ISSN 2221-6421 (Print), ISSN 2223-2567 (Online)
Coden: PJSIC6 57(3) 117-174 (2014)

Pakistan Journal of Scientific and Industrial Research

Series B: Biological Sciences

Vol. 57, No.3, November-December, 2014

(for on-line access please visit web-site http://www.pjsir.org)

Published by
Scientific Information Centre
Pakistan Council of Scientific and Industrial Research
Karachi, Pakistan

Pakistan Journal of Scientific and Industrial Research
Series B: Biological Sciences

Pakistan Journal of Scientific and Industrial Research started in 1958, has been bifurcated in 2011 into:

Series A: Physical Sciences [**ISSN 2221-6413 (Print); ISSN 2223-2559 (online)**] (appearing as issues of January-February, May-June and September-October) and
Series B: Biological Sciences [**ISSN 2221-6421 (Print); ISSN 2223-2567 (online)**] (appearing as issues of March-April, July-August and November-December).

Each Series will appear three times in a year.

This Journal is indexed/abstracted in Biological Abstracts and Biological Abstracts Reports, Chemical Abstracts, Geo Abstracts, CAB International, BioSciences Information Service, Zoological Record, BIOSIS, NISC, NSDP, Current Contents, CCAB, Rapra Polymer Database, Reviews and Meetings and their CD-ROM counterparts etc.

Subscription rates (including handling and Air Mail postage): ***Local:*** Rs. 2000 per volume, single issue Rs. 350; ***Foreign:*** US$ 400 per volume, single issue US$ 70.

Electronic format of this journal is available with: ProQuest Information and Learning, 789 E. Eisenhower Parkway, P.O. Box 1346, Ann Arbor, MI 48106-1346, U.S.A.; Fax.No.+1.734.997.4268; http://www.proquest.com.

Photocopies of back issues can be obtained through submission of complete reference to the Executive Editor against the payment of Rs. 25 per page per copy (by Registered Mail) and Rs. 115 per copy (by Courier Service), within Pakistan; US$ 10 per page per copy (by Registered Mail) and US$25 per page per copy (by Courier Service), for all other countries.

Printed and Published by: PCSIR Scientific Information Centre, PCSIR Laboratories Campus, Shahrah-e-Dr. Salimuzzaman Siddiqui, Karachi-75280, Pakistan.

Editorial Address

Executive Editor

Pakistan Journal of Scientific and Industrial Research, PCSIR Scientific Information Centre,
PCSIR Laboratories Campus, Shahrah-e-Dr. Salimuzzaman Siddiqui, Karachi-75280, Pakistan
Tel: 92-21-34651739-40, 34651741-43; Fax: 92-21-34651738; Web: http://www.pjsir.org, E-mail: info@pjsir.org

Revising Subscription Rates

Despite, increasing cost we refrained from revising subscription in last ten years. But in view of tremendous increase in cost of printing material and printing and in postage rates, it becomes imperative for us to take decision on revising the rates due to present circumstances. Following are the revised Subscription rates from January 2015

Rates from 2015		
Annual Local Subscription	Rs.2500/- per volume (including postal charges)	Rs.425/- single issue (including postal charges)
Annual Foreign Subscription	US$ 450/- per volume (including postal charges by Airmail)	US$ 75/- single issue (including postal charges by Airmail)
Agency Discount	15%	15%

Executive Editor

Pakistan Journal of Scientific and Industrial Research
Series B: Biological Sciences
Vol. 57, No. 3, November - December, 2014

Contents

Short Communications

Review

Pak. j. sci. ind. res. Ser. B: biol. sci. 2014 **57** (3) 117-122

Effect of Tank Mixed Application of Ammonium Sulphate and Carfentrazone-Ethyl + Clodinafop-Propargyl + Metsulfuron-Methyl on Weeds and Yield of Wheat

Asif Tanveer*, Adnan Khalid, Muhammad Tahir and Muhammad Ather Nadeem
Department of Agronomy, University of Agriculture, Faislabad, Pakistan

(received December 19, 2013; revised July 1, 2014; accepted August 27, 2014)

Abstract. Weeds are serious problem in wheat crop. The adjuvants are used to increase the weed control spectrum of herbicide or to reduce the dose of herbicide without affecting weed control efficiency. The effect of tank mixed application of ammonium sulphate and carfentrazone-ethyl + clodinafop-propargyl + metsulfuron-methyl on weeds of wheat (*Triticum aestivum* L.) was investigated under field conditions. The experiment comprised of carfentrazone-ethyl + clodinafop-propargyl + metsulfuron-methyl @ 500, 375 g/ha alone and with 1% and 2% ammonium sulphate solution. The maximum reduction in weed density (97.29%) and dry weight (94.27%) was recorded with carfentrazone-ethyl + clodinafop-propargyl + metsulfuron-methyl @ 500 g/ha. The grain yield and yield components of wheat were affected significantly, by treatments. Maximum grain yield was obtained with carfentrazone-ethyl + clodinafop-propargyl + metsulfuron-methyl @ 500 g/ha. This treatment resulted in 37.71% more grain yield over weedy check. Addition of 1-2% (wt/v) ammonium sulphate did not enhance the activity of herbicide. Based on present study, it is concluded that use of ammonium sulphate adjuvants did not increase the efficiency of carfentrazone-ethyl + clodinafop-propargyl + metsulfuron-methyl.

Keywords: wheat, herbicide, adjuvant, ammonium sulphate, weeds

Introduction

In addition to many other factors, the low yield of wheat is attributed to serious weed infestation. Losses due to weeds have been reported from 18 to 30% (Ashiq and Cheema, 2005). In Pakistan, major weeds of wheat that cause huge economic losses are broad leaf dock (*Rumex dentatus* L.), swine cress (*Coronopus didymus* L.), emex species (*Emex spinosa* L.), canary grass (*Phalaris minor* Retz.), wild oat (*Avena fatua* L.), fumitory (*Fumaria indica* L.), lamb's quarters (*Chenopodium album* L.), field bindweed (*Convolvulus arvensis* L.), blue pimpernel (*Anagallis arvensis* L), and bermuda grass (*Cynodon dactylon* L.), (Tanveer and Ali, 2003).

Due to high competitive ability and high reproductive potential of weeds, it is imperative to check their infestation (Rehman *et al.,* 2010). Weeds problem is getting from bad to worse in wheat sown under irrigated conditions. Cropping intensity is rapidly increasing and it is impossible to control weeds with traditional method such as Dab (suicidal germination) and hand weeding. Now a days chemical weed control is preferred because it is rapid, convenient, inexpensive and more effective (Naseer-ud-Din *et al.,* 2011). Marwat *et al.* (2003) reported that chemical weed control decreased the weed infestation and increased the yield of wheat. Post-emergence herbicides are generally, absorbed through leaves. Leaf cuticle is composed of waxes and cutin that affect the herbicide absorption. The use of an adjuvant in combination with herbicide enhances the herbicide retention and leaf surface penetration through cuticle thereby, keeping the herbicide contact with plant tissues rather than edging up and rolling off and thus increases the phytotoxicity of herbicide (Zadorozhny, 2004). Adjuvants may also improve herbicide efficacy so that the concentration or total amount of herbicide required to achieve a given effect is reduced. Ammonium sulphate has many beneficial effects e.g., acts as a buffering agent, improves pesticide activity in hard water, increase efficacy of herbicide and enhances herbicide activity under stress conditions (Hatzios and Penner, 1985).

FMC X-100 herbicide is a combination of three active ingredients namely carfentrozone-ethyl, clodinafop-propargyl and metsulfuron-methyl. All these are applied

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

as a foliar spray to control broadleaf, sedges and grass weeds and are absorbed through the leaves. Ammonium sulphate is a relatively inexpensive additive for herbicides. The objective of this study was to determine whether ammonium sulphate as an adjuvant can enhance herbicide effectiveness for weed control.

Materials and Methods

The present study was carried out at agronomy research area, University of Agriculture, Faisalabad, Pakistan, during winter 2011-12. The experiment was laid out in randomised complete block design (RCBD) having four replications with a net plot size of 6 × 1.2 m. The wheat cultivar "Sehar-2006" was grown in 20 cm apart rows with a single row hand drill using 150 kg/ha seed rate. Seedbed was prepared by pulverising the soil with cultivator followed by planking. The fertiliser NPK was applied @ 100, 95, 75 kg/ha, respectively. Half dose of N and full dose of P and K was applied at the time of seedbed preparation, while remaining half dose of N was applied at the time of first irrigation. Uniform agronomic practices except treatments under study were applied to all entries of the experiment. Treatments consisted of control (weedy check), carfentrazone-ethyl + clodinafop-propargyl + metsulfuron-methyl @ 500, 375 g/ha alone and with 1% and 2% ammonium sulphate solution. The volume of spray was calibrated by spraying ordinary water on non-experimental area in the field. The herbicide carfentrazone-ethyl + clodinafop-propargyl + metsulfuron-methyl and NH_2SO_4 was applied as tank mixed postemergence spray after 1st irrigation (35 days after sowing) with hand operated knap sack sprayer fitted with flat fan nozzle. Weeds density was recorded by counting the number of weeds 14 and 28 days after spray (DAS) and at harvest from an area of $1m^2$ within the herbicide treated plots. Weeds were removed from ground surface and dried in an oven for 24 h at 70 °C and dry weight was recorded. Data on spike bearing tillers, number of grains per spike, 1000-grain weight and grain yield were recorded by following standard procedure. Analysis of variance was carried out according to Fisher's analysis of variance technique (Steel *et al.,* 1997) and least significance difference (LSD) test was used to compare the differences among treatments' means.

Results and Discussion

Total weeds density (m^{-2}). The analysis of the data showed that carfentrazone-ethyl + clodinafop-propargyl + metsulfuron-methyl alone and with ammonium sulphate as an adjuvant showed significant effect on weeds (Table 1). Maximum number of weeds (162.75, 188.0 and 166.25 m^{-2}) was found in weedy check at 14 and 28 DAS and crop harvesting, respectively, which was significantly, higher than all other treatments under study. The significantly, minimum number of weeds (11.00, 5.50 and 4.50 m^{-2}) was found in carfentrazone-ethyl + clodinafop-propargyl + metsulfuron-methyl @ 500 g/ha with maximum weed control efficacy of 93.24 %, 97.03 % and 97.29 % at 14 and 28 DAS and harvesting, respectively. The minimum weed control was found with carfentrazone-ethyl + clodinafop-propargyl + metsulfuron-methyl @ 500 g/ha + 2 % NH_2SO_4 showing 85.41 %, 88.96 % and 87.81 % weed control over weedy check at 14 and 28 DAS and harvesting, respectively. The weed count was decreased at final harvest and this decrease can be attributed to the mortality of the weeds after completing their life

Table 1. Effect of herbicide dose and ammonium sulphate as an adjuvant on total weed density (m^{-2}) and dry weight (g/m^2)

Treatments		Total weed density			
Herbicide	Adjuvant	14 DAS	28 DAS	At harvest	Dry weight (g/m^2)
Carfentrazone-ethyl + clodinafop-propargyl + metsulfuron-methyl (g/ha)	NH_2SO_4 (%)				
Weedy check	.....	162.75^{a}	188.00^{a}	166.25^{a}	86.40^{a}
500	.....	11.00^{d} (93.24)	5.50^{d} (97.07)	4.50^{e} (97.29)	4.95^{d} (94.27)
375	.....	23.00^{b} (85.87)	17.00^{bc} (90.96)	16.25^{c} (90.22)	13.80^{bc} (84.02)
500	1	23.25^{b} (85.71)	19.75^{b} (89.49)	19.00^{bc} (88.57)	16.95^{b} (80.38)
500	2	23.75^{b} (85.41)	20.75^{b} (88.96)	20.25^{b} (87.81)	13.15^{bc} (84.78)
375	1	17.50^{c} (89.25)	12.25^{c} (93.48)	12.25^{d} (92.63)	11.85^{c} (86.28)
375	2	23.50^{b} (85.56)	19.00^{b} (89.89)	18.25^{bc} (89.02)	15.15^{bc} (82.46)
LSD value		4.007	6.193	3.365	4.925

Any two means sharing same letters did not differ significantly, at 5% level of probability; Figs. in parentheses showed % decrease over weedy check; DAS = days after spray.

span. Maximum population in weedy check was due to uncontrolled growth of weeds. Minimum number of weeds in control treatments was due to effect of herbicide. These results are confirmed by those of Shehzad *et al.* (2012) and Kumar *et al.* (2011), who reported that herbicides significantly control the weeds of wheat against weedy check. The non-significant effect of the adjuvant with full dose of herbicide can be attributed to the fact that adjuvants may have no effect on efficacy of herbicides if not used at proper rate (Tu *et al.* 2001) and even the effects of the adjuvants vary with concentration (Pacanoski, 2010). The non-significant effects of adjuvant on weed control had also been reported by O' Sullivum and Bonw' (1997). However, the addition of adjuvant with reduced herbicide dose resulted in lower total weed density than use of herbicide alone at same dose. Nadeem *et al.* (2008) also reported increase in herbicide efficacy against weeds with the addition of urea as an adjuvant. The non-significant effect of ammonium sulphate as an adjuvant at higher doses and pronounced effect at lower doses are supported by the findings of Bradley *et al*. (2000).

Individual weed density. Analysis of the data showed that carfentrazone-ethyl + clodinafop-propargyl + metsulfuron-methyl alone and with ammonium sulphate significantly, reduced the *P. minor*, *C. album*, *C. didymus* and *C. arvensis* population. The data given in Table 2 showed that maximum number of *P. minor*, *C. album*, *C. didymus* and *C. arvensis* was recorded in weedy check plots at 14 and 28 DAS, respectively, which was significantly higher than all other treatments under study. The minimum number of *P. minor* (5.75 and 1.25 m^{-2}), *C. album* (0.25 and 0.50 m^{-2}), *C. didymus* (0.50 and 0.50 m^{-2}) and *C. arvensis* (0.25 and 1.00 m^{-2}) was found in carfentrazone-ethyl + clodinafop-propargyl + metsulfuron-methyl @ 500 g/ha at 14 and 28 DAS, respectively. The respective values for percent weed control were 72.29 % and 96.35 %; 99.21 % and 97.96 %; 98.46 % and 98.64 %; and 95 % and 80.95 % over weedy check. Carfentrazone-ethyl + clodinafop-propargyl + metsulfuron-methyl @ 500 g/ha was statistically at par with all other weed control treatments in respect of *P. minor*, *C. album* and *C. didymus* control. Maximum population in weedy check was due to uncontrolled growth of weeds. Minimum number of weeds in weed control treatments could be attributed to more weed mortality due to the blocking of the electron transport system resulting in destruction of the PS-II reaction

Table 2. Effect of herbicide dose and ammonium sulphate as an adjuvant on individual weed density (m^{-2}) 14 and 28 DAS.

Treatments		Individual weeds							
Herbicide	Adjuvant	*Phalaris minor*		*Chenopodium album*		*Coronopus didymus*		*Convolvulus arvensis*	
Carfentrazone-ethyl + clodinafop-propargyl+ metsulfuron-methyl (g/ha)	NH_2SO_4 (%)	14 DAS	28 DAS	14 DAS	28 DAS	14 DAS	28 DAS	14 DAS	28 DAS
Weedy check	...	20.75^{a}	34.25^{a}	31.25^{a}	24.50^{a}	32.50^{a}	36.75^{a}	5.00^{a}	5.25^{a}
500	...	5.75^{b}	1.25^{c}	0.25^{b}	0.50^{b}	0.50^{b}	0.50^{c}	0.25^{c}	1.00^{b}
		(72.29)	(96.35)	(99.21)	(97.96)	(98.46)	(98.64)	(95)	(80.95)
375		7.75^{b}	4.00^{b}	1.50^{b}	1.50^{b}	1.50^{b}	1.75^{bc}	1.00^{bc}	2.00^{b}
	...	(62.65)	(88.32)	(95.24)	(93.88)	(95.38)	(95.24)	(80)	(61.90)
500		7.00^{b}	4.00^{b}	1.50^{b}	1.50^{b}	2.00^{b}	1.75^{bc}	1.50^{b}	1.50^{b}
	1	(66.26)	(88.32)	(95.24)	(93.88)	(93.85)	(95.24)	(70)	(71.43)
500		8.25^{b}	3.50^{bc}	1.75^{b}	2.00^{b}	1.25^{b}	2.50^{b}	1.25^{bc}	1.75^{b}
	2	(60.24)	(89.78)	(94.44)	(91.84)	(96.16)	(93.16)	(75)	(68.29)
375		6.00^{b}	3.75^{b}	0.50^{b}	0.75^{b}	1.25^{b}	0.75^{c}	0.75^{bc}	2.00^{b}
	1	(71.08)	(89.05)	(98.41)	(96.24)	(96.16)	(97.96)	(85)	(61.90)
375		7.00^{b}	4.50^{b}	1.75^{b}	1.00^{b}	1.50^{b}	1.50^{bc}	1.25^{bc}	2.50^{b}
	2	(66.26)	(86.86)	(94.44)	(95.92)	(95.38)	(95.92)	(75)	(52.38)
LSD value		2.705	2.407	1.976	2.247	2.885	1.615	2.278	2.278

Any two means sharing same letters did not differ significantly at 5% level of probability; Figs. in parentheses showed % decrease over weedy check.

centre, the already known mode of action of herbicide. These results are confirmed by those of Chhokar *et al.* (2006) and Khan *et al.* (2003), who reported that herbicides significantly control narrow and broad leaf weeds of wheat over untreated check.

The addition of adjuvant at both herbicides rates and adjuvant concentrations resulted in statistically similar density of all the weeds under study. Non-significant effect of ammonium sulphate on the efficacy of glyphosate had also been reported by Faircloth *et al.* (2004). The results are however contradictory to those reported by Nadeem *et al.* (2008), who reported significant decrease in weed density with the addition of urea as an adjuvant. The contradiction in results can be attributed to the differences in adjuvant and herbicide used.

Dry weed biomass (g/m^2). Analysis of the data showed that there was a significant effect of carfentrazone-ethyl + clodinafop-propargyl + metsulfuron-methyl alone and with ammonium sulphate as an adjuvant on dry weight of weeds at harvest (Table 1). Maximum dry weight of weeds (86.40 g/m^2) was found in weedy check. The minimum dry weight of weeds (4.95 g/m^2) was found in carfentrazone-ethyl + clodinafop-propargyl + metsulfuron-methyl @ 500 g/ha. Maximum reduction in weeds biomass was due to the herbicide. The differences in weed biomass can be attributed to the differences in weed density due to various treatments. These results are in conformity with those of El-Metwally *et al.* (2010) and Arif *et al.* (2004), who reported that herbicide decreased the fresh and dry weight of weeds as compared to the weedy check. The addition of chemical additive ammonium sulphate did not influence the activity of herbicide compared with herbicide alone. The results appear to contradict the commonly accepted idea that adjuvants enhance herbicide efficacy by increasing its absorption (Zadorozhny, 2004) and can therefore, be used to reduce herbicide input in agricultural ecosystems (Pacanoski, 2010). Results of the present study suggest that ammonium sulphate as an adjuvant should not be relied upon to improve efficiency of carfentrazone-ethyl + clodinafop-propargyl + metsulfuron-methyl. Similar conclusions can also be drawn from O' Sullivum and Bonw (1997), who reported that addition of adjuvants have no significant effect on weed control in maize.

Yield components of wheat. The statistical analysis of the data showed that there was significant effect of carfentrazone-ethyl+clodinafop-propargyl+metsulfuron-methyl alone and with ammonium sulphate as an adjuvant on spike bearing tillers, number of grains per spike and 1000 grain weight (Table 3). Maximum values of these components (364.50 m^{-2}, 46.25, 46.94 g, respectively) were recorded in carfentrazone-ethyl + clodinafop-propargyl + metsulfuron-methyl @ 500 g/ha and was followed by the application of carfentrazone-ethyl + clodinafop-propargyl + metsulfuron-methyl @ 375 & 500 g/ha along with adjuvant. These results are confirmed by those of Khan *et al*. (2008) who observed that better weed control increased the nutrients availability to the crop which ultimately increased the spike bearing tillers of wheat. Nadeem *et al*. (2008) stated that number of grains per spike of wheat increased as a result of postemergence herbicide application. The increase in 1000 grain weight was possibly due to better growth and development of crop plants which resulted in more grain weight assimilation. These results are in conformity by those of Naseer-ud-Din *et al.* (2011) who observed significantly, higher 1000 grain weight with chemical weed control in wheat.

Table 3. Effect of herbicide and ammonium sulphate as an adjuvant on yield and yield parameters of wheat

Treatments		Parameters			
Herbicide Carfentrazone-ethyl + clodinafop-propargyl + metsulfuron-methyl (g/ha)	Adjuvant NH_2SO_4 (%)	Spike bearing tillers (m^{-2})	No. of grains per spike	1000-grain weight (g)	Grain yield (t/ha)
Weedy check	...	297.25^{d}	38.00^{d}	39.15^{d}	3.50^{d}
500		364.50^{a}	46.25^{a}	46.94^{a}	4.82^{a}
	...				(37.71)
375	...	328.50^{bc}	41.50^{c}	41.87^{c}	4.23^{bc}
					(20.86)
500		321.50^{c}	42.75^{bc}	42.71^{bc}	4.16^{bc}
	1				(18.86)
500		323.50^{c}	41.75^{c}	42.42^{c}	4.09^{bc}
	2				(16.85)
375		342.25^{b}	44.00^{b}	44.04^{b}	4.44^{b}
	1				(26.85)
375		320.50^{c}	41.75^{c}	41.75^{c}	4.06^{c}
	2				(16)
LSD value		18.461	2.142	1.578	0.356

Any two means sharing same letters did not differ significantly at 5% level of probability; Figs. in parentheses showed % increase over weedy check.

The addition of adjuvant had no effect on the control of individual weeds and non-significant effect can be attributed to the dose of the adjuvant with the herbicide. The adjuvants are more effective at lower concentration and higher concentration may result in no or even toxic effect (Tu *et al.,* 2001). The results are supported by the findings of O' Sullivum and Bonw (1997), who reported that addition of adjuvants had no significant effect on yield components of maize.

Grain yield/ha). Grain yield is a function of interaction of various yield components such as number of fertile tillers per unit area, number of grains per spike and 1000 grain weight. The data given in Table 3 showed that carfentrazone-ethyl + clodinafop-propargyl + metsulfuron-methyl at various rates with ammonium sulphate as an adjuvant showed significant effect on grain yield. Maximum grain yield (4.82 t/ha) was recorded in carfentrazone-ethyl + clodinafop-propargyl + metsulfuron-methyl @ 500 g/ha, which was 37.71 % more than weedy check. The minimum grain yield (3.50 t/ha) was recorded in weedy check. Higher grain yield resulted from more number of spike bearing tillers, number of grains per spike and 1000 grain weight. These results are confirmed by El-Metwally *et al.* (2010) and Singh *et al.* (2006), who reported that application of postemergence herbicides increases the grain yield of wheat. The nonsignificant differences with the addition of ammonium sulphate as an adjuvant are supported by the findings of Faircloth *et al.* (2004). The results are however, in contrast with those of Nadeem *et al.* (2008) who reported significant increase in grain yield of maize with the use of urea as an adjuvant. The differences in results can be attributed to the different types of herbicides, adjuvants and crops used in the studies.

Conclusion

It can be concluded that carfentrazone-ethyl + clodinafop-propargyl + metsulfuron-methyl @ 500 g/ha with no supplemental adjuvant performed best in reducing weeds density, biomass and increasing grain yield and yield components of wheat. Based on present findings it is recommended that ammonium sulphate should not be used as an adjuvant with carfentrazone-ethyl + clodinafop-propargyl + metsulfuron-methyl.

References

Arif, M., Awan, I.U., Khan, H.H.U. 2004. Weed management strategies in wheat (*Triticum aestivum* L.). *Pakistan Journal of Weed Science Research,* **10:** 11-16.

Ashiq, M., Cheema, Z.A. 2005. *Effective Use of Herbicides,* Weed Science Allelopathy Laboratory, University of Agriculture, Faisalabad, Pakistan.

Bradley, P.R., Johnson W.G., Smeda R.J. 2000. Response of sorghum (*Sorghum bicolor*) to atrazine, ammonium sulphate and glyphosate. *Weed Technology,* **14:** 15-18.

Chhokar, R.S., Sharma, R.K., Chauhan, D.S., Mongia, A.D. 2006. Evaluation of herbicides against *Phalaris minor* in wheat in north-western Indian plains. *Weed Research,* **46:** 40-49.

El-Metwally, I.M., Abd El-Salam, M.S., Tagour, R.M. H. 2010. Nitrogen fertilizer levels and some weed control treatments effects on barley and associated weeds. *Agricultural and Biological Journal of North America,* **1:** 992-1000.

Faircloth, W.H., Monks, C.D., Patterson, M.G., Wentze, G.R., Delaney, D.P, Sanders, J.C. 2004. Cotton and weed response to glyphosate application with sulfur containing additives. *Weed Technology,* **18:** 404-411.

Hatzios, K.K., Penner, D. 1985. Interactions of herbicides with other agrochemicals in higher plants. *Reviews of Weed Science,* **1:** 1-63.

Khan, IA., Hassan, G., Marwat, K.B., Daur, I. 2008. Efficacy of some pre and post emergence herbicides on yield and yield components of canola. *Pakistan Journal of Botany,* **40:** 1943-1947.

Khan, M.H., Hassan, G., Khan, N., Khan, M.A. 2003. Efficacy of different herbicides for controlling broadleaf weeds in wheat. *Asian Journal of Plant Sciences,* **2:** 254-256.

Kumar, S., Angiras, N.N., Rana, S.S. 2011. Bio-efficacy of clodinafop-propargyl + metsulfuron methyl against complex weed flora in wheat. *Indian Journal of Weed Science,* **43:** 195-198.

Marwat, M.I., Ahmad, H.K., Marwat, K.B., Hassan, G. 2003. Integrated weed management in wheat-III tillers M^{-2}, productive tiller M^{-2}, spikelets spike, grain $spike^{-1}$, 1000 grain weight and grain yield. *Pakistan Journal of Weed Science Research,* **9:** 23-31.

Naseer-ud-Din, G.M., Shehzad, M.A., Nasrullah, H.M. 2011. Efficacy of various pre and post-emergence herbicides to control weeds in wheat. *Pakistan Journal of Agricultural Sciences,* **48:** 185-190.

Nadeem, M.A., Ahmad, R., Khalid, M., Naveed, M., Tanveer, A., Ahmad, J.N. 2008. Growth and yield response of autumn planted maize (*Zea mays* L.) and its weeds to reduced doses of herbicide application in combination with urea. *Pakistan*

Journal of Botany, **40:** 667-676.

Pacanoski, Z. 2010. Role of adjuvants on herbicide behavior: a review of different experiences. *Herbologia,* **11:** 67-79.

O' Sullivan, J., Bonw, W.J. 1997. Effects of timing and adjuvants on the efficacy of reduced herbicides rates for sweet corn (*Zea mays*). *Weed Technology,* **11:** 486-489.

Rehman, A., Cheema, Z.A., Khaliq, A., Arshad, M., Mohsan, S. 2010. Application of sorghum, sunflower and rice water extract combinations helps in reducing herbicide dose for weed management in rice. *International Journal of Agriculture and Biology,* **12:** 901-906.

Shehzad, M.A., Nadeem, M.A., Iqbal, M. 2012. Weed control and yield attributes against post-emergence herbicides application in wheat crop, Punjab, Pakistan. *Global Advanced Research Journal of Agricultural Science,* **1:** 007-016.

Singh, R., Dhiman, S., Rana, N.S., Kumar, S., Singh, V.K., Singh, R.G. 2006. Efficacy of dicamba alone and in combination with isoproturon on wheat (*Triticum aestivum* L.) and associated weeds. *Indian Journal of Agronomy,* **51:** 139-141.

Steel, R.G.D.,Torrie, J.H., Dickey, D. 1997. *Principles and Procedures of Statistics: A Biometrical Approach,* pp.172-177, 3rd edition, McGraw Hill Book Co. Inc., New York, USA.

Tanveer, A., Ali, A. 2003. *Weeds and Their Control,* pp. 162, Higher Education Commission, Islamabad, Pakistan, Press Manager, H-9, Islamabad, Pakistan.

Tu, M., Hurd, C., Randall, J.M. 2001. *The Nature Conservancy, Weed Control Methods Handbook: Tools and Techniques for Use in Natural Areas,* 533 pp.,Wild and Invasive Species Team, Centre for Invasive Species and Ecosystem Health, University of Gorgia, USA.

Zadorozhny, V. 2004. Herbicide based strategies for maize to prevent development of resistance in weeds in Ukraine. In: *Proceedings of 14th Australian Weed Conference, Weed Management: Balancing People, Planet, Profit,* B. M. Sindel and S. B. Johnson (eds.), pp. 290-293, Wagga, Wagga, New South Wales Australia.

Pak. j. sci. ind. res. Ser. B: biol. sci. 2014 **57** (3) 123-128

Enhanced Amylase Production by *Fusarium solani* in Solid State Fermentation

Yasser Bakri*, Mohammed Jawhar and Mohammed Imad Eddin Arabi
Department of Molecular Biology and Biotechnology, AECS, PO Box 6091, Damascus, Syria

(received January 29, 2014; revised May 7, 2014; accepted July 15, 2014)

Abstract. The present study illustrates the investigation carried out on the production of amylase by *Fusarium* species under solid state fermentation. All the tested *Fusarium* species were capable of producing amylase. A selected *F. solani* isolate SY7, showed the highest amylase production in solid state fermentation. Different substrates were screened for enzyme production. Among the several agronomic wastes, wheat bran supported the highest yield of amylase (141.18 U/g of dry substrate) after 3 days of incubation. Optimisation of the physical parameters revealed the optimum pH, temperature and moisture level for amylase production by the isolate as 8.0, 25 °C and 70%, respectively. The above results indicate that the production of amylase by *F. solani* isolate SY7 could be improved by a further optimisation of the medium and culture conditions.

Keywords: agro-industrial wastes, α-amylase, *Fusarium* spp., solid state fermentation

Introduction

α-amylase (EC 3.2.1.1, a-1,4-glucan-4-glucano-hydrolase), is an extra cellular enzyme, which catalyses the endocleavage of the a-1,4-glycoside linkages and the release of short oligosaccharides and a-limit dextrin. This enzyme is used commercially for the production of sugar syrups from starch, which consist of glucose, maltose, and higher oligosaccharides (Reddy *et al*., 2003). It is also extensively used in starch of liquefaction and paper, food, pharmaceutical and sugar industries. Although, amylases can be obtained from several sources, such as plants and animals, the enzymes from microbial sources generally meet industrial demand (Nwagu and Okolo, 2011; Pandey *et al*., 2001).

Fungal amylases are preferred to plant enzymes due to their short growth period, higher productivity and thermostability (Mishra *et al*., 2008). However, fungal growth and amylase production are dependent on growth conditions, such as type and concentration of carbon and nitrogen sources, metal ion requirement, pH and temperature of growth (Ghasemi *et al*., 2010; Cherry *et al*., 2004). Though many microorganisms can grow on a wide range of carbon and nitrogen sources, it is economically more viable to utilise the cheap and easily available resources as substrates for amylase production (de Castro and Sato, 2013; Pandey *et al*., 2001). Industrial enzymatic hydrolysis is influenced by a number of factors amongst which are environmental conditions of pH, temperature and presence of metal ion (Riaz *et al*., 2007).

Fungal amylase is preferred for use in formulation for human or animal consumption involving application under acidic condition and around 37 °C. Studies on fungal amylase especially in the developing countries have concentrated mainly on filamentous fungi probably because of the ubiquitous nature and non-fastidious nutritional requirements of these organisms (Padmini *et al*., 2012; Guimaraes *et al*., 2006).

Fusarium is a large genus of filamentous fungi, and most of *Fusarium* species are harmless saprobes and relatively abundant members of the soil microbial community (Alazem, 2007; Summerell *et al*., 2001; Onyika *et al*., 1993). These species have the ability to produce different enzymes under fermentation conditions (Kikot *et al*., 2010; Pekkarinen *et al*., 2000). Cereal grains showed cavities and furrows in endosperm of starch granules, evidencing damage caused by amylases (Jackowiak *et al*., 2002). This ecological habitat of the fungus, however, implies that *Fusarium* would be a useful resource of extracellular enzymes. Solid state fermentation (SSF) is widely established for the production of enzymes from filamentous fungi (Zaferanloo *et al*., 2014; de Castro and Sato, 2013). Morphology and physiology of these molds enable them to penetrate and colonise various solid substrates (Vijayaraghavan *et al*., 2011). SSF utilises various agroindustrial wastes as substrate that acts both physical support and source

*Author for correspondence; E-mail: ascientific@aec.org.sy

of nutrients (Pandey *et al*., 2001). In addition, the use of SSF for enzyme production has many advantages over submerged fermentation due to its simple technique, low capital investment, lower levels of catabolite repression and better product recovery (Considine *et al*., 1989). However, filamentous fungi are the best adapted for SSF. The hyphal mode of fungal growth and their good tolerance to low water activity and high osmotic pressure conditions make fungi efficient and competitive in natural microflora for bioconversion of solid substrates (Padmini *et al*., 2012; Pandey *et al*., 2001).

The objectives of the present study were, (i) to investigate the ability of *Fusarium* species to produce amylase, and (ii) to find out optimum condition for enzyme production under solid state fermentation.

Materials and Methods

Microorganism. *Fusarium* spp., isolates were obtained from wheat seeds showing disease symptoms from different locations in Syria. They were identified morphologically according to Nelson *et al*. (1983). Emphasis was placed on selecting isolates that induce differential reactions on specific wheat genotypes (Alazem, 2007), leading to the selection of 21 monosporic isolates (eight belonging to *F. culmorum*, six to *F. verticillioides*, four to *F. solani* and three to *F. equiseti*) used in this study (Table 1). The cultures were maintained on silica gel at 4 °C until needed.

Optimisation of cultural conditions. Enzyme production was carried out as reported by Bakri *et al*. (2008). The fermentation medium consisted of: (g/L) $Na_2HPO_4.2H_2O$ 10; KCl 0.5; $MgSO_4.7H_2O$ 0.15, and yeast extract 5, as a nitrogen source. The influences of different lignocellulosic materials *viz*., wheat bran, wheat straw, corn cobs hulls, soya cake and cotton seed cake on amylase production were tested (Fig. 1). Fresh fungal spores were used as inoculum and 1 mL spore suspension (containing around 10^6 spores/mL) was added to sterilised medium and incubated at 30 °C for 5 days. Various physical parameters such as pH (4, 5, 6, 7, 8 and 9), temperature (20, 25, 30, 35, 40 and 45 °C) and moisture level (50, 55, 60, 65, 70, 75, 80, 85 and 90%) were optimised by conventional methods for maximal enzyme production.

Extraction of amylase. Flasks were removed after cultivation and the enzyme was extracted by adding 25 mL of 0.1 M phosphate buffer (pH 5) to the cultures.

Table 1. Amylase production by *Fusarium* spp., in solid state fermentation

Isolate	Amylase (U/g)
F. culmorum	
SY1	45.5f
2	55.36d
3	54.6d
4	51.13e
6	76.13b
12	52.00e
13	40.40g
14	66.80c
F. verticillioides	
SY5	47.40f
9	51.40e
10	44.80f
15	54.30d
16	70.95bc
17	39.25g
F. solani	
SY7	118.35a
8	38.70g
11	43.50f
20	41.70f
F. equiseti	
SY22	33.60h
23	45.10f
24	58.80d

Values within a column followed by different letters are significantly different at P<0.001 according to Newman-Keuls test.

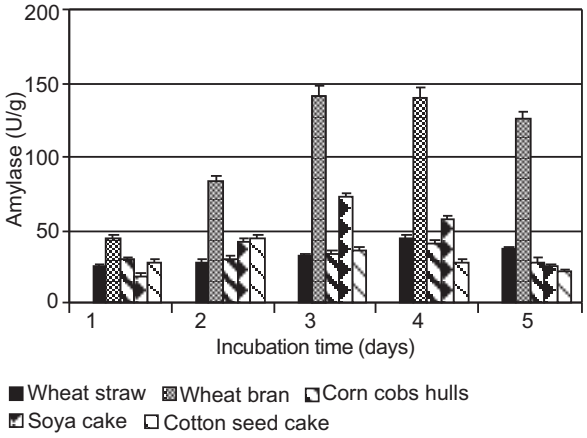


Fig. 1. Effect of lignocellulosic materials (wheat straw, wheat bran, soya cake and cotton seed cake) and incubation time on amylase production by *Fusarium solani* SY7.

The mixtures were shaken for 1.5 h on a magnetic stirrer. The supernatant was obtained by centrifugation (8000 × g for 15 min) followed by filtration through Whatman No. 1 filter paper and the filtrate was used as a crude enzyme preparation.

Assay of amylase: α-amylase activity was determined as described by Okolo *et al*. (2001). Reaction mixture contained: 1% soluble starch, 1.25 mL; 0.1 M acetate buffer (pH 5.0), 0.25 mL; and appropriately diluted crude enzyme extract, 0.25 mL. After 10 min of incubation at 50 °C, liberated reducing sugars (glucose equivalent) were estimated by the dinitrosalicylic acid method of Miller (1959). One unit (IU) of α-amylase is defined as the amount of enzyme that releases 1 μmol of glucose equivalent per min under the assay conditions and enzyme activity is expressed in terms of IU per gram dry fermented substrate.

Statistical analysis. The experiments were repeated twice and the means were analysed statistically with the analysis of variance (STAT-ICTF, 1988) with used to test for differences in amylase production among *Fusarium* isolates.

Results and Discussion

Amylase production from *Fusarium* species. The results showed that all the *Fusarium* species were capable of producing amylase. Significant differences ($P<0.001$) in the mean yield values were detected among isolates, with values being consistently higher in the isolates *F. solani* SY7 and *F. culmorum* SY6 (mean value 118.35 and 76.13 IU/g, respectively), whereas, low enzyme activity of 33.6 and 39.25 IU/g were detected for *F.equiseti* SY 22 and *F. verticillioides* SY17, respectively (Table 1). From this collection, *F. solani* SY7 isolate was selected for further studies.

Influence of some wastes on amylase production by *F. solani* SY7. Figure 1 shows that the highest amylase production (141.18 IU/g) was obtained on wheat bran after 3 days of incubation, whereas, soya cake and cotton seed cake exhibited low amylase production. These results might be attributed to the fact that the presence of readily available substrates has been noted to influence the biosynthesis of many extracellular enzymes *via* catabolite repression mechanism (Vijayaraghavan *et al*., 2011; Teodoro and Martins, 2000). Wheat bran was found to be the best substrate for α-amylase production by a thermophilic fungus *Humicola lanuginose* (Singh *et al*., 2009).

Influence of initial pH on amylase production by *F. solani* SY7. Since microorganisms are sensitive to the concentration of hydrogen ions present in the medium, pH is considered an important factor that determines the growth, morphology and product formation (Weiland, 1988). Figure 2 demonstrates that α-amylase production was significant over a wide range of pH values and was maximum at pH 8.0, which indicates that the selected isolate prefers alkaline conditions for better enzyme production. This was a rare occurrence because most fungal amylase required slightly acidic pH (4.5-6.0) (Okolo *et al*., 2001). Alva *et al.* (2007) observed two peaks optima in amylase production at initial pH 5.8 and 9.0 by *Aspergillus* sp., JGI 12. On the other hand, amylase production by *A. flavus* isolate FSS 60 was found to be best at pH 9.0 (Bakri *et al*., 2009). Thus, development of an optimal pH control strategy is helpful in obtaining higher enzyme productivity by the fungal strains.

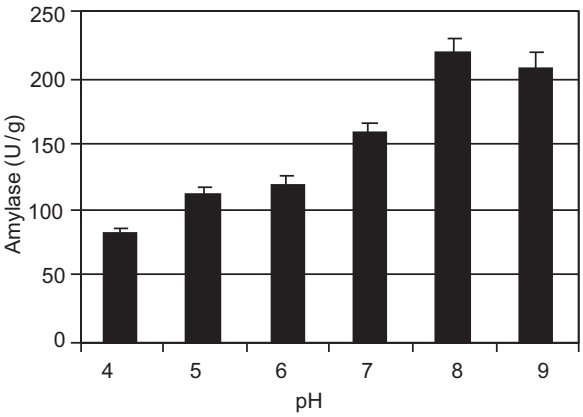


Fig. 2. Effect of pH degree on amylase produced by *Fusarium solani* SY7 grown on wheat bran under solid culture.

Influence of initial temperature on amylase production. Among the fungi, most amylase production studies have been conducted with mesophilic fungi within a range of temperature (25-37 °C) (Pandey *et al*., 2001). Figure 3 demonstrates the effect of temperature on α-amylase production, where the optimum temperature for maximum α-amylase production was 25 °C. Decrease in enzyme yield at lower or elevated temperatures resulted from the reduced metabolic activity and impaired action of the cell membrane of the fungus. The influence of temperature on the production of crude amylase showed that enzyme production decreased progressively with

increase in temperature (Fig. 3). Above 25 °C, there was a reduction in the amylase production. It is reported that the best amylase production in *A.niger* is at room temperature (Varalakshmi *et al*., 2009) and reported 30 °C be the best for amylase production by *Penicillium fellutanum* and *A. flavus*, respectively (Hernández *et al*., 2006; Okolo *et al*., 2001).

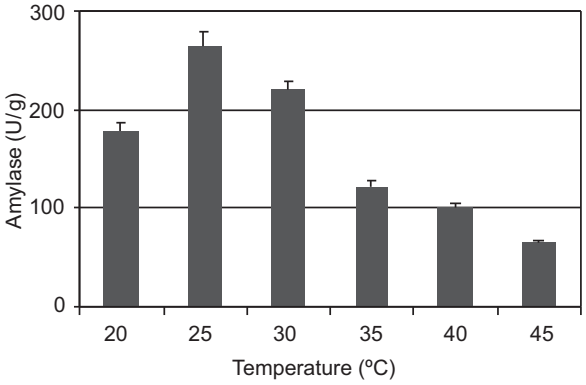


Fig. 3. Effect of temperature on amylase production by *Fusarium solani* SY7.

Influence of moisture level on amylase production. The results showed that the moisture level at 70% yielded the highest amylase production (Fig. 4). The critical importance of moisture level in SSF media and its influence on the biosynthesis of enzymes has been attributed to the interference of moisture in the physical properties of solid particles. Sodhi *et al*. (2005) reported that higher moisture level decreases porosity, changes wheat bran particle structure, promotes development of stickiness, reduces gas volume and exchange and decreases diffusion, which results in lowered oxygen transfer and reduction in enzyme production. On the other hand, lower moisture content reduces the solubility of nutrients present in solid substrate, decreases the degree of swelling and increases water tension (Ramachandran *et al*., 2004). Pandey *et al*. (1994) reported that with low water availability fungi suffer modification in their cell membranes leading to transport limitations and affecting microbial metabolism. Based on the present results, moisture at 70% seems to result in a compromise among water availability, substrate swelling and oxygen diffusion effect, favouring amylase production by *F. solani* SY7.

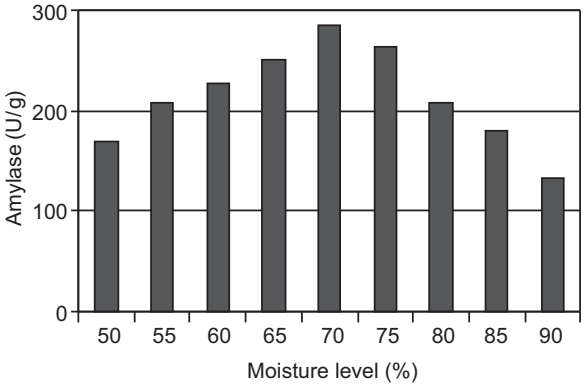


Fig. 4. Effect of moisture (%) on amylase production by *Fusarium solani* SY7.

Conclusion

The present study reveals that *F. solani* SY7 isolate proved to be an efficient producer of α-amylase under improved conditions. Wheat bran could be used as a less expensive substrate for efficient amylase production (141.18 U/g) after 3 days of incubation. The culture conditions can easily be modified to enhance the productivity of the enzyme formation that will facilitate the scale-up processes for biomass production.

References

Alazem, M. 2007. Characterization of Syrian *Fusarium* Species by Cultural Characteristics and Aggressiveness, *M.Sc. Thesis*, pp. 1-72, Faculty of Agriculture, University of Damascus, Syria.

Alva, S., Anumpama, J., Savla, J., Chiu, Y.Y., Vyshali, P., Shruti, M., Yogeetha, B.S., Bhavya, D., Purvi, J., Ruchi, K., Kumudini, B.S., Varalakshmi, K.N. 2007. Production and characterization of fungal amylase enzyme isolated from *Aspergillus* sp., JGI 12 in solid state culture. *African Journal of Biotechnology*, **6:** 576-581.

Bakri, Y., Magali, M., Thonart, P. 2009. Isolation and identification of a new fungal strain for amylase biosynthesis. *Polish Journal of Microbiology*, **58:** 269-273.

Bakri, Y., Jawhar, M., Arabi, M.I.E. 2008. Improvement of xylanase production by *Chochliobolus sativus* in solid state fermentation. *Brazilian Journal of Microbiology*, **39:** 602-604.

Cherry, H.M., Towhid, H., Md., Anwar, M.N. 2004. Extracellular glucoamylase from the isolate *Aspergillus fumigatus. Pakistan Journal of Biological Sciences*, **7:** 1988-1992.

Considine, P.J., Buckley, R.J., Griffin, T.O., Tuohy, M.G., Coughlan, M.P. 1989. A simple and inexpensive method of solid-state cultivation. *Biotechnology and Technology*, **3:** 85-90.

de Castro, R.J.S., Sato, H.H. 2013. Synergistic effects of agroindustrial wastes on simultaneous production of protease and α-amylase under solid state fermentation using a simplex centroid mixture design. *Industrial Crops and Products*, **49:** 813-821.

Ghasemi, Y., Rasoul-Ami, S., Ebrahimine, A., Zarrini, G., Kazemi, A., Mousavi-Khorshidi, S., Ghoshoon, M.B., Raee, M.J. 2010. Halotolerant amylase production by a novel bacterial strain, *Rheinheimera aquimaris*. *Research Journal of Microbiology*, **5:** 144-149.

Guimaraes, L.H.S., Nogueira, C.S.P., Michelin, M., Rizzatti, A.C.S., Sandrim, V.C., Zanoela, F.F., Aquino, A.C.M.M., Junior, A.B., Polizeli, M.L.T.M. 2006. Screening of filamentous fungi for production of enzymes of biotechnological interest. *Brazilian Journal of Microbiology*, **37:** 474-480.

Hernández, M.S., Rodríguez, M.R., Guerra, N.P., Rosés, R.P. 2006. Amylase production by *Aspergillus niger* in submerged cultivation on two wastes from food industries. *Journal of Food Engineering*, **73:** 93-100.

Jackowiak, H., Packa, D., Wiwart, M., Perkowski, J., Busko, M., Borusiewicz, A. 2002. Scanning electron microscopy of mature wheat kernels infected with *Fusarium culmorum*. *Journal of Applied Genetics*, **43:** 167-176.

Kikot, G.E., Hours, R.A., Alconada, T.M. 2010. Extracellular enzymes of *Fusarium graminearum* isolates. *Brazilian Archives of Biology and Technology*, **53:** 779-783.

Miller, G.L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugars. *Analytical Chemistry*, **31:** 426-428.

Mishra, S., Behera, N. 2008. Amylase activity of a starch degrading bacteria isolated from soil receiving kitchen wastes. *African Journal of Biotechnology*, **7:** 3326-3331.

Nelson, P.E., Toussoun, T.A., Marasas, W.F.O. 1983. *Fusarium Species: An Illustrated Manual for Identification*, The Pennsylvania State University Press, University Park, PA, USA.

Nwagu, T.N., Okolo, B.N. 2011. Extracellular amylase production of a thermotolerant *Fusarium* sp. isolated from Eastern Nigerian soil. *Brazilian Archives of Biology and Technology*, **54:** 649-658.

Okolo, B.N., Ire, S., Ezeogu, L.I., Anyanwu, C.U., Odibo, F.J.C. 2001. Purification and some properties of a novel raw starch-digesting amylase from *Aspergillus carbonarius*. *Journal of the Science, Food and Agriculture*, **81:** 329-336.

Onyika, N.B.N., Nelson, P.E. 1993. The distribution of *Fusarium* species in soils planted to millet and sorghum in Lesotho, Nigeria and Zimbabwe. *Mycopathologia*, **121:** 105-114.

Padmini, D.N., Bhattacharya, S., Das, A., Rajan, S.S. 2012. Solid-state fermentation and characterization of α-amylase from a rhizospheric isolate of *Aspergillus flavus* associated with *Mangifera indica*. *Annals of Biological Research*, **3:** 4082-4090.

Pandey, A., Soccol, C.R., Rodriguez-Leon, J., Nigam, P. 2001. *Solid State Fermentation in Biotechnology*, Asiatech Publishers, Inc., 221 pp., New Delhi, India.

Pandey, A., Ashakumary, L., Selvakumar, P., Vijayalakshmi, K.S. 1994. Influence of water activity on growth and activity of *A. niger* for glucoamylase production in solid state fermentation. *World Journal of Microbiology and Biotechnology*, **10:** 485-486.

Pekkarinen, A., Mannonen, L., Jones, B.L., Niku-Paavola, M.L. 2000. Production of proteases by *Fusarium* species grown on barley grains and in media containing cereal proteins. *Journal of Cereal Science*, **31:** 253-261.

Ramachandran, S., Patel, A.K., Nampoothiri, K.M., Chandran, S., Szakacs, G., Soccol, C.R., Pandey, A. 2004. Alpha amylase from a fungal culture grown on oil cakes and its properties. *Brazilian Archives of Biology and Technology*, **47:** 309-317.

Reddy, N.S., Nimmagadda, A., Sambasiva Rao, K.R.S. 2003. An overview of the microbial α-amylase family. *African Journal of Biotechnology*, **2:** 645-648.

Riaz, M., Perveen, R., Javed, M.R., Nadeem, H., Rashid, M.H. 2007. Kinetic and thermodynamic properties of novel glucoamylase from *Humicola* sp. *Enzyme and Microbial Technology*, **41:** 558-564.

Singh, R.K., Kumar, S., Kumar, S. 2009. Production of α-amylase from agricultural byproducts by *Humicola lanuginosa* in solid-state fermentation. *Current Trends in Biotechnology and Pharmacy*, **3:** 172-180.

Sodhi, H.K., Sharma, K., Gupta, J.K., Soni, S.K. 2005. Production of a thermostable α-amylase from *Bacillus sp*. PS-7 by solid state fermentation and its synergistic use in the hydrolysis of malt starch for alcohol production. *Process Biochemistry*, **40:** 525-534.

STAT-ITCF, 1988. *Manuel d'utilisation*, Institute

Techniques des Cereales et des Fourrages 210 pp., Services des Etudes Statistiques, Boiyneville, France.

Summerell, B.A., Leslie, J.F., Backhouse, D., Bryden, W.L., Burgess, L.W. 2001. *Fusarium*: *Paul E. Nelson Memorial Symposium*, 388 pp., 1st edition, American Phytopathological Society Press, St. Paul Minnesota, USA.

Teodoro, C.E.D., Martins, M.L.L. 2000. Culture conditions for the production of thermostable amylase by *Bacillus* sp. *Brazilian Journal of Microbiology*, **31:** 298-302.

Varalakshmi, K.N., Kumudini, B.S., Nandini, B.N., Solomon, J., Suhas, R., Mahesh, B., Kavitha, A.P. 2009. Production and characterization of alpha amylase from *Aspergillus niger* JGI 24 isolated in Bangalore. *Polish Journal of Microbiology*, **58:** 29-36.

Vijayaraghavan, P., Remya, C.S., Prakash, Vincent, S.G. 2011. Production of α-amylase by *Rhizopus microsporous* using agricultural by-product in solid state fermentation. *Research Journal of Microbiology*, **6:** 366-375.

Weiland, P. 1988. Principles of solid state fermentation. In: *Treatment of Lignocellulosics with White Rot Fungi*, F. Zadrazil and R. Reiniger (eds.), pp. 64-66, Elsevier Applied Science, London, UK.

Zaferanloo, B., Bhattacharjee, S., Ghorbani, M.M., Mahon, P.J., Palombo, E.A. 2014. Amylase production by *Preussia minima*, a fungus of endophytic origin: optimization of fermentation conditions and analysis of fungal secretome by LC-MS. *BMC Microbiology*, **14:** 55. http://www.biomedcentral.com/1471-2180/14/15

Pak. j. sci. ind. res. Ser. B: biol. sci. 2014 **57**(3) 129-135

Nutritional Evaluation of Nigerian Dried Okra (*Abelmoschus esculentus*) Seeds

Henry Niyi Ogungbenle* and Esther Funmilayo Arekemase
Department of Chemistry, Ekiti State University, P.M.B. 5363, Ado-Ekiti, Nigeria

(received January 16, 2013; revised October 9, 2013; accepted October 10, 2013)

Abstract. The proximate, functional properties, *in-vitro* multi enzyme protein digestibility and amino acid compositions of Nigerian dried okra (*Abelmoschus esculentus*) seeds were evaluated. The ash, moisture, fat, crude fibre, crude protein and carbohydrate of the okra seeds were: 4.8%, 13.5%, 39.9%, 8.82%, 26.4% and 6.62%, respectively. The water and oil absorption capacities were: 220% and 200% which makes okra seeds exhibit a high water retention capacity. The least gelation concentration was 8% while, emulsion capacity was 45.5% and foaming capacity and stability were: 12% and 2%, respectively. The *in vitro* protein digestibility was 69.3%. Glutamic acid was the most concentrated amino acid (121.1 mg/g crude protein) while, cystine (10.0 mg/g crude protein) was the least concentrated amino acid. The total amino acid was 706 mg/g crude protein.

Keywords: functional properties, digestibility, amino acid, okra seeds

Introduction

Okra (*Abelmoschus esculentus*) is a popular and important vegetable in tropical and warm temperate regions of the world. It belongs to the mallow family and contains a sticky mucilage that helps to improve digestion and may be used in the treatment of gastritis, gastric ulcer and liver/gal bladder cleansing (Daniluk, 2012). Okra is originated from tropical Africa and Asia (Kochhar, 1986) and it is widely cultivated and commonly consumed non-leafy vegetable which provides essential minerals, β carotene and vitamin B_6 required for the body growth, vision and maintenance of skin (Daniluk, 2012; Choudhury, 1977). The fruit when freshly harvested and washed can be eaten raw without cooking especially during traditional naming ceremony among 'yorubas' in south west of Nigeria. The west African okra is an annual or biannual crop which does not grow much taller than about 2 m with few tender branches. The *A. esculentus* is highly polymorphic species and the stem is woody and hairy when mature (Kochhar, 1986). The leaves alternate up to 30 cm in length, 3-5 lobes. The fruit is greenish in colour, hairy, somewhat spiny, round and short pointed pyramidal shape. It is about 10-25 cm in length and 2-3 cm in diameter. The number of seeds in a pod vary from about 20 to 150, depending on the fruit size. The seeds are 4-5 mm in diameter, dark green to grey-black, rounded with conspicuous caruncle (Irvine,1969). Okra thrives in a well drained sandy loam with a pH of 6-6.8 and is moderately tolerant to salinity and usually adapts to high temperatures, but heat coupled with low humidity slows down its growth considerably (Irvine, 1969). Okra seed is a good source for extracting greenish yellow oil useful for both domestic and industrial purposes. It also have so many medicinal uses apart from its nutritional values. These include: elimination of toxins and excess cholesterol from the body, reduction of colorectal cancer (Daniluk, 2012). Bile largely soaks up acids and toxins from the body due to fairly high fibre content during the process of digestion, the soluble pectin-based mucilaginous fibre in okra binds with this toxic bile and allows it to be eliminated quickly. If these toxic materials stay in the bowel too long may be reabsorbed causing inflammation thereby leading to bowel disease. This same process eliminates excess cholesterol from the body thereby lowering serum cholesterol levels and reducing the risk of heart disease. It helps to maintain body immune system functioning and blood sugar regulation. The significance of the present study is to determine the proximate, functional properties, *in vitro* protein digestibility and amino acids of okra seeds. The data generated would provide new nutritional literature.

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

Materials and Methods

The dried okra pod samples were purchased from Oja Oba Market in Ado-Ekiti, Ekiti State, Nigeria. Seeds were separated from the pods then sun dried for 5 days and blended into flour. The flour was packaged into air tight polyethylene bag for further analyses.

The proximate analysis of the sample for total ash, moisture, crude fibre and ether extract were carried out using the method described by AOAC (2005). The nitrogen content was determined by micro Kjedahl method described by Pearson (1976) and nitrogen content was converted to protein by multiplying by a factor 6.25. The carbohydrate content was determined by method of difference:

% carbohydrate = [100 - (% moisture + % ash + % crude fibre + % crude fat + % crude protein). All determinations were done in triplicates.

The method of Sathe *et al*. (1982) was used to determine gelation property with slight modification. The water and oil absorption capacities of the sample were determined as described by Beuchat (1977). The emulsion capacity and stability were determined by the method of Inklaar and Fortuin (1969) while, foaming capacity and stability were determined by method of Coffmann and Garcia (1977). The protein solubility as a function of pH was determined by method described by AOAC (2005). The graph of protein solubility (%) against pH was plotted using the data obtained. The multi enzyme digestibility was determined using the method of Hsu *et al*. (1977). Aqueous suspension 50 mL of the sample (6.25 mg sample per mL) in distilled water was adjusted to pH 8.0 with 0.1 M HCl and/or NaOH, while stirring in a 37 °C water bath. The multi enzyme solution (1.6 mg trypsin, 3.1 mg chymotrypsin and 1.3 mg peptidase per mL) was maintained in an ice bath and adjusted to pH 8.0 with 0.1 M HCl/ or NaOH. 5 mL of the protein multi enzyme solution was then added to the protein suspension which was being stirred at 37 °C. A rapid decline in pH occurred immediately. The pH drop was recorded automatically over a 10 min period and 15 min using a pH meter. The multi enzyme digestibility was calculated using the regression equation of Hsu *et al*. (1977) as follows:

$$Y = 210.46 – 18.10x$$

where:

Y = *in vitro* digestibility (%), x = pH of the sample suspension after 10 and 15 min digestion with the multi enzyme solution.

The amino acid profile was determined using the method described by Spackman *et al*. (1958). The sample was dried to constant weight, defatted using Soxhlet extractor and hydrolysed in sealed glass ampoule at 105 °C ± 5 °C for 22 h using 7 mL of 6 M HCl. The hydrolysate was evaporated in a rotary evaporator and loaded into the Technicon sequential multi sample amino acid analyser (TSM, Taryton, USA).

Estimation of isoelectric point (Pi). The estimation of the isoelectric point (PI) for a mixture of amino acids was calculated using the equation below:

$$IPm = \Sigma IPiXi$$

where:

IPm = the isoelectric point of the i^{th} amino acid in the mixture and Xi = the mass or mole fraction of the i^{th} amino acid in the mixture (Olaofe and Akintayo, 2000).

Estimation of quality of dietary protein. Total amino acid scores were calculated based on the whole hen's egg amino acid profile (Paul *et al*., 1976), while, the essential amino acid scores were calculated using the following (FAO/WHO, 1973):

Amino acid score = amount of amino acid per test protein [mg/g] amount of amino acid per protein in reference pattern [mg/g].

Predicted protein efficiency ratio (P-PER*) was determined using one of the equations developed by Alsmeyer *et al*. (1974) as follows:

$$P\text{-}PER^* = - 0.468 + 0.454(Leu) – 0.105(Tyr)$$

Results and Discussion

Table 1 presents the result of the proximate analysis of okra seeds. The value of ash in okra seeds is higher than those of *Moringa oleifera* leaves (2.40%), stem (0.70%) and root (2.05%) reported by Olaofe *et al*. (2013), quinoa flour (Ogungbenle, 2003), *Luffa cylindrica* (Olaofe and Aremu, 2008), white melon and benniseed (Ogungbenle, 2006) but lower than that of gourd seeds reported by Ogungbenle (2006). The ash content of any food substance gives an idea of the mineral present in the food. The moisture content obtained from okra seeds (13.5%) is higher than those of bennissed, pearl millet and quinoa flour reported by Oshodi *et al*. (1999). Okra seeds will have a low shelf life than bennissed, pearl millet and quinoa flour due to high level of moisture and hence, inhibit the growth of microorganism. The value of crude protein in okra seeds is fairly high (26.4%) which makes it useful as supplement for starchy foods that contains carbohydrate but low in protein. The value of crude protein in okra seeds is lower than those of *L. cylindrica* (32.7%) reported by Olaofe and Aremu (2008), gourd seed (30.9%), white melon (37.9%) and

Table 1. Proximate composition of dried okra seeds

Parameters	% DM
Ash	4.81
Moisture	13.5
Crude protein	39.9
Crude fat	8.82
Crude fibre	26.4
Carbohydrate (by difference)	6.62
Protein digestibility	69.3

DM = dry material.

yellow melon (28.6%) reported by Ogungbenle (2006) but considerably higher than those of benniseed, pearl millet and quinoa, respectively, as reported by Oshodi *et al*. (1999). The value of crude fibre (8.82%) of okra seed is higher than those reported for 6 varieties of African yam bean flour by Adeyeye *et al*. (1994), kidney bean (2.68%) as reported by Olaofe *et al*. (2010), *Terminalia catappa* (4.94%), reported by Nzikou *et al*. (2010) and 2.5% *L. cylindrica* (Olaofe *et al*., 2008) but lower than that of quinoa flour (9.50%) (Ogungbenle, 2003). It suggests that okra seeds would provide an added dietary fibre in the diet. The high value of fibre reported for okra seeds can improve its digestibility and absorption processes in the large intestine. Crude fat of okra seeds (6.67%) is lower than those of *L. cylindrica*, 32.7% (Olaofe *et al*., 2008), *M. bellicosus* (52.73%) and *Z. variegatus* (13.3%) reported by Adeyeye (2011), benniseed, 44.30% (Ogungbenle, 2003), *T. catappa* (51.8%) reported by Nzikou *et al*. (2010) but comparable with that of six varieties of African yam bean flour (Adeyeye *et al*., 1994) and scarlet runner bean (5.3-6.9%) reported by Aremu *et al*. (2005) but higher than those of quinoa flour 6.30% (Ogungbenle, 2003), kersting's groundnut (4.9%) bambara ground-nut (6.7%), cowpea (1.8-2.1%), respectively, reported by Aremu *et al*. (2005). Okra seeds cannot replace *L. cylindrica* and benniseed as source of oil in food industries. Carbohydrate content in okra seeds which was determined by difference is 39.9% thus making it a fair source of carbohydrate which is essential for energy production in the human body as a result of oxidation especially, when okra seeds consumed would provide similar function. Carbohydrate content in okra seeds is slightly higher than those of *Cochlospermus religiosum* (10.12±0.12%), *Sesamum radiatum* (23.67± 0.13%) reported by Ogungbenle *et al*. (2005) and *L. cylindrica* (13.6%) reported by Olaofe and Aremu (2008), 16.02% reported for *T. catappa* (Nzikou *et al*., 2010) but considerably lower than those of quinoa flour (Ogungbenle, 2003) and pearl millet (Oshodi *et al*., 1999). Table 1 also shows the *in vitro* protein digestibility of okra seeds. The average digestibility of okra is 69.3%, this value is lower than those obtained for African nutmeg (78.4%) reported by Ogungbenle (2011), African yam bean flour (hulled seed and dehulled seeds) (Adeyeye *et al*., 1996) and *Afzelia africana* (71.5%) reported by Ogungbenle and Omaejalile (2010) implying that they are more digestible in the body than okra seed. Digestibility of protein and bioavailability of its constituent amino acids are very important factors in determining the protein quality (FAO/WHO, 1991; Hsu *et al*., 1977). This is true because not all proteins are digested, absorbed and utilised to the same extent (FAO/WHO, 1991). Wallace *et al*. (1971) have reported that heat treatment of legume protein and protein-containing flours improves digestibility. To improve the digestibility of okra seeds it could require heat treatment. In Figure 1, Y is *in vitro* digestibility (%) and X is the pH of the sample solution after 15 min digestion with the multienzyme solution. It also shows that, as the time of heating increases, the protein digestibility of okra seeds increases, which confirms the earlier observation reported by Wallace *et al*. (1971). The trend of curve obtained for okra seeds agrees with that of Hsu *et al*. (1977).

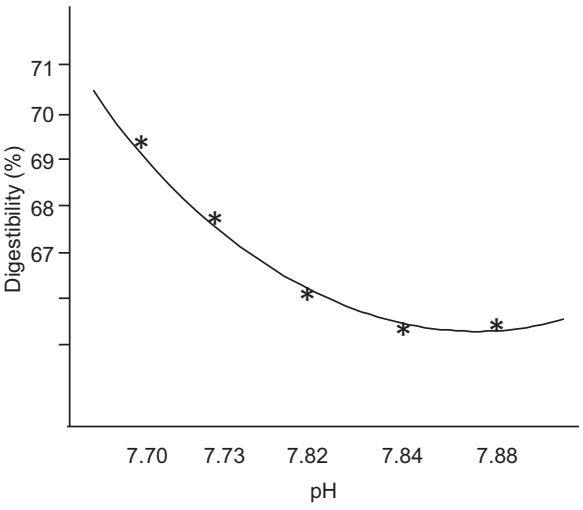


Fig. 1. The graph of protein digestibility against pH.

Table 2 shows the functional properties of dry okra seeds. The water absorption capacity 220% was lower than that of cowpea flour (246%) reported by Olaofe *et al*. (1993) but considerably higher than those of gourd seed, white and yellow melon, benniseed, bulma cotton seed reported by Ogungbenle (2006) and quinoa flour (147.00%) reported by Ogungbenle (2003). The high water absorptivity exhibited by the okra seeds may be useful in the formulation of some foods such as sausage, doughs, baked products etc. The sample was found to have oil absorption capacity of 200%. This value is lower than that of sunflower (270%) reported by Lin *et al*. (1974) but higher than most legumes such as melon seeds (122.0%) (Olaofe *et al*., 1984) and pigeon pea flour (89.70%) (Oshodi and Ekperigin, 1989). The value is also lower than those of *Afzelia africana* (588.49%) reported by Ogungbenle and Omaejalile (2010) and C*ucumeropsis edulis* (242.1%) reported by Ige *et al*. (1984). Oil absorption capacity is important since oil acts as a flavour retainer and increases the mouth feel of foods (Kinsella, 1976). Since okra seed has a higher oil absorption capacity than benniseed, pearl millet, quinoa flour (Oshodi *et al*., 1999), which indicates that okra seeds would be a better flavour retainer. The foaming capacity (12%w/v) is low thereby making okra not to be attractive for products like cakes or whipping toppings where foaming is important. Foaming capacity is lower than those of kidney bean seed (30.5%) reported by Olaofe *et al*. (2010) and 68% of pigeon pea flour (Oshodi and Ekperigin, 1989), quinoa flour (Ogungbenle, 2003), benniseed, pearl millet (Oshodi *et al*., 1999) but higher than 8.5% for *A. africana* (Ogungbenle and Omaejalilie, 2010) and fluted pumpkin seeds (10.8%) reported by Fagbemi and Oshodi (1991). The least gelation concentration of okra seeds is 8% w/v. This is lower than that of *A. Africana* seeds (6.00% w/v) reported by Ogungbenle and Omaejalile (2010). The result was better than the values reported for quinoa flour 16%w/v (Ogungbenle, 2003) and for fluted pumpkin seed (36% w/v) reported by Fagbemi and Oshodi (1991). The ability of protein to form gels and provide a structural matrix for holding water, flavour, sugar and other food ingredients is useful in food applications and in new product development, thereby providing an added dimension to protein functionality. The low value for the least gelation concentration of okra seeds indicates that it would assist in good setting of stews. Emulsion capacity (45.5%) of okra seeds is lower than those reported for pigeon pea flour (49.4%) reported by Oshodi and Ekperigin (1989), gourd seed, white and yellow melon, benniseed and bulma cotton seed (Ogungbenle, 2006).

Table 2. Functional properties of dried okra seeds

Functional properties	(%)
Water absorption capacity	220
Oil absorption capacity	200
Foaming capacity	12.0
Foaming stability	2.00
Emulsion capacity	45.5
Emulsion stability	67.0
Least gelation concentration (w/v)	8.00

The amino acid composition of okra seeds in mg/g crude protein is shown in Table 3. Glutamic acid was the most concentrated amino acid in okra seeds with a value of 121 mg/g crude protein. Glutamic acid is essential for brain metabolism and metabolism of other amino acids. Aspartic acid was next with a value of 86.5 mg/g crude protein. Leucine has the value of 68.5 mg/g crude protein, which helps to reduce muscle protein breakdown, promotes healing of skin and broken tissues. The result followed the trend for six varieties of dehulled African yam bean flour (Adeyeye, 1997) where, glutamic and aspartic acids were the most concentrated amino acids. This observation corroborates with that reported for cooked walnut seeds (Ogungbenle, 2009). The values for glutamic acid (121 mg/g crude protein) and aspartic acid (86.5 mg/g crude protein) are lower than the glutamic acid (151.6 mg/g crude protein) and aspartic acid (89.5 mg/g crude protein) in cooked walnut reported by Ogungbenle (2009). Cystine was the least concentrated amino acid with the value of 10.0 mg/g crude protein. This trend was also observed in white, yellow melon (Ogungbenle, 2006), where cystine was also the least concentrated amino acid. The value of cystine in okra seeds is 1.41% of the total amino acid composition. The total amino acid (TAA) of okra seeds was 705.8 mg/g crude protein. The value obtained is higher than those raw (439 mg/g crude protein), steeped (432 mg/g crude protein), germinated (464 mg/g crude protein) (Adeyeye, 2009) and *L. cylindrical* reported by Olaofe *et al*. (2008) but lower than that obtained in six varieties of dehulled African yam bean flour (Adeyeye, 1997). Okra seeds can be said to be considerably rich source of essential amino acids. Total essential amino acid (with histidine) had a value of 376 mg/g crude protein and 354 mg/g crude protein (without histidine)

Table 3. Essential amino acid profile of okra seeds (mg/g crude protein)

Amino acid	Value	Suggested essential amino acid score pattern	Amino acid scoring based on whole hen's egg
*Histidine[a]	21.4	-	0.89
*Arginine[a]	50.1	-	0.82
[+]Aspartic acid[a]	86.5	-	0.81
Valine[a]	36.1	0.73	0.48
Methionine[a]	10.4	-	0.34
Isoleucine[a]	25.7	0.64	0.46
Leucine[a]	68.5	0.93	0.83
Phenylalanine[a]	42.0	-	0.84
Met + Cys	-	0.58	-
Phe + Tyr	-	1.22	-
TEAA	-	-	-
With histidine	376	-	-
Without histidine	354	-	-
% TEAA	-	-	-
With histidine	53.2	-	-
Without histidine	50.2	-	-
Pi (calculated)	3.98	-	-
P-PER*	2.31	-	-

TEAA = total essential amino acids; [a] = essential amino acid; * = basic amino acids; [+] = acidic amino acids.

with percentage TEAA value of 53.2 and 50.2%, respectively, making it to form the bulk of amino acid in the okra seed (%TEAA > % TNEAA). The sample is therefore, rich in essential amino acids. TNEAA obtained in okra seeds is considerably lower than that obtained for dehulled African yam bean flour 327.2-453.8 mg/g crude protein (Adeyeye, 1997) and 409.9 mg/g crude protein for *A. africana* (Ogungbenle and Omaejalile, 2010). Total essential amino acids with and without histidine is lower than the value 110-603 mg/g crude protein obtained for African yam bean flour (Oshodi *et al*., 1995) but higher than the values 357.3 and 337.3 mg/g crude protein for periwinkle meat (Ogungbenle and Omowole, 2012) and African nutmeg (313.9 and 292.0 mg/g cude protein) reported by Ogungbenle and Adu (2012). The iso-electric point (P_I calculated) for the sample was 3.98. The value obtained is comparable with that of some selected oil seeds 2.8-6.3 (Olaofe and Akintayo, 2000). The value of P_i is useful in predicting when their will be a quick precipitation of protein isolate from biological samples (Olaofe and Akintayo, 2000). The predicted protein efficiency ratio (P-PER*) of okra seeds was 2.31 while, experimentally determined PER usually ranged from 0.00 for a very poor protein to a maximum possible of just over 4 (Muller and Tobin, 1980). PER obtained was higher than that of *L. cylindrica* (Olaofe and Aremu, 2008) meaning that okra seeds will be more utilised in the body due to increased digestibility than *L. cylindrica*. Table 3 shows the amino acid scoring pattern based on the essential amino acids. Leu + Cys was observed to be the limiting amino acid with a value of 0.58. Therefore, in order to fulfill the daily needs for the essential amino acids (EAA) in okra seeds, 100/58 or 1.72 times as much of okra seeds would have to be consumed if it is to be the sole protein in the diet.

Table 4 also indicates the non-essential amino acid scores of okra seeds based on whole hen's egg amino acid profile. Methionine (0.34) is the limiting amino acid in the sample. The factor will then be 100/34 or 2.94 times protein of okra seeds that would be taken where it would be needed as the sole protein source. This followed the trend observed in raw, steeped and fermented millet reported by Adeyeye (2009). The study is limited to the assessment of the nutritional qualities of the whole fruit and future studies on fatty acids, lipid analysis, physico chemical properties of the oil, sugar level and mineral analysis are identified.

Table 4. Non-essential amino acid profile of okra seeds (mg/g crude protein)

Amino acid	Value	Suggested essential amino acid	Amino acid scoring based on whole hen's egg
*Lysine	34.8	0.63	0.56
Threonine	31.5	-	0.62
Serine	27.9	-	0.35
[+]Glutamic acid	121	-	1.07
Proline	31.5	-	0.83
Glycine	41.1	-	1.37
Alanine	36.5	-	0.67
Cystine	10.0	-	0.56
Tyrosine	31.7	-	0.79
% TNEAA	46.9	-	-

TNEAA = total non essential amino acid; * = basic amino acids; [+] = acidic amino acids.

Conclusion

Okra seed is rich in some essential amino acids, but its high level of moisture implies low shelf life of the fresh seeds. The fairly high fibre content may aid quick

digestion process in the stomach. It is also a rich source of carbohydrate and protein which is essential for both human and livestock development. The high water and oil absorption capacities exhibited by okra seeds can be an added advantage in the formulation of foods and also as a flavour retainer. The use of okra seeds in food processing should be encouraged because of its high nutritional potentials.

References

Adeyeye, E.I. 2011. Fatty acid composition of *Zonocerus variegatus, Microtermes bellicus* and *Anacadium occidental* kernel. *International Journal of Pharma and Bio Sciences*, **2:** 135-144.

Adeyeye, E.I. 2009. Intercorrelation of amino acid quality between raw,steeped and germinated millet grains. *Pakistan Journal of Scientific and Industrial Research,* **52:** 122-129.

Adeyeye, E.I. 1997. Amino acid composition of six varieties of dehulled African yam bean flour. *International Journal of Food Sciences and Nutrition*, **48:** 345-351.

Adeyeye, E.I., Oshodi, A.A., Ipinmoroti, K.O. 1994. Functional properties of some varieties of African yam bean (*Sphenostylis sternoecarpa*) flour II. *International Journal of Food Sciences and Nutrition*, **45:** 115-126.

Alsmeyer, R.H., Cunningham, A.E., Hapich, M.L. 1974. Equations to predict PEF from amino acid analysis. *Food Technology*, **28:** 34-38.

AOAC, 2005. *Official Methods of Analysis*, 15th edition, Association of Official Analytical Chemist, Washington, DC., USA.

Aremu, M.O., Olaofe, O., Akintayo, E.T. 2005. Nutritional qualities assessment of the presence of hull in some Nigerian underutilized legume seeds. *Bulletin of Pure and Applied Sciences*, **24:** 47-52.

Beuchat, L.R. 1977. Functional and electrophoretic characteristics of succinylated peanut flour. *Journal of Agricultural and Food Chemistry*, **25:** 258-261.

Choudhury, B. 1977. *Vegetables*: *India, the Land and the People*, 214 pp., 5th edition, National Book Trust, New Delhi, India.

Coffman, C.W., Garcia, V.V. 1977. Functional properties and amino acid content of a protein isolated from mung bean flour. *International Journal of Food Science and Technology*, **12:** 473-484.

Daniluk, J. 2012. Health benefits of okra and a curry recipe to heal inflammation. http://www.chatelaine.com/health-benefits-of-okra.

Fagbemi, T.N., Oshodi, A. 1991. Chemical composition and functional properties of full fat fluted pumpkin seed flour. *Nigerian Food Journal*, **9:** 26-32.

FAO/WHO, 1991. *Protein Quality Evaluation*: Report of the Joint FAO/WHO Expert Consultation, FAO Food and Nutrition Paper 51, held in Bethesda MD., USA, December 1989, FAO, Rome, Italy.

FAO/WHO, 1973. *Energy and Protein Requirements*: Report of a joint FAO/WHO Adhoc Expert Committee. FAO Nutrition Meeting Report Serial No.52 and World Health Organisation Technical Report Series No. 522, WHO, Geneva, Switzerland.

Hsu, H.W., Vavak, D.L., Satterlee, L.D., Miller, G.A. 1977. A multienzyme technique for estimating protein digestibility. *Journal of Food Science*, **42:** 1269-1273.

Ige, M.M., Ogunsua, A.O., Oke, O.L. 1984. Functional properties of the proteins of some Nigerian oil seeds: conophor seeds and the three varieties of melon seeds. *Journal of Agricultural and Food Chemistry*, **32:** 822-825.

Inklaar, P.A., Fortuin, J. 1969. Determining the emulsifying and emulsion-stabilizing capacity of protein meat additives. *Food Technology*, **23:** 103-107.

Irvine, F.R. 1969. *West African Crops*, 272 pp., 3rd edition, Oxford University Press, London, UK.

Kinsella, J.E. 1976. Functional properties of protein foods: a survey. *CRC Critical Review in Food Science and Nutrition*, **7:** 219-280.

Kochhar, S. 1986. *Tropical Crops. A Textbook of Economic Botany.* Macmillan Publishers Limited, London, UK.

Lin, M.J.Y., Humbert, E.S., Sosulski, F.W. 1974. Certain functional properties of sunflower meal products. *Journal of Food Science*, **39:** 368-370.

Muller, H.G., Tobin, G. 1980. *Nutrition and Food Processing*, 320 pp., Croom Helm, London, UK.

Nzikou, J.M., Mvoula-Tsieri, M., Pambou-Tobi, N.P.G., Ndangui, C.B., Kimbonguila, A., Silou, Th., Linder, M., Scher, J., Desobry, S. 2010. Proximate composition and physicochemical characteristics of seed and oil from *Terminalia catappa* L. and the kinetics of degradation of the oil during heating. *Australian Journal of Basic and Applied Sciences*, **4:** 2039-2047.

Olaofe, O., Adeyeye, E.I., Ojugbo, S. 2013. Comparative study of proximate amino acids and fatty acids of *Moringa oleifera* tree. *Elixir Applied Chemistry*, **54:** 12543-12554.

Olaofe, O., Famurewa, J.A., Ekuagbere, A.O. 2010. Chemical and functional properties of kidney bean

seed flour. *Inernational Journal of Chemical Sciences*, **3:** 51-69.

Olaofe, O., Aremu, M.O. 2008. Chemical evaluation of the nutritive value of smooth *Luffa cylindrica* seed's kernel. *Electronic Journal of Food Chemistry*, **7:** 3444-3452.

Olaofe, O., Akintayo, E.T. 2000. Prediction of isoelectric points of legume and oil seed proteins from their amino acid compositions. *The Journal of Techno-science*, **4:** 49-53.

Olaofe, O., Adeyemi, F.O., Adediran, G.O. 1994. Amino acid and mineral compositions and functional properties of some oilseeds. *Journal of Agricultural and Food Chemistry*, **42:** 878-881.

Olaofe, O., Umar, Y.O., Adediran, G. 1993. The effect of nematicides on the nutritive value and functional properties of cowpea seeds. *Food Chemistry*, **46:** 337-341.

Ogungbenle, H.N., Adu, T. 2012. Proximate composition and functional properties of dehulled African nutmeg. *Pakistan Journal of Scientific and Industrial Research*, **55:** 80-85.

Ogungbenle, H.N., Omowole, B.M. 2012. Chemical, functional and amino acids composition of periwinkle meat. *International Journal of Pharmaceutical Science Review and Research*, **13:** 128-132.

Ogungbenle, H.N. 2011. Chemical, *in-vitro* digestibility and fatty acids composition of African nutmeg. *Annals of Science and Biotechnology*, **2:** 46-51.

Ogungbenle, H.N., Omaejalile, M. 2010. Functional properties and anti-nutritional properties, *in-vitro* protein digestibility and amino acid composition of dehulled *A. Africana* seeds. *Pakistan Journal of Scientific and Industrial Research*, **53:** 265-270.

Ogungbenle, H.N. 2009. Chemical and amino acid composition of cooked walnut flour. *Pakistan Journal of Scientific and Industrial Research*, **52:** 130-133.

Ogungbenle, H.N. 2006. Chemical composition, functional properties and amino acid composition of some edible seeds. *La Rivista Italia Sostasse Grasse*, **83:** 71-79.

Ogungbenle, H.N., Oshodi, A., Oladimeji, M. 2005. Chemical, energy evaluation of some underutilised legume flours. *La Rivista Italia Sostasse Grasse*, **82:** 204-208.

Ogungbenle, H.N. 2003. Nutritional evaluation and functional properties of quinoa (*Chenopodium quinoa*) flour. *International Journal of Food Science and Nutrition*, **54:** 153-158.

Oshodi, A., Ogungbenle, H.N., Oladimeji, M. 1999. Chemical composition, functional properties and nutritionally valuable minerals of benniseed, pearl millet, quinoa seed flour. *International Journal of Food Science and Nutrition*, **50:** 325-331.

Oshodi, A., Ipinmoroti, K., Adeyeye, E.I., Hall, G.M. 1995. Amino and fatty acid composition of African yam bean (*Sphenostylis stenocarpa*) flour. *Food Chemistry*, **53:** 1-6.

Oshodi, A.E., Ekperigin, M.M. 1989. Functional properties of pigeon pea (*Cajanus cajan*) flour. *Food Chemistry*, **34:** 187-191.

Paul, A., Southgate, D., Russel, R. 1976. *Mc Cance and Widdowson's The composition of Foods*, 4th edition, The Royal Society of Chemistry, HMSO, London, UK.

Pearson, D. 1976. *Chemical Analysis of Foods*, 7th edition, Churchill Livingstone, London, UK.

Sathe, S.K., Deshpande, S., Salunkhe, D.K. 1982. Functional properties of lupin seed proteins. *Journal of Food Science*, **47:** 491-497.

Spackman, D.H., Stein, W.H., Moore, S. 1958. Automatic recording apparatus for the use in the chromatography of amino acids. *Analytical Chemistry*, **30:** 1190-1206.

Wallace, G.M., Bannatyne, W.R., Khaleque, A. 1971. Studies on the processing and properties of soy milk. Effect of processing conditions on the trypsin inhibitor activity and the digestibility *in-vitro* of proteins in various soy milk preparations. *Journal of The Science of Food and Agriculture*, **22:** 526-531.

Pak. j. sci. ind. res. Ser. B: biol. sci. 2014 **57** (3) 136-139

Fatty Acid Composition of Certain Oil Seeds from Nigeria

Ibironke Adetolu Ajayi[a]*, Julius Sergio Marchini[b], Jose Ernesto Dos-Santos[c] and Julia Keiko Sakamoto Hotta[c]

[a]Industrial Chemistry Unit, Chemistry Department, Faculty of Science, University of Ibadan, Nigeria
[b]Mass Spectrometry Laboratory, Brazil
[c]Laboratoris de Nutricao do Hospital das Clinicas de Riberao Preto, Brazil

(received December 7, 2012; revised May 16, 2013; accepted June 27, 2013)

Abstract. Fatty acids of certain oil seeds from Nigeria were determined by gas liquid chromatography and their identification was based on comparison by authentic samples. Seeds studied in this study were *Aframomum danielli* K. Schum, *Arachidis hypogeal* L., *Glycine max* L., *Elaesis guineensis* var. tenera, *Piper guineense* Thonn. ex Schumach and *Treculia africana* Decne. ex Trec. The fatty acid composition of the oil seeds showed that they (except *E. guineensis*) all contained more of unsaturated fatty acids ranging from 62.80% to 86.70% for *P. guineense* and *A. hypogeal* (L.), respectively. The oils with the exception of *A. danielli* (7.50%) and *E. guineensis* (3.30%) contained linoleic acid, which is an essential fatty acid with cholesterol-lowering activity in high amount in the range of 23.10% (*A. hypogeae*) to 34.10% (*T. africana*) with *G. max*, having the highest percentage of 56.40%. Four of the oils also contained linolenic acid in the range of 1.20% for *A. danielli* to 21.60% for *P. guineense*.

Keywords: fatty acids, gas liquid chromatography, oil seeds

Introduction

Seeds have nutritive and calorific values which make them necessary item in diets. They are also good sources of edible oils and fats, which are essential nutrients (Odoemelam, 2005). Vegetable oils provide energy and essential linoleic and linolenic acids that are responsible for growth (Fasina *et al.*, 2006). Advances in nutrition research has led to awareness of beneficial and harmful effects of various dietary fats and oils (Dunford, 2001).

Seeds of *A. danielli* K. Schum are used as a traditional food spice among the Edo and Niger delta people of Nigeria, and also as an anti-inflammatory agent by rubbing of the alcohol and petrol extracts on the allergic and eczematous swelling. *A. hypogeae* (L.) is a leguminous plant that is mainly grown for its seeds. They are eaten raw, roasted and can be made into a paste used in soups and stew. *Glycine max* (L.) Merr, popularly known as soybean, is a legume that is increasingly consumed for economical and nutritional reasons. In Nigeria, it is usually roasted, dehulled, grounded and used as additives in making infant cereal and soy milk; they can also be made into paste and used in soups and stew. The palm kernel (*Elaeis guineensis* var. tenera) is taken from the oil palm; it is surrounded by an edible reddish oily palm. It can be eaten raw or with roasted or cooked maize. *P. guineense* Thonn. ex Schumach., is a seed that is not commonly eaten in Nigeria; it is closely by related to cubeb pepper, black pepper and long pepper. *T. africana* Decne. ex Trec., seeds are aromatic and have a flavour much like groundnut. They are eaten raw, roasted, boiled or fried more usually to stews.

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

The present study was conducted to compare the fatty acid composition of different oils extracted from different seeds that are available in Nigeria, in order to establish a similarity/difference between them and also to determine their potential and hence their possible usage for edible or industrial purposes. There are literature reports on various works that have been carried out on some of these seeds (Ajayi, 2008; Onyeike and Acheru, 2002; Garcia *et al.*, 1998; Oderinde and Ajayi, 1998; Kindu *et al*., 1987); however, there is no report on the comparative study of the fatty acids of these seed oils.

Materials and Methods

Plant materials and sample collection. The family, scientific, local, english names and abbreviations of the seeds, whose oil extracts are being examined in this study are given in Table 1. These seeds were purchased from local markets in Ibadan, Oyo State, Nigeria. The seeds were identified in the herbarium unit of the Botany department, university of Ibadan, Ibadan, Nigeria, where vouchers of each specimen were already deposited. These seeds were chosen out of interest.

Table 1. Scientific family, english and local names of the plants investigated

Scientific name	Family	English name	Local name	Abbreviation
Aframomum danielli K. Schum	Zangiberaceae	Alligator pepper	Atare aja	AD
Arachidis hypogeae L.	Fabaceae	Groundnut	Epa	AH
Glycine max. (L.) Merr	Fabaceae	Soybean	Ewa soya	GM
Piper guineense Thonn. ex Schumach.	Piperaceae	West African pepper	Kale, masoro	PG
Treculia africana Decne. ex Trec.	Moraceae	Achi	Ukpo	TA
Elaeis guineensis var. tenera	Palmae	Palm kernel	Ekuro	EG

Sample preparation and extraction. The seeds were deshelled manually by cracking to remove the kernels. The kernels were then ground to powder in a hammer mill and stored in air tight sample bottle in a refrigerator (4 °C) until needed for analysis. Seed oils were extracted with *n*-hexane for 8 h using a Soxhlet extractor. The solvent was removed completely and the oils obtained were used for this study. All chemicals used were supplied by British Drug House (BDH).

Fatty acid analysis. Fatty acid analysis of the seed oils was carried out at the Mass Spectrometry Laboratory, University of Sao Paulo, Ribeirao Preto, Brazil. The methyl ester of the raw oil was prepared according to Idouraine *et al.* (1996) with some slight modifications. Oil-solvent mixture was evaporated to dryness under nitrogen and then transesterified with H_2SO_4 in the presence of methanol for 2 h at 7 °C. To the resulting fatty acid methyl ester was added 40 mL of water after which the organics were extracted with petroleum ether (40-60 °C) and then dried under nitrogen. The fatty acid methyl esters were redissolved in hexane and analysed in a gas chromatograph (Shimadzu™ GC-17A) coupled to mass spectrometer (Shimadzu GCMS-QP5000™), under the following conditions: injector temperature: 250 °C; interface temperature: 270 °C; oven temperature 80 °C 1600 increasing to 160 °C and to 240 °C at 2 °C per 0.5 min; column pressure: 70 KPa, split ratio: 1:50. The column used was the DB-wax 250 (30 m×0.25 mm from J & W Scientific). The internal standards used were heptadecanóico acid (C_{17}: 0) and the methyl ester of tricosanóico acid (C_{23}: 0) (from Supelco™). To identify the peaks, fatty acid component FAME Mix 37 standard (Supelco™) was used from which the dilutions were made and used to construct the calibration curve in 6, 8 and 10 µg/µL F. It was not possible to detect any fatty acid at concentration below 6 µg/µL.

Results and Discussion

Fatty acid composition of the investigated oils is presented in Table 2. Nine fatty acids have been identified in six oil samples; these are lauric, myristic, palmitic, palmtolenic, stearic, oleic, linoleic, linolenic and arachidonic acids. Palmitic acid was the main saturated component in all the seed oils ranging from *A. hypogeae* (9.80%) to *P. guinesis* (26.00%). Even though *E. guineensis* kernel oil contains palmitic acid (10.70%), it has $C_{12::0}$ acid (44.90%) as its main fatty acid. Three of the oils contain palmitolenic acid; this ranged from *A. danielli* (1.20%) to *E. guineensis* (22.00%). All the oils with the exception of *A. danielli* (7.50%) and *E. guineensis* (3.30%) contain linoleic acid in high amount in the range of 23.10% (*A. hypogeal*) to 34.10% (*T. africana*) with *G. max*, having the highest percentage that is 56.40%. Studies on human subjects using diets rich in linoleic acid showed that, in the groups provided with higher amounts of soybean oil (50% linoleic acid content), the mortality rate due to coronary artery decreases significantly (Younis *et al.*, 2000). Ajayi (2009) reports oleic and linoleic acids as the main fatty acids in some seed oils from Nigeria. Sánchez-Manchado *et al.* (2004) also reports the range of 16.10±3.31% to 69.11±9.01% as the polyunsaturated fatty acids (PUFA) contents of some processed edible seaweeds studied.

Most vegetable oils are very good sources of linoleic acid but only very few oils contribute significant amount of linolenic acid in the diet (Longvah *et al.*, 2000). Among the oils examined, *A. danielli*, *G. max*, *P. guineense* and *T. africana* were found to contain linolenic acid ranging from 1.20% for *A. danielli* to 21.60% for *P. guinesis*; this is nutritionally significant. The consumption of Perilla oil which contains 57% linolenic acid has been reported in literature to improve learning ability, retinal function and suppression of carcinogenesis, metastasis, thrombosis and allergy (Longvah *et al*., 2000). The

Table 2. Fatty acid composition (%)[a] of the seed oils

Fatty acid composition	*A. danielli*	*A. hypogeae*	*G. max*	*P. guineense*	*T. africana*	*E. guineensis*
$C_{12:0}$	-	-	-	-	-	44.90
$C_{14:0}$	1.20	-	-	4.50	0.20	22.00
$C_{16:0}$	21.50	9.80	10.70	26.00	20.60	10.70
$C_{16:1}$	1.10	-	-	0.80	1.20	-
$C_{18:0}$	2.70	3.50	3.50	4.20	16.50	3.60
$C_{18:1}$	63.40	63.60	20.70	9.90	26.20	15.50
$C_{18:2}$	7.50	23.10	56.40	31.00	34.10	3.30
$C_{18:3}$	1.20	-	8.60	21.60	1.30	-
$C_{20:4}$	1.40	-	-	1.80	-	-

[a] = percentage by weight of total fatty acid identified as FAME.

Table 3. Oleic, linoleic, MUFA[a], PUFA[b], UFA[c], SAFA[d], oleic/linoleic, MUFA/PUFA, SAFA/UFA contents of the seed oils

Parameters	*A. danielli*	*A. hypogeae*	*G. max*	*P. guineense*	*T. africana*	*E. guineensis*
MUFA[a]	64.50	63.60	20.70	10.70	27.40	15.50
PUFA[b]	10.10	23.10	65.00	54.40	35.40	3.30
UFA[c]	74.60	86.70	85.70	65.10	62.80	18.80
SAFA[d]	25.40	13.30	14.20	34.70	37.30	81.20
Oleic/Linoleic	8.45	2.75	0.37	0.32	0.77	4.70
MUFA/PUFA	6.39	2.75	0.32	0.20	0.77	4.70
SAFA/UFA	0.34	0.15	0.17	0.53	0.59	4.32

[a] = monounsaturated fatty acids; [b] = polyunsaturated fatty acids; [c] = unsaturated fatty acids; [d] = saturated fatty acids.

consumption of *P. guineense*, if the oil is non-toxic, may probably has the same effect that Perilla oil has. *A. danielli*, *G. max*, *P. guineense* and *T. africana* may be considered for highest nutritional significance because of the presence of linolenic acid. Two of the oils; *A. danielli* and *P. guineense* contain arachidonic acid. Sanchez-Machado *et al.* (2004) also reports the presence of arachidonic acid in seaweeds. Generally, the percentage level of unsaturation in the oils is high except for *E. guineensis* kernel oil; it varied between 62.80% in *T. africana* and 86.70% in *A. hypogeal*. The percentage unsaturated fatty acids reported for three of the oils in this study, mainly *A. hypogeal*, *A. danielli* and *G. max* is higher than the one reported in literature for *T. occidentalis* (Ajayi *et al.*, 2004).

Oleic, linoleic, monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA), unsaturated fatty acid (UFA), saturated fatty acid (SAFA) oleic/linoleic, MUFA/PUFA, SAFA/UFA contents of the seed oils are presented in Table 3. The saturated/unsaturated ratio of the *E. guineensis* kernel oil is 4.32; while, it is less than 1 in all the other oils. This suggests that all the examined oils (except *E. guineensis* kernel oil) could probably be suitable as edible oil. The MUFA/PUFA ratio of three of the oils is greater than 1; this shows that half of the oils contain more of PUFA than MUFA. Half of the oils also have their oleic to linoleic acid ratio to be greater than 1. This is of great nutritional value since polyunsaturated fatty acids and their derivatives are important essential nutritive additives in mammal, especially in humans (Stransky *et al.*, 2005; Kamal-Eldin and Yanishlieva, 2002; Ziboh *et al.*, 2002).

Conclusion

All the studied oils (except *E. guineensis* kernel) are highly unsaturated with some of them containing two of the essential fatty acids. The relatively high level of PUFA in the oil extracts (apart from *E. guineensis* kernel) may make them healthy. However, further work needs to be carried out on some of these oils to determine their toxicity.

Acknowledgement

The corresponding author is grateful to Third World Academy of Science (TWAS), Italy, for the award of Associateship fellowship to make it possible for this work to be carried out in the Mass Spectrometry Laboratory, University of Sao Paulo, Ribeirao Preto, Brazil. The Department of Chemistry, University of Ibadan, Ibadan, Nigeria is acknowledged for making its facilities available.

References

Ajayi, I.A. 2009. Comparative study of the fatty acid composition of some seed oils from Nigeria. *The African Journal of Plant Science and Biotechnology*, **3:** 59-62.

Ajayi, I.A. 2008. Comparative study of the chemical composition and mineral element content of *Artocarpus heterophyllus* and *Treculia africana* seeds and seed oil. *Bioresource Technology*, **99:** 5125-5129.

Ajayi, I.A., Oderinde, R.A., Taiwo, V.O., Agbedana, E.O. 2004. Dietary effects on plasma lipid and tissues of rats fed with non-conventional oil of *Telfairia occidentalis*. *Journal of Science Food and Agriculture*, **84:** 1715-1721.

Dunford, N.T. 2001. Health benefits and processing of lipid-based nutritionals. *Food Technology*, **55:** 38-44.

Fasina, O.O., Hallman, H., Craig-Schmidt, M., Clements, C. 2006. Predicting temperature-dependence viscosity of vegetable oils from fatty acid composition. *Journal of The American Oil Chemist's Society*, **83:** 899-903.

Garcia, M.C., Marina, M.L., Laborda, F., Torre, M. 1998. Chemical characterization of commercial soybean products. *Food Chemistry*, **42:** 325-331.

Idouraine, A., Kohlhepp, E.A., Weber, C.W., Warid, W.A., Martinez-Tellez, J. 1996. Nutrient constituent from eight lines of naked squash (*Cucurbita pepo* L.). *Journal of Agricultural Food Chemistry*, **44:** 721-724.

Kamal-Eldin, A., Yanishlieva, N.V. 2002. N-3 fatty acids for human nutrition: stability concentration, *European Journal of Lipid Science and Technology*, **104:** 825-836.

Kindu, S.F., Mgadini, B., Sondergani, B.L. 1987. A new labdane diterpenoid from the seed of *Aframum danielli*. *Journal of Natural Product*, **50:** 230-231.

Longvah, T., Deosthale, Y.G., Uday Kumar, P. 2000. Nutritional and short term toxicological evaluation of Perilla seed oil. *Food Chemistry*, **70:** 13-16.

Oderinde, R.A., Ajayi, I.A. 1998. Composition of *Entada pursaetha* seed and seed oil grown in Nigeria. *La Rivista Italiana Delle Sostanze Grasse*, **75:** 457-459.

Odoemelam, S.A. 2005. Proximate composition and selected physicochemical properties of African oil bean (*Pentaclethra macrophylla*). *Pakistan Journal of Nutrition*, **4:** 382-383.

Onyeike, E.N., Acheru, G.N. 2002. Chemical composition of selected Nigerian oil seeds and physicochemical properties of the oil extracts. *Food Chemistry*, **77:** 431-437.

Sanchez-Machado, D.I., Lopez-Cervantes, J., Lopez-Hernandez, J., Paseiro-Losada, P. 2004. Fatty acids, total lipid, protein and ash contents of processed edible seaweeds. *Food Chemistry*, **85:** 439-444.

Stransky, K., Zarevucka, M., Wimmer, Z. 2005. Gas chromatography analysis of blackcurrant oil in relation to its stability. *Food Chemistry*, **92:** 569-573.

Younis, Y.M.H., Ghirmay, S., Al-Shihry, S.S. 2000. African *Cucurbita pepo* L., properties of seed and variability in fatty acid composition of seed oil *Phytochemistry*, **54:** 71-75.

Ziboh, V.A., Cho, Y.H., Mani, L., Xi, S.D. 2002. Biological significance of essential fatty acids/ lipoxygenase-derived monohydroxy fatty acids in the skin. *Archives of Pharmacal Research*, **25:** 747-758.

Pak. j. sci. ind. res. Ser. B: biol. sci. 2014 **57** (3) 140-147

Soil Borne Fungi Associated with Different Vegetable Crops in Sindh, Pakistan

Farzana Usman[a], Muhammad Abid[a], Faisal Hussain[b]*, Shaheena Arshad Khan[a] and Jawaria Sultana[a]

[a]Dr. A.G. Lab. of Aerobiology and Plant Pathology, Department of Botany, Federal Urdu University of Art, Science & Technology, Gulshan-e-Iqbal Campus, Karachi, Pakistan

[b]Department of Agriculture & Agribusiness Management, University of Karachi, Karachi-75270, Pakistan

(received March 5, 2014; revised July 3, 2014; accepted July 10, 2014)

Abstract. Different soil-borne fungi are responsible for reducing the yield of vegetables throughout the world including Pakistan. There are several soil borne fungal pathogens which aggressively infect vegetable crops. Surveys conducted during September 2010 to October 2011, demonstrated that a great diversity of soil borne plant pathogens associated with different vegetables prevail in vegetable growing areas of Sindh such as Tando Allahayar, Mirpurkhas, Ghotaki, Khairpur, Kunri, Umerkot and Karachi, etc. Our study noted in total thirteen different genera of fungi isolated from vegetable crops (cabbage, brinjal, tomato, radish and spinach). Isolated fungi identified included *Alternaria solani, Aspergillus flavus, A. fumigatus, A. niger, A. oryzae, A. terrus, Aeromonium fusidiocles, Cladosporium* sp., *Drechselra hawaiiensis, Eurotium berbanbrum, Fusarium oxysporum, Macrophomina phaseolina, Penicillium commune, Rhizoctonia solani, Trichoderma harzianum, Ulocladium* sp., and unidentified black mycelium from the soil and roots of vegetable crops. In addition, it was found that soil is commonly infected by soil-borne fungi and eventually results in heavy losses of vegetable yield in the vegetable growing areas of Sindh province. The infection rapidly increased due to many factors such as, presence of moisture, cxcess of water and infection may be caused by winds, gales and dust storms as well as by mechanical vectors.

Keyword: vegetables, root-rot, soil borne

Introduction

Vegetables included in daily schedule of diet *viz.* sweet pepper, cauliflower, carrot, cabbage, lettuce, spinach, tomato, potato, reddish, and bottle gourd are rich in proximate composition, vitamin and mineral contents. The soil and climatic conditions of Pakistan are congenial for the production of vegetables and widely diversified agro climatic zones (Hanif *et al.,* 2006). The nature has endowed Pakistan with diverse types of climatic conditions and land for vegetable crops. Therefore, a large variety of vegetables are cultivated in Pakistan throughout the year. In excess of 63 vegetable species are grown in various parts of the country as summer and winter vegetables particularly in Sindh province, Pakistan (Athar and Bokhari, 2006). In Sindh, Mirpurkhas division is positioned atop a fertile land making conditions suitable for cropping and vegetation. The major crops and vegetables are widely cultivated in this region (Hussain *et al.,* 2012). Vegetables are divided into two groups on the basis of season including winter vegetable (cultivated during the winter months of October-March) and summer vegetables (cultivated during the month of April-September). Some vegetables plants have no particular time for sowing including cucumber, radish etc. (Ali, 2000).

Vegetables are important food and highly beneficial ingredients which can be successfully utilised to build up and repair the body. They are valued mainly for their high carbohydrate, vitamin and mineral contents (Hanif *et al.,* 2006). The yield of vegetables is reducing gradually every year due to the soil-borne fungi. It is facing several biotic problems and is under threat due to soil borne pathogens in all over vegetable growing areas. Soil-borne plant diseases cause significant damage to almost all crops particularly to the vegetables (Usman *et al.,* 2013).

Infection of the vegetable plants in the field may occur at any time during the growing season. Early infections caused seedling blight and later infections caused foliar blight, stem lesion, vine rot, fruit rot and root and crown

*Author for correspondence; E-mail: faisal.botanist2011@gmail.com

rot (Usman *et al.,* 2013). Islam and Babadoost (2002) and Lee *et al.* (2001) reported that in the vegetable crops of different areas of Sindh province including Karachi (Malir, Sharafi Goth, Memon Goth and Gadap Town), Kunri, Mirpurkhas, Ghotaki, Tando Allahyar and Digri show heavy losses and several symptoms including wilting stunted growth, chlorosis, and blotch on vegetable crops. Fatima *et al.,* (2009) indicated that *Alternaria alternata*, *A. citri*, *Aspergillus niger*, *A. flavus, Aspergillus* sp., *Cladosporium cladosporioides*, *Drechslera australeinsis*, *Fusarium solani*, *Fusarium* sp., *Geotrichum candidum, Penicillium* sp., *Phytophthora capsici* and *Rhizopus stolonifer* are responsible for postharvest deterioration of fresh fruits and vegetables.

The yield of vegetables is reducing gradually every year due to the soil-borne and root rot pathogens. Soil borne and root rot pathogens cause significant damage to almost all crops particularly to the vegetables. The association of root-knot with soil borne and root rot such as *Macrophomina phaseolina*, *Fuasrium* sp., and *Rhizoctonia solani* is causing diseases in different vegetable crops particularly chilli, brinjal, okra, tomato and spinach (Farzana *et al.,* 2013; Hussain *et al.,* 2013c; Maqbool *et al.,*1988). The soil borne root infecting fungi like *Macrophomina phaseolina* is reported to produce charcoal rot, damping off, root rot, stem rot, pod rot in more than 500 plant species (Sheikh and Ghaffar, 1992; Sinclair, 1982) with more than 67 hosts recorded from Pakistan alone (Mirza and Qureshi, 1978). Soil borne plant pathogens cause significant crop losses in chilli crop alone in Sindh. Root rot fungi including *Fusarium* sp., *Macrophomina phaseolina, R. solani*, *Phytophthora* root rot and *Alternaria* spp., are causing heavy losses in chilli and other crops (Hussain *et al.,* 2013a; 2013b; Hussain and Abid, 2011).

The objectives of the present study were; 1) to survey the various fungi infecting (soil borne and root) vegetables, 2) to compare the fungal composition of assemblages in soil borne and root rot of vegetables in seven different localities of Sindh province, and 3) to measure the infection % of the fungal assemblages.

Materials and Methods

Collection and isolation of fungi. The root rot fungi of vegetables including cabbage (*Brassica oleracea* L.), brinjal (*Solanum melongena* L.), tomato (*Lycopersicon esculentum* Mill.), radish (*Raphanus sativus* L.) and spinach (*Spinacia oleracea* L.) showing wilting, stunted growth, chlorosis and blotches were collected from Sindh province including Karachi, Tando Allahayar, Mirpurkhas, Ghotaki, Khairpur, Kunri and Umerkot from September 2010 to October 2011. The infected root samples were cut into small pieces up to 1.5 to 2 cm and surfaces were sterilised by 1% Ca $(OCl)_2$ for 1 min and these pieces were transferred on potato dextrose agar (PDA) medium and Czapek's agar medium containing antibiotic (Penicillin and Streptomycin) drops. The petri dishes were incubated for 3-6 days at 28 °C. Infection % was calculated with the help of following formula:

$$\text{Infection \%} = \frac{\text{Number of plants infected by a pathogen}}{\text{Total number of plants}} \times 100$$

Method of soil sampling. A total of 55 soil samples were collected between September 2010 and October 2011, from different locations of Sindh including Karachi, Tando Allahayar, Mirpurkhas, Ghotaki, Khairpur, Kunri and Umerkot. All samples were collected randomly from locations and they were associated with different vegetable fields particularly cabbage, brinjal, tomato, radish and spinach. About 300 g of soil was collected in polythene bags, tagged with name of vegetable and location, for each sample and taken to the laboratory for further analysis.

Soil dilution technique. One gram of soil was suspended in 9 mL of sterilised distilled water with the dilution of 1:10, followed by the dilutions of 1:100, 1:1000 and 1:10000. One mL aliquot sample was poured in sterilised petri plates containing potato dextrose agar (PDA) medium. Three replicates per sample were placed. The dishes were incubated at 30 °C. The colonies of fungi on plates were counted and identified with the help of Singh *et al.* (1991). The number of colonies of each fungus was multiplied by the dilution factor which shows total number of propagules/g of soil (Waksman and Fred, 1922).

Identification of fungi. Isolated fungi were examined by using 10 × and 40 × magnifications on the microscope to identify hyphae, sporangia, sporangiophores, conidia, conidiophores and some other morphological characters including growth pattern, colony texture and growth rate of the colonies on PDA (Promputtha *et al.,* 2005). Standard manuals or refernces including (Singh, 1991; Nelson *et al.,* 1983; Domsch *et al.,* 1980; Sutton, 1980; Ellis, 1976; 1971; Barnett and Hunter, 1972) were also used for the confirmation of various species.

Results and Discussion

Fungi isolated from roots. Twelve fungi were isolated from infected samples of soil collected from different vegetable crops (Table 1). Ten different fungi were

Table 1. Fungi isolated from infected soil and roots of different vegetables collected from different areas of Sindh province, Pakistan

Host		Name of fungi	
Scientific name	Common name	Root	Soil
Brassica oleracea L.	Cabbage	*Aspergillus oryzae, Aeromonium fusidiocles, Alternaria solani*, Cladosporium* sp., *Eurotium berbanbrum, Fusarium oxysporum*, Macrophomina phaseolina*, Rhizoctonia solani*, Ulocladium* sp.	*Aspergillus flavus*, A. fumigatus, A. niger*, Fusarium oxysporum*, Macrophomina phaseolina*, Penicillium commune*, Rhizoctonia solani**
Solanum melongena L.	Brinjal	*Alternaria solani*, Fusarium oxysporum*, Macrophomina phaseolina*, Rhizoctonia solani*, Penicillium commune*, Trichoderma harzianum**	*Aspergillus flavus*, A. niger*, A. terrus, Fusarium oxysporum*, Macrophomina phaseolina*, Penicillium commune*, Rhizoctonia solani*, Trichoderma harzianum**
Lycopersicon esculentum Mill.	Tomato	*Fusarium oxysporum*, Macrophomina phaseolina*, Rhizoctonia solani**	*Alternaria solani*, Aspergillus flavus*, A. niger*, Drechselra hawaiiensis, Fusarium oxysporum*, Macrophomina phaseolina*, Rhizoctonia solani**
Raphanus sativus L.	Radish	*Fusarium oxysporum*, Penicillium commune*, Rhizoctonia solani**	*Aspergillus niger*, Fusarium oxysporum*, Macrophomina phaseolina*, Rhizoctonia solani**
Spinacia oleracea L.	Spinach	*Fusarium oxysporum*, Macrophomina Rhizoctonia solani* phaseolina*,*	*Aspergillus flavus*, A. fumigates, Drechselra hawaiiensis, Fusarium oxysporum*, Macrophomina phaseolina**

= * major fungal disease.

isolated from roots of cabbage crop. Among these: *Fusarium oxysporum, Macrophomina phaseolina* and *Alternaria solani* were predominant with mean values of 65, 53 and 40.57%, respectively as compared to other species including *Rhizoctonia solani*, *Aspergillus orzae, Ulocladium* sp., *Aeromonium fusidiocles, Cladosporium* sp., and *Eurotium berbanbrum.* The occurrence of these three fungi was maximum in samples collected from Tando Allahyar (75%), Khairpur (71%) and Ghotaki (68%), respectively, and minimum (6%) from Mirpurkhas region. These fungi were maximum in samples collected from Kunri (67 and 65%), Tando Allahyar and Khairpur (66%), respectively, and minimum (7%) from Mirpurkhas (Table 2).

The combined infection result of tomato, radish and spinach roots (Fig. 1) showed that *Fusarium oxysporum* was predominant with mean value of 58% as compared to other species *Penicillium commune, Rhizoctonia solani* and *Macrophomina phaseolina.* On the basis of regions, comparison the occurrence of these fungi was maxi-mum in the samples from Kunri (69 and 63%), Tando Allahyar (67%) and Karachi (63%), respectively, and minimum (17%) from Khairpur region (Table 2).

Table 3 shows the results of ANOVA for the fungal infection % on roots samples collected from various

Table 2. Infection % of different fungi isolated from roots of vegetable at various localities of Sindh province, Pakistan

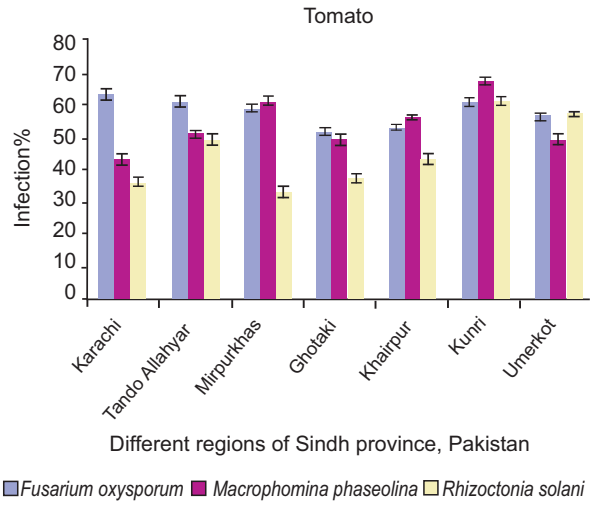
Isolated fungi	Root diseases infection %				
	Cabbage	Brinjal	Tomato	Radish	Spinach
Aeromonium fusidiocles	16.29	0	0	0	0
Alternaria solani	40.57	41.86	52.29	0	0
Aspergillus oryzae	32.70	0	0	0	0
Cladosporium sp.	15	0	0	0	0
Eurotium berbanbrum	12.43	0	0	0	0
Fusarium oxysporum	65	60.71	58	58	53.14
Macrophomina phaseolina	53	52.29	53.71	0	54.14
Penicillium commune	0	27.57	0	28.29	0
Rhizoctonia solani	40	39.57	45.14	45.86	42.29
Trichoderma harzianum	0	15.29	0	0	0
Ulocladium sp.	22.43	0	0	0	0
Unidentified black mycelium	12.86	10.86	0	0	0

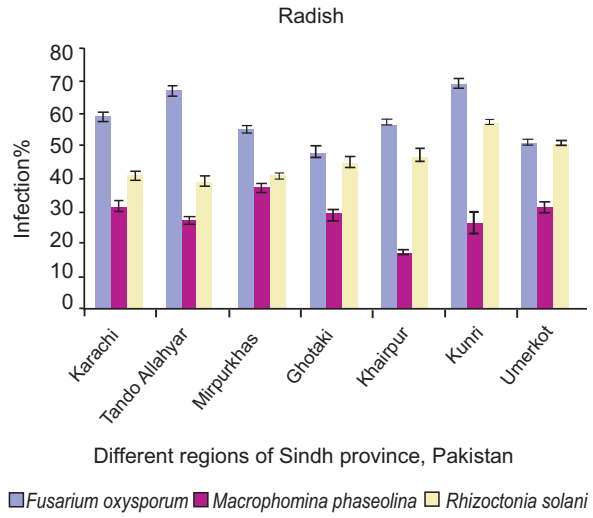
localities of Sindh. Twelve fungal species including *Alternaria solani, Aspergillus oryzae, Aeromonium fusidiocles, Cladosporium* sp., *Eurotium berbanbrum, Fusarium oxysporum, Macrophomina phaseolina, Penicillium commune, Rhizoctonia solani, Trichoderma harzianum, Ulocladium* sp., and unidentified black mycelium showed highly significant differences among localities.

The infection result of brinjal roots showed that *Fusarium oxysporum, Macrophomina phaseolina* and *Alternaria solani* were predominant with mean values of 60.71, 52.29 and 41.86%, respectively, as compared to other species including *Trichoderma harzianum, Penicillium commune* and *Rhizoctonia solani (*Fig. 2).

Table 3. F-ratios derived from ANOVA for fungal infection % of roots

Fungi species	F-ratio	P-value	$LSD_{0.05}$
	Cabbage		
Aspergillus oryzae	206.35	0.0000***	3.71
Aeromonium fusidiocles	70.11	0.0000***	2.92
Alternaria solani	72.67	0.0000***	3.81
Cladosporium sp.	98.84	0.0000***	2.63
Eurotium berbanbrum	28.03	0.0000***	2.24
Fusarium oxysporum	28	0.0000***	3.54
Macrophomina phaseolina	19.14	0.0000***	3.67
Rhizoctonia solani	76.16	0.0000***	3.65
Ulocladium sp.	46.02	0.0000***	2.65
Unidentified black mycelium	26.43	0.0000***	2.35
	Brinjal		
Alternaria solani	76.33	0.0000***	4.07
Fusarium oxysporum	12.48	0.0000***	3.47
Macrophomina phaseolina	74.75	0.0000***	3.05
Rhizoctonia solani	83.78	0.0000***	3.45
Penicillium commune	48.03	0.0000***	3.7
Trichoderma harzianum	27.29	0.0000***	2.37
Unidentified black mycelium	12.86	0.0000***	2.15
	Tomato		
Fusarium oxysporum	13.70	0.0000***	3.20
Macrophomina phaseolina	32.37	0.0000***	4.06
Rhizoctonia solani	55.46	0.0000***	4.12
	Radish		
Fusarium oxysporum	39.92	0.0000***	3.77
Penicillium commune	13.28	0.0000***	4.68
Rhizoctonia solani	23.86	0.0000***	3.71
	Spinach		
Fusarium oxysporum	44.5	0.0000***	3.48
Macrophomina phaseolina	29.42	0.0000***	3.63
Rhizoctonia solani	54.57	0.0000***	3.34





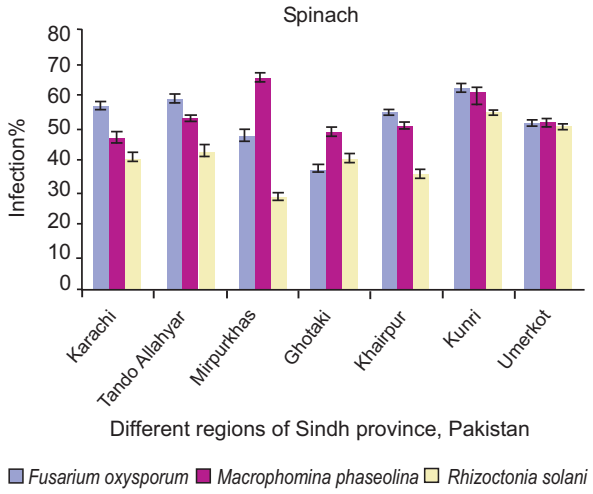


Fig. 1. Infection % of different fungi isolated from the roots of tomato, radish and spinach.

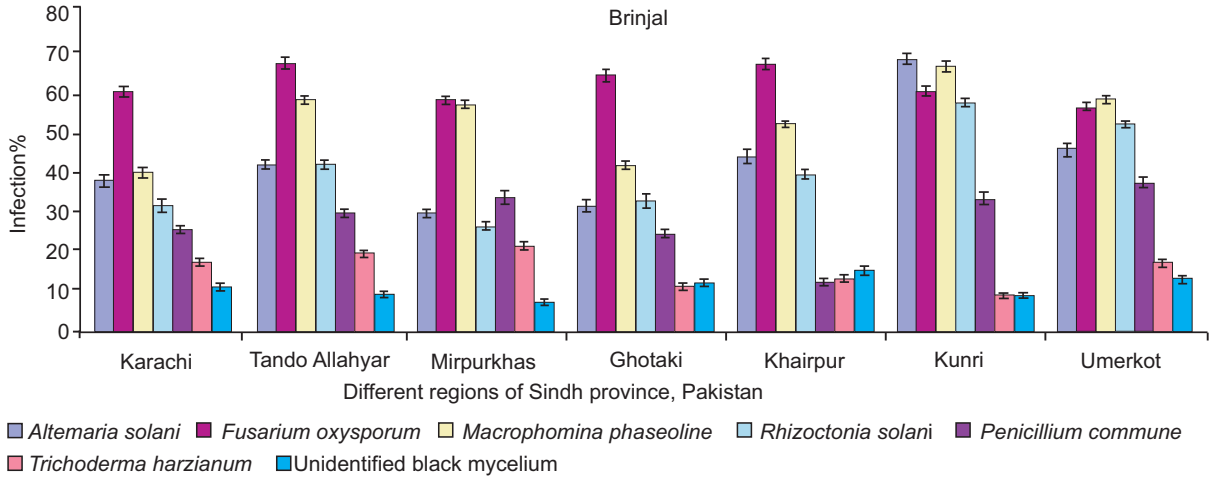


Fig. 2. Infection % of different fungi isolated from the roots of Brinjal.

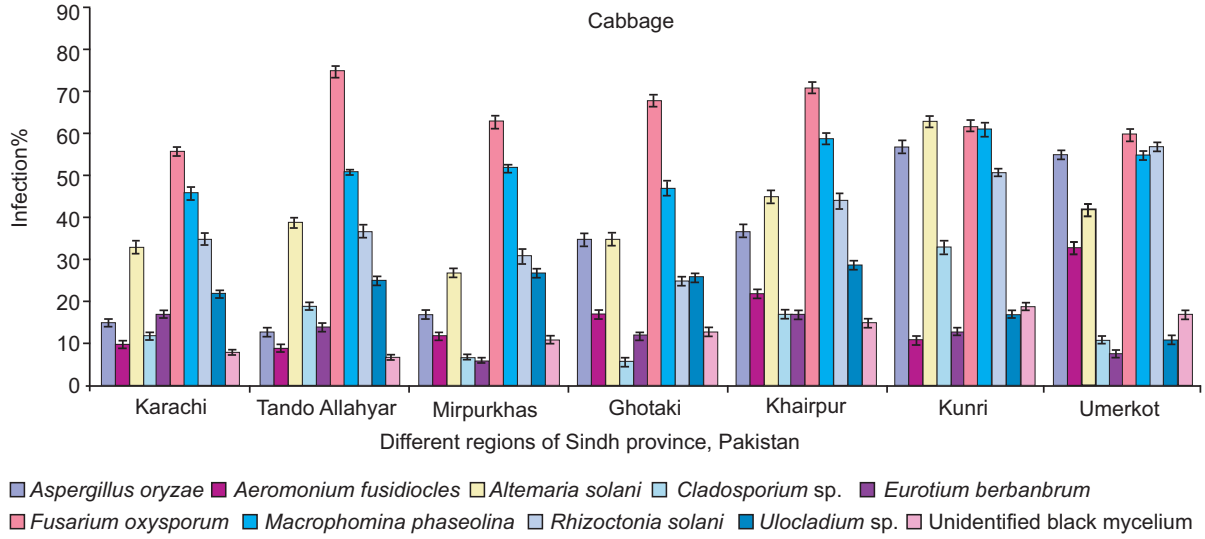


Fig. 3. Infection % of different fungi isolated from the roots of Cabbage.

All twelve species are pathogenic on all vegetable particularly tomato, radish, spinach brinjal and cabbage, crops. (Fig. 1-3).

Fungi isolated from soil. Twelve fungi were isolated from infected samples of soil collected from different vegetable crops. There are seven different fungi isolated from roots of cabbage crop. Among these *Aspergillus flavus, Fusarium oxysporum* and *Aspergillus niger* were predominant with mean values of 58, 56.29 and 38.43%, respectively, as compared to other species such as *Penicillium commune*, *Aspergillus fumigatus, Macrophomina phaseolina* and *Rhizoctonia solani.* The occurrence of these three fungi was maximum in samples collected from Umerkot (72 and 71%), Kunri (67%) and Mirpurkhas (66%), respectively, and minimum (11%) from Ghotaki region. The infection result of brinjal roots showed that *Aspergillus flavus, A. niger* and *Fusarium oxysporum* were predominant with mean values of 51.29, 39 and 37%, respectively, as compared to other species including *Aspergillus terrus, Penicillium commune, Trichoderma harzianum, Rhizoctonia solani* and *Macrophomina phaseolina.* These fungi were found maximum in samples collected from Kunri (61%), Umerkot (57%) and Karachi (56%), respec-tively, and minimum (10%) from Khairpur (Table 4).

The combined infection result of tomato, radish and spinach roots showed that *Fusarium oxysporum* and *Macrophomina phaseolina* were predominant with

Table 4. Mean and Standard error of different fungi isolated from soil of vegetable at various localities of Sindh province, Pakistan

Name of fungi	Different fungi isolated from soil							
	KHI	TAND	MPK	GHO	KHA	KUN	UME	Grand mean
			Cabbage					
Aspergillus flavus	34±1.61	57±2.12	61±2.17	52±2.37	63±2.67	67±2.16	71±1.90	58±4.50
A. fumigatus	41±1.69	22±2.83	17±2.04	11±0.75	21±2.83	13±0.75	9±0.75	19.14±4.08
A. niger	27±2.86	47±1.41	33±1.73	39±1.40	25±2.86	47±1.41	51±2.37	38.43±3.92
Fusarium oxysporum	56±2.12	61±2.17	66±2.16	39±1.40	34±1.74	66±2.16	72±1.90	56.29±5.46
Macrophomina phaseolina	31±2.86	23±2.86	34±1.74	29±2.86	37±1.40	41±1.69	23±2.86	31.14±2.57
Penicillium commune	23±2.86	12±0.75	11±0.75	17±2.04	21±2.86	19±2.04	16±2.04	17±1.68
Rhizoctonia solani	46±1.41	27±2.86	23±2.86	29±2.86	37±1.40	33±1.73	31±2.86	32.29±2.83
			Brinjal					
Aspergillus flavus	56±2.12	50±2.37	46±1.41	48±1.41	41±1.69	61±2.17	57±2.12	51.29±2.65
A. niger	41±1.69	35±1.40	34±1.40	37±1.40	31±2.86	42±1.69	53±2.37	39±2.75
A. terrus	19±2.04	11±0.75	17±2.04	16±2.04	10±0.75	13±0.75	17±2.04	14.71±1.29
Fusarium oxysporum	37±1.40	41±1.69	33±1.40	19±2.04	27±2.86	53±2.37	49±1.41	37±4.51
Macrophomina phaseolina	29±2.86	27±2.86	31±2.86	54±2.37	17±2.04	22±2.86	33±1.40	30.43±4.44
Penicillium commune	17±2.04	19±2.04	17±2.04	20±2.04	18±2.04	16±2.04	15±2.04	17.43±0.65
Rhizoctonia solani	33±1.40	29±2.86	27±2.86	24±2.86	19±2.04	35±1.40	41±1.69	29.17±2.77
Trichoderma harzianum	17±2.04	29±2.86	34±1.40	31±2.86	30±2.86	29±2.86	25±2.86	27.86±2.08
			Tomato					
Alternaria solani	35±1.40	19±2.04	22±2.86	27±2.86	29±2.86	33±1.40	41±1.69	29.43±2.88
Aspergillus flavus	56±2.37	53±2.37	50±1.41	57±2.37	47±1.41	53±2.37	44±1.41	51.43±1.78
A. niger	33±1.40	27±2.86	28±2.86	31±2.86	39±1.40	30±2.86	35±1.40	31.86±1.58
Drchselra hawaiiensis	29±2.86	27±2.86	31±2.86	25±2.86	17±2.04	11±0.75	19±2.04	22.71±2.74
Fusarium oxysporum	57±2.37	51±2.37	63±2.17	48±1.41	53±2.37	57±2.37	66±2.16	56.43±2.43
Macrophomina phaseolina	37±1.45	31±2.86	35±1.40	36±1.45	29±2.86	12±0.67	17±2.04	28.14±3.72
Rhizoctonia solani	65±2.17	57±2.37	44±1.41	41±1.69	48±1.41	33±1.40	39±1.40	46.71±4.17
			Radish					
Aspergillus niger	37±1.45	39±1.40	31±2.86	28±2.86	33±1.40	41±1.69	19±2.04	32.57±2.84
Fusarium oxysporum	57±2.37	45±1.41	61±2.17	35±1.40	37±1.45	31±2.86	36±1.45	43.14±4.41
Macrophomina phaseolina	27±2.86	19±2.04	18±2.04	27±2.86	26±2.86	39±1.40	48±1.41	29.14±4.08
Rhizoctonia solani	17±2.04	11±0.52	18±2.04	27±2.86	29±2.86	31±2.86	33±1.40	23.17±3.15
			Spinach					
Aspergillus flavus	78±2.50	65±2.17	57±2.37	67±2.16	71±1.90	47±1.37	61±2.17	63.71±3.78
A. fumigatus	29±2.86	15±2.04	11±0.52	10±0.52	27±2.86	35±1.40	31±2.86	22.57±3.89
Drechselra hawaiiensis	33±1.40	41±1.69	27±2.86	29±2.86	21±2.04	17±2.04	35±1.40	29±3.12
Fusarium oxysporum	57±2.37	82±2.50	71±1.90	69±2.16	78±2.50	66±2.17	61±2.17	69.14±3.35
Macrophomina phaseolina	31±2.86	47±1.37	45±1.41	40±1.69	38±1.45	41±1.69	36±1.45	39.71±2.04

KHI = Karachi, TAND = Tando Allahyar, MPK= Mirpurkhas, GHO = Ghotaki, KHA = Khairpur, KUN= Kunri, UME = Umerkot.

average mean value of 56 and 32%, respectively, as compared to other speciese i.e. *Alternaria solani, Aspergillus flavus, A. fumigatus, A. niger, Rhizoctonia solani* and *Drechselra hawaiiensis.* On the basis of regions' comparison, the occurrence of these fungi was maximum in the samples Tando Allahyar (82%), Khairpur (78%) and Mirpurkhas (71%), respectively, and minimum (10%) from Ghotaki region (Table 4).

Table 5 shows the results of ANOVA for the fungal infection % on soil samples collected from various localities of Sindh. Eleven fungal species including *Alternaria solani, Aspergillus flavus, A. fumigatus, A. niger, A. terrus, Drechselra hawaiiensis, Fusarium oxysporum, Macrophomina phaseolina, Penicillium commune, Rhizoctonia solani* and *Trichoderma harzianum* showed high significant differences among localities. Nine species are pathogenic on all vegetables particularly cabbage, brinjal, tomato, radish and spinach crops. In brinjal *Penicillium commune* showed non-significant difference than other vegetables.

Meteorological conditions such as high temperature and low humidity during the summer contribute to fewer fungi

Table 5. F-ratios derived from ANOVA for fungal infection % of soil

Fungi species	F-ratio	P-value	$LSD_{0.05}$
	Cabbage		
Aspergillus flavus	31.87	0.0000***	6.11
A. fumigatus	33.08	0.0000***	5.30
A. niger	24.40	0.0000***	5.93
Fusarium oxysporum	53.89	0.0000***	5.56
Macrophomina phaseolina	7.97	0.0000***	6.97
Penicillium commune	4.59	0.0006***	5.84
Rhizoctonia solani	9.94	0.0000***	6.71
	Brinjal		
Aspergillus flavus	13.16	0.0000***	5.46
A. niger	14.63	0.0000***	5.37
A. terrus	4.40	0.0009***	4.57
Fusarium oxysporum	37.38	0.0000***	5.52
Macrophomina phaseolina	21.77	0.0000***	7.11
Penicillium commune	0.70	0.6453ns	5.77
Rhizoctonia solani	10.58	0.0000***	6.35
Trichoderma harzianum	4.49	0.0007***	7.31
	Tomato		
Alternaria solani	11.45	0.0000***	6.35
Aspergillus flavus	5.49	0.0001***	5.69
A. niger	3.17	0.0087**	6.62
Drechselra hawaiiensis	8.86	0.0000***	6.88
Fusarium oxysporum	8.56	0.0000***	6.20
Macrophomina phaseolina	24.99	0.0000***	5.56
Rhizoctonia solani	40.39	0.0000***	4.90
	Radish		
Aspergillus niger	13.49	0.0000***	5.78
Fusarium oxysporum	35.79	0.0000***	5.51
Macrophomina phaseolina	22.16	0.0000***	6.47
Rhizoctonia solani	13.89	0.0000***	6.32
	Spinach		
Aspergillus flavus	22.34	0.0000***	5.98
A. fumigatus	23.82	0.0000***	5.96
Drechselra hawaiiensis	15.13	0.0000***	5.98
Fusarium oxysporum	15.35	0.0000***	6.38
Macrophomina phaseolina	9.30	0.0000***	5.01

while in the rainy season the concentration of fungi is significantly increased in the soil (Kakde *et al.,* 2001). It is interesting to note that in Karachi, located in southern Sindh, studies on airborne mycobiota (Rao *et al.,* 2009; Afzal *et al.,* 2004) have demonstrated that the aerospora is dominated by *Aspergillus flavus, A. niger* and *Alternaria solani*. Thus, the atmospheric mycobiota trend to correspond with the soil of vegetable fungal dominance.

These results confirms the findings of Hussain *et al.* (2013a); Usman *et al.* (2013);Islam and Babadoost (2002) and Lee *et al.* (2001) . The most frequent associated fungi isolated from the soil of vegetables are *Alternaria solani, Aspergillus flavus, A. fumigatus, A. niger, A. oryzae, A. terrus, Aeromonium fusidiocles, Cladosporium* sp., *Drechselra hawaiiensis, Eurotium berbanbrum, Fusarium oxysporum, Macrophomina phaseolina, Penicillium commune, Rhizoctonia solani, Trichoderma harzianum,* and *Ulocladium* sp., etc. These results prove that these fungi were most prevalent in the soil of fields and also found to be responsible for most of the decline of the vegetable crops.

This preliminary study provides basis for the determination of fungi from root and soil losses of vegetables which are most demanded in Pakistan. A detailed and investigative survey is required to establish the soil and root resistance strategies to reduce the losses both in terms of economic and food supply especially caused by fungi.

References

Afzal, M., Mehdi, F.S., Siddiqui, Z.S. 2004. Effect of relative humidity and temperature on airborne fungal allergens of Karachi city. *Pakistan Journal of Biological Sciences,* **7:** 159-162.

Ali, M. 2000. *Dynamics of Vegetable Production, Distribution and Consumption in Asia,* Asian Vegetable Research and Development Center (AVRDC), Tainan, Taiwan.

Athar, M., Bokhari, T.Z. 2006. Ethnobotany and production constraints of traditional and commonly used vegetables of Pakistan. *Journal of Vegetable Science,* **12:** 27-38.

Barnett, H., Hunter, B.B. 1972. *Illustrated Genera of Imperfect Fungi,* 241 pp., 3rd edition, Burgess Publishing Co., Minneapolis, Minnesota, USA.

Domsch, K.H., Gams, W., Anderson, T.H. 1980. *Compendium of Soil Fungi,* vol. **I**, 860 pp., Academic Press, London, UK.

Ellis, E.B. 1976. *More Dematiaceous Hyphomycetes,* 507 pp., Commonwealth Mycological Institute, Kew, UK.

Ellis, M.B. 1971. *Dematiaceous Hyphomycetes,* 608 pp., Surrey, Kew, CMI., UK.

Fatima, N., Batool, H., Sultana, V., Ara, J., Ehteshamul-Haque, S. 2009. Prevalence of post-harvest rot of vegetables and fruits in Karachi, Pakistan. *Pakistan Journal of Botany,* **41:** 3185-3190.

Hanif, R., Iqbal, Z., Iqbal, M., Hanif, S., Rasheed, M. 2006. Use of vegetables as nutritional food: Role in human health. *Journal of Agricultural and Biological Science,* **1:** 18-22.

Hussain, F., Shaukat, S.S., Abid, M., Usman, F., Akbar, M. 2013a. Pathogenicity of some important root-rot fungi to the chilli crop and their biological control. *International Journal of Biology and Biotechnology,* **10:** 101-108.

Hussain, F., Shaukat, S.S., Abid, M., Usman, F., Akbar, M. 2013b. Filamentous fungi infecting fruits and leaves of *Capsicum annuum* L. in lower Sindh. *International Journal of Biology and Biotechnology,* **10:** 109-116.

Hussain, F., Shaukat, S.S., Abid, M., Usman, F., Akbar, M. 2013c. Control of *Meloidogyne javanica* and *Fusarium solani* in chilli (*Capsicum annuum* L.) with the application of chitin. *Pakistan Journal of Nematology,* **31:** 165-170.

Hussain, F., Shaukat, S.S., Abid, M., Usman, F. 2012. Some important medicinal plants associated with the vegetation in District Mirpurkhas, Sindh. *International Journal of Biology and Biotechnology,* **9:** 405-420.

Hussain, F., Abid, M. 2011. Pests and diseases of chilli crop in Pakistan: A review. *International Journal of Biology and Biotechnology,* **8:** 325-332.

Islam, S.Z., Babadoost, M. 2002. Effect of red light treatment of seedlings of pepper, pumpkin and tomato on occurrence of *Phytophthora* damping-off. *HortScience,* **37:** 678-681.

Kakde, U.B., Hemalata, U.K., Aarti, A.S. 2001. Seasonal variation of fungal propagules in a fruit market environment, Nagpur (India). *Aerobiologia,* **17:** 177-182.

Khalil, I.A., Rehman, S. 1977. Ber (*Zizyphus jujuba* Mill.) as a nutritive fruit. *Journal of Science and Technology,* (Univ. Peshawar). **1:** 126-128.

Lee, B.K., Kim, B.S., Chang, S.W., Hwang, B.K. 2001. Aggressiveness to pumpkin cultivars of isolates of *Phytophthora capsici* from pumpkin and pepper. *Plant Disease,* **85:** 497-500.

Maqbool, M.A., Hashmi, S., Ghaffar, A. 1988. Problem of root-knot nematodes in Pakistan and strategy for their control. In: *Advances in Plant Nematology.* Proceeding of United States-Pakistan International Workshop on Plant Nematology, M.A. Maqbool *et al.* (eds.), pp. 229-240, NNRC, University of Karachi, Pakistan.

Mirza, J.H., Qureshi, M.S.A. 1978. *Fungi of Pakistan,* 311 pp., University of Agriculture, Faisalabad, Pakistan.

Nelson, P.E., Toussoun, T.A., Marasas, W.F.O. 1983. *Fusarium Species: An Illustrated Manual for Identification,* 206 pp., The Pennsylvania State University Press, University Park, PA., USA.

Promputtha, I., Jeewon, R., Lumyong, S., McKenzie, E.H.C., Hyde, K.D. 2005. Ribosomal DNA fingerprinting in the identification of non sporulating endophytes from *Magnolia liliifera* (Magnoliaceae). *Fungal Diversity,* **20:** 167-186.

Rao, T.A., Sheikh A.H., Ahmed, M.. 2009. Airborne fungal flora of Karachi. *Pakistan Journal of Botany,* **41:** 1421-1428.

Sheikh, A.H., Ghaffar, A. 1992. Effect of fungicide on the survival of sclerotia of *Macrophomina phaseolina* at different soil manure levels and its infection on guar (*Cyamopsis tetragonoloba*). *Proceeding of National Symposium on Status of Plant Pathology in Pakistan,* (3-5 Dec. 1991), A. Ghaffar, and S. Shahzad (eds.), pp. 267-271, Department of Botany, University of Karachi, Karachi. 75270, Pakistan.

Sinclair, J.B. 1982. *Compendium of Soybean Diseases,* 104 pp., 2nd edition, American Phytopathological Society and University of Illinois, IL, USA.

Singh, K., Frisvad, J.C., Thrane, U., Mathur, S.B. 1991. *An Illustrated Manual on Identification of Some Seed-Borne Aspergilli, Fusaria, Penicillia and Their Mycotoxins,* 133 pp., 1st edition, Danish Government Institute of Seed Pathology for Development Countries, Denmark.

Sutton, B.C. 1980. *The Coelomycetes. Fungi Imperfecti with Pycnidia, Acervuli and Stromata,* 696 pp., Commonwealth Mycological Institute, Kew, Surrey, UK.

Usman, F., Shaukat, S.S., Abid, M., Hussain, F. 2013. Rhizosphere fungi of different vegetables and their antagonistic activity against pathogenic fungi of brinjal and spinach. *International Journal of Biology and Biotechnology,* **10:** 255-259.

Waksman, S.A., Fred, E.B., 1922. A tentative outline of the plate method for determining the number microorganisms in the soil. *Soil Science,* **14:** 27-28.

Pak. j. sci. ind. res. Ser. B: biol. sci. 2014 **57**(3) 148-154

Isolation and Characterisation of Chitin and Chitosan from Local Sources

Tayyaba Aftab[a], Asad ullah[b], Muhammad Khalid Iqbal[a]*, Rauf Ahmed Khan[a] and Muhammad Nawaz Chaudhry[b]

[a]CEPS, PCSIR Laboratories Complex, Lahore-54600, Pakistan

[b]College of Earth and Environmental Sciences, University of the Punjab, Lahore, Pakistan

(received December 9, 2013; revised April 10, 2014; accepted April 15, 2014)

Abstract. In this study, indigenous shrimp (*Gadus morhua*) and blue crab (*Portunus pelagius*, male & female) from Karachi coastal area were collected. The flesh was extracted to use for eating and the discarded waste was converted to an environment-friendly value-added product chitosan in chemical process after minor modification of DMCPA protocol. Four chitosan samples of shrimp head shells, blue crab leg shells, claw shells and carapace were obtained. The physicochemical and functional properties i.e., colour, degree of deacetylation, moisture, ash contents, nitrogen, viscosity, water and fat binding capacities were evaluated. Comparative study showed good percentage yields of chitosan from crab leg and shrimp head shells as 25.67% and 22.06%, respectively. Moisture, ash and nitrogen contents were in acceptable ranges. The colour of blue crab leg shell was off-white, while other three were light-yellow. Difference in degree of deacetylation (DD) was significant. The DD was 77% in crab leg shell, 61.6% shrimp head shells, 25.5% crab claw shell and 20.4% for crab carapace chitosan samples. Viscosity values were low (41-116 cPs). Water and fat binding capacity were in range of 494-521 % and 378-428 %, respectively.

Keywords: chitosan, moisture, blue crab, adsorption, viscosity

Introduction

The coastline area of Pakistan stretches over 1046 km from Gwadar bay to Sir Creek, providing a fishing zone of 240,000 square kilometers. Meanwhile, inland fisheries represent great potential especially in water-logged zones spread over 50 million hectares in streams. The marine fisheries play an important role in Pakistan's economy being multimillion export sector. During the year 2010, Pakistan exported seafood products worth $300 million, mainly to China and other Far Eastern countries (Parihar, 2011). In view of this, a great interest has been increased for possible utilisation of fisheries by-products and processed left-over as a promising resource for their valuable protein, sugars and mineral contents, instead of an unacceptable hazardous waste problem (Diaz-Visurraga *et al*., 2010; Kim and Mendis, 2006). In order to tackle this growing environmental issue, an increased focus is being given to isolate value-added materials like chitin and its useful derivative chitosan from waste (Du *et al*., 2009; 2008).

Chitosan is one of the biodegradable, nontoxic, environmental friendly and natural polymer extracted from different types of mollusks, insects, marine diatoms, algae, fungi, yeasts, shrimps, crabs, and other crustacean species at industrial scale (Kurita, 2006; Tharanathan and Kittur, 2003; Yanga *et al*., 2000). Insoluble chitin is converted into a soluble derivative, chitosan after chemical treatment (Iqbal *et al*., 2005).

These natural polymers encompass the intrinsic properties as an effective colour absorbent because commercial polymers and ion exchange resins are of Petroleum based materials, which are not environment-friendly. In recent years, an interest for natural and low cost polymers is increasing day by day as recent investigations have approved an effective role of chitin and chitosan in various fields of biotechnology, chemistry, environmental safety, medicine, food processing, paper, textile production, cosmetics, agriculture and wastewater treatment (Alves and Mano, 2008). Chitosan is used as flocculant, coagulant and antimicrobial due to chelating properties and adsorbs the colour and heavy metals of effluents (Zaku *et al*., 2011; Zeng *et al*., 2008).

In order to surmount the complexity connected to wastewater containing dyes, resistant to aerobic digestion is necessary to remove the colour from industrial effluents. The adsorption of dyes by chitosan is an effective and economic method for water decontamination and reuse for irrigation purposes.

*Author for correspondence;
E-mail: khalid_khichi2000@yahoo.com

The objectives of the present study was to isolate chitosan from indigenous fishery crustacean waste (shrimp head shell, crab leg shells, crab claw shells, and crab carapace), to compare its physicochemical and functional characteristics.

Materials and Methods

Raw material collection and sample preparation. Two local crustaceans white jinga shrimp (*Gadus morhua*) maximum length of 15 cm and blue swimming crab (*Portunus pelagius*) 20 cm long male and 15 cm female were collected from Arabian Sea, Karachi coastal area and used to extract chitin. The flesh/meat was separated from waste. In case of white shrimps only head shell was taken and for blue crab (male + female) further segregation into shells of legs, claws and carapaces was done to get four different raw materials (Fig. 1a-f) to isolate chitin and chitosan.

These raw materials were scrapped free of loose tissues, washed with tap water, sun dried for two days, ground, sieved to 250µm size and kept separately in air tight plastic bags at room temperature.

Isolation of chitosan. The extraction was carried out by an alkali-acid chemical treatment method including four steps of demineralisation, decolourisation, deproteinisation and deacetylation called DMCPA protocol.

Demineralisation. The sun-dried crustacean shells were treated with 0.7 N HCL, 1:15 (w/v) for 30 min at room temperature with constant stirring. Filtered through vacuum filtration and placed under tap water for 30 min until the filtrate became neutral. The pH was noted, then the material was oven dried at 60 °C and weighed.

Decolourisation. The crustacean shells after demineralisation step were soaked in acetone at ambient temperature for 10 mins, allowed to dry in dark for 2 h. The shells were bleached for 15 mins by 0.315% (v/v) sodium hypochlorite (NaOCl) containing 5.25% available chlorine. A ratio of 1:10 w/v was used to accomplish the bleaching process, excessively washed under tap water and oven dried at 60 °C.

Deproteinisation. The decolourised shells after bleaching were treated with 1.2 N NaOH solution in ratio of 1:10 (w/v) for 2.5 h at 70-75 °C providing constant stirring. Excessively washed under tap water and oven dried at 60 °C for 4 h.

Fig. 1. **(a)** White jinga shrimp (*Gadus morhua*); **(b)** Blue swimming crab (*Portunus pelagius*); **(c)** White ginga; **(d)** Blue crab legs; **(e)** Blue claws; **(f)** Blue crab carapace.

Deacetylation of chitin. Chitosan is deacetylated derivative of chitin, so the removal of acetyl group from raw chitin is required. For this purpose raw chitin was

treated with 50% NaOH in ratio 1:13 (w/v) and autoclaved under 15 psi pressure at 121 °C temperature for 15 min, excessively washed under tap water until neutral. Hot distilled water was used for rinsing of the product, filtration and oven dried at 60 °C for 24 h.

Analytical analysis. These four isolated samples of chitosan were further characterised for moisture and ash (AOAC, 2005), degree of deacetylation (Kasaai *et al*., 2000), viscosity (Huang *et al*., 2011), colour (Tajik *et al*., 2008), water and fat binding capacity (Wang and Kinsella, 1976). All the analyses were performed in triplicate to assurance the precision and accuracy of the whole experimental work. To investigate the surface characteristics of chitosan, fourier transform infrared (FTIR, Perkin-Elmer spectrophotometer spectrum one) and elemental analysis (CHN and S) were performed by using the elemental CHNS instruments.

Results and Discussion

Colour. The colour of isolated chitosan from crab leg shells was off white, while the other three samples were light yellow in colour. Brownish to light yellow colour was observed by Tajik *et al.* (2008) and whiter to less red by Youn *et al.* (2007).

Percentage yield of chitosan. The percentage yields of chitin and chitosan was calculated from dry raw shrimp and crab waste. In case of crab claws and carapaces, high percentage weight loss was associated with excessive CO_2 gas emission with effervescence and undesirable foam that is a proof of high mineral content loss during demineralisation process (DP).

$$CaCO_3 + 2HCl \rightarrow CaCl_2 + CO_2\,(g) + H_2O$$

Similarly, after DP process weight loss was highest in shrimp head shells (47.81-24.91=22.90%), which indicated high protein content. Highest weight loss (28.26%) for crab carapaces was due to loss of pigments present on the shell. Demineralisation results are in compliance with Bolat *et al*. (2010), who found 34.32% yield out of 60% dry waste of fresh crab (*Potamon potamios*), but a final yield of chitosan was quite higher for the white shrimp and blue crab as shown in Fig. 2a.

For shrimp head shells chitosan yield (22.06%) was found in equality to that of brine shrimp (23.1%), mentioned by Tajik *et al*. (2008), but higher than (15%), the yield extracted from local Bangladesh shrimp waste mentioned. Yield of chitosan from (25.67%) crab leg shells, followed by shrimp head shells (22.06%), crab carapaces (13.81%) and finally crab claws (11.53%). Blue crab claw shells exhibited the lowest percentage yield.

It is repeatedly shown in literature that the percentage yield of chitosan in shells waste of different marine species differs extensively, depending on the type of species and the process conditions (Abdou *et al*., 2008; Chandumpai *et al*., 2004; Synowiecki and Al-Khateeb, 2003). The raw material with higher amount of crude chitin yield higher chitosan. According to No *et al.* (2003) difference in the properties of the prepared chitosan depends on the type of marine species and the ways of production.

Moisture content. In all extracted samples the observed moisture was less than 10% as stated by KFDA in 1995. No significant variation in moisture content of chitosan isolated from shrimp head shell (5.5%) and crab leg shell (5.6%), was observed, while some fluctuation in crab claw (6.1%) and crab carapace (3.9%), shown in Fig. 2b was observed that could be attributed to the hygroscopic nature of chitosan (Ocloo *et al.*, 2011).

Ash content. The ash content of four chitosan samples was in the range of 1.05 to 2.6%. Tajik *et al*. (2008) have reported that the commercial best grade chitosan contains about 1.18% ash. Literature is available showing higher values e.g., 4.05% in local brine shrimp in Bangladesh. Fig. 2c clearly shows that crab leg shell had lowest ash content following by shrimp head shell.

Nitrogen content. Nitrogen contents of four different chitosan samples were shown in Table 1. In 1995, No and Meyers reported a similar range 7.06% to 7.97% nitrogen in chitosan samples. Other elemental analysis of chitosan sample showed carbon range 39.59%, 41.78%, 45.73%, 50.10%, hydrogen range 4.48%,

Table 1. Elemental analysis and colour of four isolated chitosan samples

Chitosan source	Nitrogen	Carbon	Hydrogen (%)	C/N	Colour
Head shells (shrimp)	7.20	41.78	5.10	5.78	Light yellow
Leg shells (crab)	7.16	39.59	4.48	5.53	Off white
Claw shells (crab)	7.15	45.73	5.13	6.39	Light yellow
Carapaces (crab)	7.73	50.10	6.75	6.48	Light yellow

Values were rounded off up to two decimal places.

5.10%, 5.13%, 6.75% and accordingly the C/N ratio 5.53, 5.78, 6.39, 6.48 with a small difference among four extracted samples (Fig. 2d).

Degree of deacetylation. Being an important property of the chitosan samples, the degree of deacetylation value affects other parameters such as biodegradability, chemical reactivity and solubility of chitosan. In the present study, chitosan isolated crab leg shells and shrimp head shell had good degree of deacetylation as 77% and 61.6%, respectively. The results by claw shell and carapace chitosan were not within the reported values in literature. Degree of deacetylation depends on raw material, the extraction method, sample preparation and experimental conditions (Khan *et al.*, 2002). A range of 30-90 percent degree of deacetylation was reported by Martino *et al.* (2005).

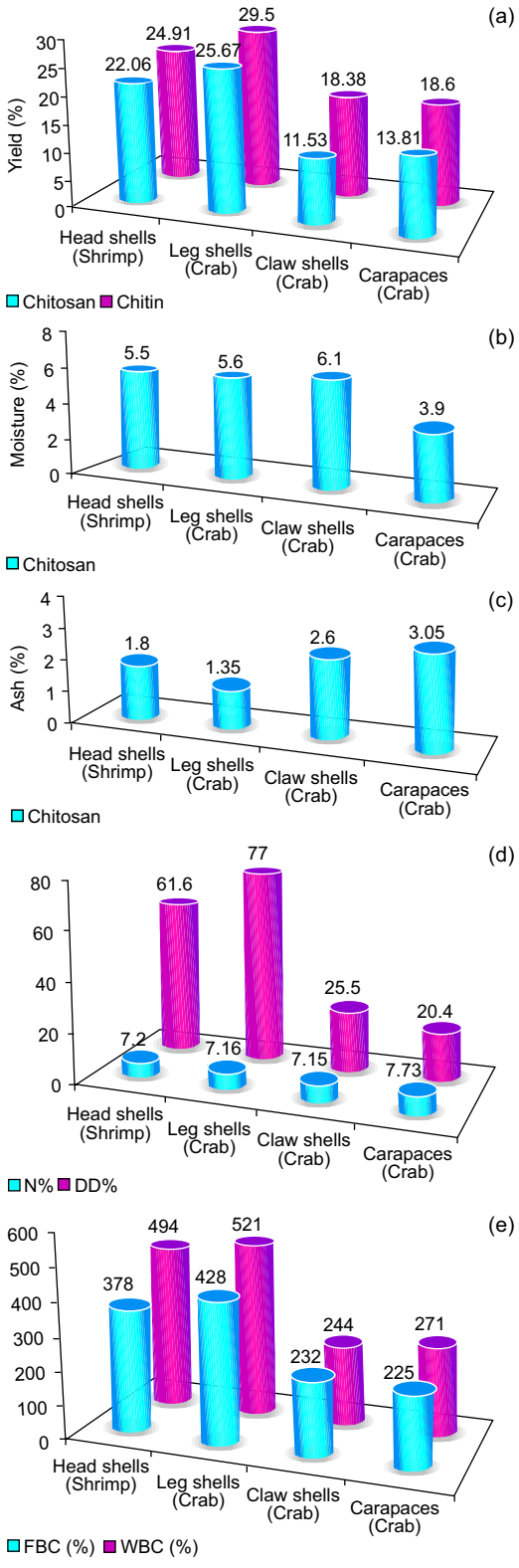
Viscosity. In the present study the values of viscosities in four isolated chitosan samples were lower ranging from 41 to 116.3 cPs (Table 2). Reduction in viscosity may be due to the use of bleaching agent or after protein extraction (Moorjani *et al.*, 1975). The viscosity range of 26-360 cPs for chitosan is reported by No *et al.* (2000). Similarly, in another study by Bough *et al.* (1978) a wider range of viscosity (60 to 5110 cPs) was reported. It was observed that crab leg and shrimp shell chitosan have higher values of viscosity than carapace and claw shell chitosan. Far lower viscosities of the samples indicate their limited applicability in food, cosmetic and medicine industry because of difficulties in handling, poor suspension and thickening agents (Tajik *et al.*, 2008).

Water and fat binding capacity. The water binding capacity value of four chitosan samples were in the range of 244% to 521%. It was observed that chitosan obtained from crab leg shells exhibited more capacity to bind water, on the other hand shrimp head shell chitosan with 494% WBC (water binding capacity) show comparable result to crab leg shells. Whereas, crab claw shells and crab carapace have 244% and

Table 2. Viscosity of four isolated chitosan samples

Chitosan source	Viscosity (cPs)
Head shells (shrimp)	97 ± 1.52
Crab leg shells	116 ± 0.57
Crab claw shells	41 ± 1.15
Crab carapaces	54 ± 1.15

Mean ± standard deviation of triplicate determinations.



N=nitrogen; DD=degree of deacetylation; FBC=fat binding capacity; WBC=water binding capacity.

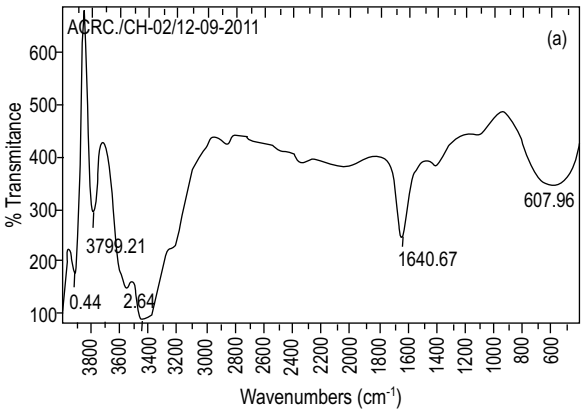
Fig. 2. Percentage yields of four isolated chitin and chitosan samples.

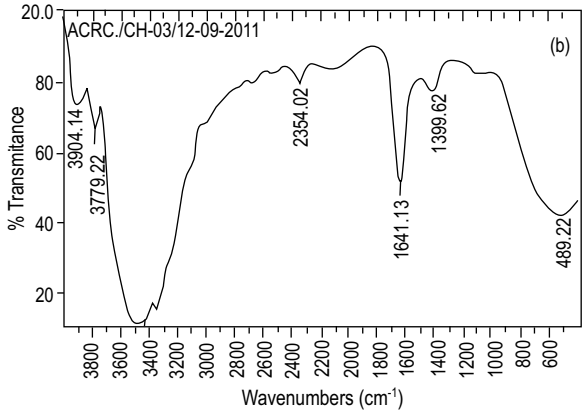
270% water binding capacities, respectively, while working on five commercial chitosans from shrimp and crab reported the range for WBC as 58% to 805%, which satisfies the low results given by crab claw shells and crab carapace chitosan samples in Fig. 2e. For different crustaceans ranges for water binding capacity (WBC) value are claimed as 355%–611% (No *et al.*, 2000); 58%–805% for shrimp chitosan and 491%–555% for crab chitosan (Kucukgulmez *et al*., 2011).

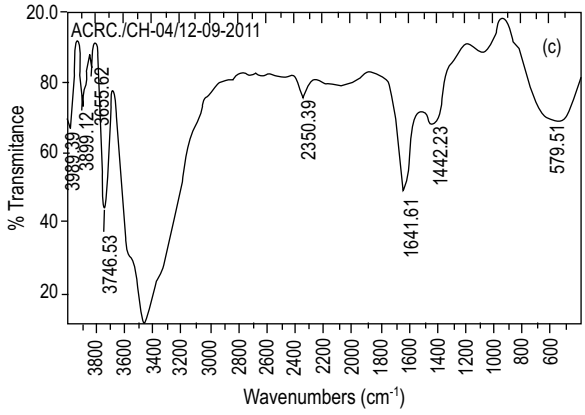
The values of FBC (fat binding capacity) of the chitosan samples were in the range of 378% to 428% excluding crab claw shells and crab carapace. These FBCs by Chitosan1 (shrimp head shell) and crab leg shells were found higher than those reported by No *et al*. (2003), but lower than findings for crawfish (706%) and for commercial crab (587%). The value 370% for shrimp chitosan is in good resemblance to shrimp head shell results. Crab leg shells with 428% is also in accordance with the value (490.10%) of crab chitosan by Ocloo *et al*. (2011).The lower values by crab claw shells (claw) and crab carapace (carapace) chitosan samples show their less capacity to bind fat, hence low applicability. The decreased viscosity as observed may be a cause of decrease in fat binding capacities in crab claw shells and crab carapace chitosan samples.

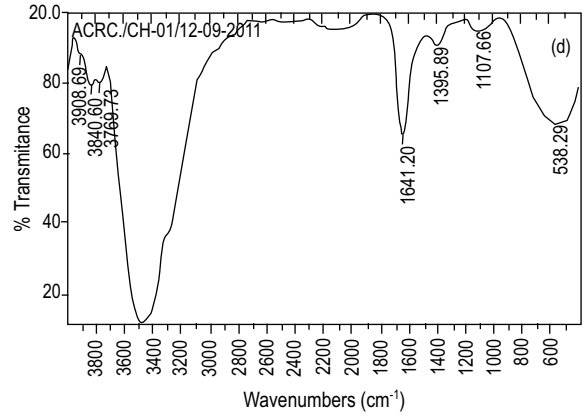
FTIR analysis. Figure 3 (a,b,c,d,e) presents the results of fourier transform infrared spectroscopy for chitosan samples isolated from shrimp head waste, crab leg shells, crab claws crab carapaces and pure chitosan, respectively. All infrared spectra are significantly similar to each other in regard of characteristic peaks region 3300-3500 cm^{-1}, 1400-1650 cm^{-1} and 500-600 cm^{-1} which is consistent to previous researchers. Some characteristic peaks of chitosan in literature are quoted as 1070 cm^{-1} for C–O stretching, 1570 cm^{-1} for N–H bending vibration of 1° amides, 1555 cm^{-1} for N–H bending vibration of amide II band, 1655 cm^{-1} for –C=O stretch vibration of 2° amide I band and 3300 cm^{-1} for $–CH_3$, $–CH_2$ bands (Lee *et al*., 2005).

Intermolecular –H bands are mostly attributed to the broad peak at about 3300 cm^{-1}, as reported by Kucukgulmez *et al.* (2011). Similarly, at 600 cm^{-1} out plane bending –OH vibrations were noted. The peaks in 1380-1442 cm^{-1} region represented the asymmetric and symmetric bending vibrations associated with $–CH_3$ groups. According to the study of Li *et al.* (1998) the bands at 1415 cm^{-1} indicate the –C–H bending vibrations of $–CH_2$.









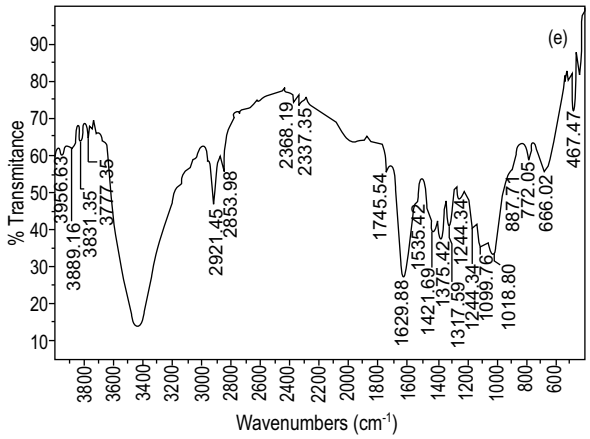


Fig. 3. FTIR spectrum of (a) isolated shrimp head shell chitosan; (b) isolated crab leg shells chitosan; (c) isolated crab claw shells chitosan; (d) isolated crab carapace chitosan; (e) pure chitosan.

Conclusion

Chitin and its derivative chitosan were isolated from waste of two local crustaceans and its various physicochemical and functional characteristics of prepared chitosan samples were investigated. In this way, the environmental pollution may be reduced by an attractive route of seafood waste management and the broad spectrum in industrial sector. On the basis of present study it was concluded that chitosan can be used as ecofriendly adsorbent.

References

Abdou, E.S., Nagy, K.S.A., Elsabee, M.Z. 2008. Extraction and characterization of chitin and chitosan from local sources. *Bioresource Technology*, **99:** 1359-1367.

Alves, N.M., Mano, J.F. 2008. Chitosan derivatives obtained by chemical modifications for biomedical and environmental applications. *International Journal of Biological Macromolecules*, **43:** 401-414.

AOAC, 2005. *Official Methods of Analysis of AOAC International*, 18th edition, AOAC International, Gaithersburg, Maryland, USA.

Bolat, Y., Bilgin, S., Gunlu, A., Izci, L., Koca, S.B., Cetinkaya, S., Koca, H.U. 2010. Chitin-chitosan yield of freshwater crab (*Potamon potamios*, Olivier 1804) shell. *Pakistan Veterinary Journal*, **30:** 227-231.

Bough, W.A., Salter, W.L., Wu, A.C.M., Perkin, B.E. 1978. Influence of manufacturing variables on the characteristics and effectiveness of chitosan products. I- Chemical composition, viscosity and molecular-weight distribution of chitosan products. *Biotechnology and Bioengineering*, **20:** 1931-1943.

Chandumpai, A., Singhpibulporn, N., Faroongsarng, D., Sornprasit, P. 2004. Preparation and physicochemical characterization of chitin and chitosan from the pens of the squid species, *Loligo lessoniana* and *Loligo formosana*. *Carbohydrate Polymers*, **58:** 467-474.

Diaz-Visurraga, J., García, A., Cárdenas, G. 2010. Lethal effect of chitosan-Ag (I) films on *Staphylococcus aureus* as evaluated by electron microscopy. *Journal of Applied Microbiology*, **108:** 633-646.

Du, W.L., Niu, S.S., Xu, Y.L., Xu, Z.R., Fan, C.L. 2009. Antibacterial activity of chitosan tripolyphosphate nanoparticles loaded with various metal ions. *Carbohydrate Polymers*, **75:** 385-389.

Du, W.L., Xu, Z.R., Han, X.Y., Xu, Y.L., Miao, Z.Y. 2008. Preparation characterization and adsorption properties of chitosan nanoparticles for eosin Y as a model anionic dye. *Journal of Hazardous Materials*, **153:** 152-156.

Huang, X.Y., Bin, J.B., Bu, H.T., Jiang, G.B., Zeng, M.H. 2011. Removal of anionic dye eosin Y from aqueous solution using ethylenediamine modified chitosan. *Carbohydrate Polymers*, **84:** 1350-1356.

Iqbal, J., Tirmizi, S.A., Mirza, M.L. 2005. Adsorption status of some transition metal ions on pretreated fish scales. *Journal of The Chemical Society of Pakistan*, **27:** 77-81.

Kasaai, M.R., Arul, J., Charlet, G. 2000. Intrinsic viscosity-molecular weight relationship for chitosan. *Journal of Polymer Science Part B: Polymer Physics*, **38:** 2591-2598.

Khan, T.A., Peh, K.K., Chng, H.S. 2002. Reporting degree of deacetylation values of chitosan: the influence of analytical methods. *Journal of Pharmacy and Pharmaceutical Sciences*, **5:** 205-212.

Kim, S.K., Mendis, E. 2006. Bioactive compounds from marine processing byproducts – A review. *Food Research International*, **39:** 383-393.

Kucukgulmez, A., Celik, M., Yanar, Y., Sen, D., Polat, H., Kadak, A.E. 2011. Physicochemical characterization of chitosan extracted from Metapenaeus stebbingi shells. *Food Chemistry*, **126:** 1144-1148.

Kurita, K. 2006. Chitin and chitosan: functional biopolymers from marine crustaceans. *Marine Biotechnology* (*New york*), **8:** 203-226.

Lee, M.Y., Hong, K.J., Kajiuchi, T., Yang, J.W. 2005. Synthesis of chitosan-based polymeric surfactants and their adsorption properties for heavy metals and fatty acid. *International Journal of Biological Macromolecules*, **36:** 152-158.

Li, Q., Weng, S., Wu, J., Zhou, N. 1998. Comparative study on structure of solubilized water in reversed micelles. 1 FT-IR spectroscopic evidence of water/AOT/n-heptane and water/NaDEHP/n-heptane systems. *The Journal of Physical Chemistry B*, **102:** 3168-3174.

Martino, A.D., Sittinger, M., Risbud, M.V. 2005. Chitosan: a versatile biopolymer for orthopaedic tissue-engineering. *Biomaterials*, **26:** 5983-5990.

Moorjani, M.N., Achutha, V., Khasim, D.I. 1975. Parameters affecting the viscosity of chitosan from prawn waste. *Journal of Food Science and Technology*, **12:** 187-191.

No, H.K., Lee, S.H., Park, N.Y., Meyers, S.P. 2003. Comparison of physicochemical, binding and anti-bacterial properties of chitosans prepared without and with deproteinization process. *Journal of Agricultural and Food Chemistry*, **51:** 7659-7663.

No, H.K., Lee, M.Y. 1995. Isolation of chitin from crab shell waste. *Journal of The Korean Society of Food Science and Nutrition*, **24:** 105-113.

Ocloo, F.C.K., Quayson, E.T., Adu-Gyamfi, A., Quarcoo, E.A., Asare, D., Serfor-Armah, Y., Woode, B.K. 2011. Physicochemical and functional characterization of radiation-processed shrimp chitosan. *Radiation Physics and Chemistry*, **80:** 837-841.

Parihar, M.M. 2011. Export to (EU) markets: The EU authorities have once again declined Pakistan the opportunity to export fish to their market. *Dawn Newspaper*, November 27.

Synowiecki, J., Al-Khateeb, N.A. 2003. Production, properties and some new applications of chitin and its derivatives. *Critical Reviews in Food Science and Nutrition*, **43:** 145-171.

Tajik, H., Moradi, M., Rohani, S.M.R., Erfani, A.M., Jalali, F.S.S. 2008. Preparation of chitosan from brine shrimp (*Artemia urmiana*) cyst shells and effect of different chemical processing sequences on the physiological and functional properties of the product. *Molecules*, **13:** 1263-1274.

Tharanathan, R.N., Kittur, F.S. 2003. Chitin−the undisputed biomolecule of great potential. *Critical Reviews in Food Science and Nutrition*, **43:** 61-87.

Wang, J.C., Kinsella, J.E. 1976. Functional properties of novel proteins: alfalfa leaf protein. *Journal of Food Science*, **41:** 286-292.

Yang, J., Shih, I.I., Tzeng, Y., Wang, S. 2000. Production and purification of protease from a *Bacllius subtilis* that can deproteainize crustacean wastes. *Enzyme and Microbial Technology*, **26:** 406-413.

Youn, D.K., No, H.K., Prinyawiwatkul, W. 2007. Physical characteristics of decolorized chitosan as affected by sun drying during chitosan preparation. *Carbohydrate Polymers*, **69:** 707-712.

Zaku, S.G., Emmanuel, S.A., Aguzue, O.C., Thomas, S.A. 2011. Extraction and characterization of chitin; a functional biopolymer obtained from scales of common carp fish (*Cyprinus carpio* l.): A lesser known source. *African Journal of Food Science*, **5:** 478-483.

Zeng, D., Wu, J., Kennedy, J.F. 2008. Application of a chitosan flocculants to water treatment. *Carbohydrate Polymers*, **71:** 135-139.

Pak. j. sci. ind. res. Ser. B: biol. sci. 2014 **57** (3) 155-160

Impact of a Widely Cultivated Tree (*Moringa oleifera*) on the Health of Commercially Important Hybrid Catfish

Kabir Mohammed Adamu* and Idris Mohammed Ahmed
Department of Biology, Ibrahim Badamasi Babangida University, Lapai, P.M.B. 11, Lapai, Niger State, Nigeria

(received January 31, 2014; revised July 10, 2014; accepted July 18, 2014)

Abstract. Plantations of the tree *Moringa oleifera* often lead to increas levels of leaf dust in nearby freshwater environments, and there is concern that, this could have a negative impact on catfish, which are important for aquaculture. Therefore, this study, determined the biochemical alterations in serum, liver and kidney of hybrid catfish (*Clarias gariepinus* (♀) × *Heterobranchus bidorsalis* (♂) exposed to sub-lethal concentrations of *M. oleifera* leaf dust in a static renewable bioassay system during a medium term exposure period. The fish (mean length, 16.33 cm, mean weight, 9.90 g) were exposed to 0.16, 0.12, 0.08, 0.04 and 0.00 mg/L concentrations of the plant leaf dust in triplicate exposure. After 21-days of exposure period, the fish were sacrificed for the biochemical parameters: glucose, total protein, albumin, globulin, cholesterol, triglyceride, γ-glutamyltransferase, alanine aminotransferase, aspartate aminotransferase and lactate dehydrogenase. Fish exposed to leaf dust showed significant differences ($P<0.05$) in levels of gamma glutamyltransferase serum, liver and kidney, and also in levels of albumin and cholesterol ($P<0.05$), in the liver and kidney. The levels of glucose and triglycerides were significantly different ($P<0.05$), in the liver and kidney, respectively, while, the other parameters were not significantly different ($P>0.05$), in the liver and kidney, respectively, in the test fish. Other parameters were not significantly different ($P>0.05$) in their respective tissue-organs. Ipso-facto, the alteration in biochemical parameters of hybrid catfish exposed to *M. oleifera* leaf dust was concentration dependent with 0.16 mg/L showing the highest negative alterations thus fish exposed to concentrations above 0.16 mg/L for longer durations may suffer impaired health effects.

Keywords: biochemical parameters, hybrid catfish, *Moringa oleifera*

Introduction

Plant materials are virtually inexhaustible source of structurally diverse biologically active substance (Ujvary, 2000), where some plants contain compounds of various classes that have insecticidal, pesticidal and molluscidal properties. One of such plants is *Moringa oleifera,* commonly known as 'Zogalle' in northern Nigeria, and one of the most useful and widely cultivated tropical trees. According to Foidl *et al.* (2001) every part of this plant is used in traditional medicine. However, the plant has also been reported to have negative effects on the health of a variety of fish species, including *Heterobranchus bidorsalis* (Olufayo and Akinpelu, 2012), *Nile tilapia,* and *Oreochromis niloticus* (Ayotunde *et al.* 2010). The tree's components are also known to affect rats (Omobuwajo *et al.,* 2011; Hamza, 2010; Tende *et al.,* 2010; Oluduro and Aderiye, 2009; Ara *et al.,* 2008; Jaiswal *et al.,* 2007) and chicken (Ogbe and Affiku, 2012). Studies have also shown that the tree has the potential of insecticidal activity against *Cnaphalacrosis medicinalis* (Muralidhara Rao *et al.,* 2010), as antimicrobial agent (Anwar and Rashid, 2007; Lockett *et al.,* 2000) and treating human infectious disease (Arekemase *et al.,* 2011).

According to Rand *et al.* (1995) in order to extrapolate meaningful, relevant and ecologically significant results in aquatic toxicity research, not only appropriate test but also appropriate organisms should be used. The hybrid catfish, *Clarias gariepinus* (♀) × *H. bidorsalis* (♂) is one of the commercially important species of fish for rapid aquaculture expansion in Nigeria and Africa, as they constitute one of the main fish species of economic importance. Thus, the study address the question of to what extent the health of catfish might be impacted by varying levels of leaf dust in the water, derived from *M. oleifera,* which are commonly planted near waters where catfish are held.

To assess fish health, some biochemical parameters were used to evaluate the functional capacity of several critical organ systems. The levels of these parameters: glucose,

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

triglycerides, albumin, globulin, total protein, cholesterol, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, and γ-glutamyltransferase in blood, liver and kidney were used. Therefore, this study was aimed at ascertaining the effect of *M. oleifera* leaf dust on the selected biochemical parameters of hybrid catfish after a medium term, 21-day exposure period in a static semi renewable bioassay system; in order to monitor the health status and provide basic information on the effects of the *M. oleifera* leaf dust on the test fish.

Materials and Methods

Two hundred and twenty juveniles of hybrid catfish, mean±standard error total length and weight of 16.33± 0.50 cm and 9.90±1.25 g, respectively, were procured from Premier Fish Farm, Chanchaga, Niger State, Nigeria. The fish were held in Biology laboratory in large aquaria of 80 L capacity with clean borehole water for 3 weeks. Thereafter, they were transferred to the experimental plastic aquaria (8 fish/30 L aquarium) in three replications. The fish were fed 3.0 mm coppens fish feed twice daily during the acclimatisation and exposure periods. Leaves of *M. oleifera* were obtained from state low-cost housing estate, Lapai, Nigeria; which were air dried for 30 days, ground into powder, sieved and stored in a sealed plastic container for use. The concentrations of *M. oleifera* used for experiment (0.16, 0.12, 0.08, 0.04 and 0.00 mg/L) dust were ascertained after preliminary and acute toxicity tests investigations into what concentrations could realistically occur in the environment, where the catfish are typically held.

At the end of the 21-day exposure period, the fish were sacrificed to obtain blood, liver and kidney. Liver and kidney supernatants and serum were obtained from the sample following the procedures described by Adamu and Nwadukwe (2013). The selected biochemical parameters were determined in accordance to the standardised methods of the manufacturer's instruction manual of the commercial kits such as total protein- Biuret method (Tietz, 1995), albumin-bromocresol green method (Doumas *et al.,* 1971), globulin-calculating method (total protein-albumin), triglycerides- enzymatic colorimetric endpoint method (Bucolo and David, 1973), cholesterol-enzymatic endpoint method (Trinder, 1969), lactate dehydrogenase-enzyme in organic synthesis method, glucose-enzymatic colorimetric endpoint method (Trinder, 1969), alanine and aspartate aminotransferases-enzymatic method (Reitman and Frankel, 1957) and γ-glutamyltransferase - enzymatic method (IFCC, 1983).

All results were presented as means ± standard error. Data were analysed using two-way analysis of variance; thereafter, individual means were compared using Bonferroni comparison test. The differences were considered statistically significant at either $P<0.01$or $P<0.05$. All statistical analyses were performed using the software package (graphpad prism @ software version 6.0 San Diego, CA, USA).

Results and Discussion

The plant leaf dust revealed varying levels of variations in the determined biochemical parameters as presented in Table 1 after the 21-days exposure period. With exception of globulin level in fish exposed to 0.16 mg/L plant leaf dust, the serum total protein, globulin, albumin, glucose, cholesterol and triglycerides were not significantly different ($P>0.05$), compared to the control. The levels of total protein, albumin and globulin were significantly different ($P<0.05$) in the liver whereby, the kidney recorded significant difference ($P<0.05$), as the concentration of the plant leaf dust increased. The liver recorded the higher significant difference ($P<0.05$), with the kidney recording the least and no significant difference in serum. With the exception of fish exposed to 0.16 mg/L of the plant leaf dust there was no significant difference ($P>0.05$) in liver cholesterol, similarly, the liver glucose and triglycerides were not significantly different ($P>0.05$). Significant difference ($P<0.05$) was recorded in kidney glucose, cholesterol and triglycerides as the concentrations of the plant leaf dust increased.

The activities of γ-glutamyltransferase and lactate dehydrogenase were not significantly different ($P>0.05$), in the serum of the test fish, however, there were varying levels of significant difference ($P<0.05$) in the enzymes activities in the liver and kidney as the concentrations of the plant leaf dust increased. The aminotransferases (aspartate and alanine) were significantly different ($P<0.05$) in the serum, liver and kidney of the test fish as the concentrations of the plant leaf dust increased.

The highest serum, liver and kidney alanine aminotransferase activity was recorded in fish exposed to 0.16 mg/L of the plant leaf dust. According to Witthawaskul *et al.* (2003), the enzyme is remarkably specific for liver function; where it is found in particularly large amounts in the liver which plays an important role in metabolism. The significant higher activity of the enzyme may be an indication that the plant leaf dust stimulated the increased synthesis of the liver enzyme. As the

Table 1. Mean value biochemical parameters of hybrid catfish exposed to concentrations of *Moringa oleifera* leaf dust after 21 days

Biochemical parameters	Organ/ Tissue	Concentration of *M. oleifera* leaf dust (mg/L)				
		0.00	0.04	0.08	0.12	0.16
Total protein (g/dL)	Serum	11.18(3.53)^{a}	10.52(0.48)^{a}	14.49(4.45)^{a}	12.11(2.09)^{a}	13.58(1.51)^{a}
	Liver	7.77 (0.19)^{a}	20.62(3.59)^{b*}	22.42(0.70)^{b*}	21.29(2.22)^{b*}	20.07(3.44)^{b*}
	Kidney	7.55 (0.70)^{a}	7.44(0.06)^{a}	10.50(0.19)^{a}	14.27(1.15)^{b}	17.77(1.26)^{b}
Albumin (g/dL)	Serum	6.82(1.16)^{a}	7.13(0.59)^{a}	9.36(1.44)^{a}	8.11(0.33)^{a}	11.42(1.28)^{a}
	Liver	5.99(0.10)^{a}	10.74(2.52)^{b}	8.59(0.49)^{b}	15.60(0.98)^{b*}	11.21(2.37)^{b}
	Kidney	3.99(0.64)^{a}	5.27(0.27)^{a}	7.65(0.32)^{b}	8.65(1.47)^{b}	10.96(0.18)^{b}
Globulin (g/dL)	Serum	4.35(2.37)^{a}	3.38(1.08)^{a}	5.12(1.02)^{a}	3.99(1.76)^{a}	2.16(0.23)^{b}
	Liver	1.79(0.10)^{a}	9.87(1.06)^{b*}	8.84(2.20)^{b*}	5.69(1.20)^{b}	8.86(1.81)^{b*}
	Kidney	3.56 (0.07)^{a}	2.18(0.33)^{a}	2.86(0.52)^{a}	5.62(0.33)^{a}	6.81(1.44)^{b*}
Glucose (mg/dL)	Serum	152.15(11.65)^{a}	160.65(3.45)^{a}	153.30(10.60)^{a}	173.05(16.05)^{a}	168.05(0.95)^{a}
	Liver	164.40(5.10)^{a}	166.60(10.50)^{a}	166.80(0.70)^{a}	185.90(1.80)^{a}	187.05(2.55)^{a}
	Kidney	129.20(25.60)^{a}	189.25(1.25)^{b}	179.95(13.85)^{b}	176.05(5.65)^{b}	193.10(1.70)^{b}
Cholesterol (mmol/L)	Serum	6.08(0.08)^{a}	6.01(0.19)^{a}	6.17(0.21)^{a}	9.73(2.58)^{a}	7.47(0.36)^{a}
	Liver	7.61(1.25)^{a}	6.99(0.33)^{a}	7.70(1.60)^{a}	9.95(0.55)^{a}	15.27(0.16)^{b*}
	Kidney	7.08(1.26)^{a}	9.23(0.11)^{a}	8.57(1.21)^{a}	11.42(1.55)^{b*}	13.25(2.18)^{b*}
Triglycerides (mmol/l)	Serum	11.12(1.05)^{a}	9.98(0.93)^{a}	10.61(0.38)^{a}	10.70(0.16)^{a}	10.60(0.11)^{a}
	Liver	10.13(0.29)^{a}	9.79(2.19)^{a}	9.10(0.98)^{a}	8.00(0.32)^{a}	8.35(0.47)^{a}
	Kidney	6.66(0.10)^{a}	9.14(0.46)^{a}	8.99(1.53)^{a}	10.19(1.11)^{b}	11.91(1.27)^{b}
γ-glutamyltransferase (µkat/L)	Serum	8.34(2.78)^{a}	18.21(7.09)^{b}	25.89(1.30)^{b*}	25.41(8.73)^{b}	35.45(2.31)
	Liver	8.91(0.38)^{a}	20.09(5.93)^{b*}	28.36(1.28)^{b*}	34.84(0.73)^{c*}	35.97(1.08)^{c}
	Kidney	9.82(1.30)^{a}	10.61(0.56)^{a}	18.34(0.18)^{b}	24.82(0.74)^{c}	35.97(1.08)^{c}
Lactate dehydrogenase (µkat/L)	Serum	1.42(0.34)^{a}	1.62(1.35)^{a}	1.42(0.20)^{a}	1.75(0.54)^{a}	1.55(0.07)^{a}
	Liver	2.02(0.81)^{a*}	3.38(0.55)^{b*}	4.45(1.21)^{c*}	2.90(1.42)^{a*}	3.17(0.47)^{b*}
	Kidney	3.27(1.13)^{a*}	3.04(1.15)^{a*}	2.23(0.47)^{b}	1.35(1.21)^{b}	0.41(0.27)^{c*}
Alanine aminotransferase (µkat/L)	Serum	0.47(0.14)^{a}	0.64(0.03)^{a}	0.56(0.06)^{a}	0.77(0.17)^{b}	0.79(0.04)^{b}
	Liver	0.23(0.00)^{a*}	0.32(0.04)^{a*}	0.29(0.06)^{a*}	0.33(0.06)^{a*}	0.42(0.08)^{b*}
	Kidney	0.14(0.03)^{a*}	0.26(0.04)^{a*}	0.44 (0.00)^{b}	0.57(0.03)^{b}	0.63(0.13)^{b}
Aspartate aminotransferase (µkat/L)	Serum	0.50(0.17)^{a}	0.61(0.05)^{a}	0.59(0.29)^{a}	0.75(0.03)^{b}	0.84(0.12)^{b*}
	Liver	0.26(0.09)^{a*}	0.58(0.14)^{b}	0.56(0.11)^{b}	0.73(0.22)^{b}	0.59(0.08)^{b}
	Kidney	0.44(0.22)^{a}	0.53(0.14)^{a}	0.83(0.06)^{b*}	0.75(0.08)^{b}	0.58(0.14)^{a}

Standard error in parentheses, different superscript means P<0.05 across the row, while *means P<0.05 down the column.

activity of the enzyme increased in the liver, there was a corresponding increased enzyme activity in the serum, which may have been caused by leakage of the enzyme into the blood thereby, recording the higher activity in the serum. The serum as connective tissue, functions in transporting of this enzyme to the kidney for excretion. The increase in serum and kidney enzyme activity in hybrid catfish exposed to *M. oleifera* leaf dust was similar to that reported by Adamu (2009), when hybrid catfish was exposed to tobacco (*Nicotiana tobaccum*) leaf dust. By and large, the increase in kidney alanine aminotransferase activity may be due to the ability of the fish to maintain its homeostasis thereby, transporting the enzyme from the serum to the kidney for excretion. The increase in the kidney aspartate aminotransferase activity as the concentrations of the plant leaf dust increased may be attributed to the potency of the plant leaf dust; as the enzyme is specific in kidney function (Witthawaskul *et al.,* 2003). This is similar to the report of Adamu (2009) in hybrid catfish exposed to tobacco leaf dust. This increase may be indications that possibly the plant leaf dust with insecticidal, pesticidal and molluscidal properties may lead to an increase in this enzyme in the kidney. On the other hand, the increase of the enzyme in the serum may be attributed to the process of either deamination or transamination caused by the plant leaf dust. This is similar to the report of Tiwari and Singh (2004), that oleander (*Nerium indicum*) leaf dust increased the serum aspartate

aminotransferase of fish. According to Hayes *et al.* (1989), increase in the activities of transaminases (ALT and AST) in serum is an indication for liver damage and function. The activities of these enzymes recorded in this study may be indicative of initial cell injury occurring in advance of gross hepatic pathology.

This study revealed that lactate dehydrogenase was insignificantly higher in serum. The higher activity of the enzyme in the liver corresponds to the higher glucose level in the liver for metabolic function. According to Boily *et al.* (2006) the enzyme is present in almost all body tissues as it plays an important role in cellular respiration. The report of Ceron *et al.* (1997) revealed that the enzyme is released from the liver after its cellular damage resulting to its higher presence in the serum and other organs. This is not the case in this study as the activity was less in serum and kidney. Therefore, the increased liver lactate dehydrogenase may be due to an alternative aerobic glycolytic pathway in conversion of lactate to pyruvate for the production of glucose, which is a major source of energy during stress (Kori-Siakpere, *et al.,* 2012). γ-glutamyltransferase is an enzyme which is found in hepatocytes and biliary epithelial cells. The enzyme activity in the serum provides a very sensitive indicator of the presence or absence of hepatobiliary disease. This activity is significantly higher compared to the control, however, levels are within the range of its activity in hybrid catfish (Adamu and Nwadukwe, 2013) except in fish exposed to 0.16 mg/L. This has further shown that the plant leaf dust at the highest concentration used in the study impedes the proper functioning ability of the liver.

The significantly higher liver total protein level recorded in the test fish exposed to *M. oleifera* leaf dust is an indication of hyperactivity of the liver that may be attributed to the plant leaf dust as the increase in aminotransferases activities may have affected amino acid metabolism and consequently the intermediates of gluconeogenesis. Adamu *et al.* (2013) reported increased liver total protein in hybrid catfish exposed to *Jatropha curcas,* which may be due to haemo-concentration arising from the plant leaf dust. This may have been the reason for the recorded high kidney total protein in fish exposed to this plant leaf dust. Another important protein in the body is the albumin which functions in maintaining the water balance in serum, transport and store a wide variety of compounds. It is responsible for about 80% of total osmotic regulation. As the concentrations of the plant leaf dust increased the level of serum, liver and kidney albumin increased. This may be an indication that the plant leaf dust may have been responsible for the high albumin values recorded. This is also evidenced by the recorded globulin level; as it transports varieties of substances such as lipid and inorganic ions. The insignificant decreased triglyceride level recorded in the liver showed that globulin was readily available to transport them.

The insignificant hyperglycaemia recorded in this study, may be attributed to the presence of the plant leaf dust as Fletcher (1975) reported that hyperglycaemia is associated with stressful situation. The content of serum glucose in cultured fish is dependent on the type of carbohydrate feed (Adamu and Kori-Siakpere, 2011). Glucose is transported from the liver to body cells *via* the bloodstream and is made available for cell absorption *via* the hormone insulin. The fish exposed to the plant leaf dust showed higher kidney glucose values, which may be an indication of liver dysfunction as the indicator enzymes (aminotransferases) have shown. Banaee *et al.* (2008) and Agrahari *et al.* (2007) have reported hyperglycaemia in fish exposed to toxicants. Cholesterol is the principal sterol synthesised by animals; in vertebrates it is formed predominantly in the liver. The hypercholesterolemia recorded in this study may be attributed to the effect of plant leaf dust as Parrier *et al.* (1972) reported that stress or pollution may lead to hypercholesterolemia in fish. Another fatty acid substance found in animal body is triglycerides (Cornel *et al.,* 1988). During exposure periods, the role of glyceride as glucose precursor becomes more important as the liver metabolism also changes (Adamu and Kori-Siakpere, 2011). The decrease in liver triglyceride may be due to the presence of high liver glucose, since the liver does not stop the synthesis of glucose. Present studies have shown the diverse importance of *Moringa oleifera.* It is therefore, necessary to use the plant with caution as concentrations of 0.16 mg/L and above may have debilitating effects on the biochemical parameters of hybrid catfish after the 21-days exposure period reducing their yield and possible death.

References

Adamu, K.M., Nwadukwe, F.O. 2013. Growth and physiological profiles of hybrid catfish fed practical diet in different water regimes. *Wayamba Journal of Animal Science,* **5:** 743-752.

Adamu, K.M., Isah, M.C., Baba, T.A., Idris, T.M. 2013. Selected liver and kidney biochemical profiles of hybrid catfish exposed to *Jatropha curcas* leaf dust.

Croatian Journal of Fisheries, **71:** 25-31.

Adamu, K.M., Kori-Siakpere, O. 2011. Effect of sublethal concentrations of tobacco (*Nicotiana tobaccum*) leaf dust on some biochemical parameters of hybrid catfish (*Clarias gariepinus* and *Heterobranchus bidorsalis*). *Brazilian Archives of Biology and Technology,* **54:** 183-196.

Adamu, K.M. 2009. Sublethal effects of tobacco (*Nicotiana tobaccum*) leaf dust on enzymatic activities of *Heteroclarias* (a hybrid of *Heterobranchus bidorsalis* and *Clarias gariepinus*). *Jordan Journal of Biological Sciences,* **2:** 151- 158.

Agrahari, S., Pandey, K.C., Gopal, K. 2007. Biochemical alteration by monococrophos in the blood plasma of fish, *Channa punctatus* (Bloch). *Pesticide Biochemistry and Physiology,* **88:** 268-272.

Anwar, F., Rashid, U. 2007. Physico-chemical characteristics of *Moringa oleifera* seeds and seed oil from a wild provenance of Pakistan. *Pakistan Journal of Botany,* **39:** 1443-1453.

Ara, N., Rashid, M., Amran, Md.S. 2008. Comparison of *Moringa oleifera* leaves extract with atenolol on serum triglyceride, serum cholesterol, blood glucose, heart weight, body weight in adrenaline induced rats. *Saudi Journal of Biological Sciences,* **15:** 253-258.

Arekemase, M.O., Kayode, R.M.O., Ajiboye, A.E. 2011. Antimicrobial activity and phytochemical analysis of *Moringa oleifera* plant against some selected microorganisms. *International Journal of Biology,* **3:** 52-59.

Ayotunde, E.O., Fagbenro, O.A., Adebayo, O.T. 2010. Toxicity of aqueous extract of *Moringa oleifera* seed powder to Nile tilapia *Oreochromis niloticus* (Linne, 1779) fingerlings. *International Research Journal of Agricultural Science and Soil Science,* **1:** 142-150.

Banaee, M., Mirvagefei, A.R., Rafei, G.R., Majazi, A.B. 2008. Effect of sub-lethal diazinon concentrations on blood plasma biochemistry. *International Journal of Environmental Research,* **2:** 189-198.

Boily, F., Beaudoin, S., Measures, L.N. 2006. Hematology and serum chemistry of harp (*Phoco groenlandica*) and hooded seals (*Cystophora cristata*) during the breeding season, in Gulf of St. Lawrence, Canada. *Journal of Wild Life Diseases,* **42:** 115-132.

Bucolo, G., David, H. 1973. Quantitative determination of serum triglycerides by the use of enzymes. *Clinical Chemistry,* **19:** 476-482.

Ceron, J.J., Sancho, E., Ferrando, M.D., Gutierrez, C., Andreu, E. 1997. Changes in carbohydrate metabolism in the eel *Anguilla anguilla* during short term exposure to diazinon. *Toxicological and Environmental Chemistry,* **60:** 201-210.

Cornel, L.H., Duffield, D.S., Joseph, B.E., Stark, B. 1988. Hematology and serum chemistry value in the beluga (*Delphinapterus leucas*). *Journal of Wildlife Diseases,* **24:** 220-224.

Doumas, B.T., Watson, W.A., Biggs, H.C. 1971. Albumin standards and management of serum albumin with bromocresol green. *Clinica Chimica Acta,* **31:** 87-96.

Fletcher, G.L. 1975. The effects of capture 'stress' and storage of whole blood on red blood cells, plasma protein, glucose and electrolytes of the winter flounder (*Pseudopleuronectes americanus*). *Canadian Journal of Zoology,* **53:** 197-206.

Foidl, N., Makkar, H.P.S., Becker, K. 2001. The potential of *Moringa oleifera* for agricultural and industrial uses. In: *The Miracle Tree: The Multiple Uses of Moringa,* L.J. Fuglie (ed.), pp. 45-76, Church World Service, Dakar, Senegal.

Hayes, R.B., Sheffet, A., Spirtas, R. 1989. Cancer mortality among a cohort of chromium pigment workers. *American Journal of Industrial Medicine,* **16:** 127-133.

Hamza, A.A. 2010. Ameliorative effects of *Moringa oleifera* Lam seed extract on liver fibrosis in rats. *Food Chemistry and Toxicology,* **48:** 345-355.

IFCC, 1983. Methods for the measurement of catalytic concentration of enzymes, Part 4: IFCC methods for γ–glutamyltransferase. *Journal of Clinical Chemistry and Clinical Biochemistry,* **21:** 643-646.

Jaiswal, D., Kumar Rai, P., Kumar, A., Metha, S., Watal, G. 2009. Effect of *Moringa oleifera* Lam. leaves aqueous extract therapy on hyperglycemic rats. *Journal of Ethnopharmacology,* **123:** 392-396.

Kori-Siakpere, O., Adamu, K.M., Okobi, I.J. 2012. Sublethal effects of chromium on enzymatic activities of the African catfish: *Clarias gariepinus* (Burchell, 1822). *Notulae Scientia Biologicae,* **4:** 24-30.

Lockeett, C.T., Calvert, C.C., Grivetti, L.E. 2000. Energy and micronutrient composition of dietary and medicinal wild plants consumed during drought: Study of rural Fulani, northeastern Nigeria. *International Journal of Food Science and Nutrition,* **51:** 195-208.

Muralidhara Rao, D., Anithal, S., Aravinda, A., Karunakai, B., Devanna, N. 2010. Pharmaceutical investigation and biopesticidal activity of *Jatropha curcas* L. seed oil on digestive enzymatic profiles of *Cnaphalacrosis*

medinalis (*Rice leaf folder*) *and* (*Helicoverpa amnigera* (cotton boll worm). *International Research Journal of Pharmacy*, **1:** 194-200.

Ogbe, A.O., Affiku, J.P. 2012. Effect of polyherbal aqueous extracts (*Moringa oleifera,* Gum Arabic and wild *Ganoderma lucidum*) in comparison with antibiotic on growth performance and haematological parameters of broiler chicken. *Research Journal of Recent Sciences,* **1:** 10-18.

Oluduro, A.O., Aderiye, B.I. 2009. Effect of *Moringa oleifera* seed extract on vital organs and tissue enzymes activities of male albino rats. *African Journal of Microbiology,* **3:** 537-540.

Olufayo, M., Akinpelu, O. 2012. Haematology and Gill pathology of *Heterobranchus bidorsalis* Exposed to sub lethal concentration of *Moringa oleifera* leaf extract. *Journal of Agriculture and Biodiversity Research,* **1:** 18-24.

Olugbemi, T.S., Mutayoba, S.K., Lekule, F.P. 2010. *Moringa oleifera* leaf meal as a hypocholesterolemic agent in laying hen diets. *Livestock Research for Rural Development,* **22:** Article no. 84. http://www.Irrd22/4/olug22084.htm.

Omobuwajo, O.R., Alade, G.O., Akanmu, M.A., Obuotor, E.M., Ossan, S.A. 2011. Microscopic and toxicity studies on the leaves of *Jatropha tanjorensis*. *Journal of Pharmacy and Pharmacology,* **5:** 12-17.

Perrier, H., Perrier, C., Gudefin, Y., Gras, J. 1972. Adrenaline-induced hypercholesterolemia in the rainbow trout (*Salmo gairdneri* Richardson): a separate study in male and female trout and the effect of adrenergic blocking agents. *Comparative Biochemistry and Physiology, Part A: Physiology,* **43A:** 341-347.

Rand, G.M., Well, P.G., Mc Carthy, L.S. 1995. Introduction to aquatic toxicology. In: *Fundamentals of Aquatic Toxicology: Effects, Environmental Fate, and Risk Assessment,* G.M. Rand (ed.), Taylor and Francis, Washington, DC., USA.

Reitman, S., Frankel, S. 1957. A Colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology,* **28:** 56-63.

Tende, J.A., Ezekiel, I., Dikko, A.A.U., Goji, A.D.T. 2010. Effect of ethanolic leaves extract on blood glucose levels of *Streptozocin* induced diabetics and normoglycemic wistar rats. *British Journal of Pharmacology and Toxicology,* **2:** 1-4.

Tietz, N.W. 1995. *Clinical Guide to Laboratory Tests,* pp. 518-519, 3rd edition, W.B. Saunders, Philadelphia, PA., USA.

Tiwari, S., Singh, A. 2004. Piscicidal activity of alcoholic extract of *Nerium indicum* leaf and their biochemical stress response on fish metabolism. *African Journal of Traditional Complementry and Alternative Medicines,* **1:** 15-29.

Trinder, P. 1969. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Annals of Clinical Biochemistry,* **6:** 24-25.

Ujvary, I. 2000. Semi-natural products and related substances as alleged botanical pesticides. *Pest Management Science,* **56:** 703-705.

Witthawaskul, P., Panthong, A., Kanjanerpothi, D., Taesothikul, T., Lertprasertsuike, N. 2003. Acute and sub acute toxicities of saponin mixture isolated from *Schefflera leucantha* viguier. *Journal of Ethnopharmacology,* **89:** 115-121.

Pak. j. sci. ind. res. Ser. B: biol. sci. 2014 **57** (3) 161-162

Short Communication

Antimicrobial Activity and Physical Characteristics of Oil Extracted from Alligator Pepper Seed *(Aframomum melegueta)* Cultivated in Owo, Ondo State, Nigeria

Aladekoyi Gbenga* and Itunnu Olubunmi Shakpo

The Department of Food Science & Technology, Rufus Giwa Polytechnic, P.M.B, 1019, Owo, Ondo State, Nigeria

(received February 15, 2013; revised January 16, 2013; accepted January 28, 2014)

Abstract. Antimicrobial activity of oil from alligator pepper (*Aframomum melegueta*) was tested against five pathogenic organisms, *Klebsiella pneumonia, Salmonella typhi., Bacillus cereus, Escherichia coli* and *Staphylococcus aureus.* The zone of inhibition of *K. pneumonia* was found to be 0.90 ± 0.01 mm, *E. coli* 0.70 ± 0.01 mm, *S. typhii* 0.60 ± 0.01 mm, *B. cereus* 0.04 ± 0.02 mm and *Staphylococcus* sp., 0.80 ± 0.02 mm. The oil was found to have more effect on *Klebsiella pneumonia,* followed by *Staph. aureus, E. coli, S. typhii* and *B. cereus* in descending order. These results show that the oil can effectively eliminate *K. pneumonia* and *Staph aureus* from human system if effectively administered in appropriate proportion.The physical characteristics are: %yield is 16.3522 ± 0.01%, specific gravity is 0.9051 ± 0.01, refractive index is 1.3335 ± 0.01, viscosity is 0.2327 ± 0.02 and colour is deep-yellow.

Keywords: alligator pepper, antimicrobial activity, zone of inhibition, pathogenic organisms

Alligator pepper (*Aframomum melegueta*) is a tropical herbaceous perennial plant with both medicinal and nutritive values found commonly in rainforest. It is widely spread across tropical Africa including Nigeria, Liberia, Sierra Leone, Ghana, Cameroon, Cote D'ivoire and Togo. The constituents of essential oil, extracted by hydrodistillation from the seeds of *A. melegueta* contain two sesquiterpene hydrocarbons, humelene and caryophllene, their oxides and five non-terpenoids (Ajaiyeoba and Ekundayo, 1999). Its seeds have pungent peppery taste due to aromatic ketones (Gala, 1996). The phytochemicals obtained from the seeds of *A. melegueta* posses active ingredients that may be exploited for local development of antimicrobials (Oyegade *et al.,* 1999). Gilani and Rahman (2005) and Sommons (1956) have studied traditional/herbal medicines and their uses.

Aframomum has been used in many herbal medicinal formulas. The seeds when ground into a soft paste shows antibiotic properties (Enti, 1988). Oladumoye (2007) and Okwu (2004) have studied that extracts from the seed of *A. melegueta* have potent antiseptic or bactericidal properties, therefore, have been used in treating wounds and preventions of infections caused by pathogenic bacteria.

*Authour for correspondence; E-mail: gbengu7@yahoo.com

The aim of this work was to evaluate the antimicrobial activities of oil extracted from alligator pepper (*A. melegueta*) against some pathogenic organisms.

The matured alligator pepper seeds were purchased from market and sundried for 3-4 h for two days with the pod. After complete dryness, the pod was removed and the seeds were separated manually and all particles were removed. The seeds were milled mechanically by small milling machine in the market and stored in a clean bottle for extraction process.

The physical characteristics which include percentage yield, specific gravity, refractive index, viscosity and colour were carried out by AOAC (2000).

The results presented in the Table 1 show that it has antimicrobial activity against all the tested organisms as indicated by zones of inhibition. The oil showed greater antimicrobial activities in *K. pneumonia* and *Staph. aureus* which indicate that they were more sensitive to the effect of the oil than other organisms. It has ability to kill the organism and eliminate it, if the appropriate proportion is applied.

The physical parameters have been presented in Table 2. The results of this work suggests that the seed oil from *A. melegueta* have broad spectrum activity. Higher antimicrobial activity of the oil was observed on

Table 1. Antimicrobial activity of oil from alligator pepper (*A. melegueta*)

Sample	Organism	Zones of inhibition (mm)
Oil extract	*Klebsiella pneumonia*	0.90 ± 0.01
	Escherichia coli	0.70 ± 0.01
	Staphylococcus aureus	0.80 ± 0.02
	Salmonella typhi	0.60 ± 0.01
	Bacillus cereus	0.40 ± 0.02

± SD of triplicate results.

Table 2. Physical parameters of oil from alligator pepper (*A. melegueta*)

Parameters of oil extract	Results
% Yeild	16.3522 0.01
Specific gravity	0.9051 0.01
Refractive index	1.3335 0.01
Viscosity(Pas/sec)	0.2327 0.02
Colour	Deep-yellow

± SD of triplicate results.

K. pneumoniae, *S. typhi* and *E. coli.* This indicates that if the oil is smeared or robbed into the affected skin (dermal), it can eliminate pathogenic organisms from the tissues due to its easy penetration into the skin as revealed by its physical characteristics. Adequate proportion can be made into capsules pharmaceutically for easy administration to cure both throat and intestinal pathogenic organisms. This is higher than the earlier results obtained by Oladunmoye and Dada (2007), Oyagade *et al*. (1999) and Akpulu *et al*. (1994) for the ethanol and ether extracts, which were not mainly oil extract. The antimicrobial effect of *A. melegueta* may be due to the phytochemical constituents presents in it (Oyagade *et al*., 1999). *A. melegueta* seeds are rich in phytonutrient such as flavonoids, phenolic compound, tannins, saponins, terperniods, cardiac glycosides and alkaloids which are very concentrated in the oil extracts.

A. melegueta seed oil has the tendency to be stored for a long time, it is edible, and non-drying oil. The oil also showed antimicrobial activity against all the tested bacterial strains even the gram negative pathogens. *A. melegueta* therefore, can be used in healthcare delivery system particularly in the developing economies.

References

AOAC, 2000. *Official Methods of Analysis International, Official Method 920.57,* vol. **11**, 17th edition, Gaithersburg, MD., USA.

Ajaiyeoba, E.O., Ekundayo, O. 1999. Essential oil constituents of *Aframomum melegueta* (Roscoe) Schum seeds (alligator pepper) from Nigeria. *Flavour and Fragrance Journal,* **14:** 109-111.

Akpulu, L.N., Dada, J.D., Odama, E.L., Galadima, H. 1994. Antibacterial activity of aqueous extract of some Nigerian medicinal plants. *Nigerian Journal of Botany,* **7:** 45-48.

Enti, A.A. 1988. *The Rejuvenating Plants of Tropical Africa, Aphrodisiace, Sterility, Impotence, Infertility,* A.K. Adoh (ed.), The North Scale Institute, Atlanta, GA., USA.

Gala, A.M. 1996. Antimicrobial activity of 6-paradol and related compounds. *Pharmaceutical Biology,* **34:** 64-69.

Gilani, A.H., Rahman, A.U. 2005. Trends in ethnopharmacology. *Journal of Ethnopharmacology,* **100:** 43-49.

Okwu, D.E. 2004. Phytochemicals and vitamin content of indigenous spices of south eastern Nigeria. *Journal of Sustain Agriculture and Environment***, 6:** 30-37.

Oladunmoye, M.K., Dada, E.O. 2007. Comparative studies on the antimicrobial activity of leaf extracts from *Aframomum melegueta* and antagonistic activity of isolates from refus on some selected pathogenic bacteria. *Research Journal of Botany,* **2:** 95-101.

Oyagade, J.O., Awotoge, O.O., Adewumi, J.T., Thorpe H.T. 1999. Antimicrobial activity of some Nigerian medicinal plants. *Bioscience Research Communications,* **11:** 193-197.

Sommons, D.C. 1956. Efik divination, ordeals and omens. *Southwestern Journal of Anthropology,* **12:** 223-228.

Pak. j. sci. ind. res. Ser. B: biol. sci. 2014 **57** (3) 163-164

Short Communication

New Record of *Anomala rugosa* Arrow, 1899 (Coleoptera: Scarabaeidae: Rutelinae) from the Fauna of Pakistan

Zubair Ahmed[a]*, Carsten Zorn[b] and Imran Khatri[c]

[a]Department of Zoology, Federal Urdu University of Arts, Science and Technology, Karachi, Pakistan
[b]Sülzer Strasse 52, 17179 Gnoien, Germany
[c]Department of Entomology, Sindh Agriculture University, Tandojam, Pakistan

(received June 6, 2013; revised October 4, 2013; accepted October 14, 2013)

Abstract. A new country record of *Anomala rugosa* Arrow, 1899 (Scarabaeidae: Rutelinae) is presented. Several specimens are recorded from the provinces of Swat and Lower Dir, Pakistan. Sexual dimorphism of this species is briefly commented.

Keywords: *Anomala rugosa,* Rutelinae, Scarabaeidae, Pakistan, new record

The members of the subfamily Rutelinae are a diverse group and exhibit a worldwide distribution. The genus *Anomala* Samouelle, 1819 comprises of approximately 270 Palaearctic species (Zorn, 2006). *Anomala rugosa* is widely distributed in the Himalayan region: India (Himachal Pradesh, Uttarakhand, Sikkim, Darjeeling), Nepal, Bhutan (Chandra *et al.,* 2012c; Zorn, 2006) and has recently been reported also from Madhya Pradesh and Chhattisgarh in Central India (Chandra *et al.,* 2012b; Chandra and Gupta, 2012a; 2012d). Here, the first record of this species in Pakistan is presented. A single female of *A. rugosa* Arrow, 1899 (Fig. 1) was collected in Thrai village, Tehsil Timergara, District Lower Dir, Khyber Pakhtoonkhwa, Pakistan during July 2012. Moreover, Pakistani specimens of this species are also present in the collection of Natural History Museum of Erfurt (Germany) and in the personal collection of Carsten Zorn. There are several males and females labelled "NW-Pakistan, Prov. Swat, 71°90'N 35°70'E Madyan, 1400m, at light 19.06-04.07.1971, leg. C. Holzschuh".

Anomala rugosa is closely related to *A. varicolor* Gyllenhal, 1817 and allied species. It differs in external morphology as well as in the shape of genitalia. *A. rugosa* shows an interesting sexual dimorphism which is absent in all related *Anomala* species: the elytra of females are densely punctate and partly rugose, with distinct minute setae. In males, the elytra are more sparsely punctate and shining, with less distinct setae. Moreover, there is a distinct difference in the coloration of the sexes: females have yellowish brown elytra with dark markings, whereas the elytra in males are nearly entirely black. A more detailed description of *A. rugosa,* is given by Arrow (1917).

Fig. 1. Female of *Anmala rugosa* Arrow, 1899.

*Authour for correspondence; E-mail: zbrahmed36@gmail.com

Acknowledgement

We are greatly indebted to Denis Keith (France), and Aleš Bezdìk (Czech Republic) for their assistance in identification of specie and for providing the literature, and Paul Lago (USA), who has very kindly corrected the language of the manuscript.

References

Arrow, G.J. 1917. Coleoptera: Lamellicornia Part II (Rutelinae, Desmonycinae, and Euchrinae). In: *The Fauna of British India, Including Cyelon and Burma,* A. E. Shipley and G. A. K. Marshall (eds.), 387 pp., 5 pls., Taylor and Francis, London, UK.

Chandra, K., Gupta, D. 2012a. An inventory of scarab beetles (Coleoptera: Scarabaeidae) of Achanakmar-Amarkantak Biosphere Reserve, Chhattisgarh, India. *Intenational Journal of Science and Nature,* **3:** 886-891.

Chandra, K., Gupta, D. 2012b. Diversity and relative abundance of pleurostic Scarabaeidae (Coleoptera) in Achanakmar-Amarkantak Biosphere Reserve, Central India. *World Journal of Zoology,* **7:** 147-154.

Chandra, K., Gupta, D., Uniyal, V.P., Bharadwaj, M., Sanyal, A.K. 2012c. Studies on Scarabaeid beetles (Coleoptera) of Govind Wildlife Sanctuary Garhwal, Uttarakhand, India. *Biological Forum-An International Journal,* **4:** 49-55.

Chandra, K., Khan, S., Gupta, D. 2012d. New records to the species diversity of family Scarabaeidae and Hybosoridae (Coleoptera: Scarabaeoidea) of Jabalpur, Madhya Pradesh (India). *Academic Journal of Entomology,* **5:** 28-36.

Zorn, C. 2006. Anomalini. In: *Catalogue of Palaearctic Coleoptera,* I. Löbl, and A. Smetana (eds.), vol. **3**, pp. 276-277, Applo Books, Stenstrup, Denmark.

Review

Pak. j. sci. ind. res. Ser. B: biol. sci. 2014 **57** (3) 165-174

Some Aspects of Developmental Trends in Chemical Modification and Transformation of Starch: Products Preparation and Potential Applications

Bode Daramola

Department of Food Technology, Federal Polytechnic, Ado-Ekiti, Ekiti State, Nigeria

(received January 21, 2014; revised June 19, 2014; accepted August 28, 2014)

Abstract. Starch, a non-depleting bioresource has a myriad of applications both in the food and non-food industries. These applications are made possible by starch modification technology. Chemical method of starch modification, the most versatile tool in accomplishing the preparation of copious starch speciality products or by-product which could serve as feed stock or precursor for the preparation of allied products is reviewed. The applicabilities of the speciality starch produced by chemical modification presented in many research papers are surveyed and pooled together and reviewed in this paper. This study suggests the myriads of opportunity that exist using chemical method of starch modification and transformation that have immerse applications in both, food and non-food industries. A projection on the future of chemical method of starch modification is highlighted.This review will motivate readership to seek accurate detail knowledge on chemical method of starch modification and transformation for technological and economic advancement.

Keywords: chemical modification, starch speciality products, transformation

Introduction

Starch is the most important reserve carbohydrate and one of the most abundant organic chemicals on earth (Jane *et al*., 1994). It could be found in the leaves of green plants in the plastids, more importantly, starch is synthesised in any plant and stored abundantly in seeds, grains, roots and tubers of many plants. The principal commercial sources of starch are roots and tubers (cassava, potato and arrow root), cereals (maize, rice and wheat), fruits (banana, plantain and breadfruit) and pith of plants (sago palm). Starch is not region-selective, as a result all countries are endowed with the possibility of abundant supply of starch provided there is vegetation. The abundance of starch spurs scientists to search for food related and non-food applications of starch. Unfortunately, starch in its native form has limited industrial applications whether in food and non-food industries. Interestingly, the properties of native starch can be altered using minor modification such that positive attributes can be greatly improved and/or negative characteristics diminished by slight and relative simple modifications (BeMiller, 1997). The primary reasons for starch modification have been tabulated by BeMiller (1997). Yaacob *et al.* (2011) reported that from 1969 to 2001, there have been more than 23,000, scientific articles in food science and technology. Therefore, it is vivid that a comprehensive review of chemical aspect of starch modification for food and non-food applications cannot be accomplished within the limited space of this article. However, it is important to make a list of some texts notably, Wurzburg (1986); Mentzer *et al.* (1984) and Rutenberg (1980) that could furnish pioneer technology details and lead references on starch modification. Beyond the content and period of the cited references, a lot of research progress has been reported in many articles. Therefore, it would be informative and educative to pool some of these articles into one piece in a review.

This study aimed at brief review of developments in chemical method of starch modification and transformation for both food and non-food applications, and some of the manipulations employed to enhance or conferred the product-value-addition. This will motivate readership to seek further knowledge on details of technological accomplishment for preparation of value added products and organic-compliant substitute from starch.

Chemical method of starch modification. Inherent properties of native starch could be changed for industrial compilation and the basic modification types practiced in the US is presented in Table 1. However, of all the methods listed, chemical process offers the highest number of modification opportunities, hence focussed herein.

E-mail: daramola_bode@yahoo.co.uk

Table 1. Modification methods and probable conferred attributes

Types of modification	Probable attribute conferred
I. *Chemical modification*	
A. Derivatisation	
1. Monostarch substitution (etherification and esterification, including polymer grafting)	Improved paste clarity
2. Cross linking (*via* distarch esterification)	Increase paste stability, lower tendency to retrograde, increase water solubility, improved water solubility.
B. Acid thinning/hydrolytic depolymerisation	Increase clarity/increase in digestibility
C. Dextrinisation (depolymerisation and *trans*-glycosylation	Increase in digestibility
D. Oxidation (bleaching and depolymerisation)	Increase in clarity
E. Hydrolysis (malto-dextrins, glucose syrup, glucose etc. - both acid and enzyme-catalysed.	Increase in digestibility
II Physical modification	
A. Pre-gelatinised process	Cold water solubility
B. Preparation of cold-water swelling starch	
III Genetic	
A. Waxy starch	Variable quality profile
B. High-amylose starch	

Source: BeMiller (1997).

The characteristics cooks of native starch, irrespective of the source are undesirable for many industrial applications. This is because, they are susceptible to retrogradation, syneresis, unwanted viscosity as a result of their inability to withstand the typical industrial processing conditions such as extreme temperature, pH, high shear rate, and freeze thaw variation encountered during manufacture of food and allied products. Some of the positive attributes conferred by modification of starch in foods are reported in a review by Daramola and Falade (2006). Since this article captions chemical method of starch modification, it is necessary to explain in brevity the chemical aspect of starch modification outlined in Table 1.

Acid thinning. Acid thinning involves hydrolysis of starch by applications of both organic and inorganic acids to break starch molecular chains namely: amylose and amylopectin. Some of the inorganic acids used are hydrochloric acid, sulphuric acid and phosphoric acid (Whistler *et al*.,1984), and organic acids anhydride used include succinic acid anhydride, acetic acid anhydride, (Wang *et al*., 1997; Agboola *et al*., 1991).

Oxidation. This involves the use of reagents such as sodium hypochlorite, potassium permanganate and sodium chlorite. All can be used in both acidic and alkaline medium (Kweon *et al.,* 2001).

Derivatisation. As listed in Table 1, derivatisation can be divided into 2 sub-groups:

(1) Starch monosubstition in nature of etherification and esterification as well as forms of polymer grafting. Any substitution of the hydroxyl moiety on the molecular chains of starch by alkoxide and alkanoate is referred to as etherification and esterification, respectively. Examples of this type of modification is acetylation of starch (Daramola and Adegoke, 2007; Lawal, 2004).

(2) Another example is starch citrates (Agboola *et al.,* 1991) and propyl-etherified starch (Teramoto *et al.,* 2003). In addition, starch ethers could be cationised or anionised, a described procedure has been reported by Kweon *et al.* (1997). Also, preparation of starch succinates by reactive extrusion has been reported by Wang *et al.* (1997). Starch can be modified to carry charges positive (cation) or negative (anion) or both. When cation and anion are combined on a single starch it is termed amphoteric starch. Amphoteric starches, could be etherified, esterified, or grafted starches which simultaneously, contain cationic groups and anionic groups. Generally, the cationic groups could be amino, ammonium, sulphonium or phosphonium. Presently, tertiary amino groups or quaternary ammonium groups are the most important industrial positive group (moiety). Anionic groups are usually carboxyl groups, phosphate groups or sulphonate groups (Yang *et al.*, 2007). A cheap reagent such as alum (double salt) has been used to modify starch as reported in an earlier study by Daramola and Aina (2007).

Starch cross-linkage. Starch cross-linkage could be accomplished using bi-functional compounds, which are capable of reacting simultaneously, with two or more hydroxyl groups in starch forming cross-linked starch. Examples of such compounds are phosphorus oxychloride, epichlorohydrin and sodium trimetaphosphate used mostly for food grade cross-linked starches (Radley, 1976). Cross-linkage facilitates intra and intermolecular bonds at random locations in the starch granules for their stabilisation. As a result of the intra and intermolecular

linkages, paste of cross linked starch are more resistant to shear, and acidic conditions (Majzoobi *et al.,* 2009). All the starch derivatives have amazing technological applications as illustratively summarised in Table 2.

Fundamental factors affecting starch modification using chemical method. The factors affecting starch modification using chemical method can be divided into three classes:

(1) The primary factors which include processing variables such as temperature, solid solvent ratio, stirring rate, concentration of solvent, time and pressure.

(2). The secondary factors are type or chemical nature of solvent (e.g. acid, alkanol, alkaline, esterification and etherification reagents). Nature of chemical is majorly affected by the chemical or inherent functional groups. Added to these methods of processing are: use of reactive extrusion (Wang *et al*., 1997), microwave heating (Shogren and Biswas, 2006) and use of ionic liquids among others. Also in this group, the use of catalysts, initiator, promoters and modifiers.

(3) The tertiary factors involves: high technology, involving manipulation of active site in molecular chains and granules of starch and combination of chemical method for modification of starch. For example, it is possible to combine cross-linkage method with ionic method in one of the modification. Another one is chemical modification of starch in solvent free environment (Aburto *et al.,* 2005).

Instrumentation in chemical method of starch modification. The commonest analytical instruments for characterisation of modified starch accomplished using chemical method are viscosity analyses (RVA). RVA profiles, the pasting characteristics such as peak viscosity, set back viscosity, final viscosity, pasting temperature and time of starch. Their technological interpretation signifies the extent of conferred modification (Meadow, 2002; Delcour *et al.,* 2000; Deffenbaugh and Walker, 1990; 1989).

Similarly, the thermal characteristics could be studied using a differential scanning calorimeter (DSC). Some of the evaluated parameters are: onset temperature, peak temperature, completion temperature and gelatinisation enthalpy. With the understanding that the two molecular chains, amylose and amylopectin are folded into granule and the fact that there are no two starch granules that are exactly same in terms of size, shape and configuration. Therefore, starch morphological characteristics are studied using scanning electron microscopy (SEM) (Jane *et al.,* 1994; Jing-ming and Sen-lin, 1990). Besides morphological characteristics, SEM gives insight to location and conformation of hilum that contain the cavity through which chemical moiety passes into the amylose and amylopectin chains. Other importance of SEM on starch granules involves revelation on integrity of starch granule to hydration and dehydration and vapour pressure. It also reveals relationship between starch granules disruption and physicochemical properties of starch.

Structural characteristics of starch could be evaluated using fourier transform-infrared (FTIR). FTIR is important in revealing degree of substitution and the substituent chemical moiety. Other analytical instruments include; x-ray diffraction analytics, and nuclear magnetic resonance. Convectional tests carried out on starch are paste clarity, swelling capacity, solubility, tensile strength, stress, elongation at break, and tear strength. Other assessed parameters are apparent viscosity, freeze thaw stability, gel strength and thermal stability analysis (Chung *et al.,* 2004).

Preparation of starch noodle. Besides the additives application of starch in food preparations, where it could function as binding or adhesion, clouding and dusting agents. Other functions include flowing aid, antistaling and gelling agents. Starch is also used to glaze, mould, shape and thicken during food preparations. Examples of food in which starch performed the itemized functions have been tabulated by Ihekoronye and Ngoddy (1985), as reviewed by Daramola and Falade (2006).

Beyond the uses of speciality starch as additives as previously explained, food can be produced using whole starch. Noodles can be produced from starch. Although, starch is void of gluten, pregelatinised starch is used as binder mixed with ungelatinised starch to facilitate extrusion or sheeting to produce noodles. Basic protocol for the commercial production of bihon-type noodles can be found in literature (Tam *et al*., 2004).

Food noodles can be prepared from flour or starch, provided the product has noodles qualities that are defined by visual attributes, cooking and eating qualities, transparency and glossiness. In cooked starch noodles, the noodles should remain firm and not sticky on standing after cooking. These characteristics have positive correlation to noodles texture and mouth feel acceptance.

Starch transformation. ***Precursor of starch sweeteners and other organic chemicals.*** Sweetener can be produced from starch using varieties of acids (Daramola and Falade,

Table 2. Starch derivatives and potential applications

Modified starch/description	Modification type	Domain of application	Leading references
Complexes of oppositely charged ionic starches e.g., polyelectrolyte complexes	Amphoteric	Medicine: dialysis, ultrafiltration anti-thromobogenic agent	Willett, 1995
Oxidized starch	Oxidation H_2O_2 Cu^{2+} ($CuSO_4$) catalyst Fe^{2+} ($FeS\ O_4$)	Paper industry: surface sizing coating and binding	Parovuori *et al.,* 1995
Carboxymethylation of starch propyl-etherification of starch	Etherification alkaline Sodium-mono chlorocetate 1-bromo propane	Biodegradable polymer for coating for food and pharmaceutical products	Yaacob *et al.,* 2011; Teramoto *et al.,* 2003
Amphotenic starches	Amphoteric starches: Etherification, Esterification, Grafting	Paper making additives, for retention, and strength properties	Yang *et al.,* 2007
Cross-linked starch	Cross-linkage phosphoryl chloride	Food additives: thickners, colloidal stabilizer, water retention, agents for food and Non-food waste H_2O treatment	Majzoobi *et al.,* 2009; Wang *et al.,* 2005
Cross-linked, cationised anionised amphoteric	Esterification, Etherification	To bind heavy metals from waste water waste treatment	Carmona-Garcia, *et al.,* 2009
Cross-linked hydrophilic polymers super absorbent	Graft co-polymeric cation	Surgical pad pharmaceutical, domestic, sanitary use, soil condition	Xiao *et al.,* 2010; Liu *et al.,* 1996
Complexation of modified starch with pigment	Complexation with pigment	Improvement of optical and pant ability performance of water	Kuuti *et al.,* 2010
Etherification and cross-linkage	Cationization graft-copolymerization	Water treatment as flocculant in place of alum	Yang *et al.,*2007; Sharma *et al.,* 2006
Cross-linkage of starch	High performance elastomer (Tyre-making)		Song *et al.,* 2010; Qing *et al.,* 2006
Aerogels based microcellular foams	Microcellular starch-for production of synthetic foam	Alterative material Xu *et al.,* 2005	Xu *et al.,* 2005; Glenn and Irving, 1995

2006). Some of the obtained products are glucose, maltose, dextrin and maltose. These sweeteners are superior on the basis of dearth of physiological abnormalities associated with sweetener of synthetic origin.

Starch sweeteners have been produced from starch using acids such as hydrochloric acid, sulphuric acid and phosphoric acid. Each of the acids has its advantages and limitation. For instance, hydrochloric acid is an inorganic acid and it hydrolyse starch and the hydrolysis is complete in comparison to hydrolysis carried out by using phosphoric acid. Nevertheless, sweeteners obtained from hydrochloric acid hydrolysed starch require treatment and other purification processes. However, phosphoric acid hydrolysed starch can be used directly for biological production of ethanol without purification. The application or use of phosphoric acid rather than hydrochloric acid presents some advantages. Phosphoric acid is non-volatile and safer to handle in comparison to hydrochloric acid. Since, phosphoric acid is less severe in comparison to hydrochloric acid, it could be speculated that the application of phosphoric acid should be accompanied by lesser by-products. The hydrolysed starch or sweetener can be diluted on addition to foods or beverages, the phosphoric acid concentration falls to values as low as 0.01-0.05% resulting to a pleasant taste of acidity at pH values of about 3.0. More importantly, if the hydrolysate is to be used in fermentation, there is no need to eliminate the phosphoric acid. Interestingly, neutralisation with ammonia leads to ammonium phosphate, an important supplement for fermentation growth (Fontana *et al.*, 2008). The products from starch hydrolysis, called hydrolysates are made of molecules of different length, consequently, in addition to neutra-lisation and purification, the hydrolysates are fractionated using chromatographic techniques. Three distinct products namely, glucose, maltose and oligosaccharides fractions are recovered. This is one of the starch processing methods patented (Masuda *et al.*, 1995). Besides being used as sweetener, the hydrolysate is also an ingredient for formulation of tablet coats (Czarnecki and Belniak, 2009).

Aside, from application of acids to hydrolyse starch, alkaline such as sodium hydroxide, potassium hydroxide can be used to depolymerise starch at high temperature approximately 240 °C for a period not less than 60 min to yield organic compounds such as glycol and oxalic acids (Verendel *et al.,* 2011). It is important to state that some of the end-products of starch hydrolysis notably glucose and maltose could be further modified and transformed to organic chemicals such as ethanol, hydroxyl methyl furfural (Zhang *et al*., 2012), and fructose (Roman-Leshkov *et al.*, 2010), lactic acid and other feedstock chemicals.

Some miscellaneous modified starch products: Modified starch has applications in many different industries (Table 3) that range from food, drug and medicine to non food industries such as mining, building and electronic industries. Some of these are illustrated hereunder: other than cooking, native starch can be treated or modified to become granular, cold water soluble. The preparation of this kind of speciality of starch using alcoholic-alkaline treatment had been reported by Chen and Jane (1994). Another procedure that used liquid ammonia and ethanol was also described by Jackowski *et al.* (2002). Other novel product include functional modification of starch by esterification of the hydroxyl group by introduction of a chromophoric moiety leading to development of a thermostable and photo-responsive system on starch. A procedure of this kind had been reported by Chandran *et al.* (2012). A starch benzyl ether of high degree of substitution has a unique property of being not gelatinised when cooked at 100 °C. However, when prepared at super atmosphere in a steam injection cooker at 150 °C, dispersion fluid sol results, which when cast gives resistant films.

Most chemical modification of starch is based on material science properties. Starch could also be modified for exhibition of certain nutritional functionality other than dietary fibre. Starch could be conjugated to non-starchy substances thereby, lending significant changes in starch nutritional functionality. This was demonstrated by the work of Hatton *et al.* (1995), that changed the functional properties of carboxymethyl potato starch conjugated with whey proteins. The covalently linked carboxymethyl potato starch-whey was characterised by improved thermal stability and reduced retrogradation in comparison to unmodified starch. Also the conjugated starch-whey product possessed retinol binding ability present in β- lactoglobulin present in whey and α-lactoalbumin present in whey known to participate in lactose synthetase reaction. One of the reasons for turning to starch for synthesis of starch-materials is its biodegrability. This is expressed in the capability of microorganisms to decompose polymeric materials to low molecular compounds, such as carbondioxide and nitrogen, so that carbon returns to the ground/soil or atmosphere. Beyond this, today research is focussing on dissembling the blocks coupled within functionalised starch. This claim has been demonstrated in the research of Auzely-Velty and Rinaudo (2003), for the synthesised starch-

Table 3. Spectrum of modified starch utilising industries with example of application

Industry	Application
Food processing	Pie filling, padding, mixes, fruit juice sources, bulking agent, molds, fluidifying agent maintenance of viscosity in acid foods e.g., fruit juice, decrease syneresis in ice-cream or refrigerated foods. Hard candy gum drops
Paper/board/corrugating	Pigment retention on paper (wet-end addition), as surface sizing agent coating
Brewing	as adjunct
Textile/warp sizing	Text finishing agents to add weight, smoothness and stiffness
Pharmaceuticals	Cosmetics: tooth paste, body cream or lotion Pharmaceutical tablets; binder, filler, disintegrant Body powders
Building pots	Binder for foundries
Agriculture	Soil conditioners
Adhesives	Corrugated boards paper bag Guming applications
Crude oil mining	Oil drilling mud
Briquetting	Domestic and industrial heating
Packaging	Plastics High temperature stable hybrid nylon e.g., meat roasting nylon; Edible films Preparation of surgeon gloves

betaine derivatives using disopropylcarbodiimide and 4-dimethylaminopyridine as coupling agents in an aprotic polar solvent, which carry cleavable cationic groups derived from naturally occurring glycine-betanine. The improvement in this study, lies in lower toxicity and larger biodegradability in comparison to the traditional cationic surfactant compounds.

Some modification enhancement manipulations. Although BeMiller (1997) in an earlier review predicted that there could not be approval of new reagents for derivation of starch, for safety concerns. However, as knowledge expands, new reagents with special attributes are discovered and other process enhancement manipulation. Two examples shall be discussed in brevity.

Use of ionic liquids. For example, effort on the use of ionic liquids as solvent for reaction-medium for starch has started to receive attention. Ionic liquids are a new technology considered to be green chemistry because of their biodegradability, and low toxicity. Added to this, ionic liquids have a spectrum of compound characteristics through a broad selection of anions and cations combi-nations. Other advantages inherent to ionic liquids are non-flammability and thermal stabilities and enhancement of reaction rates with higher selectivity, an example of such study is an early work of Stevenson *et al.* (2007) on changes in structure and properties of starch of four botanical sources dispersed in ionic liquid. Besides, acting as solvent, ionic liquids have been reported to exhibit catalytic property. This was demonstrated by acylation of maltodextrin in ionic liquid (Biswas *et al.*, 2009). This process obviates the use of catalyst during acylation of maltodextrin with its economic and technical advantages.

Application of enhanced microwave heating. Starch can be hydrolysed using microwave field. The advantage of this form of heating lies in dissipation of heat inside the medium, which raises the energy of the molecules rapidity. This heating mode is different from convectional heating because all the molecules in the heated system receive same amount and rate of heat readily energized and resulting to higher reaction rates and greater selectivity. The afore-described process could be enhanced by the addition of metallic halides such as sodium chloride, potassium chloride, iron III chloride to the microwave heating solvent or medium. The importance of the inorganic salts could be demonstrated by the amount of (88-109 wt %) glucose hydrolysed from starch using hydrochloric acid for a period of 240 s at a temperature of 145 °C, while the control sample without salt (all other conditions were same), yielded less glucose (78 wt %) not earlier than a period of 600s as demonstrated in the study of Kunlan *et al.* (2001).

Future prospects of chemical method of starch modification. Since chemical modification offers the most versatile opportunities of tailoring starch granules to fit for a particular properties/purpose/application, the future appears bright regarding the relevance of chemical modification of starch for products or preparation of speciality starch for food and non-food applications than is known presently. Another important reason for good prospects for continuous progress or advancement in

chemicals modification of starch is the peculiarities of starch as a bioresource as outlined in Table 4. Additional projection on the future of chemical method of starch modification and prospective application is as follows: The future of chemical method of starch modification would be addressed from the two broad domain of application namely: (1) food application and (2) non-food application. Regarding food application perspective, there are no indications that new chemicals would be approved for modification of starch to be used in food processing. This is in agreement with the prediction of BeMiller (1997). However, any new chemical to be used for modification of starch for food would undergo strict examination to have passed safety test for approval. Beyond this, chemical modification processes for starch could be enhanced by combination with use of novel solvents such as ionic liquid as medium of reaction and catalytic agent (Biswas *et al.,* 2009). Also chemical modification could be combined with physical process such as irradiation. Regarding chemical modification of starch for application in peripheral food and non-food applications, there exists an open end opportunity. Chemical modification of starch in preparation of edible packaging materials used in fast foods and institutional catering and short-time, and self degradative packaging that could be used as packaging materials in bottling of table water and flocculating agents. Such products could be prepared using starch and long chain fatty acids of different unsaturation and alkylation. Polymeric materials have inexhaustive applications list in bioprocessing. The aim of this section is to articulate the need and the possibility of using starch to replace the other materials produced from non-starchy or petrochemical products. Most especially, that biodegradability is of priority in material science, and polymer technology in present day technology. In separation technology, molecularly imprinted polymers (MIPs) have been applied in adsorptive separation as selective chromatographic materials and in the field of solid phase extraction. MIP is a synthetic porous polymer with selective and specific recognition ability of the binding cavities to target molecules by non-covalent interactions such as hydrogen bonding, electrostatic interactions π-π and hydrophobic forces as reported by Seechamnanturakit and Suede (2012).

Table 4. Starch bioresource peculiarities

Starch sources is non-regional specific
Starch is abundant
Renewable/sustainability
Ecofriendly or non-toxic
Amenable to modification in comparison to cellulose and chitin much available bioresource
Inexpensive
Biodegradable
Availabilities of many reaction sites
Modification is not high technology
Procedure is less labourious and operational hazard
Non-depleting

In addition, verse opportunities await application of starch modified to meet application as immobilised enzymes with respect to reversibly soluble-insoluble polymer. Presently, all the polymers used in enzyme immobilisation are synthetic (Dourado *et al.,* 2002) consequently, it would be more friendly to use biodegradable polymer of starch origin. Finally, chemically modified starch would find place in other stimuli-responsive polymers, sometimes referred to as smart or intelligent polymers which are presently dominated by synthetic polymers. Stimuli-responsive polymers have a series of biological applications, which include tissue engineering, drug release systems, biological sensors, temperature and light responsive films or optical sensors, intelligent microfluidic switches and diagnostic devices (Milichovsky, 2010) consequently an important tool in automation of bioprocess.

Conclusion

The admirable potentials of chemical modification technology to produce speciality starch with both, food and non-food applications are reviewed. This by no means exhaust the modification and transformation of starch for both food and non-food industrial applications. It is anticipated that this review would motivate readership to seek detail and accurate technology knowledge on know-how-accomplishment to develop domestic starch for import substitution for technological and economic benefits.

References

Aburto, J., Alric, I., Borredon, E. 2005. Organic solvent free transesterification of various starches with lauric acid methyl ester and triacyl glycerides. *Starch/Starke,* **57:** 145-152.

Agboola, S.O., Akingbala, J.O., Oguntimein, G.B.1991. Production of low substituted cassava starch acetate and citrates. *Starch/Starke,* **43:** 13-15.

Auzely-Velty, R., Rinaudo, M. 2003. Synthesis of starch derivatives with labile cationic group. *International Journal of Biological Macromolecules* **31:** 123-129.

BeMiller, J.N. 1997. Starch modification: challenges and

prospects. *Starch/Starke,* **49:** 127-131.

Biswas, A., Shogren, R.L., Willet, J.L. 2009. Ionic liquid as a solvent and catalyst for acylation of maltodextrin. *Industrial Crops and Products*, **30:** 172-175.

Carmonai-Garcia, R., Sanchez-Rivera, M.M., Mendez-Montealvo, G.., Bello-Perez, L.A., Garza-Montoya, B. 2009. Effect of the cross-linked reagent type on some morphological, physicochemical and functional characteristics of banana starch (*Musa paradisiaca*). *Carbohydrate Polymers,* **76:** 117-122.

Chandran, A., Kuriakose, S., Mathew, T. 2012. Thermal and photoresponsive studies of starch modified with 2-(5-(4- Dimethylamino-benzylidine-4-oxo-2-thioxo-thiazolidine)-3-yl) acetic acid. *International Journal of Carbohydrate Chemistry*, **2012:** Article ID 356563, pp. 8, http://dx.doi.org/10.1155/2112/566563.

Chen, J., Jane, J. 1994. Preparation of granular cold-water-soluble starches by alcohol-alkaline treatment. *Cereal Chemistry,* **71:** 618-622.

Chung, H.J., Woo, K.S., Lim, S.T. 2004. Glass transition and enthalpy relaxation of cross-linked corn starches. *Carbohydrate Polymers,* **55:** 9-15.

Czarnecki, W., Belniak, P. 2009. Tablets coated with the novel starch hydrolysate emulsion. *Acta Poloniae Pharmaceutica,* **66:** 429-431.

Daramola, B., Adegoke, G.O. 2007. Production and Partial characterisation of food grade breadfruit acetylated starch. *Journal of Food, Agriculture and Environment,* **5:** 50-54.

Daramola, B., Aina, J.O. 2007. Effects of alum on pasting and some physicochemical properties of cassava (*Manihot esculentum*) Starch. *World Journal of Diary and Food Sciences,* **2:** 18-22.

Daramola, B., Falade, K.O. 2006. Enhancement of agronomical values: upstream and downstream opportunities for starch and starch adjuncts. *African Journal of Biotechnology,* **5:** 2488-2494.

Deffenbaugh, L.B., Walker, C.E. 1990. Use of the rapid visco-analyzer to measure starch pasting properties, Part II: effect of emulsifiers and sugar-emulsifier interactions *Starch/Starke,* **42:** 89-95.

Deffenbaugh, L.B., Walker C.E. 1989. Use of the rapid visco-analyser to measure starch pasting properties. *Starch/Starke,* **41:** 461-467.

Delcour, J.A., Vansteelandt, J., Hytheir, M., Abecassis, J. 2000. Fractionation and reconstitution experiments provide insight into the role of starch gelatinization and pasting properties in pasta quality. *Journal of Agricultural and Food Chemistry,* **48:** 3774-3778.

Dourado, F., Bastos, M., Mota, M., Gama, F.M. 2002. Studies on the properties of celluclast/eudragist L-100. *Journal of Biotechnology,* **99:** 121-131.

Fontana, J.D., Mitchell, D.A., Molina, O.E., Gaitan, A., Bonfim, T.M.B., Adelmann, J., Grzybowski, A., Passos, M. 2008. Starch depolymerisation with diluted phosphoric acid and application of the hydrolysate in astaxanthin fermentation. *Food Technology and Biotechnology*, **46:** 305-310.

Glenn, G.M., Irving, D.W. 1995. Starch-based microcellular foams. *Cereal Chemistry*, **72:** 155-161.

Hattori, M., Yang, W., Takahashi, K. 1995. Functional changes of carboxymethyl potato starch by conjugation with whey protein. *Journal of Agricultural and Food Chemistry,* **43:** 2007-2011.

Ihekoronye, A.I., Ngoddy, P.O. 1985. *Integrated Food Science and Technology*, Macmillan Eduction London, UK.

Jackowski, R., Czuchajowska, Z., Baik, B.K. 2002. Granular cold water gelling starch prepared from chickpea starch using liquid ammonia and ethanol. *Cereal Chemistry*, **79:** 125-128.

Jane, J.L., Kasemsuwan, T., Leas, S., Zobel, H., Robyt, F.T. 1994. Anthology of starch granule morphology by scanning electron microscopy. *Starch/Starke,* **4:** 121-129.

Jing-ming, L., Sen-lin, Z. 1990. Scanning electron microscope study on gelatinisation of starch granules in excess water. *Starch/Starke,* **42:** 96-98.

Kunlan, L., Lixin, X., Jun, L., Junp, P., Guoying, C., Zuwei, X. 2001. Salt-assisted acid hydrolysis of starch to D-glucose under microwave irradiation. *Carbohydrate Research,* **331:** 9-12.

Kuuti, L., Putkisto, K., Hyvarinen, S., Peltonen, S., Koivunen, K., Paulapuro, H., Tupala, J., Leskela, M., Virtanen, T., Maunu, S.L. 2010. Starch-hybrid fillers for paper. *Nordic Pulp and Paper Research Journal,* **25:** 114-123.

Kweon, D.K., Choi, J.K., Kim, E.K., Lim, S.T. 2001.

Adsorption of divalent metal ions by succinylated and oxidized corn starches. *Carbohydrate Polymers,* **46:** 171-177.

Kweon, M.R., Sosulski, F.W., Han, H.S. 1997. Effect of aqueous ethanol cationization on functional properties of normal and waxy starches. *Starch/Starke,* **49:** 202-207.

Lawal, O.S. 2004. Composition, physicochemical properties and retrogradation characteristics of native, oxidized, acetylated and acid-thinned new cocoyam (*Xanthosoma sagittifolium*) starch. *Food Chemistry*, **87:** 205-218.

Liu, M., Cheng, R., Wu, J. 1996. Preparation and swelling properties of super-absorbent polymer. *Chinese Journal of Polymer Science,* **14:** 48-56.

Majzoobi, M., Radi, M., Farahnaky, A., Jamalian, J., Tongdang, T. 2009. Physico-chemical properties of phosphoryl chloride cross-linked wheat starch. *Iranian Polymer Journal*, **18:** 491-499.

Masuda, T., Kawano, K., Miyawaki, I. 1995. Process for production of starch sugars. *United States Patent*, patent No. 5391299/Date Feb. 21, 1995.

Meadow, F. 2002. Pasting process in rice flour using rapid visco analyser curves and first derivatives. *Cereal Chemistry,* **79:** 559-562.

Milichrovsky, M. 2010. Water-A key substance to comprehension of stimuli- responsive hydrated recticular systems. *Journal of Biomaterials and Nanobiotechnology,* **1:** 17-30.

Parovuori, P., Hamurien, A., Forssell, P., Autio, K., Poutanen, K. 1995. Oxidation of potato starch by hydrogen peroxide. *Starch/Starke,* **47:** 19-23.

Qing, Q., Wu, Y., Tian, M., Liang, G., Zhang, L., Jun, M. 2006. Modification of starch for high performance elastomer. *Polymer,* **47:** 3896-3903.

Radley, J.A. 1976. *Examination and Analysis of Starch and Starch Products*, pp. 220, Applied Science Publishers Ltd., London, UK.

Roman-Leshkor, Y., Moliner, M., Labinger, J.A., Davis, M.E. 2010. Mechanism of glucose isomerisation using a solid lewis acid catalysts in water. *Angewandte Chimie International Edition,* **49:** 8954-8957.

Rutenberg, M.W. 1980. Starch and its modification. In: *Handbook of Water Soluble Gums and Resins,* R.L. Davidson (ed.), Chap 22, McGraw-Hill, New York, USA.

Seechamananturakit, V., Suedee, R. 2012. The synthesis and characterisation of retinol-molecularly imprinted polymers as a selective sorbent in solid-phase extraction. *International Journal of Applied Science and Technology,* **2:** 81-93.

Sharma, B.R., Dhuldhoya, N.C., Merchant, V.C. 2006. Flocculants-an eco-friendly approach. *Journal of Polymers and the Environment,* **14:** 195-202.

Song, S-S., Qi, H-B., Wu, Y-P. 2010. Preparation and properties of water absorbent composites of chloroprene rubber, starch and sodium acrylate. *Polymers for Advanced Technologies*, **33:** 1778-1785.

Shogren, R.L., Biswas, A. 2006. Preparation of water-soluble and water-swellable starch acetates using microwave heating. *Carbohydrate Polymers*, **64:** 16-21.

Stevenson, D.G., Biswas, A., Jane, J., Inglett, G.E. 2007. Changes in structure and properties of starch of four botanical sources dispersed in the ionic liquid 1-buty1-3-methyllilidazolium chloride. *Carbohydrate Polymers,* **67:** 21-31.

Tam, L.M., Corke, H., Tan, W.T., Li, J., Collado, L.S. 2004. Production of bihon-type noodles from maize starch differing in amylose content. *Cereal Chemistry,* **81:** 475-480.

Teramoto, N., Motoyama, T., Yosomiya, R., Shibata, M. 2003. Synthesis thermal properties and biodegradability of propyl-etherized starch. *European Polymer Journal,* **39:** 255-261.

Verendel, J., Church, T.L., Andersson, P.G.. 2011. Catalytic one-pot production of small organics from polysaccharide. *Synthesis*, **11:** 1649-1677.

Wang, L., Shogren, R.L., Willett, J.L. 1997. Preparation of starch succinates by reactive extrusion. *Starch/Starke,* **49:** 116-120.

Whistler, R.L., BeMiller, J.N., Paschall, E.F. 1984. *Starch: Chemistry and Technology,* pp. 543-574, 2nd edition, Academic Press, Miami, Florida, USA.

Willett, J.L. 1995. Complexes of oppositely charged ionic starches. *Starch/Starke,* **47:** 29-32.

Wurzburg, O.B. 1986. Forty years of industrial starch research. *Cereal Food World*, **31:** 897-903.

Xiao, C.M., Tan, J., Xue, G.N. 2010. Synthesis and properties of starch-g-poly (maleic anhydride-co-

vinyl acetate). *Express Polymer Letters,* **4:** 9-16.

Xu, J., Zhou, J., Hanna, A.M. 2006. Melt-Intercalated starch acetate nanocomposite foams as affected by type of organoclay. *Cereal Chemistry*, **82:** 105-110.

Xu, S., Feng, S., Peng, G., Yushan, A., Wanh, J. 2005. Removal of Pb(II) by cross-linked amphoteric starch containing the carboxymethyl group. *Carbohydrate Polymers*, **60:** 301-305.

Yaacob, B., Mohd Amin, C.I., Hashim, K., Abu Bakar, B. 2011. Optimization of reaction conditions for carboxymethylated Sago starch. *Iranian Polymer Journal,* **20:** 195-204.

Zhang, M., Zhang, S., Ju, B., Yang, J. 2007. A review of amphoteric starches in preparation and application. *The Proceedings of The 3rd International Conference on Functional Molecules,* pp. 31-35.

Pakistan Journal of Scientific and Industrial Research
Series B: Biological Sciences
Volume 57
Contents

Series B: Biological Science
Vol. 57, No. 1, March - April, 2014

Short Communications

Vol. 57, No. 2, July - August, 2014

Review

Vol. 57, No. 3, November - December, 2014

Short Communications

Review

Pakistan Journal of Scientific and Industrial Research
Series B: Biological Sciences
Volume 57
Author Index

Pakistan Journal of Scientific and Industrial Research
Series B: Biological Sciences
Volume 57
Subject Index

Pakistan Journal of Scientific and Industrial Research

PCSIR - Scientific Information Centre

PCSIR Laboratories Campus, Shahrah-e-Dr. Salimuzzman Siddiqui, Karachi - 75280, Pakistan

Ph: 92-21-34651739-42, Fax: 92-21-34651738, E-mail: info@pjsir.org Website: www.pjsir.org

EXCHANGE FORM

We wish to receive Pakistan Journal of Scientific and Industrial Research Ser. A: Phys. Sci: and/or Ser. B: Biol. Sci. in exchange of :

Name of Journal: _____
Frequency: _____
Subjects Covered: _____
Institution: _____
Address: _____
_____
_____

Signature: _____
Name: _____
Designation: _____
Date: _____
E-mail: _____
Fax: _____
Phone: _____

Pakistan Journal of Scientific and Industrial Research

PCSIR - Scientific Information Centre

PCSIR Laboratories Campus, Shahrah-e-Dr. Salimuzzman Siddiqui, Karachi - 75280, Pakistan

Ph: 92-21-34651739-42, Fax: 92-21-34651738, E-mail: info@pjsir.org & pcsir-sic@cyber.net.pk, Website: www.pjsir.org

SUBSCRIPTION FORM

I / we wish to subscribe to 'Pakistan Journal of Scientific and Industrial Research' Ser. A: Phys. Sci: and/or Ser. B: Biol. Sci. The filled in proforma is being returned for compliance.

Subscriber's data:
Name: _____
Address: _____
_____
_____
E-mail: _____
Fax: _____
Phone: _____

Signature: _____
Order Membership No. (if any): _____

Tick the relevant box: ☐ Send invoice ☐ Bill later on ☐ Cheque for .....enclosed

RevisedSubscription Rates:
Local: Rs. 2500/ = per volume; Rs. 425/ = per copy
Foreign: US$ 450/ = per volume; US$ 75/ = per copy

Payment should be made through cross cheque in favour of Pakistan Journal of Scientific and Industrial Research and mailed to the Director PCSIR - Scientific Information Centre, PCSIR Laboratories Campus, Shahrah-e-Dr. Salimuzzaman Siddiqui, Karachi-75280, Pakistan.