the grade and recovery because it allows the middling as well as gangue particles to remain in pulp thus producing a cleaner concentrate of greater purity.

It was observed that stibnite mineral did not float satisfactorily with xanthate until it was activated. However, it readily floats after activation with lead ions. Activation is, in fact, due to formation of more stable molecules of lead sulphide at the mineral surface by the adsorption of lead ions on the crystal lattice of stibnite (Pen'kov, 2001). The lead

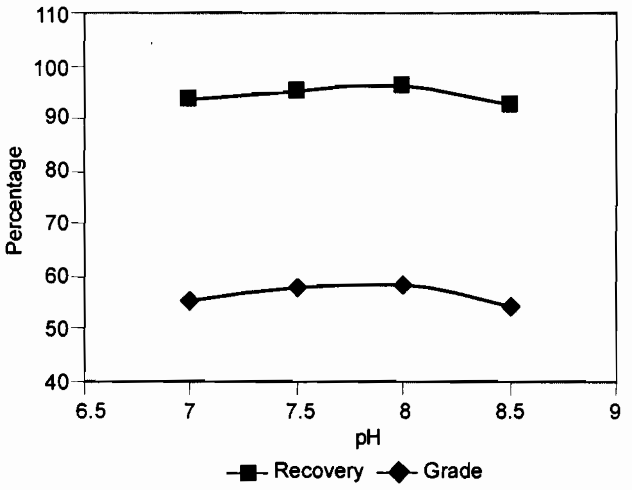

Fig. 4. Effect of pH.

sulphide, so deposited at the mineral surface, reacts readily with xanthate to form insoluble lead xanthate which being hydrophobic, can easily attach with air bubbles. Results obtained using different quantities of activator are given in Fig. 6. It is obvious that the grade and recovery increase with increase in the amount of activator up to 1500 g/ton and further increase is not effective. It is due to the fact that, with less amount of activator, all the stibnite minerals present in the ore are not activated and as the quantity of activator is increased, the grade and recovery increase until all the stibnite minerals are activated.

Results obtained using different quantities of depressant are presented as Fig. 7. Stibnite was readily floated by potassium amyl xanthate after activation but the problem was to keep down the gangue material. This difficulty was overcome by using sodium silicate as gangue depressant. It increases the selectivity of flotation by rendering the gangue minerals hydrophilic, thus preventing their flotation. It was observed that, quartz, the main gangue mineral present in antimony ore of Krinj, along with other gangue minerals such as clay and silicates were depressed by it. However, it was noted that with the increase in the amount of depressant, initially the grade improved but after 300 g/ton, the grade and recovery decreased probably due to the over coating of some middling particles. Larger amount of depressant was required at rougher stage, as the gangue minerals were present in excess, while, only slight amount of it gave better results at the cleaning stage.

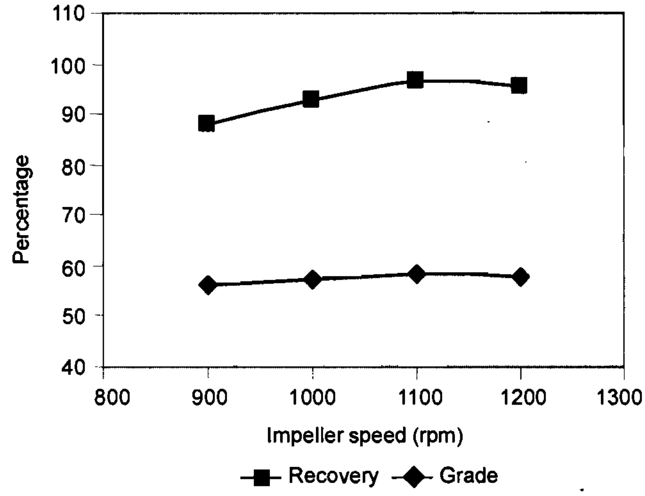

Fig. 5. Effect of impeller speed.

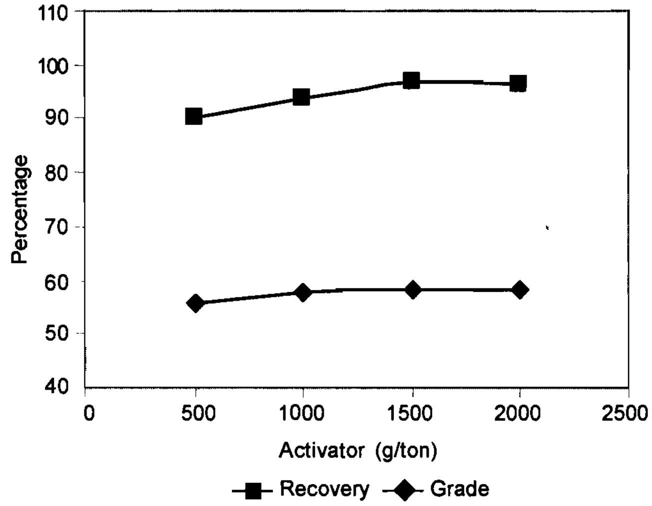

Fig. 6. Effect of activator.

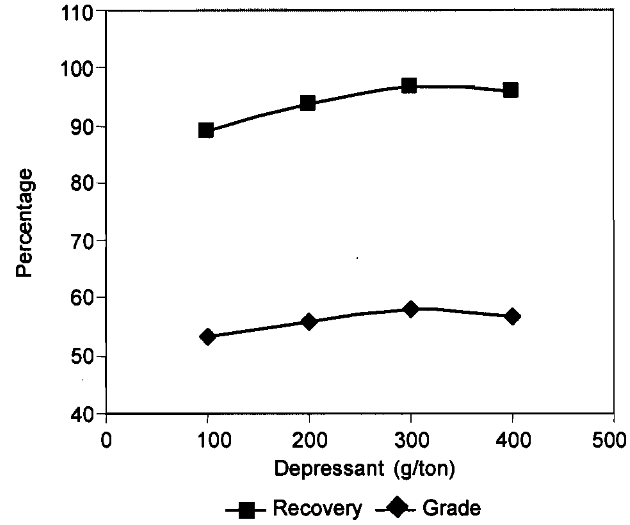

Fig. 7. Effect of depressant.

The amount of collector used for antimony flotation and its effect on grade and recovery is presented in Fig. 8. It is evident that better results were achieved at a dosage of 150 g/ton. The reason is that the collector ionizes in solution and produces positively charged ions of alkali metals and negatively charged xanthate ions which are then adsorbed on the surface of the sulphide minerals to form a monomolecular layer due to the chemical forces between the polar group of the collector and the mineral surface resulting in insoluble metal xanthates which are hydrophobic and aerophilic (Wills, 1992). It is notable, on the other hand, that excessive amount of collector adversely affects the grade and recovery of stibnite minerals due to the development of multi-layers of collector on the mineral particles which reduce the selectivity (Crozier, 1992). It was observed that at rougher stage, potassium amyl xanthate coats all the liberated as well as partially exposed particles. But at cleaning stage, there was no need for the addition of collector as mineral particles were already coated.

Frother, being heteropolar, is adsorbed on the air-water interface due to its surface active action thus reducing the surface tension of water and stabilizing the mineralized air-bubbles. Results obtained using different quantities of frother are presented in Fig. 9. It is apparent that a dosage of 50 g/ton gives reasonable grade and recovery; further increase shows no significant improvement in this regard.

Conditioning time has a pronounced effect on the grade and recovery of the concentrate. It allows the surfaces of the mineral particles to react with the reagents (Zhang and Shang, 2002). Results obtained for the effect of different conditioning time intervals are shown in Fig. 10. A conditioning time of 20 min was found to be sufficient for the contact with the mineral particles for optimum recovery and grade. It appears that on prolonged conditioning, the conditioner coating on the mineral particles gets peeled off resulting in lower flotation grade and recovery. It was also observed that short conditioning time requires more collector as it remains unable to coat all the mineral particles present in the pulp, in a short time.

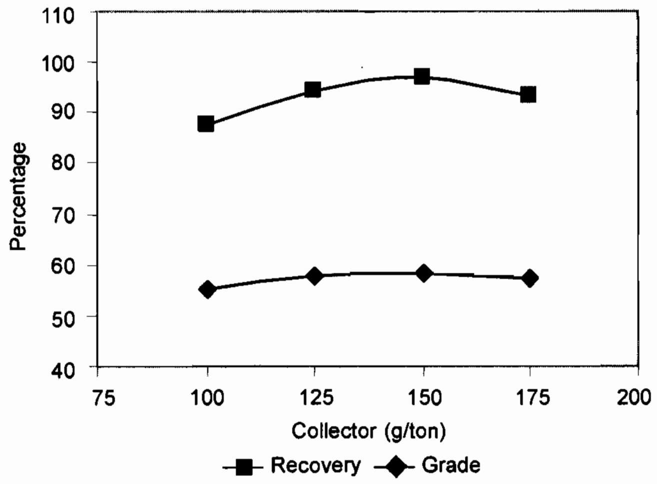

Fig. 8. Effect of collector.

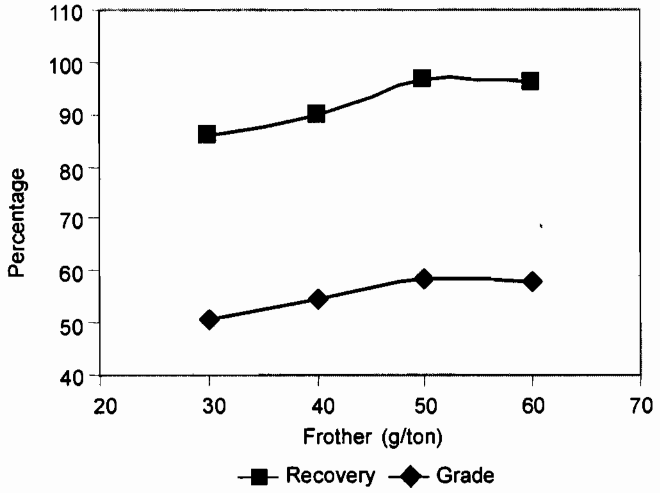

Fig. 9. Effect of frother.

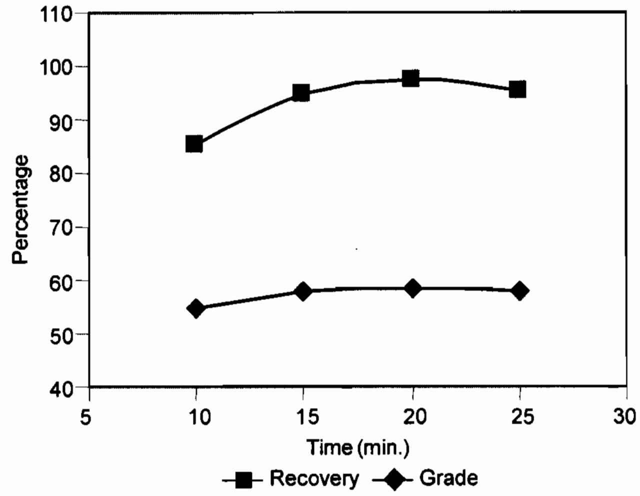

Fig. 10. Effect of conditioning time.

Conclusion

Antimony ore of Krinj area of Chitral comprises mainly of stibnite as economic mineral. Depending upon its nature, the antimony ore of this area can be upgraded by two stage flotation technique to produce antimony concentrate of required grade with acceptable recovery. Gravity concentration technique cannot be applied to this ore consisting of finely disseminated grain texture. The flotation technique is the most suitable for its upgradation. Antimony content of this ore is large enough to be exploited and flotation can produce antimony concentrate of about 62% Sb with 95% recovery. The obtained concentrate can be utilized for the production of chemicals and extraction of metal.

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Review

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Regulation of Arachidonic Acid Metabolism in the Human Fetus and the Neonate

Sheikh Arshad Saeed[a]*, Muhammad Atif Waqar[a], Areeba Jawed[b], Rushna Pervez Ali[b] and Muhammad Anwar Waqar[a]

[a]Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Karachi - 75270, Pakistan

[b]Department of Biological and Biomedical Sciences, The Aga Khan University, Karachi, Pakistan

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Abstract. The human fetus exists in an environment in which there is an apparent over-abundance of prostaglandins (PGs). Neonates are also believed to contain high concentrations of PGs. Since both fetus and the neonate have a significant potential for prostaglandin catabolism, it may be inferred that some benefits accrue from a prostaglandin rich environment and that prostaglandins (PGs) are serving important roles in both intrauterine and early extrauterine life. Prostaglandins are formed from non-esterified arachidonic acid (AA) by the action of cyclooxygenase (COX). AA is also metabolized by way of lipoxygenase enzyme pathway. Products of this pathway are known to modulate prostaglandin biosynthesis. Little information is available concerning these pathways in fetal and neonatal tissues. In this review article, the results of studies designed to evaluate AA metabolism in the fetus and the neonate are described. In addition, AA metabolism in uterine and intrauterine tissues is also considered, since the products of such metabolism are important for normal fetal growth and development.

Keywords: arachidonic acid metabolism, inhibitors/stimulators, the neonate, uterine, intrauterine tissues

Concentrations of prostanoids in fetal and neonate plasma.

Fetal circulation. Information on the concentrations of hormones in the plasma of human fetuses has been limited to measurements in umbilical plasma obtained at delivery. Recently, however, prostaglandin concentrations have been determined in blood samples obtained by fetoscopy, at 16-20 weeks of gestation.

The prostaglandins measured in the study were prostaglandin E_2 (PGE_2) and 6-keto prostaglandin $F_{1\alpha}$, (6-keto $PGF_{1\alpha}$), the nonenzymatically formed product of prostacyclin degradation. $PGE_{2\alpha}$ output was higher than PGF_2 and the concentrations of PGE_2 and $PGF_{2\alpha}$, were greater in both fetal and maternal outputs when compared with primary prostaglandins (Greystoke *et al.*, 2000). Strikingly, the concentrations of 6-keto-$PGF_{1\alpha}$, in fetal plasma are manifold greater than the concentrations in the maternal circulation. More recently, the effects of cyclooxygenase-l (COX-1) and COX-2 contributions to basal and interleukin-1 (IL-1) beta-stimulated prostanoid synthesis in human neonatal cerebral microvascular endothelial cells has also been investigated (Steinert *et al.*, 2002). These findings are suggestive that prostacyclin may serve an important role in the fetus during early pregnancy. Whether prostacyclin circulating in the fetus is influencing fetal organogenesis or is exerting a tonic effect on the placental vascular bed is uncertain. Prostacyclin circulating in the fetus may be a part of a biological protection mechanism for the fetus. Prostanoids, the products of cyclooxygenase (COX) pathway appear to be important regulators of blood flow in neonate. It has been demonstrated that COX activity in cultured endothelial cells in micro vessels from autopsy specimens of neonatal human cerebral cortex and cerebellum (22-26 week gestational age) resulting in production of vasodilator prostanoids, prostacyclin (6-keto-$PGF_{1\alpha}$) and PGE_2 from arachidonic acid (AA) (Barden *et al.*, 2004; Parfenova *et al.*, 2002).

Umbilical circulation. Concentrations of PGE_2, $PGF_{2\alpha}$ and 13, 14-dihydro-15-keto- $PGF_{2\alpha}$, (PGFM; the major circulating metabolite of $PGF_{2\alpha}$) in umbilical plasma are greater than in maternal plasma. On the other hand, maternal and fetal plasma concentrations of 6-keto-$PGF_{1\alpha}$ and thromboxane B_2 (TXB_2, the degradation product formed from TXA_2) are similar (Mitchell *et al.*, 1980). Concentrations of PGE, PGF and PGFM are all significantly raised in umbilical plasma obtained after the onset of labour indicating that labour is a stimulus to prostaglandin production by the fetal placental unit. Umbilical plasma concentration of 6-keto-$PGF_{1\alpha}$ and TXB_2, however, are unaffected by labour. A significant arterio-venous difference exists across the umbilical circulation for prostaglandin E_2 (PGE_2) with higher concentrations in venous plasma. This arterio-venous difference exists both before and after the onset of labour. In normal ovine pregnancy, arterial levels of

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*Author for correspondence; E-mail: arshad.saeed@iccs.edu prostacycline I_2 (PGI_2) are increased, which may in part reflect increased uteroplacental production. Moreover, the gravid ovine uterus also appears to produce PGE_2 and metabolize $PGF_{2\alpha}$, (Magness *et al.*, 1990). Similar arterio-venous plasma differences cannot be demonstrated for PGF, PGFM, 6-keto-$PGF_{1\alpha}$ or TXB_2. The finding that PGE concentrations are higher in umbilical venous blood than in umbilical arterial blood has been considered as suggestive that PGE in the fetal circulation is, at least partly, placental in origin.

Later studies suggest that the sensitivity of ductus arteriosus to PGE_2 is decreased by oxygen exposure. Cytochrome P_{450} affects the potency by causing constriction of the ductus arteriosus, as inhibitors of cytochrome P_{450} cause ductus to relax (Olley and Coceani, 1987). Hence, the high PGE concentration in the umbilical circulation at birth reflects an intrauterine environment in which potency of the ductus arteriosus would be maintained with PGE being dominant over PGF.

Neonatal circulation. The first report describing plasma prostaglandin levels in the neonatal period came from Siegler *et al.* (1977) who measured PGE in cord blood after term delivery and in peripheral plasma at 2-3 days of age and throughout childhood. The plasma level of PGE was found to be significantly lower at 2-3 days of age compared with values in cord blood which increased continuously, thereafter until adult life. These results must be interpreted with caution since the adult plasma levels of PGE described were approximately ten-fold greater than accepted values. In a study of the possible relationship between prostaglandin and respiratory distress syndrome, plasma levels of PGE and PGF were measured in a control group of infants over the first ten days of life following preterm delivery. No difference was found in circulating levels of either PGE or PGF between the first and the tenth day after delivery. These authors did not comment on the sustained high levels of PGE and PGF during the period of ductal closure, when enhanced prostaglandin catabolism would be expected. Using radio-immunoassay techniques, it has been found that circulating concentrations of PGE in neonates born at term are significantly reduced by the sixth day of extra uterine life compared with levels/at birth. Mean concentrations of PGF and PGFM also are lowered in the first week of life. Quite a different pattern appears for circulating concentrations of 6-keto-$PGF_{1\alpha}$ and TXB_2 in the prenatal period (Mitchell *et al.*, 1981a). By six days, neonates, born at term, have higher circulating levels of both 6-keto-$PGF_{1\alpha}$ and TXB_2 than at birth. Infants born before term, but uncomplicated by major diseases, also have plasma concentrations of PGE, PGF and PGFM on the sixth day of life similar to those infants born at term. Delivery before term is not, therefore, associated with obvious difference in capacity for prostaglandin biosynthesis or metabolism in the neonatal period. It has been found that prostaglandin concentrations in the plasma of pre-term infants are raised above those of adults for at least 60 days. Importantly, concentrations of PGE in neonatal plasma decline more rapidly than concentrations of other prostaglandins and this reduction may play an active or facilitatory role in closure of the ductus arteriosus. It should be noted that prostacyclin and TXA_2 have little action on the ductus arteriosus. COX-1 and COX-2 develop unevenly in the ductus while both enzymes contribute to PGE_2 formation at term; COX-1 is the major isoform in the prematures. COX-2, however, may acquire greater importance before-term following physiological and pathophysiological stimuli (Coceani *et al.*, 2001). In pregnancy related hypertension, increase in synthesis of TXA_2 occurs early during pregnancy.

Metabolism of AA. *Cyclooxygenase pathway.* The potential for prostanoid (essentially prostaglandin and thromboxane) biosynthesis by human fetal tissues has been evaluated in detail (Ringseis *et al.*, 2007; Leonhardt *et al.*, 2003). This study reports that human fetal tissues were obtained from pregnancies in the first and second trimesters of gestation. Tissues were minced and superfused. The method of tissue superfusion allows prostanoids (formed acutely due to the traumatisation of tissues) to be removed before commencing timed collections under steady state conditions. The results of this study show that the rate of formation of 6-keto-$PGF_{1\alpha}$ by all tissues studied was generally greater than the rate of formation of PGF_2 or PGE_2. The rate of formation of 6-keto-$PGF_{1\alpha}$ was highest in aorta. This is not surprising as 6-keto-$PGF_{1\alpha}$ is a metabolite of prostacyclin. Prostacyclin formation was greatest in vascular tissues since the intimal lining is considered to be a major site of prostacyclin biosynthesis. Furthermore, vascular tissue from fetuses of other animal species has been shown to produce PGs predominantly which serve to prevent platelet adhesion and clumping. Intriguingly, the second highest rate of formation of 6-keto-$PGF_{1\alpha}$ was by fetal stomach. Decidua produced 12 to 28 times more prostaglandins than placenta and fetal membranes with 6-keto $PGF_{1\alpha}$ as the main metabolite (Wetzka *et al.*, 1993). In adults it is thought that prostacyclin may act in the stomach to have a cytoprotective effect. The fetal lung and adrenal also produce prostacyclin although at lower rates. The adult lung has been thought to be a major source of prostacyclin. Formation of prostacyclin by the human fetal adrenal is of interest since prostacyclin is a potent stimulant of adenylate cyclase activity and hence may be of importance in regulating steroid hormone formation. The capacity for the production of PGE_2 and $PGF_{2\alpha}$, is higher in the secretory phase of endometrium than in the proliferative phase and the maximum formation of

PGE_2 and $PGF_{2\alpha}$ was found in the mid secretory phase and the late secretory phase, respectively (Ishihara *et al.*, 1986). In general, in the other tissues investigated, the rate of production of $PGF_{2\alpha}$ was greater than that of PGE_2.

Lipoxygenase pathways. The first detailed evaluation of AA metabolism by way of lipoxygenase pathways in human fetal tissues has also been described (Keeney *et al.*, 1998; Schafer *et al.*, 1996; Saeed and Mitchell, 1983). Human fetal tissues were obtained after voluntary termination of pregnancy between 12 and 18 weeks of gestational age. Tissues were minced, homogenized prior to incubation with radio-labelled AA. Products were extracted and subjected to thin layer chromatography and various lipoxygenase products, were determined. All tissues investigated formed lipoxygenase derivatives of AA. It was found that liver was a major source of lipoxygenase metabolites. The high rates of conversion of AA to lipoxygenase metabolites in the fetal tissues are similar to the rates of conversion of AA to lipoxygenase metabolites in adult rat liver (Capdevila *et al.*, 1981).

Although absolute identification of the products formed is not available, lipoxygenase products formed by human fetal tissues had chromatographic mobilities identical with (5S)5-hydroxy-6, 8, 11, 14-eicosatetraenoic acid (5-HETE) and 12-HETE, respectively. The production of prostaglandins and HETEs by pregnancy specific human tissues was investigated in a short-term culture system. The main AA metabolite in all tissues from lipoxygenase pathway was 12-HETE (Wetzka *et al.*, 1993). It is interesting to note that the formation of 5-HETE which reflects the biosynthesis of the precursor 5-hydroperoxyeicosatetraenoic acid (5-HPETE) which is an essential intermediate in the formation of leukotrienes (Samuelsson *et al.*, 1980). Both 12-HPETE and 15-HPETE have been shown to inhibit prostacyclin formation and hence the production of 12-HETE by tissues may well form part of a self regulatory mechanism of AA metabolism. Prolonged exposure to HETEs may compromise the anti thrombotic and vasodilator properties of endothelium by reducing its capacity to produce eicosanoids including PGI_2 (prostacyclin). 12-HETE released by activated platelets and macrophages reduced prostacyclin formation in the bovine aortic endothelial cultures by as much as 70% (Hadjiagapiou and Spector, 1986). However, the rate of formation of prostacyclin also has been shown to be enhanced by certain leukotrienes. The relative rates of formation of different lipoxygenase derivatives may, therefore, be of importance in the regulation of prostacyclin formation by fetal tissues.

Metabolism of AA in uterine and intrauterine tissues. ***Cyclooxygenase pathway.*** Using the technique of tissue superfusion (Table 1), a concerted series of experiments have been performed to evaluate the production of prostanoids by uterine and intrauterine tissues. Amnion is a significant source of PGE and indeed PGE_2 is the major prostanoid synthesized by most tissues. Substantial formation of TXB_2 occurs in deciduas Vera and placenta. The mean rate of formation of PGE_2 by amnion tissue after labour (Meadows *et al.*, 2003; Macchia *et al.*, 1997) was found to be higher than before labour, although the difference was not statistically significant. Subsequently, it was demonstrated that there is a significant increase in

Table 1. Rates of production of prostanoids by intrauterine tissues superfused *in vitro*

		Production of prostanoid (ng/mg per g dry wt)			
	Prostanoid	Amnion	Chorion	Decidua	Placenta
Tissues obtained after spontaneous vaginal delivery	Prostaglandin E	13.17±2.21	2.89±0.46	1.72±0.24	2.02±0.38
	Prostaglandin $F_{2\alpha}$	0.83±0.19	0.51±0.12	0.49±0.09	0.66±0.13
	Thromboxane B_2	1.59±0.37	0.61±0.19	2.12±0.38	4.94±0.39
	6-keto prostaglandin $F_{1\alpha}$	6.31±2.40	2.43±0.64	1.46±0.43	1.33±0.39
Tissues obtained at elective Caesarean section	Caesarean section				
	Prostaglandin E	9.62±1.62	3.13±0.59	2.50±0.57	2.48±0.47
	Prostaglandin $F_{2\alpha}$	0.74±0.19	0.76±0.20	0.80±0.25	0.82±0.24
	Thromboxane B_2	2.42±0.79	0.88±0.26	2.76±1.09	4.84±1.05
	6-keto 6-keto-				
	Prostaglandin $F1_{\alpha}$	2.37±0.65	1.76±0.40	1.41±0.38	1.11±0.21

values are mean ± S.E.M. for ten individual determinations prostaglandin synthase in amnion during labour (Okazaki *et al.,* 1981). It is widely considered that the biosynthesis of PGE$_2$ by fetal membranes and in particular amnion is vital in the events culminating in the onset and maintenance of labour. Classical excitatory effect of PGE and PGF is followed by hyper polarization. This restricts the response to a single contraction and decreases the frequency of subsequent contractions. The amplitude of the hyper-polarization decreases during labour allowing contraction frequency to increase. Its persistence at this time ensures complete relaxation between each single robust contraction preventing spasm of the uterus that would restrict blood flow to the fetus during delivery (Parkington *et al.,* 1999). Cyclic adenosine monophosphate (cAMP) may be involved in the labour induced by PGE$_2$ and cyclic guanosine monophosphate (cGMP) in that induced by oxytocin (Nagata *et al.,* 1988). The low rates of formation of PGE$_2$ by the tissues are consistent with an environment in which the production of substances with vasoconstrictor activity should be minimized. However, PGE from villous trophoblast can influence the function of many leucocytes by raising intracellular cAMP concentrations and hence might be important in maintenance of pregnancy (Kelly *et al.,* 1995). Prostacyclin formation by various intrauterine tissues may provide a tonic stimulus to uteroplacental blood flow and hence be protective of fetal development.

Data are also available suggesting that cervix is a major source of prostaglandins, particularly of the E series and it has been hypothesized that softening and dilation of the cervix at term are dependent upon locally formed prostaglandins. Such an action is a protective mechanism for the fetus since without cervical softening and dilatation, the onset of labour would result in contractions and the fetus would be pressed against an inflexible structure. The combination of cervical ripening with intracervical PG gel application and induction of labour by extra-amniotic PG gel under epidural anesthesia is an efficient and safe method for treatment of intrauterine fetal death (Cromi *et al.,* 2007; Neilson, 2007). Prostaglandin formation has also been demonstrated in myometrium.

Lipoxygenase pathways. Human uterine and intrauterine tissues have the potential to form lipoxygenase metabolites of AA (Saeed and Mitchell, 1982a). The major lipoxygenase product formed by human amnion, deciduas vera and placenta has been found to be 12-HETE. The chorion produces only a trace amount of 12-HETE. It has been postulated that, since various HETEs are potent chemotactic agents for human neutrophils, eosinophil and macrophages, production of these metabolites by human intra-uterine tissues may serve to regulate leukocyte and/or macrophage infiltration during pregnancy and parturition (Mitchell *et al.,* 1983). Such infiltration occurs in cervical tissue during cervical ripening and it has been demonstrated that cervix produces lipoxygenase derivatives of AA (Saeed and Mitchell 1982b) that also reside in the myometrium (Erkinheimo *et al.,* 2000; Mitchell *et al.,* 1983). The biosynthesis of lipoxygenase derivatives of AA by placental tissue may be critical in the maintenance of fetal hemostasis since changes in formation of prostacyclin and/or prostanoids in this tissue could lead directly to changes in utero-placental blood flow. Hence, the production of lipoxygenase metabolites in these tissues may be considered of utmost importance for fetal well being.

Regulation of the metabolism of AA by inhibitory factors. ***Inhibition of prostanoid biosynthesis.*** In 1977, Saeed *et al.,* demonstrated the existence of circulating inhibitors of prostaglandin synthase. These were named "endogenous inhibitors of prostaglandin synthase" (EIPS). EIPS activity in the plasma of pregnant women was demonstrated (Mitchell *et al.,* 1981b) to be significantly lower during the third trimester of pregnancy (Brennecke *et al.,* 1982). A significant fall in the activity of EIPS in amniotic fluid during labour has been demonstrated (Fig. 1, 2) (Saeed *et al.,* 1982). This is a key

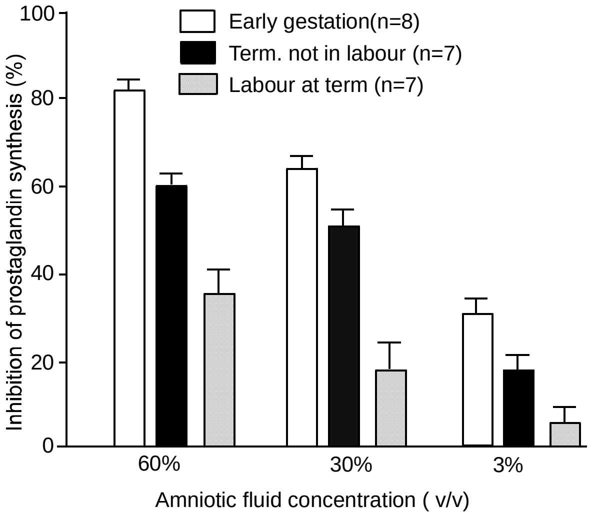

Fig. 1. The inhibition of prostaglandin synthesis by human amniotic fluid in relation to gestation and labour. (Results are presented as mean (±SE) inhibitory activities for amniotic fluid (at three concentrations) obtained at early gestation (15-17 week), term gestation and at spontaneous labour. At all three stages, concentrations of amniotic fluid through inhibitory activity of samples at term was less than the activity at early gestation ($p=0.05$) and samples obtained in labour demonstrated lower inhibitory activity than either early ($p<0.001$) or term ($p<0.01$) samples).

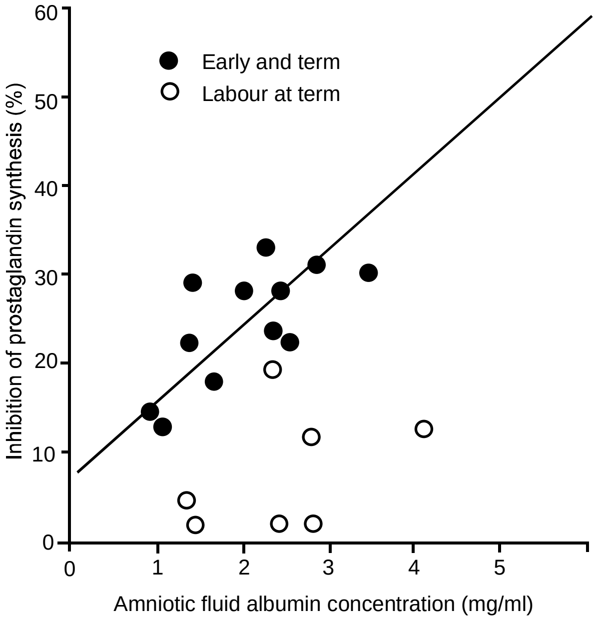

Fig. 2. The relationship of prostaglandin synthesis inhibition by human amniotic fluid to albumin concentration in the samples, at an assay concentration of 3% (v/v) amniotic fluid. (Samples obtained at early gestation and at term, not in labor, showed a significant ($p<0.01$) linear correlation ($r=0.90$) between inhibitory activity and albumin concentration. Samples obtained at labour did not exhibit a significant correlation ($r = 0.390$, $p > 0.30$) between inhibitory activity and albumin concentration).

observation since amniotic fluid bathes the amnion, it is the key structure in the mechanisms of the onset of human labour through its production of PGE_2. (Das *et al.,* 2007; Murthy and Kennea, 2007; Saeed *et al.,* 1982) Hence it is possible that the biosynthesis of prostaglandins is tonically inhibited throughout pregnancy and that such inhibition is withdrawn at the onset of labour. This inhibition is of benefit to fetus since it prevents labour before term and may reduce excessive prostaglandin formation near the utero-placental vascular bed which could lead to vascular constriction. The fetus has less EIPS activity in its plasma than the adult; this is true both in sheep and in man. Umbilical plasma has less EIPS activity than adult plasma although no arteriovenous differences have been found (Gold *et al.,* 2006). EIPS concentration in the neonate increases gradually during the first month of life to reach adult levels by 1-2 months of life. A reciprocal relationship was found between plasma EIPS levels and previously reported plasma prostaglandin concentrations. This result supports the role for EIPS in the control of prostaglandin biosynthesis in humans (Friesen and Innis, 2006; Saeed *et al.,* 1977). Interestingly this is just the opposite of circulating prostaglandin levels. More recently it has been shown that amniotic fluid inhibits PG production at the level of PG synthase enzymes. Endogenous prostaglandin inhibitors in amniotic fluid may play a role in maintaining uterine quiescence throughout gestation and its withdrawal at term may be involved in the initiation of labour (Orlov *et al.,* 1996; Saeed *et al.,* 1982, 1977).

Inhibition of lipoxygenase activities. An endogenous inhibitor of lipoxygenase activity has been described (Saeed *et al.,* 1980). No substantial information is available on such activity during pregnancy and parturition, although we have obtained results that such an inhibitor is present in human amniotic fluid. If this inhibitor does circulate in pregnant women, it may serve to regulate lipoxygenase activities and hence may be important in preventing the potential deleterious effects of 12-HPETE and 15-HPETE on prostacyclin within the uterus.

Inhibition of phospholipase activities. The elegant studies of Johansen *et al.* (2000) and Flower and Blackwell (1979) have suggested that glucocorticoids act to inhibit prostaglandin formation by inhibition of phospholipase activities. The mediator of this effect has been named macrocortin or lipomodulin. Human fetal adrenal tissue has been shown to respond to glucocorticoids by inhibition of prostaglandin formation in a manner consistent with the formation of lipomodulin (Mitchell *et al.,* 1982). This finding may be significant for the understanding of the regulation of adrenal growth and the secretion of steroids by the tissue. Moreover, it may be of importance in the understanding of the regulation of regression of the fetal zone of the adrenal during early neonatal life. The suppression of prostaglandins that have vasodilatory properties could provide a mechanism whereby the blood supply to the inner fetal zone of the adrenal is reduced or completely abolished and hence the fetus would regress rapidly. Glucocorticoids down regulate COX-1 gene expression and prostacyclin synthesis in fetal pulmonary artery endothelium (Jun *et al.,* 1999). It has been demonstrated that glucocorticoids affect prostaglandin formation by human amnion cells in monolayer culture. Interestingly, human myometrial cells in monolayer culture do respond to glucocorticosteroid by reduced formation of prostaglandin. The latter observation is particularly interesting since the major prostaglandin formed by the myometrium is prostacyclin (Abel and Kelly, 1979), an inhibitor of uterine activity in sheep (Lye and Challis, 1982). Hence an increased rate of glucocorticosteroid biosynthesis during labour may act to reduce the rate of biosynthesis of a uterine relaxant and thus allow the effects of uterotonic prostaglandins to be dominant. Studies conducted by McLaren *et a1.* (1996) indicate that glucocorticoid induced PG production is due to increased formation of prostaglandin H synthase-2 (PGHS-2) isozyme in ovine cotyledon. The presence of glucocorticosteroids sensitive within fetal and uterine environments provides another regulatory mechanism for prostanoid formation during the pregnancy and parturition.

Regulation of AA metabolism by stimulatory factors. ***Circulating substances.*** A variety of substances found in the maternal circulation have been reported to stimulate prostanoid formation. The substances include oxytocin, bradykinin and estrogens (William and El-Tahir, 1980). In systems using isolated human endometrial fragments, progesterone has been shown to inhibit PG production markedly. Data collected by Kelly and Smith (1987) shows that the inhibition of PG production shown by progesterone, acting on secretory phase endometrium cultured as tissue fragments, is reversible by the receptor blocking antiprogestins. Whether the substances have tonic effects on prostaglandin biosynthesis by uterine tissues is unknown. Chorionic renin may have a novel role in the regulation of amnion cell $PGE_{2\alpha}$ production that is independent of angiotensin formation (Lundin-Schiller and Mitchell, 1991). Recent findings indicate that histamine may act as a local regulator of PGA_2 and $PGF_{2\alpha}$ production in human term decidua and may involve interaction with IL-1 (Schrey *et al.,* 1995). It seems somewhat unlikely that such substances would play a major role in maintaining fetal homeostasis since the stimulation of any prostanoid by these substances would occur not only within the uterus but also in other maternal tissues. Moreover recent data suggest that human chorionic gonadotropin (hCG) may also have a biological role in the regulation of PG synthesis in early human placenta (North *et al.,* 1991). In addition, Jones and Challis (1990) support the possibility of paracrine stimulation corticotropin-releasing hormone (CRH) and adrenocorticotropin (ACTH) of PG production in intrauterine tissues and suggest that in part, the effects of CRH on placental PG output might be mediated through ACTH.

Substances in uterine and intrauterine tissues. Uterine and intrauterine tissues contain cytosolic factors that cause a stimulation of prostaglandin biosynthesis (Saeed and Mitchell, 1982c). The stimulation of biosynthesis is different not only for different prostaglandins but also between dif-ferent uterine tissues. Indeed the nature of the stimulation is also different. At present no data are available concerning the presence of such stimulation of prostaglandin biosynthesis within intrauterine tissues which may provide yet another regulatory mechanism for AA metabolism. Parturition in the sheep is preceded by an increase in the synthesis of prostaglandins by intra-uterine tissues. Prostaglandin H synthase (PGHS) is the central enzyme in prostanoid production (McLaren *et al.,* 1996). Since prostacyclin biosynthesis is increased during pregnancy, it is likely that a specific stimulant of prostacyclin formation is present within intrauterine tissues. Pregnancy affects preferential changes in the sub-cellular distribution of PGI synthase in myometrial cells. Relative to its PGI synthase content pregnant myometrium contained twice as much PGH synthase as non-pregnant myometrium (Moonen *et al.,* 1984). It would then seem possible that a reduced activity of such a stimulant could lead to a chronic reduction in uteroplacental blood flow and thus lead to growth retardation and pregnancy-induced hypertension. Given the multitude of effects of prostacyclin within the body the finding of a stimulant of prostacyclin biosynthesis within intrauterine tissues has wider significance since the characterization of such a substance may eventually permit clinical treatment with the substance. Developing the clinical use of eicosanoid-related drugs and assessing the potential use of these drugs require a 3-phase approach: reducing the complications in the treatment of neonates with ductus-dependent congenital heart diseases and primary pulmonary hypertension requiring PGE_1, PGE_2 and PGI_2 therapy; conducting clinical trials of the synthesis inhibitors and receptor antagonists of TXA_2 and LT that have already been used in the treatment of adult patients with bronchial asthma; and evaluating the efficacy of new modulators of eicosanoid biosynthesis, such as eicosapentaenoic acid and anti-allergy drugs, in the treatment of eicosanoid-related diseases in children (Shimizu, 1998).

The biosynthesis and release of AA metabolites (prostaglandins and lipoxygenase products) within the human uterus and by the fetus are extremely complex. It likely involves a series of inhibitory and stimulatory factors that include a combination of different products of AA metabolism. However, given the clinical importance of prostaglandin formation by the fetus and the uterus, it is of importance that studies are conducted to characterize the ultimate regulator of prostaglandin formation.

Our ability to modulate the formation of prostaglandins and lipoxygenase products during pregnancy will have major clinical implications since prostaglandins do have several protective actions on the uterus and in particular on the fetus and the neonate. On the other hand, it should be recognized that interference with the normal pattern of AA metabolism could have disastrous consequences. Hence, there is a great need for extensive basic scientific studies to be conducted before cautious clinical trials of any of the substances described in this review can be considered.

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