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Differentiation of unfrozen and frozen–thawed kuruma prawn (*Penaeus japonicus*) from the activity of β-hydroxyacyl-CoA-dehydrogenase (HADH) in aqueous extracts

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Changes in the β-hydroxyacyl-CoA-dehydrogenase (HADH) activity of kuruma prawn (*Penaeus japonicus*) muscle due to freezing treatment were investigated. The enzyme was extracted by immersion of kuruma prawn meat in phosphate buffer (0·1 M, pH 6·0) at 25°C for 15 min. After juice filtration, the enzyme activity was assayed using acetoacetyl-CoA as substrate and measured spectrophotometrically at 340 nm. No significant differences were found among HADH mean activity values of prawn meat frozen at temperatures ranging from −10 to −196°C. The HADH activity was significantly higher ($p < 0{\cdot}001$) in frozen–thawed prawns than that in unfrozen animals because during freezing there is a release of HADH. Storage time in crushed ice did not influence thawed prawns' HADH activity. However, slight differences were found in unfrozen samples. Therefore, the determination of the HADH activity may be a useful method to differentiate between unfrozen and thawed prawn.

INTRODUCTION

Prawns or shrimp are among the most valuable of seafoods. They are frozen in large quantities because they are highly perishable. The most common US market form is headless, raw, frozen prawn (Anonymous, 1986), but in other countries (e.g. Spain), whole, raw, fresh or frozen are the most common forms. Many consumers prefer fresh prawns although they are two or three times more expensive than the frozen ones. Thus, some retailers sell frozen and thawed prawns for fresh prawns. Several methods have been developed to reduce and control this fraudulent practice in fish. Gould (1971) and Vincenzo *et al.* (1985) designed some enzymic methods, but they needed to apply a tedious electrophoresis, which may be unfeasible for routine monitoring. Other authors (e.g. Barbagli & Crescenzi, 1981; Werkmeister & Demmer, 1986; Kitamikado *et al.*, 1990) developed enzymic methods without electrophoresis, but, as far as the authors are aware, none have been used commercially. This may be due to the fact that the method of Barbagli & Crescenzi (1981) was only applicable to trouts, and the Kitamikado *et al.* (1990) technique was useful for many whole fish species, but it could not distinguish between unfrozen or thawed fish fillets because this method is based on the activity of neutral β-*N*-acetylglucosaminidase, located in the red blood cells.

Hamm & Gottesman (1982) and Gottesman & Hamm (1983) developed an enzymic method, based on the release of β-hydroxyacyl-CoA-dehydrogenase (HADH) during freezing/thawing, to distinguish thawed from unfrozen meat. García de Fernando *et al.* (1992) and Hoz *et al.* (1992) modified the former technique and showed that it differentiates between fresh and thawed trout (*Salmo gairdneri*) and crawfish (*Procamburus clarkii*) meat. The purpose of the present study was to determine the usefulness of the HADH activity to differentiate thawed from unfrozen prawns.

MATERIALS AND METHODS

Raw materials

Three-hundred and thirty kuruma prawns (*Penaeus japonicus*) of around 20 g were obtained in a fish farm located in Huelva (Spain) and transported to the laboratory in crushed ice within 10 h after being caught.

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Storage of samples

A portion (140) of shell-on prawns was kept in crushed ice and another portion (190) was divided into five groups. The groups were frozen respectively at −10°C (10 samples), −18°C (150), −35°C (10), and −80°C (10) or immersed in liquid nitrogen (−196°C) (10). After 1 week of frozen storage, samples were thawed at 4°C overnight.

Assay for HADH activity

To know the effect of the storage time in crushed ice on the (HADH) activity of unfrozen or thawed (frozen at −18°C) prawns, sampling was done after 0, 1, 2, 4 and 6 days of crushed ice storage.

Peeled prawn tail (head and shell removed, but not deveined) was used for determining the HADH activity according to the method previously reported by García de Fernando *et al.* (1992): portions of prawn tails (*c.* 2 g) were immersed in two volumes (ml) of phosphate buffer (0·1 M, pH 6·0) at 25°C and maintained in a water bath at the same temperature for 15 min. The juice obtained was filtered through Whatman paper No. 2. The filtrate was assayed for HADH activity, mixing in a methacrylate disposable semimicro spectrophotometric cell (light pathway 1 cm; capacity 1 ml), in the following order: 34 μl of juice, 70 μl of EDTA (34·4 mM) and 880 μl of phosphate buffer (0·1 M, pH 6·0). The mix was maintained at room temperature for 3 min and eventually, 20 μl of NADH (1·5 mM), and 20 μl of acetoacetyl-CoA (5·9 mM) were added. Immediately, the absorbance at 340 mm was measured every 0·5 min, up to 3 min. The HADH value was calculated (HADH value = $-10^4 \times$ slope of the line resulting from spectrophotometric measurement at 340 nm at different times).

Statistical analyses

Data were analysed by variance analyses, and the differences between means were established using the Scheffe-F test.

RESULTS AND DISCUSSION

The HADH mean activity values of kuruma prawn (*Penaeus japonicus*) muscle frozen at −10, −18, −35, −80 or −196°C are shown in Table 1. HADH mean activity values ranged from 78 to 104 at −10°C and −196°C, respectively. No significant differences were found among HADH mean activity values of prawn meat frozen at the above described temperatures.

Table 1. HADH activity (mean ± standard deviation) of thawed kuruma prawn (*Penaeus japonicus*) muscle previously frozen at different temperatures

Temperature (°C)	HADH value	Number of samples
−10[a]	78 ± 22[c]	10
−18[a,b]	101 ± 26	150
−35[a]	87 ± 14	10
−80[a]	101 ± 13	10
−196[a]	104 ± 27	10

[a] HADH value was determined immediately after thawing.
[b] Thawed prawns were kept in crushed ice for 0, 1, 2, 4 or 6 days before analysis.
[c] No significant differences ($p < 0.05$) were found.

Table 2 shows the effect of the storage time in crushed ice on the HADH mean values of prawn muscle. These mean values ranged from 87 to 115 and from 35 to 58 in thawed and unfrozen prawn muscle, respectively. Storage time did not influence thawed prawns' HADH activity values. Slight differences were found in unfrozen samples due to the consistent increase observed throughout storage (i.e. from 35 to 57). This increasing trend may be explained by the progressive meat deterioration. Indeed, the thawed and unfrozen prawns stored 6 days in crushed ice, showed a clear spoilage (mainly ammonia odour and meat softening). However, the HADH value (Table 2) of thawed prawns were not affected by the storing time.

To establish the usefulness of the HADH activity values to differentiate between unfrozen and thawed prawns, the data of Tables 1 and 2 (190 thawed and 140 unfrozen) were combined and statistically analysed (Table 3).

The HADH activity values ranged from 21 to 189 (mean ± standard deviation, 99 ± 26) and from 8 to 129 (46 ± 25) in the thawed and unfrozen prawn meat, respectively. When data from unfrozen and thawed samples were statistically compared, HADH mean values of samples were found to be significantly different at the level $p < 0.001$. According to the HADH mean values and their standard deviations, the following criterion was established; samples with HADH

Table 2. HADH activity (mean ± standard deviation) of frozen (−18°C)—thawed and unfrozen—kuruma prawn (*Penaeus japonicus*) muscle stored in crushed ice

	Storing days				
	0	1	2	4	6
Thawed	101 ± 26[a] (n = 70)	95 ± 35[a] (n = 20)	115 ± 18[a] (n = 20)	87 ± 15[a] (n = 20)	99 ± 42[a] (n = 20)
Unfrozen	35 ± 19[a] (n = 40)	42 ± 24[a,b] (n = 25)	51 ± 24[a,b] (n = 25)	53 ± 28[a,b] (n = 25)	57 ± 22[b] (n = 25)

n = number of samples.
[a,b] Values in a row with different superscripts differ significantly ($p < 0.05$).

Table 3. HADH activity (mean ± standard deviation) of unfrozen and thawed kuruma prawn (*Penaeus japonicus*) muscle

	HADH value	Number of samples
Unfrozen	46 ± 25[a]	140
Thawed	99 ± 26[b]	190

[a,b] Values followed by different letter differ significantly ($p < 0.001$).

values lower than 70 are unfrozen, those with HADH values higher than 80 are thawed and values ranging from 70 to 80 are uncertain. With this criterion, the thawed samples tested would be classified as 8% unfrozen, 11% uncertain and 81% properly as such, while the unfrozen prawns would be as 7% thawed, 7% uncertain and 86% properly as unfrozen.

Toldrá *et al.* (1991) reported that the HADH activity determination was not useful to differentiate between unfrozen or thawed pork meat frozen previously at temperatures above −12°C. This observation was not confirmed by García de Fernando *et al.* (1992) working with trout (*Salmo gairdneri*), but HADH values of prawns frozen at −10°C were lower than, although not significantly different from, those of other freezing temperatures, which partially supports the opinion of Toldrá *et al.* (1991). In any case, these temperatures should not be commercially used. Shrimp stored at −12°C begin to show quality deterioration after 3 months and are inedible after 10 months, whereas shrimp stored at −40°C had the appearance of fresh frozen shrimp and were of good quality after 12 months (Banks *et al.*, 1977). Shrimps stored at −18°C are of very good quality, and it is doubtful that the extra energy costs involved with producing lower storage temperatures would be offset by any benefit gained with only a slightly higher quality product (Wheaton & Lawson, 1985). For this reason the temperatures around −18°C are mainly used by the seafood industry. Thus, if the criterion described above (<70 HADH activity value to unfrozen, 70–80 to uncertain and >80 to thawed prawn tails) is used to differentiate between unfrozen and thawed, in the frozen samples the following classification is obtained only at −18°C: 8% as unfrozen, 8% as uncertain and 84% as thawed.

The storage time at −18°C did not affect the HADH activity (data not shown), since prawns (20 each batch) stored for 1 and 6 months had HADH mean activity values of 97 ± 30 and 96 ± 36, respectively.

The present study confirms the observation that the HADH activity allows us to distinguish whether fish or seafood muscle has been either unfrozen or thawed (García de Fernando *et al.*, 1992; Hoz *et al.*, 1992). However, it seems that every fish species presents different HADH activity values. The systematic use of this method for other fish and seafood is, therefore, necessary to establish the appropriate range of HADH values for every species.

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