SHORT-TERM PRESERVATION OF *BRACHIONUS CALYCIFLORUS*: EFFECTS OF STORAGE ON PROTEIN, LIPID AND CARBOHYDRATE CONTENTS

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ABSTRACT

The short-term preservation of *Brachionus calyciflorus* for 45 days at three different temperatures (4, –4 and –20°C) led to decrease in protein, lipid and carbohydrate contents in all the three cases. However, the rate of deterioration was much higher at 4C than at –4 and –20°C. At 4C, protein, lipid and carbohydrate contents reduced by 76.78, 81.11 and 62.83%, respectively, and at –4°C, these were 27.94, 37.46 and 18.42%, respectively, whereas at –20°C, the deterioration was limited to 9.28, 16.44 and 11.35%, respectively, when compared with the control values. Thus, preservation at –20°C is comparatively better as it exerts limited effect on the protein, lipid and carbohydrate contents of *B. calyciflorus*.

Keywords: Preservation, *Brachionus calyciflorus*, protein, carbohydrate, lipid

INTRODUCTION

The successful rearing of planktivorous fish larvae usually depends upon the presence of an adequate quantity of zooplankton prey immediately after first feeding (Opuszynski *et al.,* 1984; Ludwig, 1993). The failure to maintain a sufficient quantity of zooplankton can result in decreased fish survival and growth (Geiger, 1983). The production of zooplankton depends on weather; so, a continuous supply of food for the fish is not assured. The other reason for the irregular supply of plankton is its dependence on natural or induced phytoplankton blooms. The culture of plankton is not possible at all locations and the transportation of live food is also difficult (Srivastava, 2000). The quality of zooplankton is also a factor as there are seasonal variations in the biochemical composition of the plankton (Lavens and Sorgeloos, 1996). Considering all these factors, various procedures for increasing plankton abundance and suitable substitutes for living zooplankton have been and are still being sought. The preservation of live food is the reliable alternative and it has been achieved with varying degrees of success (Gunkel, 1980; Medgyesy and Wieser 1982; Fermin and Bolivar, 1994; Montaini *et al.,* 1995).

Attempt to use frozen zooplankton for the rearing of those species of fish that do not accept artificial feed goes back by a long time (Einsele, 1949). However, these attempts have not been very promising in the past (Brett, 1971; Barnabe, 1976; Sargent, 1979; Fluchter, 1980; Kainz and Gollmann, 1980), although Kentouri (1980, 1981) reported the successful

rearing of several marine species, particularly sea bass (*Dicentrarchus labrax*) with frozen plankton. Dabrowski (1984) also reported that coregonid larvae fed on live or deep-frozen zooplankton show good growth and satisfactory survival. Herring and trout assimilate more than 90% of the dry matter when fed on frozen calanoid copepod, *Calanus finmarchicus* and are healthier (Sargent, 1979) with good survival (Dabrowski, 1984) and growth (Fermin and Bolivar, 1994). James *et al.* (1995) concluded that live zooplankton (predominantly *Daphnia* spp.) do not result in better growth and survival than a frozen equivalent. The frozen plankton floats making it easier for the fish to catch (Tucker, 1992). Free amino acids are present in the frozen fluid that surround the zooplankton and these form a powerful attractant and appetite stimulant for fish (Dabrowski and Rusiecki, 1983; Mearns, 1986; Tucker, 1992). However, freezing and preservation also result in the deterioration of the nutritional values of zooplankton (Medgyesy and Wieser, 1982). The optimum conditions for freezing to minimize this deterioration need to be worked out. Many workers have studied the nutritional devaluation of frozen phytoplankton (Grima *et al.*, 1994; Lubzens *et al.*, 1995). However, little attention has been paid to evaluate the nutritional values of frozen zooplankton. The present communication is an attempt in this direction. The objective of the present study was to investigate the effect of three different temperatures (4, –4 and –20°C) on the nutritional quality of *Brachionus calyciflorus* and to find out the optimum condition of preservation.

MATERIAL AND METHODS

Organic manure - cow dung, wheat bran and poultry manure (at the ratio of 1:1:1) - was used for the monoculture of *B. calyciflorus* (Srivastava and Roy, 2007). The plankton-rich water was sieved through a 53-m plankton net. Collected zooplankton was first washed with double-distilled water to remove impurities. It was filtered through filter paper and was dried on blotting sheet. From this blot-dried plankton, 100 mg of tissue was taken as control (fresh sample) for the estimation of protein, lipid and carbohydrate, and a sufficient quantity of plankton was transferred to cryovials and preserved at 4°C in a refrigerator, and at –4 and –20°C in deep freezers. Preserved plankton samples were collected every day up to the seventh day, and on the 15th, 30th and 45th days for biochemical analysis. Three replicates were used for each treatment and the results were averaged.

Lowry method (Lowry *et al.*, 1951), Rausch method (Rausch, 1981) and anthrone method (Hedge and **Hofreiter**, 1962) were used for the estimation of protein, lipid and carbohydrate, respectively.

RESULTS

The biochemical composition of fresh sample of *B. calyciflorus* showed that protein was the main component of the body (18.43%) followed by lipid (9.37%) and carbohydrate (6.08%) on wet-weight basis (Fig. 1). This implies the suitability of this zooplankton as starter food for larviculture.

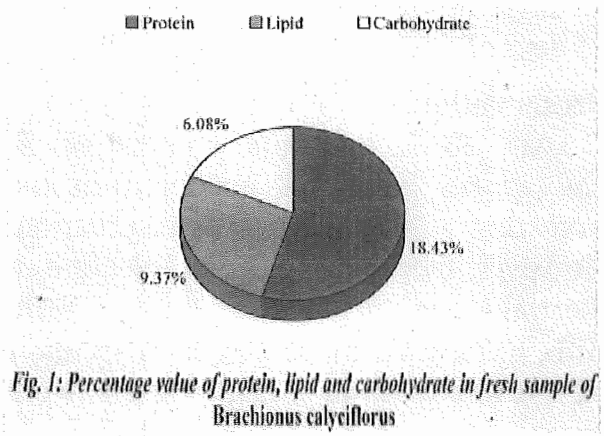

Fig. 1: Percentage value of protein, lipid and carbohydrate in fresh sample of Brachionus calyciflorus

Protein Content of *B. calyciflorus*

The protein content of control (fresh sample) was 184.3 mg g^{-1} wet weight of tissue.

Preservation at 4°C

The protein contents of *B. calyciflorus* on the first, second, third, fourth, fifth, sixth, seventh, 15th, 30th and 45th days of preservation at 4C were recorded to be 168.4, 162.6, 160.2, 135.9, 133.7, 130.6, 119.5, 98.0, 58.2 and 42.8 mg g^{-1} wet weight of tissue, respectively (Fig. 2),

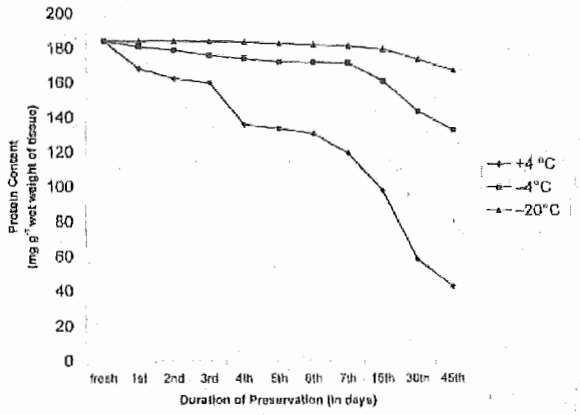

Fig. 2: Protein content of B. calyciflorus *after various durations of preservation at 4, –4 and –20°C*

thus, showing a decrease of 8.63, 11.77, 13.08, 26.26, 27.46, 29.14, 35.16, 46.83, 68.42 and 76.78%, respectively, as compared to the control value.

Preservation at –4°C

Preservation at –4°C also resulted in a decrease in the protein content. It was found to be 181.2 mg g^{-1} on the first day, 179.1 mg g^{-1} on the second day, 176.1 mg g^{-1} on the third day, 174.3 mg g^{-1} on the fourth day, 172.4 mg g^{-1} on the fifth day, 172.1 mg g^{-1} on the sixth day, 171.8 mg g^{-1} on the seventh day, 161.0 mg g^{-1} on the 15th day, 143.6 mg g^{-1} on the 30th day and 132.8 mg g^{-1} wet weight of tissue on the 45th day of preservation (Fig. 2). Thus, at –4°C, the protein content decreased by 1.68% in one day, 2.82% in two days, 4.45% in three days, 5.43% in four days, 6.46% in five days, 6.61% in six days, 6.78% in seven days, 12.64% in 15 days, 22.08% in 30 days and 27.94% in 45 days of preservation.

Preservation at –20°C

At –20°C, the protein content of *B. calyciflorus* remained unaltered up to the second day (48 hours) of preservation. However, there was a gradual decline in the value on the third, fourth, fifth, sixth, seventh, 15th, 30th and 45th days of preservation at –20C which were recorded to be 184.2, 183.8, 182.9, 182.1, 181.4, 179.8, 173.8 and 167.2 mg g^{-1} wet weight of tissue, respectively (Fig. 2). Thus, at –20C, the protein content decreased by 0.05% in three days, 0.27% in four days, 0.76% in five days, 1.19% in six days, 1.57% in seven days, 2.44% in 15 days, 5.69% in 30 days and 9.28% in 45 days of preservation.

Lipid Content of *B. calyciflorus*

The fresh sample of *B. calyciflorus* contained 93.7 mg g^{-1} lipid.

Preservation at 4°C

Lipid contents of *B. calyciflorus* on the first, second, third, fourth, fifth, sixth, seventh, 15th, 30th and 45th days of preservation at 4C were recorded to be 85.7, 80.4, 78.5, 75.2, 72.9, 68.7, 57.5, 47.6, 34.2 and 17.7 mg g^{-1} wet weight of tissue, respectively (Fig. 3), thus, showing a decrease of 8.54, 14.19, 16.22, 19.74, 22.20, 26.68, 38.63, 49.20, 63.50 and 81.11%, respectively, as compared to the control value.

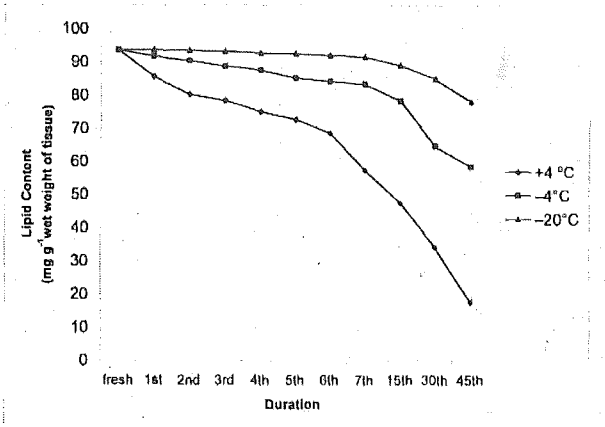

Fig. 3: Lipid content of B. calyciflorus *after various durations of preservation at 4, -4 and -20°C*

Preservation at –4°C

Preservation at –4°C also resulted in the decrease of lipid content. It was found to be 91.8 mg g^{-1} on the first day, 90.5 mg g^{-1} on the second day, 88.9 mg g^{-1} on the third day, 87.8 mg g^{-1} on the fourth day, 85.4 mg g^{-1} on the fifth day, 84.3 mg g^{-1} on the sixth day, 83.6 mg g^{-1} on the seventh day, 78.5 mg g^{-1} on the 15th day, 64.7 mg g^{-1} on the 30th day and 58.6 mg g^{-1} wet weight of tissue on the 45th day of preservation (Fig. 3). Thus, at –4°C, lipid contents decreased by 2.03% in one day, 3.42% in two days, 5.12% in three days, 6.30% in four days, 8.86% in five days, 10.03% in six days, 10.78% in seven days, 16.22% in 15 days, 30.95% in 30 days and 37.46% in 45 days of preservation, respectively.

Preservation at –20°C

At –20°C, the lipid content of *B. calyciflorus* remained unaltered in the first day (24 hours) of preservation. However, there was a gradual decline in the value on the second, third, fourth, fifth, sixth, seventh, 15th, 30th and 45th days of preservation at –20C which were recorded to be 93.6, 93.3, 92.9, 92.7, 92.1, 91.8, 89.2, 85.1 and 78.3 mg g^{-1} wet weight of tissue, respectively (Fig. 3). Thus, at –20°C, the lipid content decreased by 0.11% in two days, 0.43% in three days, 0.85% in four days, 1.07% in five days, 1.71% in six days, 2.03% in seven days, 4.80% in 15 days, 9.18% in 30 days and 16.44% in 45 days of preservation.

Carbohydrate Content of *B. calyciflorus*

Carbohydrate content in fresh sample was recorded to be 60.8 mg g^{-1} wet weight of tissue.

Preservation at 4°C

Carbohydrate contents of *B. calyciflorus* on the first, second, third, fourth, fifth, sixth, seventh, 15th, 30th and 45th days of preservation at 4C were

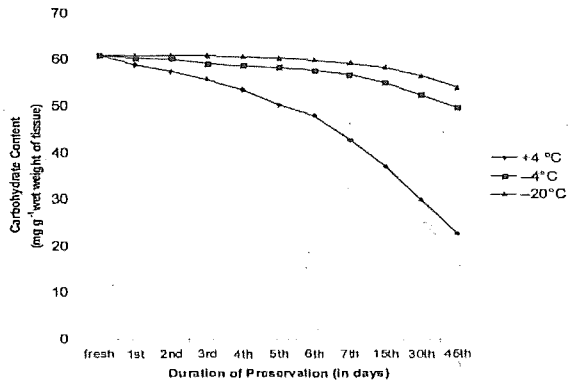

Fig. 4: Carbohydrate content of B. calyciflorus *after various durations of preservation at 4, -4 and -20°C*

recorded to be 58.9, 57.4, 55.7, 53.5, 50.1, 47.9, 42.6, 36.9, 29.8 and 22.6 mg g^{-1} wet weight of tissue, respectively (Fig. 4), thus, showing a decrease of 3.13, 5.59, 8.39, 12.01, 17.60, 21.22, 29.93, 39.31, 50.99 and 62.83%, respectively, as compared to the control value.

Preservation at –4°C

Preservation at –4°C also resulted in the decrease of carbohydrate content. It was found to be 60.4 mg g^{-1} on the first day, 60.1 mg g^{-1} on the second day, 59.1 mg g^{-1} on the third day, 58.7 mg g^{-1} on the fourth day, 58.2 mg g^{-1} on the fifth day, 57.6 mg g^{-1} on the sixth day, 56.7 mg g^{-1} on the seventh day, 55.0 mg g^{-1} on the 15th day, 52.3 mg g^{-1} on 30th day and 49.6 mg g^{-1} wet weight of tissue on the 45th day of preservation (Fig. 4). Thus, at –4°C, carbohydrate contents decreased by 0.66% in one day, 1.15% in two days, 2.80% in three days, 3.45% in four days, 4.28% in five days, 5.26% in six days, 6.74% in seven days, 9.54% in 15 days, 13.98% in 30 days and 18.42% in 45 days of preservation, respectively.

Preservation at –20°C

At –20°C, the carbohydrate content of *B. calyciflorus* remained unaltered up to the third day (72 hours) of preservation. However, there was a gradual decline in the value on the fourth, fifth, sixth, seventh, 15th, 30th and 45th days of preservation at –20C which were recorded to be 60.6, 60.2, 59.9, 59.2, 58.3, 56.5 and 53.9 mg g^{-1} wet weight of tissue, respectively (Fig. 4). Thus, at –20°C, carbohydrate content decreased by 0.33% in four days, 0.99% in five days, 1.48% in six days, 2.63% in seven days, 4.11% in 15 days, 7.07% in 30 days and 11.35% in 45 days of preservation.

DISCUSSION

The preservation of zooplankton at three different temperatures: 4, –4 and –20C in the present study has shown that in all the three cases, there is decrease in nutritional quality. However, all the three parameters studied were more severely affected at 4C than at –4 and –20C. On the 45th day of preservation, the contents of protein, lipid and carbohydrate were reduced to 76.78, 81.11 and 62.83%, respectively, at 4°C; 27.94, 37.46 and 18.42%, respectively, at –4°C; and 9.28, 16.44 and 11.35%, respectively, at –20°C when compared with the control (fresh sample). Thus, it is obvious from this study that the preservation of *B. calyciflorus* at 4C is extremely harmful for all the three important nutritional parameters – protein, carbohydrate and lipid. However, preservation at –20C is the least harmful to protein content and moderately harmful to carbohydrate content. But unfortunately, even this temperature is unable to maintain the lipid content of the sample beyond 83.56% during preservation.

The present study gets support from the findings of Srivastava (2000) who reported decrease in protein, lipid and carbohydrate contents of *Ceriodaphnia cornuta* preserved at 4C (57, 84 and 61%, respectively) and –20C (9, 55 and 26%, respectively) for 72 hours. This study also finds support from many other studies made with phytoplankton. Lubzens *et al.* (1995) reported significant reduction in certain fatty acid (16:0 and EPA) levels in *Nannochloropsis* sp. preserved at 4C. However, preservation at –20C caused only slight differences in its fatty acid profile. Similarly, Grima *et al.* (1994) also observed significant decrease in saturated

and monosaturated fatty acid contents of marine micro-alga, *Isochrysis galbana,* preserved at 4C for 30 days, but at the same time, preservation at –20C made little effect on this value. Medgyesy and Wieser (1982) also noticed that there is little difference in the nutritional value of deep-frozen zooplankton. Therefore, this study suggests that preservation of *B. calyciflorus* is suitable only at sufficiently low temperature.

In conclusion, the preservation of *B. calyciflorus* at –20C is comparatively better as it exerts limited effect on the protein and carbohydrate contents. This information would be helpful for fish farmers in managing a steady supply of live food and also in its easy transport for successful culture of larvae of finfish and shellfish.

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