

Significance of Cocoon Refrigeration on the Larval Performance of Multivoltine Mulberry Silkworm (*Bombyx mori* Linn.)

Smita Shukla, Surendra Prasad and V.B. Upadhyay

Department of Zoology,
D.D.U. Gorakhpur University Gorakhpur-273009, India

Abstract: *Bombyx mori* has an economic importance because of the commercial values of its silk that is called the queen of textiles. The silk is preferred over all other types of fibers due to its remarkable properties like water absorbency, heat resistance, dyeing efficiency and luster. The present study reveals the effect of cocoon refrigeration on the larval performance i.e., larval weight, larval duration and survival of larvae. The cocoons were consigned to low temperature at 5°C at 0, 2, 4, 6 and 8 days of prerefrigeration and refrigerated for 0, 5, 10, 15, 20, 25 and 30 days. The maximum larval weight was noticed in case of 0 day prerefrigerated-0day refrigerated cocoons. The survival of larvae was noticed maximum in case of 6 day prerefrigerated-0day refrigerated cocoons and the maximum larval duration was noticed in case of 2 day pre-refrigerated-30 day refrigerated cocoons. At low temperature, the insects will get acclimatized.

Key words: Prerefrigeration period • Refrigeration period • Larval weight • Larval duration • Survival of larvae

INTRODUCTION

Silkworm is one of the most important domesticated insects, which produces luxuriant silk thread in the form of cocoon by consuming mulberry leaves during larval period. The mulberry silkworm *Bombyx mori* L. is very delicate, highly sensitive to environmental fluctuations and unable to survive extreme natural fluctuation in temperature and humidity because of their long years of domestication since 5000 years. Thus the adaptability to environmental conditions in the silkworm is quite different from those of wild silkworm and other insects. The larval duration, larval weight and survival of larvae are the most important factor which influences the production of cocoon on commercial scale. The efforts are being made to evolve new technologies that are cost effective, labour saving and eco friendly. In order to increase the production of silk, efforts have been made to study the effect of temperature [1], relative humidity [2], photoperiod [3], artificial diet [4], X-rays [5], magnetization of eggs [6, 7], magnetization of larvae [8], magnetization of cocoon [9], incubation period of eggs [10], long term preservation of cocoon [11], global warming [12], 20-hydroxy ecdysone hormone [13] etc on the

performance of *B.mori*. Rise in temperature increase various physiological fluctuations and with a fall in temperature, the physiological activities are decreased. The variation in the environmental conditions during the last decade emphasizes the need of management of the temperature for sustainable production. The present study is an attempt to study the effect of refrigeration of cocoon on the larval performances.

MATERIALS AND METHODS

Seed Cocoon: The seed cocoon (pupa enclosed in silken case) of multivoltine mulberry silkworm *Bombyx mori* nistari, a native of west Bengal in India, was taken in the present study. The seed cocoon (pupa enclosed in silken case), obtained from the silkworm grainage Behraich, Directorate of Sericulture Uttar Pradesh and were maintained in the plywood trays (23×20×5 cm) under the ideal rearing condition in the silkworm laboratory [14] Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur. The temperature, relative humidity and photoperiod were maintained at 26±1°C, 80±5% RH and 12±1 hours light a day respectively till the emergence of moth from the seed cocoon. The moths emerged generally in the morning at

around 4 am. The trays, in which seed cocoon were kept, were suddenly illuminated by light in the morning at 4 O'clock on 9th and 10th day of spinning. The newly emerged moth, from seed cocoons, were quickly picked up and kept sex-wise in separate trays to avoid copulation. The male moths were smaller and more active than the female moths. The whole grainage operation was performed [14, 15].

Rearing of Larvae: After two consecutive days of hatching, the silkworm larvae were collected with the help of bird's feather and reared to maintain a stock culture in the silkworm laboratory at $26\pm 1^{\circ}\text{C}$, $80\pm 5\%$ RH and 12 ± 1 hour light a day. Four feeding of the small pieces of fresh and clean leaves of *Morus alba* were given to the larvae and care was taken that food always remained in excess in the rearing trays. After completion of fifth instars, the ripe worms ceased feeding and ready for spinning. Now small mountages were provided to the ripe worm. The ripe worms soon begin the mounting which was completed within three days. Thus sufficient number of cocoons was obtained.

Experimental Designs: The seed cocoon was obtained from the silkworm grainage and was maintained in the laboratory. The moths emerged were allowed to mate. After mating the female moths were allowed for egg laying. The eggs laid were transferred to the BOD incubator for hatching. After hatching the larvae were collected and reared to maintain a stock culture in the laboratory. After completion of fifth instars, the mountages were provided to the ripe worms for the formation of cocoon. Thus sufficient no. of cocoons was obtained which were used for further experiments to the refrigeration of cocoon.

Refrigeration of Cocoon: The cocoons obtained from the laboratory were refrigerated for different duration at varying conditions of pre-refrigerated periods of cocoons. The 'pre-refrigeration' period refers to be the duration between the completion of the cocoon formation and beginning time of the refrigeration of experimental cocoon. The zero day (0 day) refrigeration of cocoons refers to be the control (no refrigeration of cocoon). During the pre refrigeration period the cocoons were kept in BOD incubator maintained at optimum condition of temperature, relative humidity and photoperiod at $26\pm 1^{\circ}\text{C}$, $80\pm 5\%$ RH and 12 hours dim light a day respectively For the refrigeration of cocoon 630 cocoons were

consigned to low temperature at 5°C at '0 day' prerefrigeration period (one of the prerefrigeration conditions i.e. control). The cocoons were refrigerated for 0,5,10,15,20,25 and 30 days. For this purpose a group of 90 cocoons (30 cocoons in each of the three batches.) were released at once without any refrigeration (0 day pre refrigeration and 0 day of refrigeration) of cocoon which was taken as control. Further the rest of 540 cocoons were consigned at 5°C inside the refrigerator. After this a second group of 90 cocoons (three batches of 30 cocoons in each batch) were cold treated for 5 day refrigeration and were released from refrigeration accordingly in the groups of cocoons as in previous cases, after 10,15, 20,25 and 30 days of transferred chronically to BOD incubator maintained at optimum conditions of rearing. Three replicates of each experiment were made.

Like the above experimental designing at 0 day of pre refrigeration period, the similar series experiments were performed for the refrigeration of cocoons at 2,4,6 and 8 day of pre refrigeration periods of the cocoon. The moth emerged commonly in the morning at around 4:00 AM. The newly emerged moths were kept sex wise in separate trays to avoid copulation within the same group. Further, three batches each containing 5 good males and 5 good females were made and they were allowed to mate. After 4 hours of mating, the paired moths were decoupled manually. Further the gravid females were allowed to lay eggs on the sheet of paper. The egg laying moths were covered by open plastic cellules to prevent the intermixing of egg masses deposited by different female moths. After 24 hours of egg layings, the female moths were individually examined for their disease freeness. The disease free laying (DFLs) thus prepared, were washed with 2 % formalin for 15 minute. To increase the adhesiveness of eggs on cards and surface disinfections. Thereafter the egg sheets, with eggs laid on were thoroughly washed with running water to remove formalin the eggs were dried in BOD incubators maintained in the laboratory. After two consecutive days of hatching, the silkworm larvae were collected with the help of brush and reared to maintain a stock culture in the silkworm laboratory at $26\pm 1^{\circ}\text{C}$, $80\pm 5\%$ RH and 12 ± 1 hours light a day.

Larval weight-For the determination of larval weight 30 larvae (three batches of 10 larvae in each batch) were recorded. Three replicates of each experiment were made; the larval weight was taken on the day when fifth instar larvae stop feeding.

Larval duration-For the determination of larval duration 90 larvae (three batches of 30, larvae in each batch) was taken for observation. The time required from the hatching of larvae to the third day of spinning by the fifth instar larvae was considered as larval duration.

Survival of larvae-For determining the survival of larvae 90 larvae (three batches of 30, 3rd instar larvae in each batch) were taken under the observation. The number of larvae which attained the pupal stage was counted for the calculation of the survival of larvae as following.

$$\text{Percent survival of larvae} = \frac{\text{No of larvae pupated}}{\text{No of 3rd instar larvae taken for observation}} \times 100$$

RESULTS

Larval weight: The data obtained from table-1 clearly indicates that the duration of cold storage and pre refrigeration period of silkworm cocoon have considerable impact on the weight gained by the larvae of silkworm larvae. At all the durations (0, 2, 4, 6 and 8 days) of prerefrigeration, the weight gained by the larvae of refrigerated cocoons declined considerably with the increasing duration of refrigeration. At the prerefrigeration periods of 0, 2, 4, 6 and 8 days, the larval weight decreased from 1.700±0.007 to 1.180±0.011 g, 1.645±0.018 to 0.956±0.043 g, 1.581±0.023 to 1.101±0.045 g, 1.540±0.036 to 1.180±0.011 g, 1.645±0.018 to 0.956±0.043 g, 1.581±0.023 to 1.101±0.045 g, 1.540±0.036

to 1.198±0.014 g and 1.490±0.047 to 1.280±0.026 g, respectively with the increase in the refrigeration period from 0 to 30 days. The larval weight was (1.700±0.007 g) maximum in case of untreated cocoons and minimum (0.956±0.043 g) in case of 2 day prerefrigerated-30 day refrigerated cocoons. Two-way ANOVA indicates that both the duration of refrigeration and prerefrigeration significantly (P<0.01) influenced the weight of silkworm larvae.

Larval duration: The data obtained from table -2 clearly indicates that both the refrigeration period and prerefrigeration period of cocoon influenced the larval duration of silkworm. At all the conditions of prerefrigeration periods (0, 2, 4, 6 and 8 days) the time required from 1st instars to fifth instars larvae has been noticed to be increased considerably with the increasing duration (up to 30 days) of cold storage of cocoons. In all the conditions of cold storage i.e. 0, 2, 4, 6 and 8 days, the larval duration increased from 25.01±0.300 to 27.90±0.291 days, 24.60±0.256 to 29.72±0.283 days, 24.34±0.326 to 28.89±0.264 days, 24.10±0.048 to 28.23±0.121 days and 23.60±0.216 to 27.44±0.280 days respectively with the increase in the refrigeration period from 0 to 30 days. Maximum (29.72±0.283) larval duration was noticed in 2 days prerefrigerated – 30 days refrigerated cocoons while it was minimum (23.60±0.216) in case of non-refrigerated cocoons. The rate of increase in the larval duration was

Table 1: Effect of the refrigeration of cocoon on the weight (g) of *Bombyx mori* larvae.

Prerefrigeration Period (days)	Refrigeration Period (days)							F ₁ -ratio n ₁ =6
	0	5	10	15	20	25	30	
0	1.700±0.007	1.521±0.012	1.455±0.022	1.380±0.011	1.347±0.021	1.286±0.018	1.180±0.011	7.2313*
2	1.645±0.018	1.463±0.027	1.421±0.013	1.338±0.029	1.306±0.011	1.173±0.015	0.956±0.043	
4	1.581±0.023	1.411±0.011	1.386±0.011	1.299±0.005	1.246±0.013	1.101±0.045	N.Sd	
6	1.540±0.036	1.375±0.014	1.346±0.021	1.271±0.009	1.198±0.014	N.Sd	N.Sd	
8	1.490±0.047	1.326±0.020	1.280±0.026	N.Sd	N.Sd	N.Sd	N.Sd	

F₂-ratio= 6.3942* n₂=4

*P<0.01 N.Sd = Not Survived

Each value represents mean± S.E of three replicates.

Table 2: Effect of the refrigeration of cocoon on the duration (days) of *Bombyx mori* larvae.

Prerefrigeration Period(days)	Refrigeration Period (days)							F ₁ -ratio n ₁ =6
	0	5	10	15	20	25	30	
0	25.01±0.300	25.19±0.459	25.36±0.320	26.07±0.313	26.62±0.270	27.51±0.247	27.90±0.291	1.7143*
2	24.60±0.326	25.37±0.315	25.70±0.334	26.41±0.300	27.26±0.463	28.01±0.302	29.72±0.283	
4	24.34±0.326	25.62±0.230	26.16±0.460	27.06±0.520	27.90±0.291	28.89±0.264	N.Sd	
6	24.10±0.048	25.92±0.282	26.60±0.277	27.39±0.577	28.23±0.121	N.Sd	N.Sd	
8	23.60±0.216	26.26±0.051	27.44±0.280	N.Sd	N.Sd	N.Sd	N.Sd	

F₂-ratio= 3.5757* n₂=4

*P< 0.01 N.Sd=Not survived

Each value represents mean ± S.E. of three replicates.

Table 3: Effect of the refrigeration of cocoon on the survival (%) of *Bombyx mori* larvae.

Prerefrigeration Period (days)	Refrigeration Period (days)							F ₁ -ratio n ₁ =6
	0	5	10	15	20	25	30	
0	79.99±1.925	76.29±0.978	73.32±1.113	71.10±0.643	68.88±1.110	65.92±1.334	59.62±1.337	6.6897*
2	82.21±1.281	74.43±1.284	71.47±1.615	69.25±0.973	66.29±0.978	61.47±1.481	52.21±2.940	
4	84.81±2.892	71.10±2.313	68.14±1.957	66.28±1.612	64.07±1.334	54.06±2.063	N.Sd	
6	89.99±1.925	69.25±2.892	66.29±2.670	64.07±2.590	60.36±0.978	N.Sd	N.Sd	
8	81.10±2.939	67.03±2.063	65.18±1.957	N.Sd	N.Sd	N.Sd	N.Sd	

F₂-ratio= 5.4813* n₂=4

*p<0.01 N.Sd=Not Survived

Each value represents mean± S.E of three replicates.

almost of similar trend in all the cases of the prerefrigeration of cocoon. In 4, 6 and 8 days of prerefrigerated conditions, the pupae did not survived after 25, 20 and 10 days of refrigeration period. Two way ANOVA indicates that both the duration of refrigeration and prerefrigeration have significant (P<0.01) influence on the larval duration.

Survival of Larvae: The data presented in table 3 clearly indicates that both, the prerefrigeration period and the duration of refrigeration have very low degree of influence on the survival of larvae. For all the prerefrigeration periods i.e. 0, 2, 4, 6 and 8 days, the survival of larvae, obtained from the refrigerated cocoons, decreased with the increasing duration of refrigeration from 0 to 30 days. In all the conditions of prerefrigeration period i.e.0, 2, 4, 6 and 8 days, the survivility percentage of larvae decreased slowly from 79.99±1.925 to 59.62±1.337%, 82.21±1.281 to 52.21±2.940%, 84.81±2.892 to 54.06±2.063%, 89.99±1.925 to 60.36±0.978% and obtained from the refrigerated cocoons, decreased with the increasing duration of refrigeration. The declining trend of the survival percentage with the increasing refrigeration period was common in all the cases. Two way ANOVA indicates that both the duration of refrigeration and prerefrigeration have significant (P<0.01) influence on the survival of larvae.

The cold storage duration and prerefrigeration period both caused considerable impact on the larval performances. It is concluded that the cold storage of *B. mori* cocoon, up to 10 days, has almost no negative impact with regard to the larval performances. Thus if required, the cocoons of *B.mori* nistari can be refrigerated up to 10 days for the purpose of commercial rearing.

DISCUSSION

The duration of cold storage and the prerefrigeration period both have considerable impact on the weight gained by *B. mori* larvae. The larval weight of silkworm has been reported to vary in accordance with the variation

in the varieties of same host plant, used for feeding of *B. mori* [16]. The larval weight of *B. mori* has been noticed to be affected by the starvation of larvae [17]. Photoperiod influence larval weight [3]. Relative humidity influences weight of *B. mori* larvae [18]. Refrigeration of cocoon influence larval weight [19]. Refrigeration of eggs at blue pigmentation stage of *B. mori* influence larval weight [20]. Refrigeration of silkworm eggs at blue eye spot influence weight of silkworm larvae [21]. Refrigeration duration of *B. mori* eggs influences weight of silkworm larvae [22]. Seasonal variation influence larval weight [23]. The Artificial diet influence larval weight [4]. X-rays influence larval weight [5]. The magnetization of eggs influence larval weight [6]. Cocoon magnetization influence larval weight [7]. Effect of magnetization on larval weight of *B. mori* L [8]. Cocoon magnetization influence larval weight of *B. mori* [9]. Nutrition influence weight of *B. mori* larvae [24-25]. Protein and fiber constituent influence weight of silkworm larvae [26]. Herbal tonic influence larval weight [27]. Antibiotics influence the larval weight of *B. mori* [28].

The larval duration is an important economic parameter to measure the relative growth rates. It is well known that an ideal race is one which has a shorter larval duration resulting in low leaf consumption and avoidance of disease [29]. Temperature affects the physiological process of larval body resulting in marked seasonal variations, therefore, larval duration varied significantly due to seasonal changes [13]. Larval duration of bivoltine silkworm was more in winter but the span was comparatively less in dry, wet and summer season [14]. When the silkworm larvae were exposed to 24 hours light/day, the larval span was prolonged, resulting in good production of cocoon [15]. An increase in the temperature resulted in increased metabolic activity which ultimately decreased the larval duration [23]. The exogenous application of juvenoids prolonged the larval period [30]. The application of manta at the concentrations of 2.5 ppm. caused delay in the feeding period which resulted in the prolonged larval duration

[31]. The juvenile hormone secreted by corpora allata is responsible for preventing the metamorphosis [32]. Decrease in temperature enhances the moulting duration in silkworm [33].

The prerefrigeration period and the duration of refrigeration have very low degree of influence on the survival of larvae. Temperature variation influenced the physiological ability of insect, therefore, larval duration varied significantly due to seasonal changes [34]. The average temperature between 23.9 to 25.8°C along with 90.9% relative humidity has been proved to be favorable for the best survival of *B. mori* larvae [35]. The exposure to high temperature during the later developmental stage considerably reduced the survival rate [36]. The activity of Ache decreased significantly in head portion of the larvae after cold stress. The possible reason behind this may be that due to cold stress some inhibitory compounds are likely to be produced in larval body which leads to death [37]. Rearing of silkworm larvae at lower levels of RH resulted in lower fecundity, hatchability, pupation and higher larval mortality [38]. Varying sets of temperature and humidity affect both quantity and quality of silkworm as 40% mortality of larvae was recorded at 25°C. [39]. The air current of 1.0m/sec during 5th age rearing reduces the larval mortality [40]. The prerefrigeration and refrigeration periods significantly influenced the larval performance. The refrigeration of cocoons possibly causes certain biochemical and physiological alterations which influenced the larval performances of *B. mori* larvae.

REFERENCES

1. Upadhyay, V.B. and A.B. Mishra, 1991. Nutritional ability of bivoltine silkworm *Bombyx mori* L. larvae at higher temperature regimes. *J. Adv. Zool.*, 12(1): 56-59
2. Mishra, A.B. and V.B. Upadhyay, 1992. Nutritional efficiency of bivoltine *Bombyx mori* L. larvae at higher regimes of relative humidity. *J. Adv. Zool.*, 13(1 and 2): 16-18.
3. Mishra, A.B. and V.B. Upadhyay, 1993. Nutritional efficiency of bivoltine *B. mori* L. larvae at different photoperiod. *Proc. 80th session Indian Science Congress Assoc. Goa.*, pp: 54-55.
4. Iwarvat, Y. and Y. Ono, 1969. Rearing experiment an artificial diet composed mainly on the mulberry beat powder harvested in the late autumn and after J. *Seric. Sci.*, 38: 307-315.
5. Kanarew, G. and G.T. Cham, 1985. Effect of laser irradiation of silkworm eggs on silkworm *Bombyx mori* development and productivity. *Zhivotnov Nauki*, 22: 47-53.
6. Upadhyay, V.B. and S.K. Tripathi, 2006. Effect of the magnetization of eggs on the silk producing potential mulberry silk worm (*Bombyx mori* Linn.) *Sericologia*, 46(13): 269-278.
7. Upadhyay, V.B. and S. Prasad, 2010. Magnetization for the improvement of silk producing potential in multivoltine mulberry silkworm (*B. mori* Linn.). *The Bioscan*, 5(2): 285-289.
8. Chaugale, A.K. and N.K. More, 1992. Effect of magnetization on the developmental period and cocoon characters of the *B. mori* Linn. *Indian J. Seric.*, 31(2): 115-122.
9. Prasad, S. and V.B. Upadhyay, 2011. Biotechnological importance of cocoon magnetization with particular reference to the larval performance of multivoltine mulberry silkworm (*B. mori*) *Middle- East J. of Scientific Research*, 10(5) : 565-572.
10. Tripathi, S.K. and V.B. Upadhyay, 2005. Magnetization of eggs influences the incubation period of multivoltine mulberry silkworm. *B. mori*. *Linn. Eggs. J. Adv. Zool.*, 26(1): 24-28.
11. Rajkhowa, G., Rajesh Kumar and R.K. Rajan, 2011. Effect of long term preservation method of munga silkworm. *Mun. Ent. Zool. Vol.6, No.2. A text book on silkworm rearing technology*, Central silk board, Bangalore, India.
12. Zama, T., B. Sarmah, O. Hemchandra and J. Kalita, 2010. Global warming and its impact on the productivity of muga silkworm (*Antheraea assamensis* Helfer), 1: 199-209.
13. Prasad, S. and V.B. Upadhyay, 2012. Influence of 20-Hydroxyecdysone on the Larval Performance of Multivoltine Mulberry Silkworm (*Bombyx mori* L.) *African J. Basic & Appl. Sci.*, 4(5): 146-154.
14. Krishnaswamy, S., M.M. Narasimhanna, S. Suryanarayan and S. Kumar Raja, 1973. *Sericulture Manual-2 silkworm rearing* F.A.O. *Agric Serves Bull. Rome*, 15(92): 1-13.
15. Jolly, M.S., 1983. Organization of industrial bivoltine grainage for tropics. *Seric. Project No.3.C.S.R. and T.I. Mysore, Govt. of India*, pp: 19-20.
16. Koul, O., K. Tikku, B.P. Saxena and O.K. Atal, 1979. Growth and silk production in *B. mori* L. fed on 3 different varieties of mulberry. *Indian J. Seric.*, 18: 1-5.

17. Alagumalai, K., R. Ramraj, M. Thiravalluvan, N. Nagendran and R. ranjaly, 1991. Effect of antibiotics on larval cocoon characters and fecundity of silkworm, *Bombyx mori* L. Environment and ecology, 9(3): 795-796.
18. Upadhyay, V.B. and K.P. Gaur, 2002. Effect of ecological factors on the performance of *Bombyx mori* L. larvae. Zool. Sci India, pp: 91-104.
19. Upadhyay, V.B., R. Singh and S. Prasad, 2009. Refrigeration of cocoons influences the pupal Characteristics of multivoltine mulberry silkworm (*Bombyx mori* Linn.) J. Appl. Biosci., 35(2): 166-168.
20. Govindan, R. and T.R. Narayanswamy, 1986. Influence of refrigeration of egg of multivoltine silkworm, *Bombyx mori* L. at eye spot stage on rearing performance. Sericologia, 26(2): 151-155.
21. Swamy, T.K.N. and R. Govindan, 1987. Effect of refrigeration of eggs of pure Mysore race of silkworm, *Bombyx mori* L. at the blue stage. Entomon, 12(2): 105-107.
22. Panday, A.K. and V.B. Upadhyay, 1999. Impact of refrigeration of egg and pre- refrigeration period on the larva of *Bombyx mori* L. J. Adv. Zool, 20: 217-222.
23. Jyothermayala, D. and D. Bhartahi, 1993. Effect on the growth pattern of silkworm *B. mori* with reference to seasonal variation. Environ. Ecol., (1): 220-222.
24. Kanafi, R.R., R. Ebadi, S.Z. Mirhosseini, R.R. Seidavi, Zolfaghari and K. Etebari, 2007. A review on nutritive effect of mulberry leaves enrichment with vitamins on economic traits and biological parameters of silkworm *Bombyx mori* L. Inv. Sur. J., 4: 86-91.
25. Seidavi, A.R., A.R. Bizhannia, R. Sourati and M. Mavvajpour, 2005. The nutritional effects of different mulberry varieties on biological characters in silkworm. Asia Pac. J. Clin. Nutr., 14: 122.
26. Ghosh, M.K., R.C. Srivastava and B. Prasad, 1998. Impact of protein and fiber constituent of food leaves on larval weight of oak tasar silkworm. *Antheraea proyelei* (sturnidae: Bombycidae) UTTAR PRADESH J. Zool., 18(1): 5-8.
27. Manimuthu, M. and L. Isaiarasu, 2010. Influence of herbal tonic Aloe on the overall performance of the mulberry silkworm, *Bombyx mori* L.
28. Deehu, P.S., R. Govindan, M.C. Deraish and T.K.N. Swamy, 1997. Effect of antibiotics on growth and cocoon parameters of silkworm, *Bombyx mori* L. Mysore Journal of Agricultural Science., 31(1): 41-46.
29. Mathur, S.K. and G. Subba Rao, 1987. The saga of Murishidabad Silk industry. Indian Silk, 26(5): 16-17.
30. Akai, H., K. Kimura, M. Kiuchi and A. Shibukawa, 1985. Increase of silk production by repeated treatments with a juvenile hormone analogue. J. Seric. Sci. Jpn., 54: 297-299.
31. Kamada, M., 1992. Effect of metoprene (Manta) application on growth and cocoon production in the silkworm, *B. mori*. at different rearing temperature. J. Seric. Sci. Japan, 61(2): 116-122.
32. Baker, C.R., 2003. Hormonal control of metamorphosis. Insect Biochem., pp: 989-1002.
33. Kamili, A.S. and M.A. Masoodi, 2004. Principals of temperate sericulture, Kalyani Pub., New Delhi, pp: 1-2.
34. Das, P.K. and K. vijayaraghavan, 1990. Studies on the effect of different mulberry varieties and season on the larval development and cocoon characters of silkworm, *Bombyx mori* (L). Indian J. Seric., 29(1): 44-53.
35. Singh, H. and G.S. Mavi, 1987. Rearing of mulberry silkworm (*Bombyx mori* L) during autumn and spring seasons under the Punjab conditions. J. Ent. Res., 10(1): 79-84.
36. Rahmathulla V.K., 2012. Management of Climate factors for successful silkworm (*B. mori* L.) crop and higher silk production.
37. Soliman, F.M., L.A. El-Elaimy and H.M. Hamada, 1995. Malathion toxicity to *Gambusia affinis* and its effect on brain Ache activity. Alexandria. J. Agril. Res., 40: 227-242.
38. Hussain, M., S.A. Khan, M. Naeem and A.U. Mohsin, 2011. Effect of relative humidity on factors of seed cocoon production in some inbred silkworm (*Bombyx mori*) lines, International journal of Agriculture and Biology, 13(1): 57-60.
39. Kaleem, S., I. Mahmood, M. Ahmad, M.A.A.H.A. Bukhsh, A. Wasaya, A. Qayyum and M.A. Raza, 2011. Studies on biology of a new strain (K₂) of silkworm (*B.mori*) under different sets of temperature and humidity. J. of Animal and Plant Sciences, 21(3): 556-560.
40. Rajan, R.K. and M.T. Himantharaj, 2005. A text book on silkworm rearing technology, Central silk board, Bangalore, India.

Refractometric Analysis of Some Local Onion Cultivars (*Allium cepa* L.) Bulbs for Dehydration

^{1,2}I.B. Gashua, ^{2,4}U.S. Ukekpe and ^{1,3}A.M. Abba

¹University of Wolverhampton, School of Applied Sciences, Wulfruna, WV1 1SB. Wolverhampton, UK

²The Federal Polytechnic Damaturu, Department of Science Laboratory Technology, Damaturu, Nigeria

³Umar Suleiman College of Education, Biology Department Gashua. Yobe State, Nigeria

⁴Abubakar Tafawa Balewa University Bauchi, Chemistry Programme, Bauchi, Nigeria

Abstract: Onion is one of the oldest cultivated bulb producing plant species and second most valuable vegetable after tomatoes in the world but is subject to great production loss due to its poor storability. Refractometric analysis was carried on some randomly selected production stock of onion bulbs in Bade district of Yobe state, Nigeria. This is because, soluble solid constitute most of the content of the onion bulb and dehydrator onion cultivars are developed from lines with high dry matter contents. Although dehydration is considered as one of the oldest and important method of onion processing for storage, it is yet to be utilised in this part of the world. The results of the study showed that different cultivars have different amount of soluble solid determined by their refractive index values which varies considerably and that smaller and medium size bulbs have more dry matter content than larger bulbs. The local white and red onion bulb cultivars have the potentials for dehydration compare to the other cultivars within the location of the study area.

Key words: Dehydration • Cultivar • Refractive Index • Onion • Correlation

INTRODUCTION

Onion (*Allium cepa* L.) is an economically most important bulb vegetable crop in Nigeria cultivated mostly for its distinctive flavour and the many medicinal purposes which it serves. It is a member of the family *Alliaceae*. The world production of onion is 64.48 million tons from 3.45 million ha area with at least 175 countries involved [1].

Onion is one of the world most popular and significant vegetable grown for its pungent bulbs. The bulb of typical onion composed of concentric, fleshy, enlarged leaf bases. The outer leaves loss moisture and becomes scaly while the inner leaves generally thickened as the bulb develops. The green leaves above the bulb are hollow and arise subsequently from the meristem at the innermost point of the base of the bulb with the stem being very small and insignificant during vegetative growth [2].

The production of onion for dehydration requires cultivar with high soluble solid or dry matter content. Soluble solid/dry matter content is a very important trait related to onion flavour and storability and is often considered significant in determining onion cultivars for breeding of dehydrator varieties. Previous studies on sugar contents of onions [3] showed that, there is high positive correlation between sugar contents and dry matter found in onion bulbs and that such cultivars can be dried and packed as powder for use as spices or exported, because, if in powdered form deterioration is limited, considering the high rate of spoilage of onions during storage. Study of an alternative approach to dry matter content of dehydrated food products such as oven drying method was carried out by [4]. This can only be effective if the onion used are varieties with high soluble solid contents, thus the need to develop such cultivars through effective breeding of selected varieties.

Corresponding Author: Gashua Ibrahim B., University of Wolverhampton, School of Applied Sciences, Wulfruna, WV1 1SB. Wolverhampton, UK.

Although dehydration is considered as one of the oldest and vital method of onion processing, it is yet to be utilised in this part of the world, consequence of which leads to mass loss of produce during storage and great economic loss to the local producers and marketers. The advantages of dehydrated onion include reduction in cost of transportation due to reduced size of dehydrated onion products (Flakes and powder) and avoidance of mass loss of onion bulbs during storage due to its high perishability. In addition, dehydrated onions are more uniform in flavour than fresh once. When kept in cool, dry and moisture proof containers, it can be stored for long period of time.

The determination of dry matter content of onion cultivars is very important in tropical countries where they are mostly grown and loss due to storage is very significant as a result of high temperature and poor storage means. In consideration of these advantages, the idea of this work was conceived, with the aim of using refractometric analyses to test the evaluation of different local Onion cultivars for dehydration potentials so that breeding of such cultivars can be accepted.

MATERIALS AND METHODS

Plant Material: The plant materials for the study were Local Red, Geidam Brown, Ex-Borno and Local White onion (*Allium cepa* L.) bulbs obtained from the production stock of local farmers within Bade district of Yobe state, Nigeria.

Experimental: One hundred and twenty bulbs of each of the four different cultivars making a total of four hundred and eighty were used for the experiment. They were analysed for soluble solids in triplicate using refractometer (Bellingham and Stanley, refractometer, London) [4]. To do this, each bulb was peeled off and the fresh inner layer removed and squeezed with a hand presser to obtain the juice. Two drops of the juice was dropped on the sensor of the refractometer. The instrument was then directed toward a convenient light source to shine into the small glass window (c.a 1cm²) located at the rear of the top plate, then observe through the eye piece, by looking through the top scope. Adjust the bottom dial until the dark/light interface is seen. The position at which the demarcation line passes the scale gives the reading. The instrument requires no further adjustment. It gives reading of percentage sucrose in terms of refractive index. The prism sensor is cleaned off using fine cotton wool immersed in distilled

water after each reading. Readings were obtained in triplicate using randomised complete block design. Bulb weights were measured at the beginning of the experiment using acculab electronic balance and mettler PC 2200, Delta range.

Data Analysis: The data obtained from this study were analysed using the SPSS statistical software package. Basic statistics for all parameters (means, standard deviation, minimum and maximum values) and analysis of variance (ANOVA) and mean performance of the different cultivars were computed. The Duncan multiple range test was used to determine the level of significance which was declared at 0.05.

RESULTS AND DISCUSSION

Dehydrator Parameters: Previous workers [4], used different methods to measure solidity and dry matter contents of onion bulbs and concluded that, the refraction value of the pressed juice gives reliable information on the dry matter of the bulbs. Determination of the quality of fruits and vegetables is important for both producers and consumers and can be achieved through modern techniques which involves the use of non-destructive analytical methods such as Vis/NIR [5], spectrometers and refractometers to determine the percentage soluble solids or dry matter contents of onion bulb which is known to be highly correlated to the refractive index of the pressed juice [6].

The two most important dehydrator parameters measured in this research among other parameters are the refractive index, which gives an idea of the estimated amount of soluble solids content and the weight of the bulbs. The local white cultivar is a better cultivar compared to the others analysed, because of its high soluble solids/dry matter content indicated by the higher mean refractive index value of 1.3751, having a range of 1.3572 to 1.3801 as presented in table 1. This is followed by the local red cultivar with a mean value of 1.3705. Although these cultivars were found to be small in size with mean weight of 150.69 and 165.11 for the local white and red respectively. The Ex-Borno and Geidam brown cultivar recorded mean refractive index values of 1.3471 and 1.3470 with weight of 350.12 and 335.0 respectively. This showed that higher weight is associated with low refractive index and thus low soluble solid/dry matter contents and vice versa. It can therefore be suggested at this point that, using effective breeding selection program, these adoptable cultivars can complement

Table 1: Mean, standard deviation and range of dehydrator parameters for four local onion cultivars

Cultivar	SoV	Mean	S.D	Max	Min
Local Red	RI	1.3705	0.5321647	1.377	1.355
	Weight (g)	165.11	65.32	232.2	63.21
G. Brown	RI	1.3470	0.0012435	1.345	1.341
	Weight (g)	335.0	45.693218	487.3	135.6
Ex-Borno	RI	1.3471	0.0005123	1.347	1.341
	Weight	350.12	56.231002	498.2	120.1
Local White	RI	1.3751	0.7879156	1.3801	1.3572
	Weight (g)	150.69	80.13	186.2	50.60

SoV=Source of variation, RI=Refractive index, S.D=Standard deviation, G. Brown= Geidam Brown

Table 2: Combine analysis of variance showing mean squares for the four different cultivars

SoV	D.F	RI	Sucrose	Weight (g)
Treatment	3	0.00126088**	769.91825**	10186636.6 ^{ns}
Replication	3	0.00001936 ^{ns}	19.130703 ^{ns}	5669881.52 ^{ns}
Error	238	0.00001070	6.9524496	5337290.46

D.F=Degree of freedom, ** highly significant at p=0.01, ^{ns}= non-significant. g= grams

Table 3: Mean performance for the four different cultivars

Treatment	RI	Weight (g)	S.D
Local Red	1.3700 ^a	167.0 ^a	0.6004321 ^a
G. Brown	1.3475 ^b	340.0 ^b	0.0009651 ^b
Ex-Borno	1.3473 ^c	351.9 ^b	0.0006551 ^b
White	1.3705 ^d	159.9 ^a	0.7561120 ^a

Mean with the same superscript are not significantly different.

each other's weakness in terms of size/weight and soluble solid content (Determined by refractive index in this case), being two among the most important parameters in determining onion for dehydration.

Analysis of Variance: The combined analysis of variance with the mean squares of the cultivars studied is presented in table 2 below. The treatment mean squares for refractive index and sucrose content were observed to be highly significant and positive. This is in close agreement with previous workers [3, 7] as stated earlier, that reported highly significant correlation between percentage dry matter or soluble solids and refractive index of pressed juice of onions. However, non-significant effect was observed for weight treatment mean square. The relationship between weight/size and refractive index need to be investigated in these local onion cultivars and varieties.

Mean Performance: The mean performance is presented in table 3 for all different cultivars. It was observed that the refractive index were all significantly different from each other, suggesting that any small change in the value of the refractive index will affect the soluble solid/dry matter content of the bulbs. Although the difference in values was seen to be very small, the change in content will be large. The performance in weight did not follow

similar trend. The Local White and Red were found to be statistically similar and same applicable to the Ex-Borno and G-Brown. It also get to show here that cultivars with high bulb weight have lesser RI values which are significantly different and the reverse applied to the smaller size bulbs.

CONCLUSION

The result showed that different onion bulb types have different refractive index values and amount of soluble solid/dry matter contents with weight which varies considerably. The local white cultivar and the red proved to be more promising in terms of their suitability for dehydration considering their higher refractive index values compared to the Ex-Borno and G-Brown. These cultivars showed tendency for improvement if selection for the desirable traits will be carried out considering their adaptability to the environment and choice to the local populace.

ACKNOWLEDGEMENT

The authors wish to thank the local farmers of Bade district in Yobe state, Nigeria for their cooperation in providing valuable information regarding the local cultivars and too, the department of crop production and

horticulture, Federal University of Technology, Yola, Nigeria for allowing the use of their laboratory equipment during the course of this research.

REFERENCES

1. FAO, 2009. Food and Agricultural Organization., 2009 Area and production data. www.fao.org.
2. Shanmugasundran, S., 2001. Onion cultivation and seed production. Asian Vegetable Research and Development Centre. Learning centre training guide.
3. Kadams, A.M., 1986. Variability of sugar contents within the fleshy scales of onion bulbs (*Allium cepa* L). In the Proceedings of the 6th conference of the Horticultural society of Nigeria. University of Nigeria, Nsuka. Nigeria, pp: 56-60.
4. Teye, E., A.P. Asare, R.S. Amoah and J.P. Tetteh, 2011. Determination of the dry matter content of Cassava (*Manihot esculenta* Crantz) tubers using specific gravity method. *ARPN Journal of Agriculture and Biological Science*, 6(11): 23-25.
5. Nieuwhof, M., J.W.D.E. Bruyn and G. Frieda, 1973. Methods to determine solidity and dry matter content of onion (*Allium cepa* L.). *Euphytica* 22(1): 39-45. <http://dx.doi.org/10.1007/BF00021554>.
6. Tian, H., Y. Ying, H. Lu, X. Fu and H. Yu, 2007. Measurement of soluble solids contents in water melon by Vis/NIR diffuse transmittance techniques. *Journal of Zhejiang University Science B*, 8(2): 105-110.
7. Fosket, R.L. and G.A. Peterson, 1979. Relation of dry matter content to storage quality in some onion varieties and hybrids. In the Proceedings of the 1979 American Society of Horticultural science conference, pp: 314-318.