Food Chemistry 224 (2017) 347–352

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

The role of microorganisms in the degradation of adenosine triphosphate (ATP) in chill-stored common carp (*Cyprinus carpio*) fillets

Dapeng Li [a], Longteng Zhang [a], Sijia Song [a], Zhiying Wang [a], Chunli Kong [a], Yongkang Luo [a,b,*]

[a] Beijing Advanced Innovation Center for Food Nutrition and Human Health, College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, PR China

[b] Beijing Laboratory for Food Quality and Safety, College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, PR China

ARTICLE INFO

Article history:
Received 12 July 2016
Received in revised form 30 November 2016
Accepted 19 December 2016
Available online 21 December 2016

Chemical compounds studied in this article:
Adenosine triphosphate (PubChem CID: 5957)
Adenosine diphosphate (PubChem CID: 6022)
Adenosine monophosphate (PubChem CID: 6083)
Inosine monophosphate (PubChem CID: 8582)
Hypoxanthine ribonucleoside (PubChem CID: 6021)
Hypoxanthine (PubChem CID: 790)

Keywords:
Common carp
AMPD
ACP
ATP degradation
Microbial communities

ABSTRACT

Biochemical and microbial changes after harvest strongly affect the final quality and shelf life of fish and fish products. In this study, the role of microbes in the degradation of adenosine triphosphate (ATP), and the origin of adenosine monophosphate deaminase (AMPD) and acid phosphatase (ACP) in common carp fillets during different stages of chilled storage (at 4 °C) were investigated. The content of ATP, ADP, AMP, IMP, HxR, and Hx, the activity of AMPD and ACP, and the total count of viable, *Aeromonas*, *Pseudomonas*, H_2S-producing bacteria, and lactic acid bacteria were examined. Results indicated that the population of microbial communities in control samples increased with storage time, and *Pseudomonas* peaked on the 10th day of storage. Changes in AMPD activity were less related to the abundance of microbes during the entire storage period. However, ACP was derived from both fish muscle and microbial secretion during the middle and late stages of storage. Degradation of ATP to IMP was not affected by spoilage bacteria, but the hydrolysis of IMP, and the transformation of HxR to Hx was affected considerably by the spoilage bacteria.

1. Introduction

The common carp (*Cyprinus carpio*) is one of the most important freshwater fish with total production of 4,159,117 tons in 2014 (FAO, 2016). Common carp (*Cyprinus carpio*), commonly known as the Chinese major fresh water carp, is found to be a good source of protein. However, common carp is an extremely perishable food. Fish and fish products are more prone to spoilage and growth of food-borne pathogens than other foods with high protein content. The spoilage of common carp tends to occur rapidly with the growth and metabolism of microorganisms (Gram & Huss, 1996). Some microorganisms, such as *Pseudomonas*, *Aeromonas*, *Flavobacterium*, *Shewanella*, *Micrococcus*, and *Moraxella* are commonly involved in the spoilage of freshwater fish (Austin, 2006; Wang, Luo, Huang, & Xu, 2014; Zhang, Li, Li, Liu, & Luo, 2015). *Aeromonas* and *Pseudomonas* were found to be dominant in spoiled grass carp (*Ctenopharyngodon idellus*) (Wang et al., 2014), whereas *Pseudomonas* was the major microorganism in common carp (*Cyprinus carpio*) at the end of the shelf life (Zhang et al., 2015).

The growth of the microorganisms and ATP degradation can strongly affect the final quality of fish and fish products. ATP degradation is an important biochemical change in the post-mortem muscle of fish and shellfish. After fish death, the post-mortem evolution in fish muscle includes capture, rigor mortis, resolution of rigor mortis, autolysis and spoilage (Hamada-Sato, Usui, Kobayashi, Imada, & Watanabe, 2005). In dead fish, the muscle glycogen is consumed constantly and abundant ATP is produced

Abbreviations: ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; IMP, Inosine monophosphate; HxR, hypoxanthine ribonucleoside; Hx, hypoxanthine; AMPD, adenosine monophosphate deaminase; ACP, acid phosphatase; ALP, alkaline phosphatase; TVC, total viable counts; PCA, plate count agar; AMB, *Aeromonas* Medium Base; LAB, lactic acid bacteria; CK, control check; SG, the sterilization group.

* Corresponding author at: College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, PR China.

E-mail addresses: luoyongkang@263.net, luoyongkang@cau.edu.cn (Y. Luo).

http://dx.doi.org/10.1016/j.foodchem.2016.12.056
0308-8146/

rapidly. Then, adenosine diphosphate (ADP) and adenosine monophosphate (AMP) are generated by ATP degradation within one day. IMP, which is associated with the umami taste (a pleasant taste) of fish, is the third ATP compound to break down. IMP and its salt are widely used in food industry to enhance food flavor (Hong, Regenstein, & Luo, 2015). It is generated by adenosine monophosphate deaminase (AMPD) after the ammonia is removed from AMP. Alasalvar, Taylor, Öksüz, Shahidi, and Alexis (2002) believed that this action was totally autolytic in sea bass (*Dicentrarchus labrax*). Then, with the action of phosphatase and nucleoside phosphorylase, IMP degradation continues on a cascade reaction that produces hypoxanthine ribonucleoside (HxR) and hypoxanthine (Hx) (Howgate, 2006; Tsai, Cassens, Briskey, & Greaser, 1972). Surette, Gill, and Leblanc (1988) demonstrated that generation of Hx in Atlantic cod (*Gadus morhua*) during storage might be caused by bacterial enzymes rather than autolytic mechanisms. Hx is formed mainly as a result of the effect of nucleoside phosphorylase that is produced from spoilage bacteria (Hernandez-Cazares, Aristoy, & Toldra, 2011). Shiba, Shiraki, Furushita, and Maeda (2014) found that ATP was gradually transformed into HxR mainly due to the action of autolytic enzymes, and HxR was hydrolyzed into Hx mainly by autolytic as well as bacterial enzymes in tiger puffer fish (*Takifugu rubripes*) fillets. However, the contribution of autolytic enzymes and microorganisms in ATP catabolism of fresh water fish post-mortem has not been studied to any great extent (Hong et al., 2015; Howgate, 2005, 2006).

The amount of IMP is an important indicator of freshness of fish products and it is responsible for desirable odor and taste (Hong et al., 2015; Minami, Sato, Shiraiwa, & Iwamoto, 2011; Ocano-Higuera et al., 2011). As an irreversible process, the formation of IMP is controlled by an essential enzyme AMPD in ATP degradation (Minami et al., 2011; Ye et al., 2012). Marquez-Rios et al. (2008) isolated and purified AMPD (178 kDa) from mantle muscle of jumbo squid (*Dosidicus gigas*). The hydrolysis of IMP is controlled by two kinds of essential phosphatases such as acid phosphatases (ACP) and alkaline phosphatases (ALP). The activity of ACP in grass carp (*Ctenopharyngodon idellus*), channel catfish (*Ietaluruspunetaus*), and mud eel (*Siren lacertina*) is higher than that of ALP (Wang, Liu, Liu, Chen, & Xiong, 2010). Kuda, Matsumoto, and Yano (2002) reported that phosphatase activity at pH 10 and 11 increased with the aerobic plate count in horse mackerel (*Trachurus japonicus*) and gurnard (*Lepidotrigla microptera*) that were stored at 0 or 10 °C. However, the scientific information is limited on whether the AMPD and ACP are from the fish or from microorganisms that develop at different periods of storage (Hong et al., 2015; Howgate, 2005, 2006).

The objective of the present research work was to determine the relationship between degradation of ATP and microbial communities. In addition, the study also aimed to demonstrate the origin of AMPD and ACP in common carp fillets during different storage periods at 4 °C in terms of content of ATP, ADP, AMP, IMP, HxR and Hx, the K, Ki, H, P, Fr and G values, the activity of AMPD and ACP, and the total count of viable, *Aeromonas*, *Pseudomonas*, H_2S-producing bacteria, and lactic acid bacteria.

2. Materials and methods

2.1. Sampling preparation

Forty fresh samples of common carps, with an average weight and length of 1.41 ± 0.07 kg and 39.67 ± 2.52 cm, were obtained from aquatic product wholesale market in Beijing, China and transported to the laboratory alive in aerated foam boxes that contained water. Live carps were killed by a blow on the head, scaled, eviscerated, beheaded, washed and filleted into two pieces (size: 16.0 × 8.0 × 2 cm^3; mean weight: 277.67 ± 5.51 g). The fillets were washed immediately with cold sterile water three times. Then, the fillets were divided into two groups, one group was the control group (CK, n = 40), in which the fillets were dipped in sterile water for 10 min and another group was the sterilization group (SG, n = 40), in which the fillets were submerged in 5% methanol solution for 10 min. Ten fillets were dipped in three-liter of sterile water or 5% methanol solution. Then, the fillets of the two groups were washed with cold sterile water once and packaged in polyvinyl chloride bags. All pouches were stored in a refrigerator at 4 ± 1 °C. Three fillets from each group were taken randomly and the analyzed on 0, 6, 12, 24, and 36 h, and on 2, 3, 4, 6, 8, and 10 d.

2.2. Enumeration of microbial communities

The total number of different microbial communities was determined according to the plate colony-counting method of Wang et al. (2014). Five grams of common carp meat were weighed aseptically and homogenized (15 s) in 45 mL of sterile physiological saline (0.85%) solution with Interscience Bag Mixer (Basic Pnoramic, IUL, Barcelona, Spain) for. The homogenized samples were diluted serially (1:10) and 100 μL of each sample were spread on the surfaces of the culture medium. Total viable counts (TVC) were determined on plate count agar (PCA), and incubated at 30 ± 1 °C for 72 h. Lactic acid bacteria (LAB) and *Aeromonas sp.* were enumerated on MRS agar and *Aeromonas* Medium Base (AMB), respectively, and incubated at 30 ± 1 °C for 48 h. *Pseudomonas sp.* were enumerated on *Pseudomonas* CFC Selective Agar (CFC) at 20 ± 1 °C for 48 h. H_2S producing bacteria were enumerated on Iron Agar medium (IA) at 20 ± 1 °C for 4 days. All counts were expressed as log CFU/g. PCA, IA, and MRS agar were procured from HaiBo Biological Technology Co., Ltd. (Qingdao, China). AMB and CFC were obtained from OXOID Ltd. (Basingsioke, Hampshire, England).

2.3. Measurement of ATP related products and K, Ki, H, P, Fr and G values

Six kinds of ATP related products (ATP, ADP, AMP, IMP, HxR, and Hx) were pretreated by following a previously described method (Li et al., 2016). Nucleotides and related compounds were measured by a reverse-phase high-performance liquid chromatograph (HPLC) (Shimadzu, LC-10 ATseries, Japan) equipped with a SPD-10A (V) detector. A COSMOSIL 5C18-PAQ column (4.6 mm i.d. × 250 mm) (NacalaiTesque, Inc., Kyoto, Japan) was installed inside a thermostatic compartment for use at a temperature of 25 °C. The mobile phase was 0.05 mol/L phosphate buffer (pH of 6.8) and flow rate was 1.0 mL/min under isocratic elution conditions. The samples were filtered through a 0.22 μm membrane and analysis was carried out by injecting 50 μL of sample and detection was monitored at 254 nm. Reference standards were used to calculate the quantities of ATP, ADP, AMP, IMP, HxR, and Hx.

The K, Ki, H, P, Fr and G values were calculated by following the method of Song, Luo, You, Shen, and Hu (2012) as:

$$\text{K value } (\%) = [(\text{HxR} + \text{Hx})/(\text{ATP} + \text{ADP} + \text{AMP} + \text{IMP} + \text{HxR} + \text{Hx})] \times 100 \quad (1)$$

$$\text{Ki value } (\%) = [(\text{HxR} + \text{Hx})/(\text{IMP} + \text{HxR} + \text{Hx})] \times 100 \quad (2)$$

$$\text{H value } (\%) = [\text{Hx}/(\text{IMP} + \text{HxR} + \text{Hx})] \times 100 \quad (3)$$

$$\text{P value } (\%) = [(\text{HxR} + \text{Hx})/(\text{AMP} + \text{IMP} + \text{HxR} + \text{Hx})] \times 100 \quad (4)$$

$$\text{Fr value } (\%) = [\text{IMP}/(\text{IMP} + \text{HxR} + \text{Hx})] \times 100 \quad (5)$$

$$\text{G value } (\%) = [(\text{HxR} + \text{Hx})/(\text{AMP} + \text{IMP} + \text{HxR})] \times 100 \quad (6)$$