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INCREASED HYDROXYMETHYLFURFURAL AND DECREASED DIASTASE ACTIVITY AS INDICATORS OF DEGRADATION OF QUALITY OF HONEY

Abstract.- Fresh and naturally pure honey samples were analyzed for diastase activity and hydroxymethylfurfural (HMF) content. These samples were multifloral in origin produced by European honeybee, *Apis mellifera.* To study their sensitivity to temperature these samples were heated at 30ºC, 40ºC, 50º C, 60º C, and 70º C and their HMF concentration and diastase activities were estimated. Honey samples at storage temperature of 25ºC had diastase activity of 25.44 DN and HMF concentration of 6.15mg/kg. At 30ºC the diastase number was 27.33DN, whereas it reduced to 8.4DN at 70°C. Similarly, HMF concentration was 8.8mg/kg at 30ºC, which increased to 87.2mg/kg at 70ºC. Decreasing diastase activity and increasing HMF concentration could, therefore, be used as criteria for degrading honey quality on account of over heating and improper storage.

Key words: Diastase activity, HMF, multifloral honey.

Honey is known to show activities of quite a few enzymes including amylases, diastase, glucosidase (invertase), glucose oxidase, catalase and acid phosphatase (Crane, *The world history of beekeeping and honey hunting*, Routledge, 1999). Also protease and esterase activity have been reported. They play a vital role in the production of honey from its raw plant materials. The conversion of nectar or honeydew into honey can be brought about only by the action of certain enzymes and these are present in the glandular secretion of the bees. Diastase activity of the honey is due to an enzyme produced by the bee's hypopharyngeal glands and also in plants (Crane, Constituents and characteristics of honey. In: *A book of honey*, Oxford University Press, 1980). Another component of honey hydroxymethylfurfural (HMF), formed by the breakdown of hexose's sugars, in the presence of an acid. A small amount is present naturally even in the fresh honey (Pajuelo, *Vida-Apicola*, **71**:47-51, 1995). These two components have therefore, assumed importance in the honey quality control (Anam and Dart, *Anal.Proc.,* **32**:515-517,1995). The present reports describes the effect of temperature on the diastase and HMF concentration in multifloral honey, as markers for the quality of the honey.

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Material and methods

The study was carried out on 20 multifloral honey produced by European honeybee, Apis mellifera and collected from different localities of Pakistan. These samples were taken from honey extractor directly from hives in sealed bottles. The honey showed no signs of fermentation or granulation. For the purpose of this experiment honey samples were heated in sealed bottles for 6 hours in a thermostatic water bath at 30°C, 40°C, 50°C, 60°C and 70°C. HMF concentration and diastase activity were measured according to the standardized methods of the European Honey Commission (Bogdanov *et al., Bee World*, **80**: 61-69, 1999).

Results and discussion

Tables I and II shows the effect of temperature on diastase number and HMF content, in the honey, respectively. There was a natural variation in diastase number in fresh honey ranging 20-30 DN. The mean values of diastase activity for all samples showed a linear relationship between decreasing enzyme activity with increasing heating. The initial heating, caused an elevation of enzyme activity (Table I), with a peak at 37°C. Heating beyond 40°C caused decreased in the diastase activity. Except one honey sample, at 70°C almost all the honey samples had diastase activity below the limits set by International Honey Standards. At 25°C the honey samples, on the average, showed diastase activity 25.44 ± 0.66DN (n=20) which after heating at 30°C showed about 7% increase and at 40°C about 4% increase. When, however, these samples were heating at 50°C, 60°C, 70°C, the diastase activity was gradually decreased. For example at 70°C and

60°C the diastase activity was reduced 35% and 66.5%, respectively.

Table I.- Effect of temperature on diastase activity.

Samples	Temperatures (°C)					
	25	**30**	**40**	**50**	**60**	**70**
Sample 1	20	25	36	25	15	15
Sample 2	23	23	33	24	16	12
Sample 3	25	27	32	24	17	10
Sample 4	24	24	38	27	13	8
Sample 5	26	27	38	27	14	7
Sample 6	26	27	37	29	20	9
Sample 7	27	30	33	28	21	5
Sample 8	28	30	35	28	19	6
Sample 9	29	32	38	29	18	7
Sample 10	29	30	37	30	17	12
Sample 11	30	31	34	30	18	11
Sample 12	22	25	35	26	22	9
Sample 13	24	27	36	24	18	8
Sample 14	22	26	33	24	11	4
Sample 15	25	25	32	24	16	6
Sample 16	23	26	39	25	15	8
Sample 17	28	29	38	30	14	9
Sample 18	27	28	36	22	14	7

Similarly initial contents of HMF in fresh honey were low with a minimum amount of 5g/kg and maximum 12g/kg. When the honey samples were subjected to heating at different temperatures a gradual increase in the HMF concentration (Table II) was observed. The honey samples at room temperature of 25°C had on the average HMF concentration of 6.15±0.33 mg/kg (n=20). On heating these samples at 30°C, 40°C, 50°C, 60°C and 70°C, 43%, 205%, 523.6%, 937%, and 1314.5% increase in the HMF content was observed, respectively. The HMF concentration of honey samples heated up to 50°C approached the maximum limit set as quality criteria for fresh honey. At 60°C and 70°C, the HMF production was beyond the limits of freshness. This general qualitative behaviour of diastase activity and HMF is similar to that found in other studies (White, *J. AOAC,* **47**: 486-488, 1964; Cervantes *et al*., *Apiacta*, **35**: 162-170, 2000; Karbournioti, *Apiacta*, **36**: 177-181, 2001). Diastase is the most stable enzyme in honey. Therefore, it is usually used as an indicator of overheating and acts as quality control parameter. The amount of enzyme depends on the age of the bee (Brouwers, *J. Apicult. Res*., **21**: 193-198, 1983), the stage of colony (Huang *et al*., *Apidologie*., **20**:455-464, 1989) and beekeeping practices (Laude *et al*., *Apidologie*, **22**: 371-380, 1991). Honey from citrus as well as honey produced in warmer climates contains naturally low levels of diastase (Serra-Bonvchi *et al*., 1995; Linder *et al*., *J. Agric. Fd. Chem.*, **44**:139-140, 1996). The honey sugars particularly fructose is affected by temperature during extracting, liquefying or clarifying or by age during storage. The result is the production of HMF. A trace amount of HMF is naturally present in honey but a large increase in this compound is due to overheating (Prota *et al*., *Apic. Mod.,* **88**: 51-59, 1997; Esti *et al*., *Fd. Chem.*, **1-2**:125-128, 1997; Garcia, *Indust.Conserv.*, **69**:353-357, 1994) or to the adulteration of honey with commercial invert sugar (Kim *et al*., *Korean Apicul*., **10**:19-28, 1995) .On the other hand LaGrange and Sanders (*Cereal Fd. Wld*., **33:** 833-838, 1988) stated that honey produced in subtropical climates has a high HMF value which exceeds 40 ppm, which is maximum standard for HMF in the Codex Honey Quality Regulations (1999).

Table II.- Effect of heating on HMF in honey.

Samples	Temperatures (°C)					
	25	**30**	**40**	**50**	**60**	**70**
Sample 1	4	12	29	55	72	110
Sample 2	5	8	30	48	51	85
Sample 3	6	9	15	47	52	82
Sample 4	4	10	23	45	67	83
Sample 5	8	9	17	46	69	84
Sample 6	6	9	17	38	49	83
Sample 7	7	12	17	36	47	80
Sample 8	7	7	20	39	57	90
Sample 9	6	9	19	32	58	95
Sample 10	5	10	18	35	55	92
Sample 11	4	7	16	39	55	75
Sample 12	9	8	17	30	48	77
Sample 13	8	9	16	32	72	90
Sample 14	7	9	15	31	65	83
Sample 15	6	9	15	32	49	79
Sample 16	5	5	14	36	57	85
Sample 17	8	10	18	38	57	88
Sample 18	7	8	19	35	55	86
Sample 19	6	7	20	39	56	97
Sample 20	5	9	21	37	59	100

School of Biological sciences,
University of the Punjab,
New Campus, Lahore-54590

S. QAMER
A.R. SHAKOORI

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NEW RECORD OF A CATFISH, *CLARIAS BATRACHUS* (LINN. 1758) FROM PAKISTAN

Abstract.- An air breathing catfish, *Clarias batrachus* (Linnaeus, 1758) has been recorded for the first time from Thatta district of Sindh, Pakistan. Earlier this fish has been reported to occur in south Asian region, from Indonesia to India. It is commonly known as "Magur". It grows to about 30-45 cm and prefers swampy and weedy areas. *Clarias batrachus* is an important culturable species and is grown extensively in earthen ponds in Thailand, Bangladesh and India. There is good potential for the culture of *C. batrachus* and other catfish species in Pakistan.

Keywords: Cat fish, *Clarias*, Pakistan.

The fish fauna of Indian subcontinent was studied in detail by Day (*The fishes of India being the natural history of the fishes known to inhibit the seas and freshwaters of India, Burma and Ceylon*, vol. I and II Reprinted 1958, William Dawson and Sons, London, pp. 483-486, 1878). Taxonomic studies on freshwater fishes of Pakistan are few (Qureshi, *Common freshwater fishes of Pakistan*. A.R.C.P. Govt. Publ. pp. 61, 1965; Mirza, *Proc. Pakistan Congr. Zool*., **1**: 1-41, 1980; Mirza, In: *Biodiversity of Pakistan* (eds. S.A. Mufti, C.A. Woods and S.H. Hasan), PMNH and Florida Museum of Nat. Hist. USA. pp. 325-333, 1997; Rafique and Qureshi, In: *Biodversity of Pakistan* (eds. S.A. Mufti, C.A. Woods and S.H. Hasan). PMNH and Florida Museum of Nat. Hist. USA. pp. 335-343, 1997). Siddiqi *et al*. (*Agric. Pakistan,* **24**: 201-220, 1973) gave a check list of 46 fish species found in Keenjher lake (Distt. Thatta). Similarly Leghari *et al*. (In: *Proc. Sem. Aquat. Biodiv. Pakistan* (eds. Q.B. Kazmi and M.A. Kazmi), pp. 139-157, 1999) have recorded 30 fish species from Bakar lakes area (Distt. Sanghar), presently called as Chotiari reservoir. In Manchar lake (Distt. Dadu) a total of 32 fish species have been documented (Jafri *et al*., In: *Proc. Sem. Aquat. Biodiv. Pakistan* (eds. Q. B. Kazmi and M. A. Kazmi), pp. 63-73, 1999). Mirza (*Illustrated handbook of animal biodiversity of Pakistan.* Saad Printers, Rawalpindi, Pakistan, pp. 39-42, 1998) while discussing animal diversity of Pakistan has mentioned seven commercial catfish species. Mirza and Bhatti (In: *Proc. Sem. Aquat. Biodiv. Pakistan* (eds. Q.B. Kazmi and M.A. Kazmi), pp. 177-184, 1999) have noted a total of 34 catfish species from Pakistan.

None of the above workers have reported the presence of any species of family Claridae from Pakistan. A catfish belonging to genus *Clarias* is being reported here.

Materials and methods

During a recent visit to Thatta fish market (February 2003), a likely new catfish species was observed. A total of four specimens were obtained and preserved in ice. These were brought to the laboratory for further studies. Photographs of largest specimen were taken and morphometric and meristic counts of the four specimens were recorded. The taxonomic status of this new catfish was confirmed with the help of keys and illustrations given by different researchers (Day, *op.cit.,* 1878; Munshi *et al*., *Natural history of fishes and systematics of freshwater fishes of India.* Narendra Publ. House, Delhi, India, pp. 299-302, 1988; Talwar and Jhingran, *Inland fishes*, Vol. I and II, Oxford-IBH Publ. Co. New Delhi, India. pp. 682-688, 1991; Jayram, *The freshwater fishes of the Indian region*. Narendra Pub. House Delhi, India. pp. 301-304, 1999).

Results

Synonymy: 1758, *Clarias batrachus* (Linnaeus); 1822, *Macropteronotus magur* (Mam. & Buch.); 1828, *Clarias (marpus) batrachus* (Curv. & Vel.); 1849, *Clarias batrachus* (Jerdon); 1858, *Silurias batrachus* (Bloch); 1863, *Clarias punctatus* (Bleaker); 1878, *Clarias magur* (Day); 1941, *Clarias batrachus* (Hora); 1988, *Clarias batrachus* (Datta *et al*.); 1991, *Clarias batrachus* (Talwar and Jhingran); 1999, *Clarias batrachus* (Jayaram).

Vernacular names of *Clarias batrachus* are Magur, Mah-gur, Monghir and Kugga. It is proposed that the local name of *Clarias batrachus* be referred as "Magur".

Colour

It is greenish brown when freshly caught. Specimens preserved in formalin became grayish

Table I.- Meristic counts of *Clarias batrachus* occurring in River Indus.

Sr. No.	Total length (cm)	Total weight (g)	Dorsal fin rays	Anal fin rays	Pectoral fin rays	Pelvic fin rays	Caudal fin rays
1.	22.5	87	61	49	1/10	6	15
2.	21.5	85	60	47	1/09	6	15
3.	19.8	63	61	47	1/10	6	15
4.	19.0	47	61	47	1/10	6	15
Range	19.0-22.5	47-87	60-61	47-49	1/9-1/10	6	15

black dorsally, with white abdomen. There are 12-14 vertical lines of very small white spots visible on lateral side of body. These lines cross three horizontal lines of similar spots. Among horizontal lines the upper two are very close to each other.

Diagnostic features

Body: Elongated, anterior portion rounded while posterior half laterally compressed from the origin of anal fin (Fig. 1).

Fig. 1. *Clarius batrachus*, Magur, from River Indus.

Head: Dorso-ventrally depressed. Dorsal surface of head with sandpaper like surface, occipital process obtuse, two small depressions on mid-line, anterior one two times longer than posterior one. Distance between occipital process and origin of dorsal fin 4.2-5.3 to the length of head. Upper jaw somewhat longer, gape of mouth small, transverse, sub-terminal and ventral in position. Accessory respiratory organ developed on fourth gill arch. Gill-rakers are short, blunt spine shaped.

Barbles: Four pairs, nasal one short, one maxillary and two mandibular are comparatively longer.

Fins: Dorsal and anal fins are long and spineless. Pectoral fins having finely serrated spines. Caudal fin somewhat rounded. The range of some morphometric and meristic counts of four specimen is shown in Table I.

Discussion

Members of the genus *Clarias*, family Claridae, are widely distributed throughout south-east Asia, India and Africa (Talwar and Jhingran, 1991 *op.cit.*). In India four species namely *C. batrachus, C. dayi, C. brachysoma* and *C. dussumieri* have been recorded, but last three species have limited distribution. This is the first record of *C. batrachus* from (Sindh) Pakistan. It is a rare species and probably found in the estuarine area of river Indus. Only Talwar and Jhingran (1991 *op.cit.*) have described the presence of prominent irregular white spots on the flanks. In the present specimens, the spots are much smaller and are in the form of vertical and horizontal lines. Some variation has also been noticed in fin-ray counts. Ratio of head length and distance of origin of dorsal fin agrees with the values reported by Jayaram (1999 *op.cit.*). During earlier part of life, *C. batrachus* feeds upon micro crustaceans and worms, while in adult stage the main food consists of aquatic insects (Thakur, *Int. Rev. Ges. Hydrobiol.*, **63**: 421-431, 1978). Males are smaller than females. The fish attains maturity at the age of one year, at a total length of about 25 cm and weighing 150 – 200 g. According to Faruq *et al*. (*Bangladesh J. Fish.*, **19**: 67-70, 1996) its fecundity ranges from 3028 to 9064 (total length 18.1 – 26.2 cm, respectively), while it was enumerated 10,000 – 15,000 by Thakur (*Jap. J. Ichthyol.*, **23**: 178-180, 1976) and the eggs having yellow-brown colour. The eggs remain attached to

aquatic vegetation.

Clarius batrachus is considered as an important species for culture among Asian countries (Pillay, *Aquaculture principles and practices*. Fishing News Books. pp. 339-346, 1990). Pond culture of *C. batrachus* and *C. macrocephalus* is very popular in Thailand. Recently in Bangladesh another species *C. gariepinus*, an African catfish has been introduced as an exotic species, for pond culture (Arthur and Ahmed, *Checklist of the parasites of fishes of Bangladesh*. FAO Technical Paper, 369/1, FAO, Rome, Italy. 2000).

To enhance the production of fish in Pakistan, culture of catfish in combination with Tilapia may become a profitable venture, especially in swampy and saline waters.

Department of Fresh Water Biology and Fisheries, University of Sindh, Jamshoro-76080, Sindh, Pakistan

W.A. BALOCH
S.I.H. JAFRI

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FECUNDITY OF *OXYGASTER GORA* (HAMILTON) (CYPRINIDAE: CYPRINIFORMES)

Abstract.- *Oxygaster gora* is an indigenous small but commercially important fish species of Bangladesh. A female having a mean total length as 127.32±37.47 mm and mean total weight as 18.47±17.38 g has a reproductive potential of 23860.13±8980.21 eggs in average. The species is found to be a highly fecund one in relation to its size. The numbers of eggs are moderately correlated with the size and weight of the female and those of its ovary.

Key words: *Oxygaster*, fecundity.

Oxygaster gora (Hamilton) is commonly known as `Gora Chela' in different parts of Bangladesh (Bhuiyan, *Fishes of Dacca*, Pub. No **13**, 148 pp. 1964). This small palatable fish is available in tanks, rivers, ponds, beels and inundated flood plain throughout Bangaldesh (Islam and Hossain, *Fish. Bull*., **1**: 1-31, 1983). Works had been done on the fecundity of different indigenous fish species of Bangaldesh *e.g. Puntius* spp. (Shafi and Quddus, *Bangaldesh J. Zool*., **2**: 133-145, 1974; Mustafa *et al*., *Bangladesh J. Zool*., **10**: 92-100, 1982), *Channa gachua* and *C. punctatus* (Bhuiyan and Rahman, *Bangladesh J. Zool*., **10**: 101-116, 1982; Bhuiyan and Rahman, *J. Asiatic Soc. Bangladesh (Sci.)*, **10**: 75-81, 1984), *Anabas testudineus* (Banu *et al*., *Bangladesh J. Aquacul.,* **6&7**: 45-50, 1985; Nargis and Hossain, *J. Asiatic Soc. Bangladesh (Sci.)*, **15**: 21-29, 1988), *Puntius stigma* (Islam and Hossain, *Univ. J. Zool. Rajshahi Univ*., **9**: 69-74, 1990), *Lepidocephalus guntea* (Hossain *et al*., *The Rajshahi Univ. Studies*, Part B, **19**: 141-145, 1991), *Colisa fasciata* (Bhuiyan *et al*., *J. biol. Sci*., **3**: 189-191, 1995), *Chanda ranga* (Mortuza *et al*., *Pakistan J. Zool*., **28**: 259-260, 1996), *Nandus nandus* (Hossain *et al*., *J. biol. Sci*., **5**: 305-307, 1997), etc. The present study was aimed to estimate the fecundity of *O. gora*, and to determine the relationships between the number of eggs and total length, standard length, total weight, body depth, ovary length and ovary weight of the fish. Knowledge on fecundity of a species is very important for its conservation, management and culture.

A total of 384 specimens were collected during a period of one year (January to December 2000) of which 194 were female and 60 were gravid. These 60 females were taken to estimate the fecundity. After collection the specimens were preserved in 10% formalin. Total length (TL), standard length (SL), body depth (BD) and total weight (TW) of the specimens were recorded. Ovaries were dissected out and length (GL) and weight (GW) of the ovaries were taken. Number of eggs of each specimen was determined by Gravimetric method (Lagler, *Freshwater fishery biology*, 2nd ed. 106 pp., 1956). The gravid females were obtained only for five months (April to August) of the year, and fecundity was estimated from these gravid females.

Ovaries were found to contain mature and ripe ova of mean diameter as 2.97±0.12 and 3.7±0.39 mm which were light orange and orange in colour, respectively. Mean fecundity was calculated as 23860.13±8980.21 eggs for a female having a mean TL as 127.32±37.47 mm, mean SL as 104.96±32.98

mm, mean TW as 18.47±17.38 g, mean BD as 56.45±19.18 mm, mean GL as 48.6±17.86 mm and mean GW as 1.85±1.48 g. The number of eggs per gram of body weight of the fish was calculated as 1291.83.

The regression equations of the correlation between fecundity (F) and (i) and total length (ii) standard length, (iii) total weight, (iv) body depth, (v) ovary length and (vi)ovary weight were obtained as follows:

i) F = -2987.5±210.87 TL (r = 0.774)
ii) F = -909.21±235.98 SL (r = 0.751)
iii) F = 16832±380.49 TW (r = 0.619)
iv) F = -550.84±432.45 BD (r = 0.853)
v) F = 2875.2±431.82 OL (r = 0.734)
vi) F = 16152±4645.8 OW (r = 0.742)

Fecundity of *O. gorai* was found to increase with the increase of length and weight of the female. Number of eggs produced by the females was correlated with the body depth. In the present study it was observed that the number of eggs varied from month to month. However, variation of fecundity among the same sized fish was noticed, individual physiology of the fishes and their surroundings may be the controlling factors for such variation. Similar results had been reported from fishes like *Tilapia nilotica* (Ahmed *et al*., *J. Asiatic Soc. Bangladesh (Sci.),* **4**: 67-71, 1978), *A. testudineus* (Nargis and Hossain, *J. Asiatic Soc. Bangladesh (Sci.)*, **15**: 21-29, 1988), *N. nandus* (Hossain *et al*., *J. biol. Sci.,* **5**: 305-307, 1997). From the present result it can be concluded that *O. gora* is a highly fecund fish for its size having a single spawning season from April to August.

Department of Zoology **M. KAMRUL AHSAN**
Rajshahi University **SANGJUCTA RANI DAS**
Rajshahi 6205 **SELINA PARWEEN***
Bangladesh

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*To whom all correspondence should be addressed: