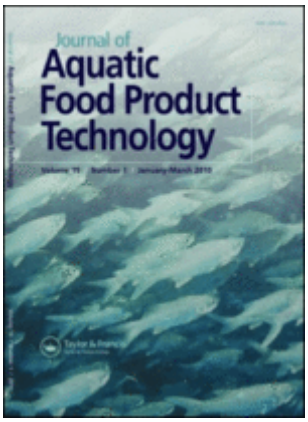

Determination of pH in pre rigor fish muscle - method matters

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Determination of pH in *pre rigor* fish muscle – method matters

ABSTRACT

The determination of *post mortem* muscle pH are often carried out in studies on how pre-slaughter handling influences the development of *rigor mortis* and the quality of the fish products. The analysis might be performed by inserting a pH-electrode into the intact flesh or using a suspension containing a minced muscle sample. Although it has been known for a long time that mincing muscle tissue accelerates glycolysis and breakdown of ATP, more recent studies quite commonly determine pH after mincing *pre rigor* muscle without including an enzyme inhibitor that arrest glycolysis. This may have resulted in anomalously low *pre rigor* muscle pH. The aim of the present work was compare the two methods of pH determination in *pre rigor* fish muscle and to highlight the importance of including the enzyme inhibitor when homogenizing *pre rigor* fish muscle prior to pH analysis.

Keywords: *pre rigor* muscle pH, fish, minced muscle, accelerated glycolysis

Introduction

Post mortem muscle pH is often determined during the conversion of muscle to meat since both the pH value at the time of slaughter, the subsequent rate of decline in pH and the ultimate pH *post rigor* will affect the quality of the meat (Lawrie and Ledward, 2006). In farmed fish, the determination of *post mortem* (pm) muscle pH have commonly been carried out in studies on how pre-slaughter handling influences the development of *rigor mortis* and the quality of the fish products (Kristoffersen et al., 2006; Lerfall et al., 2015; Mørkøre et al., 2008; Robb et al., 2000; Rucinque et al., 2018; Sigholt et al., 1997; Wilkinson et al., 2008).

The measurements of pH in pm muscle are often done by inserting a pH-electrode directly into the flesh (e.g. Bagni et al., 2007; Erikson and Misimi, 2008; Jerrett and Holland, 1998; López-Luna et al., 2014; Mørkøre et al., 2008; Morzel et al., 2003; Roth et al., 2012). Alternatively, a muscle sample is homogenized in an aqueous liquid or a minced sample may be mixed with a liquid and the pH determined in the suspension by an ordinary pH-meter (e.g. Álvarez et al., 2018; Andersen et al., 1997; Ayala et al., 2010; Bensid et al., 2014; Bugeon et al., 2003; Chang and Wong, 2012; Einen and Thomassen, 1998; Boyd et al. 1984; Fan et al., 2008; Li et al., 2015; Li et al., 2017; Matos et al., 2010; Noori et al., 2018; Sigholt et al., 1997; Wang et al., 2018). When this latter method was established many decades ago, it was recommended to mince *pre rigor* muscle in the presence of mmol/L concentrations of sodium iodoacetate to block the glycolysis by complete inhibition of glyceraldehyde-3-phosphate dehydrogenase (Bendall, 1973; Lawrie, 1953; Padieu and Mommaerts, 1960). The inclusion of this enzyme inhibitor during the homogenization is necessary when determining the pH in *pre rigor* muscle since the mincing process accelerates glycolysis and breakdown of ATP and ADP (Hamm and van Hoof, 1971; Newbold and Scopes, 1971; Nowlan and Dyer, 1974). This knowledge appears in many cases to have been lost on the way since several of the more recent studies cited above are examples of publications where pH were determined in minced fish

muscle in the *pre rigor* state without including sodium iodoacetate. This may probably have resulted in erroneously low pH value early *post mortem* due to the accelerated glycolysis.

The aim of the present work was compare the two methods of pH determination in *pre rigor* fish muscle and to highlight the importance of including the enzyme inhibitor when homogenizing *pre rigor* fish muscle prior to pH analysis.

2. Materials and methods

Six non-starved farmed Atlantic salmon (*Salmo salar* L.) and six wild Atlantic cod (*Gadus morhua* L.) which had been caught alive by demersal seine and fed frozen capelin and herring, were obtained from the Aquaculture Research Station, Tromsø. The average weight of the salmon and cod were 1.23 kg and 8.35 kg, respectively. The fish were carefully netted and killed by a blow to the head and since it was important to start the pH determinations as soon as possible *post mortem*, the fish were not bled prior to the analyses. The right side was deskinned and two muscle samples of 25 g were excised from the loin part of each fillet. One tissue sample was added an equal volume of 0.15 mol/L KCl and the other 0.15 mol/L KCl containing 5 mMol/L sodium iodoacetate (Sigma-Aldrich, Steinheim, Germany) and minced using an Ultra Thurrax (IKA T25B, IKA Werke GmbH, Germany) for 2 x 10 s at room temperature.

The pH was also determined in the intact loin part of the left fillet of fish by inserting the pH electrode into the muscle through an incision in the skin. A new incision was made at each measurement. The pH both in the homogenates and the intact muscle were determined using a WTW 330/Set-1 pH-meter (Wissenschaftliche-Technische Werkstätten, Weilheim, Germany) equipped with a double pore glass electrode (Hamilton Bonaduz AG, Bonaduz, Switzerland) and the first recordings were performed 5 - 10 min *post mortem*. The measurements carried out from 0 to 60 min pm were performed at the aquaculture station. The

fish were then gutted, iced in boxes and transported together with the homogenates to the Norwegian College of Fishery Sciences were the fish and the homogenates were stored in a cold room (4 ^{0}C) during the subsequent pH determinations.

3. Results and discussion

The *post mortem* pH of the salmon muscle determined by inserting the electrode into the intact flesh showed a stable value of around 7.2 during the first hour (Fig 1A). Eight hours pm, the pH had declined to 6.7 and an ultimate pH of approximately 6.2 - 6.3 was reached at around 28 h pm (Fig 1B). These changes in salmon muscle pH *post mortem* are similar to those observed by others (Mørkøre et al., 2008).

The minced *pre rigor* salmon muscle homogenized with 0.15 mole/L KCl containing 5 mmole/L sodium iodoacetate and in 0.15 mole/L KCl only, had both a pH of 6.8 immediately after mixing (Fig. 1A). No change in the pH occurred in the presence of iodoacetate during the subsequent storage for 46 hours showing that the inhibitor completely blocked the glycolytic process (Fig 1A and B) as described in the previously cited publications from more than 40 years ago. The pH of the muscle suspension containing only 0.15 mole/L KCl showed however a decline to about 6.6 and 6.5 after 20 and 60 min pm, respectively (Fig 1 A). No further reduction in muscle pH was observed demonstrating that the ultimate pH found by using the stick electrode was not achieved in the suspensions. This could be due inactivation of glycolytic enzymes and the dilution of the minced muscle tissue. When compared with the stable pH observed in the intact muscle during the first hour *post mortem*, the pH decline in the muscle minced in the presence of an equal volume 0.15 mmol/L KCl only, confirms that the mincing process accelerates glycolysis and ATP breakdown. Mincing the salmon muscle with an equal volume of 0.15 mole/L KCl containing 5 mmol/L iodoacetate gave pH value at time 0 which was about 0.3 pH-units lower that was recorded using the stick electrode (Fig 1A). This could

possibly be explained by the accelerating effect of mincing on the rate of glycolysis and the time elapsed before complete inactivation of glyceraldehyde-3-phosphate dehydrogenase. Mincing destroys the structural integrity of the muscle cells leading to a closer contact between enzymes, substrates and activators and thereby increased rates of glycolysis and ATP hydrolysis. The elevated concentration of freely distributed calcium is believed to be a major contributor to the increased rates (Cohen, 1987; Jeacocke, 1984; Nowlan and Dyer, 1974).

The results obtained with Atlantic cod (Fig 2A and B) were similar to the ones found for Atlantic salmon. The initial pH of 7.0 in minced cod muscle at time 0 min was approximately 0.2 units lower than found using the stick electrode. In the sample homogenized with an equal volume of 0.15 mole/L pH was reduced to 6.9 within 20 min with no further reduction during the subsequent storage of the homogenate for 46 h. As with the salmon, there was no change in pH in the cod homogenate containing iodoacetate from time 0 min to the end of the storage period of 46 h. When inserting the electrode into the intact cod muscle, a constant pH of about 7.2 was recorded the first 8 hours pm. Then it declined to 6.6 and 6.4 after 22 and 28 h, respectively. An average ultimate pH of 6.3 was found 46 h pm, confirming that the cod used in this experiment had been well fed (Kristoffersen et al., 2006).

Muscle tissue in *rigor mortis* or *post rigor* have no glycolytic activity and the pH value should therefore not be dependent on method used. We determined the muscle pH in a farmed salmon fillet stored in ice for 76 h and found a value of 6.3 independent of using a stick electrode or measuring in homogenate made with an equal part 0.15 mole/L KCl (results not shown).

4. Conclusions

The results clearly show that the pH of a *pre rigor* fish muscle should be determined by inserting the pH electrode into the intact fillet. Mincing of muscle in an equal volume 0.15 mole/L KCl accelerates the rate of glycolysis leading to an erroneously low pH early *post*

mortem. Including sodium iodoactate, an inhibitor of the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase, in the KCL solution effectively blocks the glycolysis. However, the mincing process even in the presence the enzyme inhibitor, results in a lower initial pH compared to value found by measuring directly in the intact tissue.

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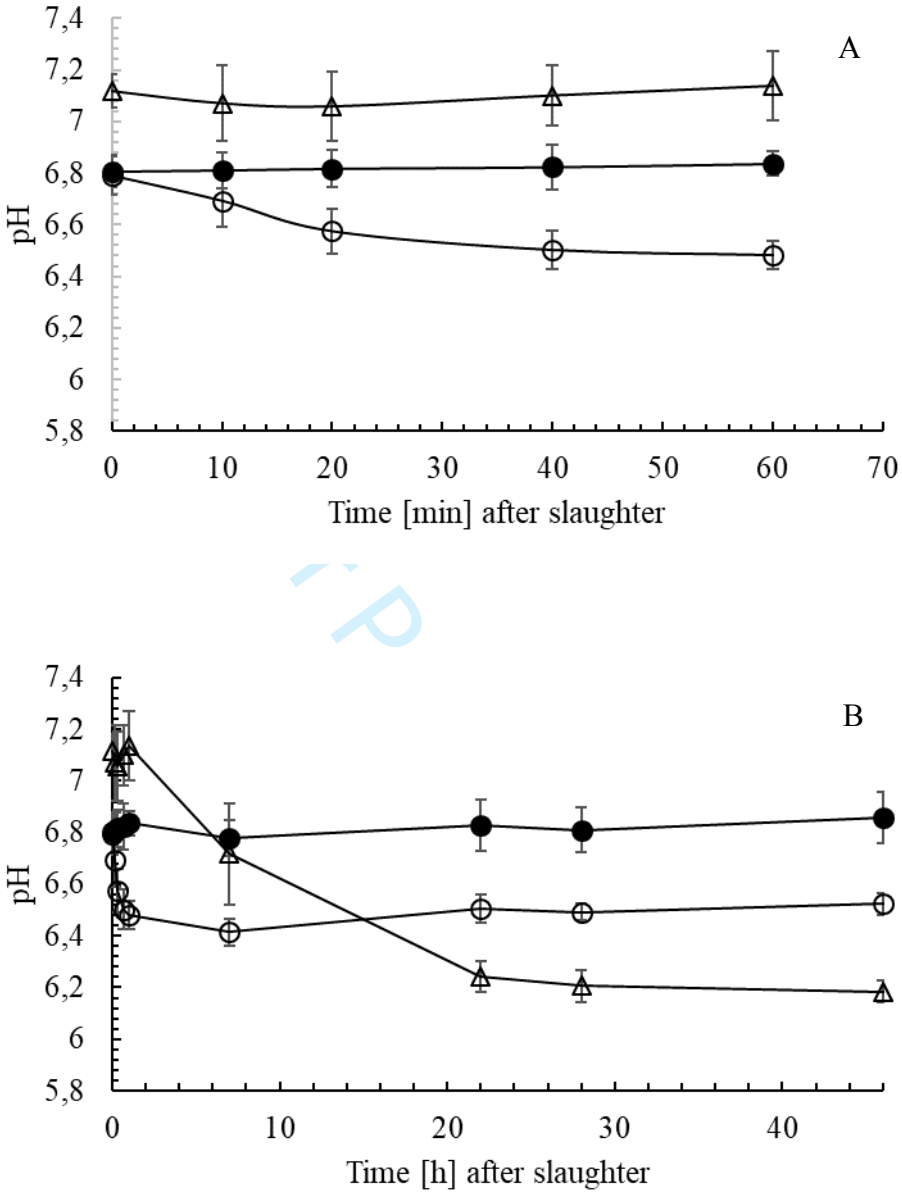
Figure 1

Development of pH in *pre rigor* Atlantic salmon (n = 6) muscle determined after mincing with an equal part 0.15 mole/L KCl-solution with (●) and without (○) sodium iodoacetate. A: First hour after slaughter. B: Until 46 h after slaughter. Muscle pH determined by inserting a pH electrode into the intact muscle (Δ). Symbols and bars represent, respectively, mean and standard deviation.

Figure 2

Development of pH in *pre rigor* Atlantic cod (n = 6) muscle determined after mincing with an equal part 0.15 mole/L KCl-solution with (●) and without (○) sodium iodoacetate. A: First hour after slaughter. B: Until 46 h after slaughter. Muscle pH determined by inserting a pH electrode into the intact muscle (Δ). Symbols and bars represent, respectively, mean and standard deviation.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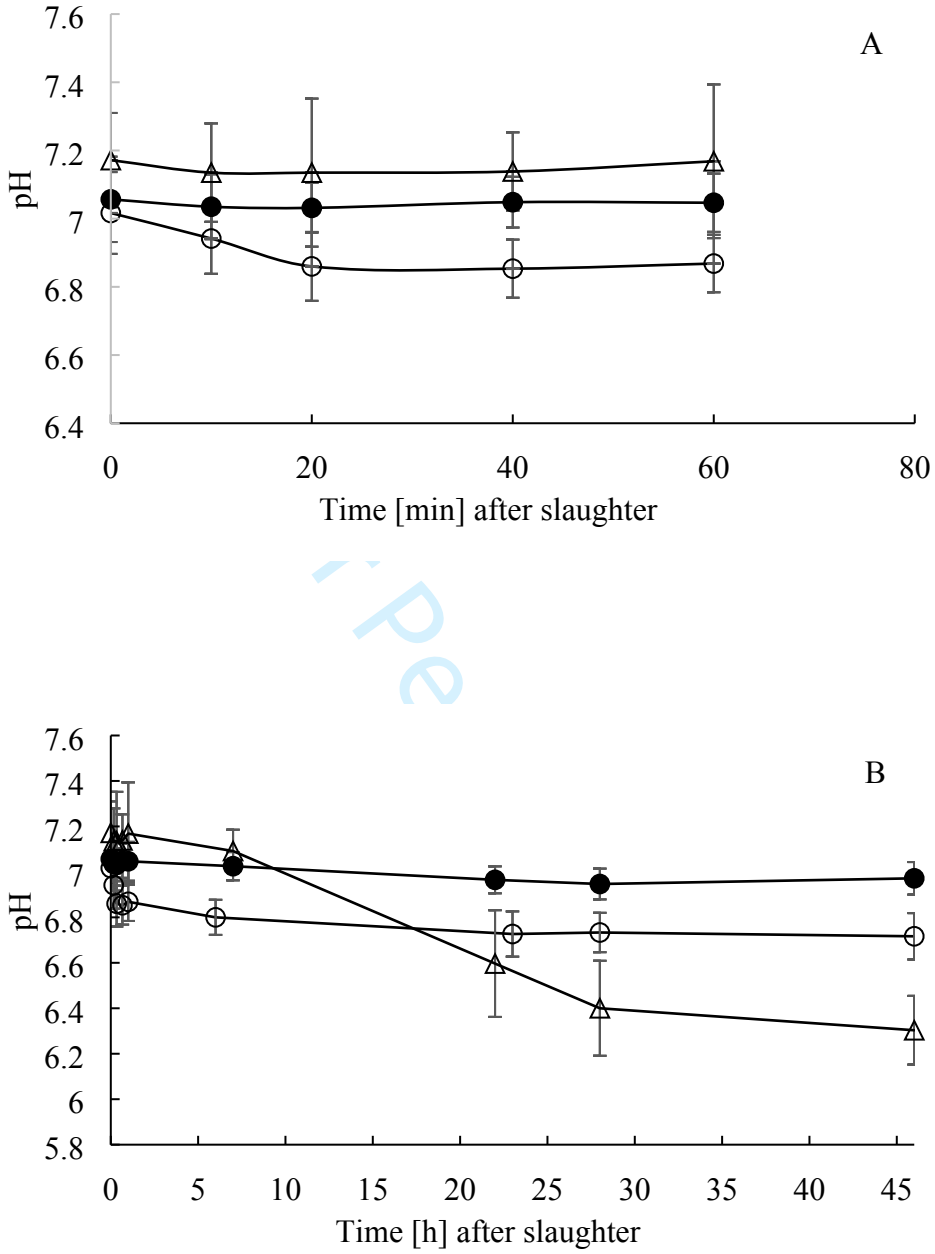
7,4
7,2
7
6,8
6,6
6,4
6,2
6
5,8
0
10
20
30
40
50
60
70
Time [min] after slaughter
B
pH
7,4
7,2
7
6,8
6,6
6,4
6,2
6
5,8
0
10
20
30
40
Time [h] after slaughter

Figure 1


A
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7.4
7.2
7
6.8
6.6
6.4
0
20
40
60
80
Time [min] after slaughter
B
pH
7.6
7.4
7.2
7
6.8
6.6
6.4
6.2
6
5.8
0
5
10
15
20
25
30
35
40
45
Time [h] after slaughter

Figure 2