

## Physicochemical and textural properties of squid (*Loligo vulgaris*) muscle treated with organic acids

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### Abstract

The aim of this study is to investigate the effects of organic acid treatments on tenderizing of squid muscle. Squid samples removed head, skin, viscera, and tentacles and cut into square pieces of 4 x4 cm were soaked into the citric and lactic acid solvents (1 and 2%) and kept in a refrigerator (4°C) for 24 h. It was found that organic acid treatments affected the physicochemical properties of squid. While acid application decreased pH values and water holding capacity, its increased cooking loss and free amino acid content. Texture profile parameters and sensory texture scores did not change significantly after acid treatments. The type and concentration of acids were not effective for all parameters except cooking loss.

**Keywords:** *Physicochemical; Organic acids; Squid; Texture*

### 1. Introduction

The squid which has commercial importance is an important member of cephalopods. The squid mantle, which contains a high amount of insoluble myostromin, has a tough flesh texture and is difficult to chew (Grygier *et al.*, 2020). Various methods such as enzyme treatment (Gokoglu *et al.*, 2017; Ketnawa & Rawdkuen, 2011; Melando *et al.*, 1997; Konno & Fukazawa, 1993; Stanley & Hultin, 1984), cooking (Sikorski & Kolodziejska, 1986), phosphate salts (Pietrasik *et al.*, 2010) and sodium chloride (Kugino & Kugino, 1995) have been studied to reduce squid toughness in previous studies.

It has been stated that the marination process in organic acid solutions was traditionally used as a means of softening and flavoring meats. These treatments decrease the mechanical resistance of meats. (Berge *et al.*, 2001). This effect occurs probably due to low pH which causes swelling of meat, increased proteolysis of cathepsin, and conversion of collagen to gelatine (Burke & Monahan, 2003). Marinades with a tenderizing capacity are particularly important in applications involving muscles rich in connective tissue. These muscles often make up the cheaper carcass cuts and the tenderizing effect of marinating offers a commercially important means of upgrading those (Burke & Monahan, 2003).

Mostly marination with acids has been applied to meat, especially beef (Berge *et al.*, 2001; Ertbjerg *et al.*, 1999; Oreskovich *et al.*, 1992; Rao & Gault, 1990; Aktas *et al.*, 2003; Ke *et al.*, 2009; Chang *et al.*, 2010) and octopus' muscle (Katsanidis, 2004; Nagai *et al.*, 2002; Katsanidis & Agrafioti,

2009). However, studies on squid muscles are very limited. The aim of this study is to investigate the effects of organic acid treatments on the physicochemical properties and texture of squid muscle.

### 2. Materials and Methods

#### 2.1. Material

Squid (*Loligo vulgaris*) was obtained from the wholesaler fish market. They were purchased just after landing and placed in a cold storage bag with ice. Before treatments head, skin, viscera, and tentacles of squids were removed, then cut into square pieces of 4 x4 cm.

#### 2.2. Organic acid treatments

Citric and lactic acids were used for treatments. Concentrations of acids were determined after preliminary trials applied to squids of their different concentrations. For preliminary trials, squid pieces were dipped into acid solutions in different concentrations between 0.1 and 5%. At last, concentrations of 1 and 2% were determined as the best in terms of sensory properties. While concentration lower than 1% was not effective on tenderness, higher ones than those of 2% caused a sour taste. In preliminary trials, samples were dipped into the solutions in different sample/ solution ratios. The best ratio was determined as 1/1 (w/v sample/solution). Keeping time in acid solutions of the samples was also determined by preliminary tests. When the samples were kept for more than 24 h, they either lost their quality or had an acidic taste.

After the preparation of 1% and 2% lactic and citric acid solutions, the squids were immersed in acid solvents (1/1 w/v) and kept in the refrigerator (4°C) for 24 hours. Untreated samples were used as a control. After all treatments analyses were conducted.

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### 2.3. Analyses

Measurement of pH was carried out by dipping the pH-meter (WTW Inolab, Weilhem, Germany) probe into diluted samples with distilled samples after homogenization (Manthey *et al.*, 1988). The centrifugation method was used to determine water holding capacity (WHC) (Hughes *et al.*, 1997). The weight of squid samples was measured before and after cooking to determine cooking losses, which was expressed as the cooked weight subtracted from uncooked weight, divided by the uncooked weight then multiplied by 100. Cooking loss was expressed as a percent. Total free amino acid content was determined by spectrophotometric method and expressed as mg glutamic acid equivalents  $\text{kg}^{-1}$  sample (Yokoyama & Hiramatsu, 2003). Texture measurements were carried out using a texture analyzer TA.XT2 (Stable Micro Systems, Godalming, U.K.) with 5 kg load cell. Texture profile analysis (TPA) was performed with a compression test. Toughness was measured using the shearing test. Hardness, springiness, cohesiveness, gumminess, and chewiness were measured. Toughness was determined by the maximum force (kg) necessary to cut the sample. Sensory analysis was conducted by a sensory panel. Six trained conducted the panel. The ability of panelists to detect differences in hardness was tested. Four reference foods from the standard hardness scale (Szczesniak & Kleyn, 1963) were presented to the panelists. Panelists having the ability to place the foods in true order were used in the panel of the present study. Squid pieces were evaluated in terms of hardness, chewiness, and elasticity attributes using nine-point descriptive scales as follows: 1= extremely hard 9= extremely tender for hardness; 1= poor chewiness 9= well chewiness for chewiness; 1= low elasticity 9= high elasticity for elasticity. Two replications of the experiment were conducted at separate times and all analyses were performed in duplicates. Analysis was conducted using the SAS software (Statistical Analysis System, Cary, NC, USA). When main effects or interactions were significant, Duncan's Multiple Range test was used.

## 3. Results and Discussion

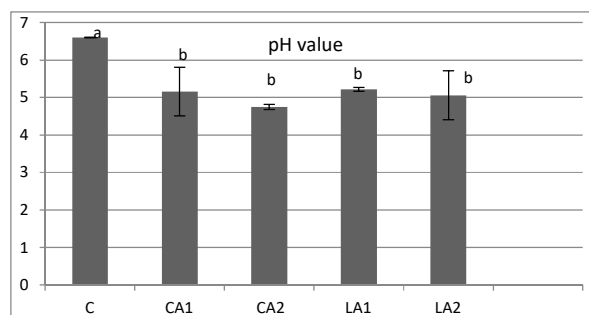
### 3.1. pH value

The pH value of meat is highly important because it has a major influence on water holding capacity, tenderness, and juiciness. Low pH in muscle causes an increase in negative charges. This neutralizes the positive protein charges and releases the water molecules. The initial pH of squid muscle was found 6.6. However, pH values of squid muscle treated with organic acids significantly ( $p < 0.05$ ) decreased as compared to control samples (Fig. 1). Differences in pH values of squid muscle treated with citric and lactic acid were not significant statistically. The acid concentration was not effective on pH value. Lower pH values were reported for beef and fish marinated with organic acids (Aktas *et al.*, 2003; Ke *et al.*, 2009; Gokoglu *et al.*, 2004). The muscle of the squid mantle differs from the muscle of fish and mammals. The muscle fibers in fish muscles run parallel to the long axis of the fish. Contrary, the muscle fibers of the squid mantle are arranged orthogonally into layers of the radial (perpendicular to the skin) and the circumferential band (parallel to the skin) sandwiched between the outer and inner tunic of the connective tissue (Otwell & Hamann, 1979).

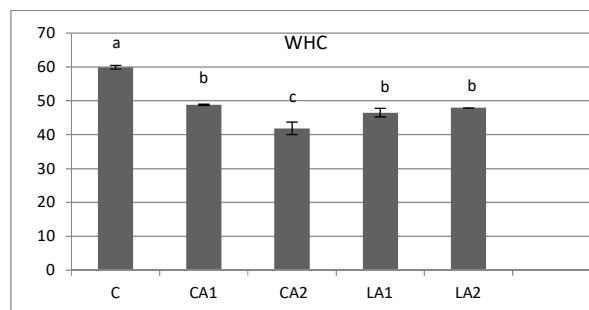
### 3.2. Water holding capacity (WHC)

The water-holding capacity of squid muscle significantly ( $p < 0.05$ ) decreased after the marination process (Fig. 2). Acid type and concentration were not effective on WHC. In fact, increase in WHC is expected in acidic conditions. Many

researchers have reported that WHC and tenderness of beef muscle are improved at acidic conditions below the typical pH of post-mortem beef muscle (pH 5.2–5.5) (Berge *et al.*, 2001; Eilers *et al.*, 1994; Ertbjerg *et al.*, 1999; Gault, 1985 & 1991; Ke, 2006; Stanton & Light, 1990). Muscle swells gradually as the pH decreases to below pH 4.5. Below pH 4.5, most of the thin filaments were extracted and the myofibrils fused together giving an amorphous, coagulated appearance at pH 4.5 (Rao *et al.*, 1989). The reason for this lies in better water binding of proteins below the isoelectric point. In this study pH of squid muscle marinated with organic acids did not reach critical values (below the isoelectric point). The decrease in water holding capacity is probably due to the denaturation of myofibrillar proteins, which play a role in water retention. Reduced WHC results from myofibrillar shrinkage, as well as from the movement of water from the myofilament space into the extra-cellular space. There are three main factors involved in the shrinkage and/or swelling of the myofibrils: the onset of rigor mortis, the decline in pH, and protein fragmentation (Ketnawa & Rawdkuen, 2011).



**Figure 1.** pH values of squid muscle treated with citric and lactic acids  
C= Control; CA1=Citric acid (1%); CA2= Citric acid (2%); LA1= Lactic acid (1%); LA2= Lactic acid (2%)



**Figure 2.** Water holding capacity of squid muscle treated with citric and lactic acids  
C= Control; CA1=Citric acid (1%); CA2= Citric acid (2%); LA1= Lactic acid (1%); LA2= Lactic acid (2%)

### 3.3. Cooking loss

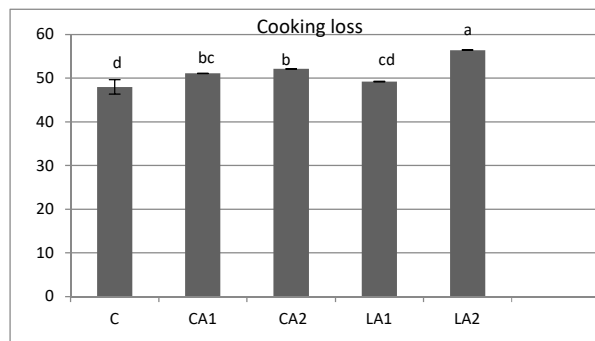
Cooking loss in squid muscle significantly increased after marination with organic acids (Fig. 3). The increase in cooking loss was observed in conjunction with a decrease in the water holding capacity after marination. There were no differences in cooking losses between treatments of citric and lactic acids. The concentration of acid was effective on cooking loss. Higher concentration resulted in higher cooking losses. The lower degree of hydration of the myofibrillar proteins at the isoelectric point was responsible for a higher moisture loss during cooking. As reported by Rao & Gault (1990) and Ke *et al.*, (2009), below pH 4.5, most of the thin filaments became extracted and the myofibrils fused together. The present study

could not be reached these pH values in squid muscle, so the cooking loss did not increase.

### 3.4. Total free amino acids

An estimate of protein degradation in seafood during storage and processing can be done by spectrophotometric determination of free  $\alpha$ -amino groups that appear when a peptide bond is cleaved. Various compounds such as ninhydrin and trinitrobenzene sulfonic acid react especially with amino groups resulting in a colorimetric reaction that can be measured spectrophotometrically and correlated to the number of peptide bonds cleaved (Jakobsen *et al.*, 2010).

The free amino acid content of squid treated with organic acids was significantly higher ( $p < 0.05$ ) than in the control samples (Fig. 4). The free amino acid concentration in samples after acid treatment significantly increased ( $p < 0.05$ ). There was no significant ( $p > 0.05$ ) effect of acid type and acid concentrations on free amino acid levels. Hinkle (2010) reported that when the pH of a muscle decreases, reaching the isoelectric point of approximately pH 5.3, the protein repulsion would be at the lowest point indicating equal amounts of positive and negative charges. As the pH becomes more acidic the balance of charges is disrupted by an increase in positive charges, causing repulsion. The same process occurs with negative charges when the pH becomes more basic. Saunders (1994) also reported that when the pH falls protein degradation starts to occur.



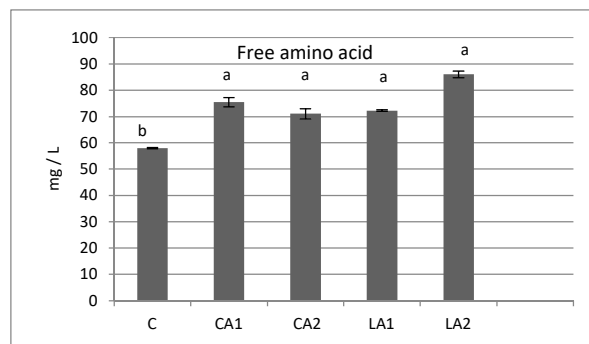
**Figure 3.** Cooking loss of squid muscle treated with citric and lactic acid  
C= Control; CA1=Citric acid (1%); CA2= Citric acid (2%); LA1= Lactic acid (1%); LA2= Lactic acid (2%)

### 3.5. Textural properties

Texture profile parameters did not change after marination with organic acids. Acid type and concentration did not affect textural parameters (Table 1). Ertbjerg *et al.* (1995) showed that lactic acid injected at a low concentration (0.3 M), led to a pH value of 5.2 which is close to the isoelectric point of the major myofibrillar proteins, did not improve beef texture, while injection at 1.0 M resulted in a meat pH of 4.6 and decreased meat toughness. Gault (1985) reported the effects of low pH on cooked meat tenderness by the addition of acetic acid solutions. Peak force was maximum at pH values around 5.0 (range 4.5 $\pm$ 5.5) and decreased sharply as the pH decreased from 4.6 to values less than 4.1. Several studies have suggested tenderness is directly influenced by water-holding capacity. Water holding capacity is the amount of water that can be held within a muscle during some form of mechanical force such as cutting, tumbling, etc.

There were no significant differences in shear force values between the samples marinated with organic acids and control (Fig. 5). Ke *et al.* (2008) reported that Warner-Bratzler shear force decreased as muscle pH was lowered to 3.52; then

shear force significantly increased as the pH was buffered back to pH 5.26. Ertbjerg *et al.* (1999) found a slight decrease in shear force over a 21-day period post-marination with lactic acid suggesting that there would be no concern for over-tenderization. Acid marination did not show a tenderization effect on squid muscle due to pH over the isoelectric point. Sensorial texture scores were not affected by acid treatments. There were no significant ( $p > 0.05$ ) differences in sensory texture scores between treatments. Similar findings were reported by Aktas and Kaya (2001).



**Figure 4.** Total free amino acid content of squid muscle treated with citric and lactic acids  
C= Control; CA1=Citric acid (1%); CA2= Citric acid (2%); LA1= Lactic acid (1%); LA2= Lactic acid (2%)

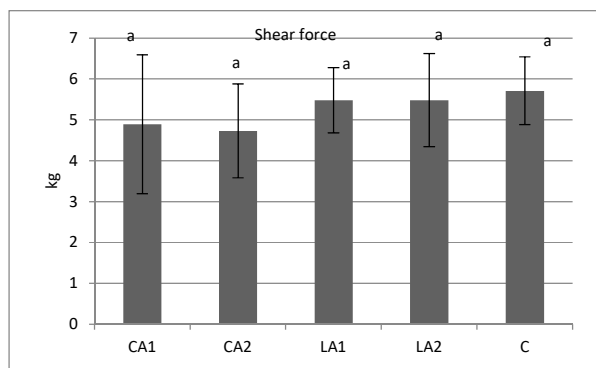
**Table 1**

Texture analysis results of squid treated with citric and lactic acids<sup>1, 2</sup>

Texture parameters (Instrumental)	Organic acids				Control
	1%	Citric acid 2%	1%	Lactic acid 2%	
Hardness (kg)	2.78 $\pm$ 1.09a	2.87 $\pm$ 0.72a	2.61 $\pm$ 0.87a	2.63 $\pm$ 0.47a	3.24 $\pm$ 0.78a
Springiness (cm)	0.89 $\pm$ 0.05a	0.83 $\pm$ 0.08a	0.85 $\pm$ 0.08a	0.88 $\pm$ 0.03a	0.82 $\pm$ 0.01a
Gumminess (kg)	2.35 $\pm$ 1.05a	1.90 $\pm$ 0.32a	2.23 $\pm$ 0.57a	2.21 $\pm$ 0.51a	2.23 $\pm$ 0.66a
Cohesiveness	0.83 $\pm$ 0.05a	0.76 $\pm$ 0.06a	0.81 $\pm$ 0.05a	0.83 $\pm$ 0.04a	0.68 $\pm$ 0.05b
Chewiness (kg)	1.87 $\pm$ 1.01a	1.97 $\pm$ 1.65a	1.79 $\pm$ 0.62a	1.94 $\pm$ 0.46a	1.84 $\pm$ 0.55a
Texture parameters (Sensory)					
Hardness	5.20 $\pm$ 2.43a	5.30 $\pm$ 2.82a	6.11 $\pm$ 1.83a	6.11 $\pm$ 2.52a	4.30 $\pm$ 0.70 a
Chewiness	5.66 $\pm$ 2.39a	5.44 $\pm$ 1.94 a	6.40 $\pm$ 2.65 a	6.20 $\pm$ 2.58a	5.30 $\pm$ 1.80 a
Elasticity	5.22 $\pm$ 2.53a	5.11 $\pm$ 2.31a	5.88 $\pm$ 2.36 a	6.88 $\pm$ 1.76a	5.00 $\pm$ 1.50a

<sup>1</sup>Each number represents the average value of each parameter

<sup>2</sup>Different letters in the same row indicate significant differences between treatments



**Figure 5.** Shear force values of squid muscle treated with citric and lactic acids  
C= Control; CA1=Citric acid (1%); CA2= Citric acid (2%); LA1= Lactic acid (1%); LA2= Lactic acid (2%)

#### 4. Conclusion

In this study, organic acid treatment of squid muscle caused changes in some physicochemical properties due to low pH values. However, it had no tenderization effect. Concentrations used in this study were not sufficient to tenderize the muscle of squid because of pH of the muscle was found over the isoelectric point. Higher concentrations need to tenderize squid muscle. However, in the preliminary trials, a sour taste was determined in squid muscle when organic acids of more than 2% were used. If squid meat will use for sour meals such as salads, higher concentrations can be used. Otherwise, it can be said that organic acid marination is not a proper tenderization method for squid.

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