Quality assessment of rainbow trout (*Oncorhynchus mykiss*) fillets during super chilling and chilled storage

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Abstract In order to evaluate the effect of super chilling (-3°C) and chilled (3°C) storage on the quality of rainbow trout fillets, total volatile base nitrogen (TVB-N), drip loss, pH, electric conductivity (EC), total aerobic count (TAC), K and related values, adenosine triphosphate (ATP) and related compounds, color and sensory score were determined and correlation between these indicators were analyzed. According to the comprehensive evaluation of TAC, K value and sensory score, the limit for acceptability of rainbow trout fillets was 5 days at 3°C and 11 days at -3°C . Additionally, the correlation coefficients between TVB-N and other freshness indicators (TAC, K value, sensory score) were relatively low. TVB-N may be inadequate for evaluating freshness changes of rainbow trout fillets compared with other indicators. Among the K and related values, H value was a better freshness indicator in rainbow trout fillets during chilled and super chilling storage for its better correlation coefficients with other freshness indicators. Super chilling storage could extend the shelf life of rainbow trout fillets by 6 days compared to chilled storage.

Keywords Rainbow trout · Super chilling storage · Chilled storage · Freshness indicator

Introduction

Fresh fish products are more susceptible to quality deterioration than most other foods because of their high levels of moisture and nutrients. Therefore, better and more advanced methods are demanded to extend the shelf life of various aquatic food products. Currently, considerable research has been focused on improving fish quality and extending shelf life, such as high-pressure preservation (Erkan et al. 2010), radiation preservation (Oraei et al. 2012), coating preservation (Arashisar et al. 2009) and vacuum package (Jeya et al. 2005), etc. Storage temperature is one of the most important factors influencing shelf life of aquatic food products. Thus, the low-temperature preservation including super chilling storage and chilled storage is widely used in fish storage.

Super chilling (also called partial freezing) is often used to describe a process whereby a food product is stored at $1\text{--}2^{\circ}\text{C}$ below the initial freezing point of the product (Claussen 2011). Lu et al. (2013) found that super chilling storage could extend the shelf life of Songpu mirror carp (*Cyprinus carpio*) by 12 days compared with chilled storage. Ando et al. (2004) reported that super chilling storage was able to maintain the color of prawns and the physical properties of the meat, and consequently offered potential as a new storage technique suitable for replacing refrigeration. Claussen (2011) suggested that super chilling enable safe, high quality and long term storage of foods. Additionally, lower energy consumption, less thawing problems, lower degree of protein denaturation and less structural damage are observed in super chilling storage compared to frozen storage.

The quality of fish deteriorates due to microbial spoilage and biochemical reactions during the postmortem period. As a result of these events, sensory quality and nutritional value of fish get deteriorated. The various compounds formed during these biochemical changes can be used as an index of freshness. Among the chemical methods, the concentrations of

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adenosine triphosphate (ATP) and its breakdown products, adenosine 5-diphosphate (ADP), adenosine 5-monophosphate (AMP), inosine 5-monophosphate (IMP), hypoxanthine riboside (HxR), and hypoxanthine (Hx), respectively, can be used as indices of fish freshness. During the early stage of storage, ATP is degraded by endogenous enzymes in fish muscle, and a series of reactions results in the conversion of ATP through several intermediates to Hx. As the degradation continues, Hx is converted to xanthine, uric acid and ring cleavage products. In a large variety of fishes, nucleotide degradation follows a well-defined process: ATP→ADP→AMP→IMP→HxR→Hx→uric acid.

Significant variations both among and within species clearly limit the practical usefulness of measuring the level of a single nucleotide breakdown product to measure freshness. Measuring the concentration of nucleotide degradation compounds as combinations can be a useful way of determining freshness. Therefore, the K value, including intermediate breakdown products, is widely used as a freshness index. Since adenosine nucleotides disappear very quickly, Karube et al. (1984) proposed the Ki value, which excludes ATP, ADP and AMP, as an index of freshness quality. H values have been described by Luong et al. (1992) as an index of freshness quality. Additionally, the G and P values are reported to be most useful with lean fish. Gill et al. (1987) also proposed the Fr value for yellow fin tuna (*Thunnus albacares*).

Rainbow trout (*Onchorynchus mykiss*) which are native to California have been one of major fresh water fish in the world. There are several studies on the quality characteristics of rainbow trout during various storage conditions (Rezaei and Hosseini 2008; Oraei et al. 2012; Arashisar et al. 2004). However, few reports covered quality changes in rainbow trout fillets during super chilling and chilled storage, and there is limited information regarding the correlations between nucleotide ratios (K, Ki, H, G, P and Fr values) and other freshness indicators in rainbow trout fillets.

The objectives of this study were to evaluate the effect of super chilling and chilled storage on the quality changes of rainbow trout fillets, by employing freshness indicators, which included total volatile base nitrogen (TVB-N), drip loss, pH, electric conductivity (EC), total aerobic count (TAC), K and related values, adenosine triphosphate and related compounds, color and sensory score, and to investigate the most reliable freshness quality indicators among the K and related values, by analyzing correlation between these indicators.

Materials and methods

Materials and pretreatment

Fresh rainbow trout (weight $1,097 \pm 47$ g, length 40 ± 1 cm) were provided by Yanqing YuDuShan Coldwater Fishery

Base and immediately transported to the laboratory alive. They were killed by a blow in the head, scaled, gutted, filleted and rinsed with tap water. Three fillets were sampled immediately (day 0), while rest of the fish fillets were packed in polyvinyl chloride bags, divided into two lots and stored at 3 and -3 °C in refrigerators (BCD-268EM, Xinfei Co., Ltd., Henan, China) immediately. Three samples from both lots were taken randomly for analysis every 3 days.

Determination of TVB-N

TVB-N was determined according to the micro-titration method as described by Hu et al. (2013).

Determination of drip loss

Measurement of drip loss of rainbow trout fillets followed the procedures described by Hong et al. (2012). Each rainbow trout fillet was weighed before storage. After the storage, the fillets were taken from polyvinyl chloride bags and the drip in the package was poured off. Then the rainbow trout fillets were blotted with a paper towel and reweighed to determine drip loss. The drip loss (%) of the fillet was calculated by the following equation:

$$\text{Drip loss(\%)} = (\text{initial weight of fillet} - \text{final weight of fillet}) / (\text{initial weight of fillet}) \times 100.$$

Determination of pH

The pH value was determined according to the method of Song et al. (2011). A 10 g sample of fish flesh was dispersed in 100 mL of distilled water and stirred for 30 min, and then the mixture was filtered. The pH value of filtrate was measured using a digital pH meter (Mettler Toledo FE20/EL20, Shanghai, China).

Determination of EC

The EC was determined as described by Yao et al. (2011). A sample of 10 g fish flesh was homogenized with 100 mL distilled water and then stirred for 30 min. The mixture was filtered and the EC of filtrate was measured using a digital EC meter (Mettler Toledo FE20/EL20, Shanghai, China).

Microbiological analysis

Fish samples were analysed following the methodology recommended by Li et al. (2011) with some modifications. A sample of 5 g rainbow trout muscle was aseptically weighed and homogenized with 45 ml of sterile 0.9 % physiological saline for 1 min. The homogenized samples (1 mL) were serially diluted in 9 mL sterile 0.9 % saline. The incubation condition used for TAC was 30 °C for 72 h.

Sensory analysis

Fish samples (both fresh and cooked) were analyzed following the methodology recommended by Hong et al. (2012) with some modifications. The samples were evaluated by nine trained panelists from the laboratory staff. The sensory evaluation was based on a five point scale to determine. Scores of separate characteristics of fillets were summed to give an overall sensory score. The maximum score was 25 and shelf-life criteria assumed that rejection would occur when the sensory attributes declined below 12.5.

Determination of color

Changes in color of Rainbow Trout muscle were determined with a fully-automatic colorimeter (ADCI-60-C, Beijing Chentaik Instrument Technology Co., Ltd., Beijing, China) according to the method of Hong et al. (2012).

ATP, related compounds, K and related values

Determinations of nucleotides and related compounds were performed according to the method of Zhang et al. (2011). The amounts of ATP and its related compounds were determined

and calculated based on standard ATP, ADP, AMP, IMP, HxR and Hx. The K, Ki, H, P, Fr and G values were calculated as follows:

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

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

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

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

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

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

Statistical analysis

All the tests were done in triplicate except microbiological which was done in duplicate and the results were expressed as mean and standard deviation. The least significant difference procedure was used to test for difference between means (significance was defined at $P < 0.05$) using the Compare Means Procedure of SPSS 17.0 (SPSS Inc., Chicago, IL).

Result and discussion

TVB-N

TVB-N has been widely used as an indicator of deterioration of fish quality. Fraser and Sumar (1998) indicated that bacterial catabolism of amino acids in fish muscle results in the accumulation of ammonia and other volatile bases. Variations in TVB-N content of the rainbow trout fillets stored at 3 and -3 °C are shown in Fig. 1a. Initially, the TVB-N value was 11.39 mg/100 g. It was similar to the report of Song et al. (2011) who indicated that the initial value of bream (*Megalobrama amblycephala*) was 12.62 mg/100 g. The TVB-N value increased more sharply at 3 °C than -3 °C during the storage. Thus, the storage temperature plays an

Fig. 1 Changes in total volatile base nitrogen (a) and drip loss (b) of rainbow trout fillets at 3 °C and -3 °C

Fig. 2 Changes in pH (a) and electric conductivity (b) of rainbow trout fillets at 3 °C and −3 °C

important role in controlling TVB-N accumulation of fish. As shown in Fig. 1a, the TVB-N value of samples stored at 3 °C increased to 20.72 mg/100 g towards 9th day whereas it was 19.34 mg/100 g at 21th day of −3 °C, indicating that super chilling storage could effectively inhibit the increase of TVB-N.

Drip loss

As one of the key indicators to measure the water holding capacity of meat protein, drip loss reflects the degree of loss of fish juiciness during the storage. Drip loss results in reduced fish quality, and the drip liquid is also a good growth medium for bacteria (Duun and Rustad 2007). The changes in drip loss of rainbow trout fillets are shown in Fig. 1b. The drip loss of samples during storage at both temperatures increased with storage time. It increased to 2.30 % and 4.05 % on the 6th day at 3 and −3 °C, respectively, indicating that the drip loss at −3 °C was higher than that at 3 °C during the storage. During super chilling storage, some of the water freezes out and the concentration of solutes in unfrozen solutions increases. This may lead to denaturation of the muscle proteins and structural damage of membranes, which can result in

increased drip loss (Duun and Rustad 2008). The result was similar to the study of Simpson and Haard (1987) who found that the drip loss of Atlantic cod (*Gadus morhua*) at −3 °C was significantly higher than at 0 °C.

pH

The variations in pH of rainbow trout fillets during storage are presented in Fig. 2a. The initial pH was 6.51, similar to the result by Ocaño-Higuera et al. (2009) for cazon fish. Nevertheless, it was lower than the observations made by Zhang et al. (2012). Zhang revealed that the initial pH of grass carp (*Ctenopharyngodon idellus*) was 6.59. According to the findings of Ocaño-Higuera et al. (2009), variations in the initial values of pH may due to the type of muscle, species, season, diet and level of activity or stress during the catch. Change in pH of both temperatures showed the same trend, in which the values decreased initially and then increased. The initial reduction of pH may be attributed to the decomposition of glycogen, ATP, creatine phosphate (Zhang et al. 2012) and the dissolution of CO₂ in the fish muscle (Manju et al. 2007). The later increase of pH value is possibly due to the production of volatile basic components, such as ammonia (Goulas

Fig. 3 Changes in total aerobic count (a) and sensory score (b) of rainbow trout fillets at 3 °C and −3 °C

Table 1 Changes in color of rainbow trout fillets at 3 °C and −3 °C

Storage time (d)		0	3	6	9	12	15
L*	3 °C	62.77±1.39 ^{Ad}	53.90±1.72 ^{Aa}	48.53±1.04 ^{Aa}	54.12±0.95 ^{Aa}	54.24±0.12 ^{Aa}	55.43±0.57 ^{Ac}
	−3 °C	62.77±1.39 ^{Ac}	58.30±0.29 ^{Ba}	58.06±0.76 ^{Ba}	62.24±1.29 ^{Bc}	61.58±1.36 ^{Bbc}	60.13±0.35 ^{Bb}
a*	3 °C	7.48±0.02 ^{Aa}	8.37±0.65 ^{Aab}	11.78±0.65 ^{Ad}	10.95±0.91 ^{Ad}	9.60±0.55 ^{Ac}	8.75±0.67 ^{Abc}
	−3 °C	7.48±0.02 ^{Aa}	7.55±0.08 ^{Ba}	8.05±0.50 ^{Ba}	7.65±0.00 ^{Ba}	7.33±0.83 ^{Ba}	8.08±1.01 ^{Aa}
b*	3 °C	20.92±0.93 ^{Ad}	20.41±0.25 ^{Ad}	16.95±0.22 ^{Ab}	18.16±0.75 ^{Ac}	17.61±0.13 ^{Abc}	15.53±0.81 ^{Aa}
	−3 °C	20.92±0.93 ^{Ac}	18.86±1.69 ^{Bb}	17.16±1.42 ^{Aa}	17.45±1.71 ^{Aab}	16.79±0.67 ^{Ba}	17.24±0.81 ^{Bab}

All values are the mean±standard deviation ($n=3$)

^{A-B} Means±SD (standard deviation) within a column sharing a common letter were not significantly different ($P>0.05$)

^{a-d} Means±SD (standard deviation) within a row sharing a common letter were not significantly different ($P>0.05$)

and Kontominas 2005). There was no significant difference in sample pH stored at both temperatures during the initial 6 days. However, fillets at −3 °C showed a slower rate of increase in pH value than at 3 °C during the later storage. It can be explained that super chilling can decrease the rate of metabolism in microorganism, inhibit the activities of endogenous enzyme and delay the damage of fish quality.

EC

As an index of the concentration of electrolytes in the muscle tissues, EC can impact body fluid balance, survival and meat quality (Ekanem and Achinewhu 2006). EC was widely used in meat research to evaluate the quality of meat. Yao et al. (2011) used EC to measure the quality of crucian carp

Fig. 4 Changes in adenosine triphosphate (ATP) and related compounds concentration of rainbow trout fillets during storage at 3 °C (a) and −3 °C (b)

Fig. 5 Changes in K, Ki, H, P, Fr and G values of rainbow trout fillets during storage at 3 °C (a) and −3 °C (b)

(*Carassius carassius*) flesh. Banach and Żywica (2010) tried to use EC to examine the types of beef meat. As shown in Fig. 2b, the initial EC value of rainbow trout fillets was 1,344 $\mu\text{S}/\text{cm}$. The EC value increased gradually until 9th day and 15th day at 3 °C and at −3 °C respectively, and then increased sharply. The significantly increase of EC value was owing to that the muscle tissues get decomposed and fluids flow out. The EC value at 3 °C was significantly higher than at −3 °C during the later storage ($P<0.05$). Similar observations were reported by Lu et al. (2013).

TAC

Changes of TAC in rainbow trout fillets are shown in Fig. 3a. The initial counts of bacteria were 3.86 log cfu/g, indicated good quality of fish used in this study. Generally the initial microbial load of freshwater fishes is different because of water condition and temperature (Song et al. 2011). TAC of rainbow trout fillets stored at both temperatures increased with the storage time, and the increase was faster at 3 °C compared to −3 °C. Similar results were reported for bream (*Megalobrama amblycephala*) during partial freezing and chilled storage by Song et al. (2012). The TAC of rainbow trout fillets was 7.56, 7.46 log cfu/g on the 6th day at 3 °C and 12th day at −3 °C, respectively, which exceeded the spoilage level of 7 log cfu/g reported by IFST (1999). These results revealed that super chilling storage effectively inhibited the microbial growth by prolonging the lag phase.

Sensory assessment

Sensory scores showed a significant decline with increasing storage time at both temperatures (Fig. 3a). After 6 days

storage period at 3 °C the fillets were found unacceptable whereas fillets at −3 °C were still acceptable. The slow rate of decrease in the sensory score for fillets at −3 °C may be attributed to the low temperature inhibiting enzyme activity and microbial growth in the rainbow trout muscle which can be compared with the reports for Songpu mirror carp (Lu et al. 2013) and bream (Song et al. 2012) that the sensory parameters of super chilled group were superior compared to chilled group.

Color

Color and appearance of muscle foods are very important in terms of consumer perception of muscle foods quality, and are dominant factors in consumer purchasing decision. Table 1 shows the changes of rainbow trout fillets color at 3 and −3 °C. The initial values of L^* (whiteness), a^* (+a red, −a green) and b^* (+b yellow, −b blue) were 62.77, 7.48 and 20.92, respectively. No significant differences in b^* value were observed between the two storage temperatures. Compared to the initial color of fillets, chilled storage samples showed a decrease in L^* and a significant rise in a^* during the initial 6 days storage, while super chilling storage samples showed no significant changes in L^* and a^* during the storage. As a result, super chilling fillets showed higher stability of color compared with chilled storage samples. L^* and b^* values of both samples exhibited an initial fast reduction followed by a growth, probably due to loss of water in the early stages of storage. The mechanisms of those changes are not entirely clear. However, drip loss (Traore et al. 2012), and lipid oxidation (Hamre et al. 2003) may be responsible for the changes of color.

Table 2 Correlation between freshness indicators of rainbow trout fillets at 3 °C and −3 °C

Freshness indicators		drip loss	TVB-N	EC	TAC	sensory score	K value	Ki value	H value	P value	Fr value	G value
drip loss	r 3 °C	1	0.962**	0.926**	0.911*	−0.952**	0.897*	0.903*	0.984**	0.899*	−0.899*	0.992**
	−3 °C	1	0.930**	0.950**	0.913**	−0.927**	0.957**	0.955**	0.952**	0.951**	−0.951**	0.857**
TVB-N	r 3 °C		1	0.981**	0.783	−0.854*	0.743	0.751	0.904*	0.746	−0.746	0.970**
	−3 °C		1	0.965**	0.856**	−0.862**	0.882**	0.885**	0.970**	0.876**	−0.880**	0.965**
EC	r 3 °C			1	0.721	−0.787	0.687	0.693	0.860*	0.691	−0.689	0.928**
	−3 °C			1	0.936**	−0.944**	0.958**	0.962**	0.997**	0.956**	−0.960**	0.922**
TAC	r 3 °C				1	−0.989**	0.961**	0.972**	0.960**	0.968**	−0.967**	0.889*
	−3 °C				1	−0.995**	0.945**	0.956**	0.915**	0.949**	−0.954**	0.754*
sensory score	r 3 °C					1	−0.946**	−0.958**	−0.981**	−0.952**	0.951**	−0.941**
	−3 °C					1	−0.951**	−0.961**	−0.924**	−0.955**	0.959**	−0.773**

TVB-N, total volatile base nitrogen; EC, electric conductivity; TAC, total aerobic count; r, means Pearson correlation

** Correlation is significant at 0.01 levels (two-tail)

* Correlation is significant at 0.05 levels (two-tail)

ATP and related compounds

The patterns of ATP degraded products of rainbow trout fillets stored at 3 and -3°C are depicted in Fig. 4a and b respectively. The main changes are the loss of IMP and increase of Hx, whereas ATP and ADP remained approximately constant in very low concentration ($<0.3\text{ }\mu\text{mol/g}$) during the storage. AMP content of both treatments declined sharply during the initial 3 days and then remained relatively low concentration ($<0.4\text{ }\mu\text{mol/g}$). The degradation from ATP to IMP could be attributed to muscle endogenous autolytic enzymes (Gram and Huss 1996) and the conversion of ATP to IMP is usually complete within 1 or 2 d. The level of IMP in rainbow trout fillets decreased to $2.16\text{ }\mu\text{mol/g}$ and $3.18\text{ }\mu\text{mol/g}$ at 3°C and -3°C , respectively on the sixth day, indicating that lower temperature inhibited the degradation of IMP. IMP was regarded as a contributor to desired flavor and disappearance of IMP has been correlated with the loss of flavor and freshness in some fish species (Mazorra-Manzano et al. 2000). As nucleotide degradation progressed, IMP was slowly degraded to HxR and Hx, which resulted in the progressive loss of the desirable fresh fish flavor (Itoh and Kimura 2002). The concentration of HxR in rainbow trout fillets stored at 3°C reached its peak level on 6th day and then decreased to $0.51\text{ }\mu\text{mol/g}$ on 15th day. The HxR content at -3°C showed a similar trend but increased more slowly reaching its peak level of $4.05\text{ }\mu\text{mol/g}$ on 12th day. The difference indicated that accumulation of HxR was delayed at -3°C compared with that at 3°C . The initial concentration of Hx was approximately $0.04\text{ }\mu\text{mol/g}$ and with storage it increased linearly, more pronounced at chilled storage. Hence the study proved that super chilling prevented the accumulation of HxR and Hx more effectively than chilled storage.

K and related values

Variations in K, Ki, H, P, Fr and G values of rainbow trout fillets during storage at 3°C and -3°C are shown in Fig. 5a and b respectively. The K and related values increased (Fr decreased) in both treatments during the storage, which is mainly attributed to the sharp decline of IMP and increase of Hx in the fish flesh. The rates of increase in K, Ki and P values were similar, due to the low concentration of ATP, ADP and AMP during the storage periods, and the large accumulation of HxR and Hx during the late period. The H value of rainbow trout fillets increased slowly compared with the K, Ki and P values. The slow increase in H value may be due to the relative high level of HxR during the storage. The G value rose gradually until days 6 and 18 at 3°C and -3°C respectively, after which it increased sharply due to the increase in Hx content in both treatments. The Fr value decreased with increasing storage period under both conditions, due to the sharp drop in IMP concentration in the fish flesh.

The correlation between freshness indicators

The correlation coefficients between indicators are showed in Table 2. Results indicated that sensory score highly correlated with TAC, drip loss, K and related values. Additionally, the correlation coefficients between TVB-N and other freshness indicators (TAC, K value, sensory score) were relatively low. TVB-N may be inadequate for indicating freshness of rainbow trout fillets compared with other indicators. Furthermore, significant correlations ($P<0.05$) were observed between H value and other freshness indicators (drip loss, TVB-N, EC, TAC and sensory score). The correlation coefficients between H value and other freshness indicators (drip loss, TVB-N, EC, TAC and sensory score) were relatively higher than K, Ki, P, G and Fr values, indicating that H value was a better freshness indicator for rainbow trout fillets during chilled and super chilling storage. This result was similar to the study of Alasalvar et al. (2002), who found that H value was a better indicator for wild sea bass (*Dicentrarchus labrax*).

Conclusion

Compared to chilled storage, super chilled samples showed higher sensory score and lower EC, TAC and TVB-N, but it could induce higher drip loss. Based on the TAC, K value and sensory assessment, the limit for acceptability of rainbow trout fillets was 5 days at 3°C (chilled storage) and 11 days at -3°C (super chilling storage). Hence super chilling storage could extend the shelf life of rainbow trout fillets by 6 days compared to chilled storage. Additionally, TVB-N is not a good indicator for evaluating freshness changes in rainbow trout fillets. Among the K and related values, H value was a better freshness indicator for rainbow trout fillets during chilled and super chilling storage.

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