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Master Thesis 2015
30 credits

Cathepsin Activity and Texture in Atlantic salmon Muscle

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Ås，May 2015

Acknowledgements

I sincerely great appreciate to all those people who contributed to or assisted for me in the process of writing this thesis.

Firstly, I am most indebted to my supervisor professor Magny S Thomassen, for her invaluable suggestion in the selection of the topic and in the preparation of this thesis. Her remarkable insights and valuable suggestions have contributed greatly to the completion. Her serious attitude toward academic research, her profound knowledge and rigorous scholarship have impressed me deeply, and will continue to exert influence on my future study and work.

Secondly, I would like to thank Vibeke Host for her invaluable help and support during the lab work.

Thirdly, thanks to Nofima AS Company and its research institution for providing the lab and salmon fish resources.

I would also like to express my sincere thanks to my parents and friends for their love, care, persistent support, understanding and encouragement. It is my pleasure to dedicate this thesis to them.

Ås, May, 2015

Abstract

Cathepsins, a family of lysosomal proteases, are believed to play a role in muscle tenderization. In the present study the activity of cathapsin B+L in Atlantic salmon muscle and a possible influence on the textural quality was studied. Total of 98 Atlantic salmon from 10 families were slaughtered and pre-rigor filleted. This salmon fillet texture was measured instrumentally at 5 days post-mortem. The cathapsin activities were measured on muscle samples frozen immediately after slaughter. Statistically differences between families were observed for the break force (BF)of Atlantic salmon muscle texture but not for the cathepsin B+L activity. This paper was aiming to explore the relationship of Cathepsin B+L activity and break force but no significant correlation was found.

Key words:

Atlantic salmon, cathepsin activity, muscle texture, break force

Contents

1. Introduction

1.1 The distribution of Atlantic salmon

The Atlantic salmon (*Salmosalar* L.) is one of the most important aquaculture species among salmonids. The Atlantic salmon is an economically important anadromous fish distributed from southern Europe to arctic waters and North America (World Wildlife Fund, 2001; Hindar et al., 2007).

The Atlantic salmon has two types which contains landlocked salmon and migratory salmon. The landlocked salmon is small and grows slower, which lives in freshwater lakes in the Atlantic, however, the life history of migratory salmon and Pacific salmon are similar, which all grow in seawater and breed in freshwater. The Atlantic salmon is distributed in the northern Atlantic and Ungava Bay, the freshwater from Ontario lake to the southern areas of the white sea and into Russia. For centuries, the Atlantic migratory route has been form the British Isles and the Scandinavian Peninsula to the north by northeast, and has provided rich food for the people there.

1.2 Atlantic salmon farming

Since the 1970s, the Atlantic salmon farming has developed rapidly and has become a most promising industry, and the scale of farming is developing rapidly. Norway is the largest producer in the world, the output of 1.18million tons in 2013(reference website 1). There were 470 Atlantic salmon hatchery existed in the world, more than 1000 farms. The growth of demand, prices, aquaculture production will continue to increase, while the Pacific salmon are farmed in Japan, Chile, the United States and Canada, but according to the statistics in 1993, the Atlantic salmon still accounts for the world total output of more than 90%.

1.3Atlantic salmon muscle quality

The Atlantic salmon muscle contains a variety of important physiological functions of minerals and trace elements such as Ca, Fe, Mn, Zn, Mg and Cu, and which are rich in the muscle and had appropriate proportion, which can be used to supplement the human body mineral nutrition dietary sources, has great edible and economic value.

Firm texture of Atlantic salmon fillets is considered as one of the most important eating quality characteristics together with colour, fat content and fatty acid composition (Rasmussen, 2001). Therefore, the softening of the salmon fillet postmortem, which is partly caused by

proteolysis(Bahuaud et al, 2010a), has an obvious effect to consumers acceptance. Further, proteolytic activity is affected by the proteases in the muscle. Lysosomal enzymes like the cathepsins and cytosolic enzymes like calpain are the most types of proteinases.

1.4 Cathepsin in muscle

The proteolytic enzymes cathepsins are thought to be involved in the hydrolysis of myofibrillar proteins in both mammalian and fish muscle (Bahuaud et al., 2008; Godiksen, Morzel, Hyldig, &Jessen, 2009). There are many lysosomal cathepsins, at present, 10 cathepsins have been purified and identified from fish and shellfish muscle and visceral, namely, cathepsin A, B, C, D, E, H, L, L-like (Jiang et al.，1994；Lee et al., 1996; Barrett et al., 1981), X (Jiang et al., 1994) and S (Pangkeyet al., 2000).But for purification and identification and the exploration of the lysosomal cathepsins, most studies focus on cathepsin B, L, Hand L-like (An H. et al., 1994Yanmashita et; al., 1990a; 1990b; 1990c;). This is because the properties of the cathepsin B, L,H, L-like and many surimi product quality are closely related. For example, cathepsin B is the most active in Pacific whiting (Merluccius productus), cathepsin L had the strongest activity in washed surimi (An H. et al., 1994). In addition, when comparing the four Pacific species distribution of cathepsin activity, it

was found that cathepsin B and L activity were high in Pacific whiting (Merluccius productus) and arrowtooth flounder (Atheresthes stomias), cathepsin H in Alaska Pollock (Theragra chalcogramma). And in the event of gel softening of Japanese flounder (Paralichthys olivaceus) large cathepsin L-like activity was also found in fish surimi (Toyohara,1993).

According to the research about the functions and characteristics of enzymatic hydrolysis of fish muscle myosin and cytoskeletal protein (Jiang et al.1996; Jiang et, 1997; al., 1994; Sherekar et al., 1988), cathepsin B, L, H, and L-like is the most critical for surimi protein degradation.

Cathepsin B, L, H and L-like have the endopeptidase activity, can act on a variety of muscle fiber proteins and other protein substrates (Jiang ST; Lee JJ; Chen HC. 1996). Cathepsin L is considered to be the most affective in the salmon fish muscle softening. Recently there were observed significant negative correlation between fillet firmness and the cathepsin L activity found in Atlantic salmon muscle (Bahuaud et al, 2010a).

And also in surimi made of Pacific whiting (Merluccius productus), cathapsin L play a very important role in protein degradation. In carp (Cyprinuscarpio) purified cathepsin L from muscle were also found, in the pH 5.0-7.0 range, not only to degrade carp myofibrillar components, such as myosin heavy chain (MHC), actin, troponin T and I, but can

continue to complete degradation of intermediate products (Ogata et al, 1998). Cathepsin L-like was found to be involved in the hydrolysis of surimi and surimi products and influenced the quality (Masaki et al.1993; Toyohara et, al., 1993).

The pH optimum of cathepsin B, L, H or L-like hydrolysis of actomyosin has been found to be 6.5 (Jiang et al., 1992) or 5.5-6.0 (Aranishi et al., 1997), 7(Aranishi et al., 1997)and 5.5 (Lee et al., 1993).Although the fin fish (Scomber japonicas) had the optimal pH for cathepsin B hydrolysis of actomyosin pH6.5-7.5, the enzyme at pH7.5 still had more than 40% of the activity (Jiang et al., 1994). One research also presented cathepsin B and cathepsin L reached the highest enzyme activity in postmortem Atlantic salmon muscle with pH 6.1 after 144 hours ice stored (Mari Øvrum Gaarder, 2011).

1.5 Breeding for salmon quality

In 1971, a program for breeding of Atlantic salmon was started in Norway. At this time growth rate, age of sexual maturation, disease resistance, and meat quality such as fat percentage, fat distribution, and flesh color was important (Gjedrem, 2000; Johnston et al., 2006). Not until lately, the importance of muscle texture and the softening after slaughter has been included in the breeding programs. Some studies on different strains of

Atlantic salmon in Norway and other farming countries have, however, now showed significant differences in fillet texture between strains (review by Mørkøre, 2008).

The importance of cathapsins in this connection was first studied by Bahuaudet al (2010). B and L activities were measured in salmon from families that had low, medium and high muscle texture after slaughter. They found a significant correlation between cathepsin L activity and texture. Due to this, more information about this is of interest to the breeding companies.

2. Material and methods

2.1 Sampling of Atlantic salmon

There were 98 Atlantic salmon samples in this experiment, which were taken from Averøy Norway, October 2012(Figure 2.1). The fish were from the family material of the breeding company Aquagen, about 10 fish from 10 different families. The fish were slaughtered at Averøy and filleted and the fillets stored on ice.

Fish weight and length were measured immediately.

Figure 2.1：Atlantic Salmon taken from Averøy Norway.

2.2 Texture measurements

Fillets texture, break force, and max force were measured instrumentally

at 5 days post-mortem using the TA-XT2 instrument (Figure 2.2).A flat-ended cylindrical probe was used, and this was pressed into the fillet at a constant speed (1 mm/s).

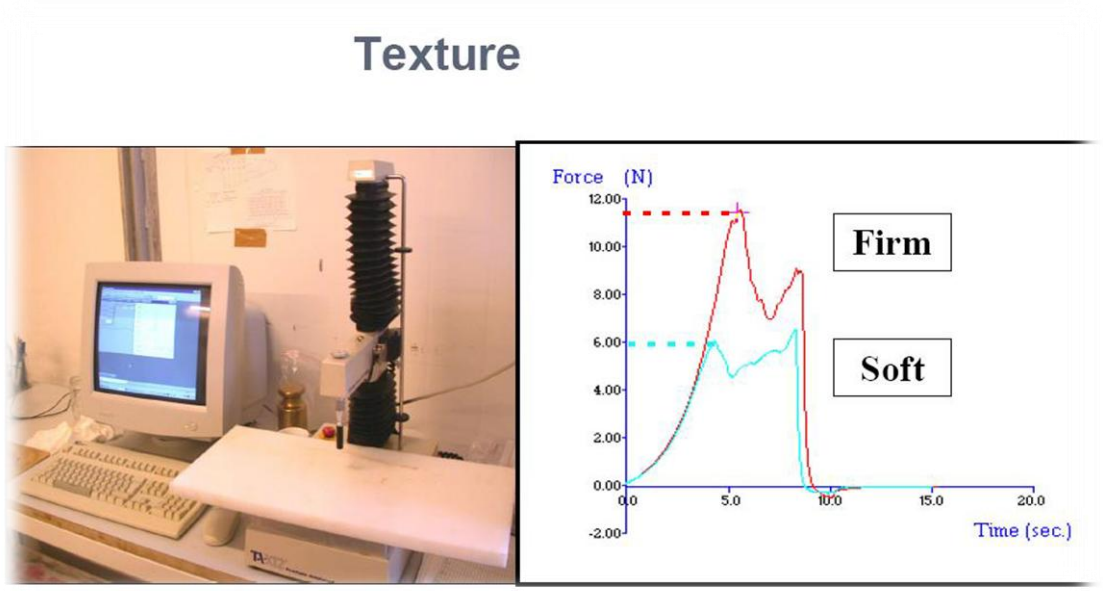

Figure2.2: A picture of the texture measuring instrument TA-TX2, and the graph showing the top point (BF = Breaking force) used as a measure of muscle hardness.

2.3 Cathapsin measurements

2.3.1 ***Extraction***

The most commonly used extraction method for determining cathepsin activities in muscle is to homogenize muscle samples in a sodium acetate buffer containing a detergent Triton X-100, followed by centrifugation of the homogenate (Figure2.3). The resulting supernatant was retained and assayed for cathepsin activity.

Figure2.3：Centrifugal machine.

2.3.2 Homogenization of muscle samples

A relatively small number of samples (12) were taken from the freezer each time, and kept on ice. Then turned on the table centrifuge and set the temperature to 4℃. Cut small pieces of each muscle sample, and weight very carefully in 300 mg (297-303) to the special homogenization tubes. Noted the weight for each sample. Added 1000 microliter of cold extraction buffer, while working on ice. Homogenized at 5000 for 20 seconds two times (Figure2.4). Centrifuged the samples using the table centrifuge at 13000 rpm for 30 min at 4℃. The supernatant was pipetted into two tubes with 350ml for each. Froze them on liquid nitrogen before storing them at -80C.

Figure2.4：Homogenization machine.

2.3.3 Extraction buffer

Weighted in 4.1 gram of Sodium-acetate, added 1 ml of Triton -100, dilution by 500 ml water, and corrected pH to 5.5.This buffer is very stable, and can be stored in fridge with 4℃.

2.3.4 Brij solution

0.1% Brij 35

Weight 0.1g Brij, dilution by 100ml water. Kept it at Fridge with 4℃.

2.3.5 Incubation buffer

Weighted 2.05g Sodium-acetate (82.03g/mol) and 0.093g Sodium-EDTA(372.24g/mol), dilution by 80ml water. Adjusted pH to 5.5. Filled up with water to 100ml. Kept in fridge with 4℃.

Added 100ul DTT to 20ml incubation buffer for daily use.

2.3.6 1M DTT

Weight 1.54g DTT, dissolved in 10ml water. Pipetted all of it and divided into 500ul tubes. Kept tubes in freezer with -20 ℃.

2.3.7 Substrate B+L

Pipetted 25ul of B+L solution from 4℃, dilution with 20ml water.

2.3.8 Stop Buffer

Weighted 9.4g mono-chloracetic acid(94.5g/mol) and 8.203g Sodium Acetate(82.03g/mol). Added 900ml water and adjusted pH to 4.3. At last filled up to 1000ml with water.

2.3.9 Standard Solution

a) 5000nm standard solution :

   Pipetted 5ul of stock solution in 4℃ and added 10ml purity water.

b) 200nm Standard solution :

   Pipetted out 400ul of 5000nm standard solution and added 9.6 ml stop solution.

c) 100nm Standard solution:

   Pipetted 750ul of 200nm Standard solution and vibrating mixing with 750ul of stop solution.

d) 50nm Standard solution:

   Pipetted 750ul of 100nm Standard solution and vibrating mixing with 750ul of stop solution.

e) 0nm Standard solution:

   Just made by piping of stop solution

2.3.10 Fluorimetrically Measurement

The activity of cathepsin B+L was measured fluorimetrically according to the method of Kirschke, Wood, Roisen and Bird(1983). Fluorescence measurement at excitation 355 nm and emission wavelength360 nm determined the release of the fluorogenic reagent

7-amido-4-methylcoumarin(Figure2.5). Substrates was used byN–CBZ–L–phenylalanyl–L–arginine–7–amido–methylcoum–arin(Z-Phe-Arg-Nmec) for cathepsin B+L activity(Bahuaud et al.,2009; Cheret, Delbarre-Ladrat, De Lamballerie-Anton, and Verrez-Bagnis, 2007; Dell Ceuninck et al., 1995;Zhao et al 2005). In all case, the assays were run in triplicates.

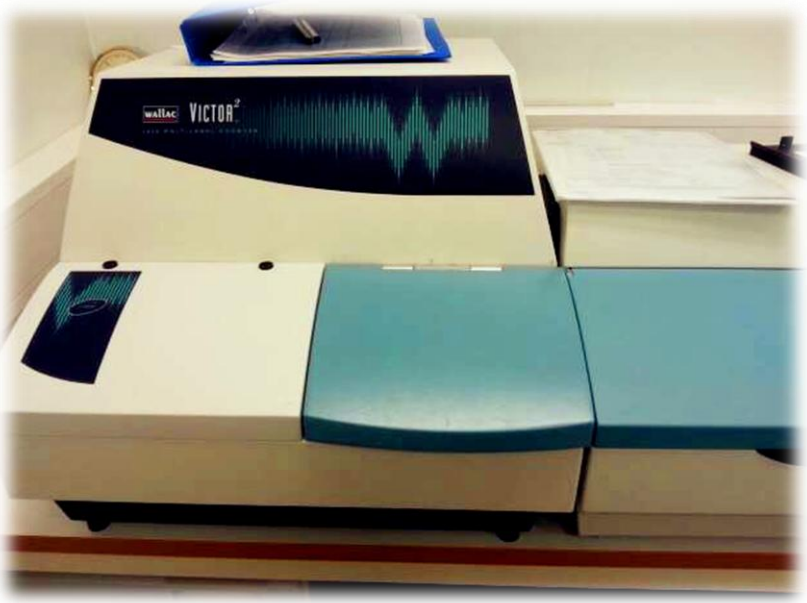

Figure 2. 5：Fluorescence measurement machine.

2.3.11 Assay

a) Turned on the water bath in 40℃. Get samples from -80℃ to 4℃. Marked glass tubes
b) Pipetted10 ul sample into tube with 200 ul incubation buffer.
c) Pipetted 40 ul Brij 35 into “a”.
d) Incubated 5 minutes in 40 ℃.

e) Pipetted200 ul substrate solution B+L into “c”.

f) Incubated 10 minutes in 40 ℃.

g) Pipetted 2.0ml stop solution into “e” and kept samples in the dark.

h) Pipetted 200 ul of “f” from each tube into the black fluorimetrically measurement plate.

i) Run the machine and measured absorbance.

2.4 Statistical analysis

Gutted weight, length, texture (break force) and enzyme activity were analyzed by one-way analysis of variance (ANOVA) using the GLM procedure of SAS computer software (SAS, 1990), with family strain as the only independent class variable. If the model was significant on a $p < 0.05$ level, the means were ranked with Duncan’s Multiple Range test. The R^2 expresses the proportion of the variance explained by the statistical model and equals the between-group sum-of-squares divided by the total sum-of-squared (Type III statistics).

For correlation analysis the *“pairs”* statement in the statistical program R was used.

3. Results

3.1 Fish material

3.1.1 Fish material overview

Fish weigh and length from 10 different salmon families were measured immediately after collected on land, and condition factor (KF) was calculated later, as presented in table 3.1.

Family NO.	Mean Weight(g)	Mean length(cm)	Mean K-Factor
55	1032±396.5	45±5.0	1.12±0.21
77	1595±349.5	52±4.0	1.14±0.05
78	1913±528.0	53±5.5	1.26±0.14
109	1469±320.0	51±3.0	1.12±0.08
112	1745±304.5	51±2.5	1.28±0.11
335	1769±503.0	52±5.5	1.20±0.07
370	1971±450.5	55±4.5	1.18±0.05
374	1583±657.5	51±6.5	1.10±0.10
403	2130±407.0	56±3.5	1.24±0.15
433	2019±526.5	54±5.0	1.24±0.12

±Number： ± standard deviation

Table 3.1: Fish weight, length and condition factor from 10 salmon families.

Here present the mean weight from 10 different fish families in figure 3.1.

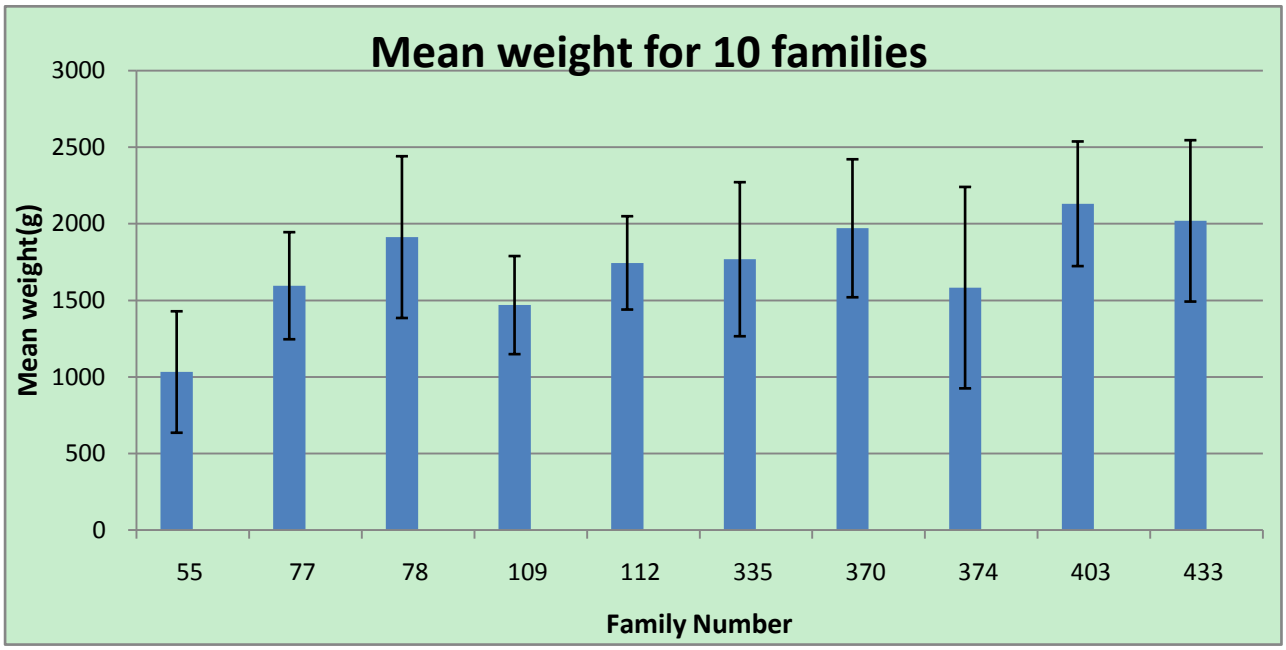

Figure 3.1 Mean weight of 10 families.

Figure 3.1 shows the significant lowest salmon mean weight in family 55with 1032g. The other mean weight of 9 salmon families were between 1469 g to 2130g.

The statistic analysis of mean weight from 10 different fish families was in figure 3.2 as below.

Means with the same letter are not significantly different.

Duncan Grouping			Mean	N	FAMILY
	A		2130.4	14	403
	A				
B	A		2018.7	9	433
B	A				
B	A		1970.5	12	370
B	A				
B	A	C	1912.8	14	78
B	A	C			
B	A	C	1768.5	12	335
B	A	C			
B	A	C	1744.6	9	112
B		C			
B		C	1595.4	9	77
B		C			
B		C	1582.9	7	374
		C			
		C	1468.9	10	109
	D		1032.1	7	55

Figure 3.2: Statistic analysis of the mean weight from 10 different fish families.

Based on the results ($P< 0.0001$, $R^2 = 30$) from SAS analysis, Figure 3.2 illustrated the statistic significantly difference between the mean weight from 10 different fish families.

3.1.2 Fish length

The mean length from 10 different fish families was showed below in figure 3.3.

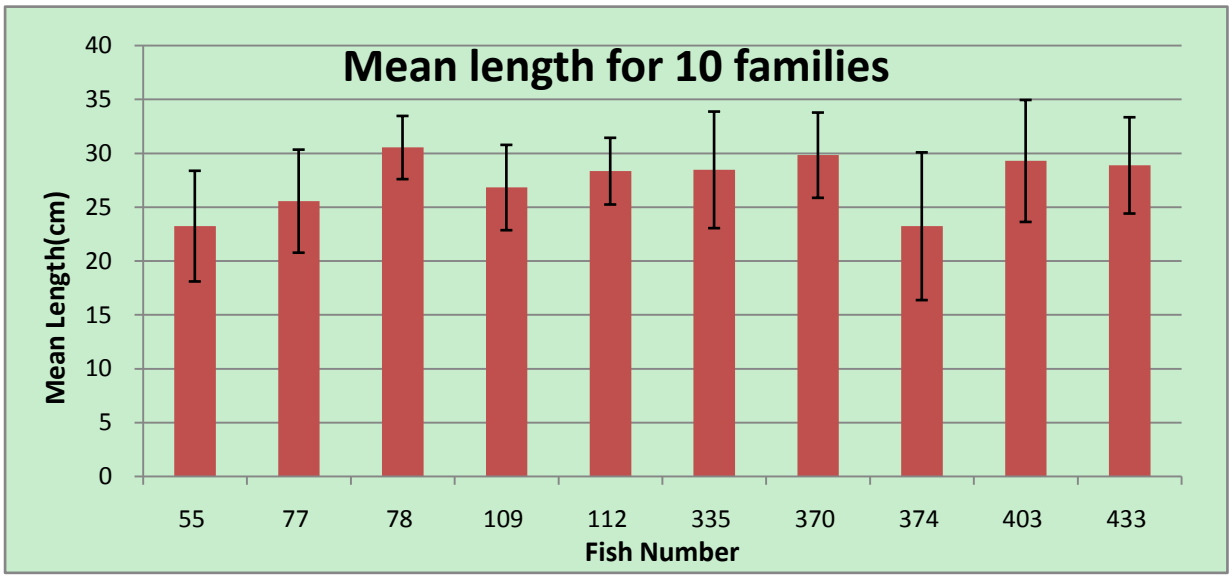

Figure 3.3 Mean length of 10 families.

Figure 3.3 present the smooth fluctuations of mean length from 10 salmon families between 45cm to 56 cm.

Here showed the statistic analysis of mean length from 10 different fish families in figure 3.4.

Means with the same letter are not significantly different.

Duncan Grouping			Mean	N	FAMILY
	A		30.542	14	78
	A				
B	A		29.832	12	370
B	A				
B	A		29.298	14	403
B	A				
B	A		28.886	9	433
B	A				
B	A		28.471	12	335
B	A				
B	A		28.349	9	112
B	A				
B	A	C	26.829	10	109
B		C			
B		C	25.565	9	77
		C			
		C	23.246	7	55
		C			
		C	23.236	7	374

Figure 3.4：Statistic analysis of mean length from 10 different fish families.

According to the results ($P = 0.0059$, $R^2 = 21$) from SAS analysis, Figure 3.4 presented the statistic significantly difference between the mean length from 10 different fish families.

3.1.3 K-Factor

K-factor is the calculation to get an indication of fish shape for trout and salmon (referenced website 2). K-factor calculated using the formula:

K-Factor = weight (gram) $\times$ 100/ length^3 (cm^3)

The value of K-Factor as below:

K-Factor<0.90 means thin fish

K-Factor= 0,95 means medium fish

K-Factor=1.0 means good quality

K-Factor=1.1 means very good quality

K-Factor> =1.2 means very oily fish

Here presented the K-Factor for 10 salmon families as showed in figure 3.5.

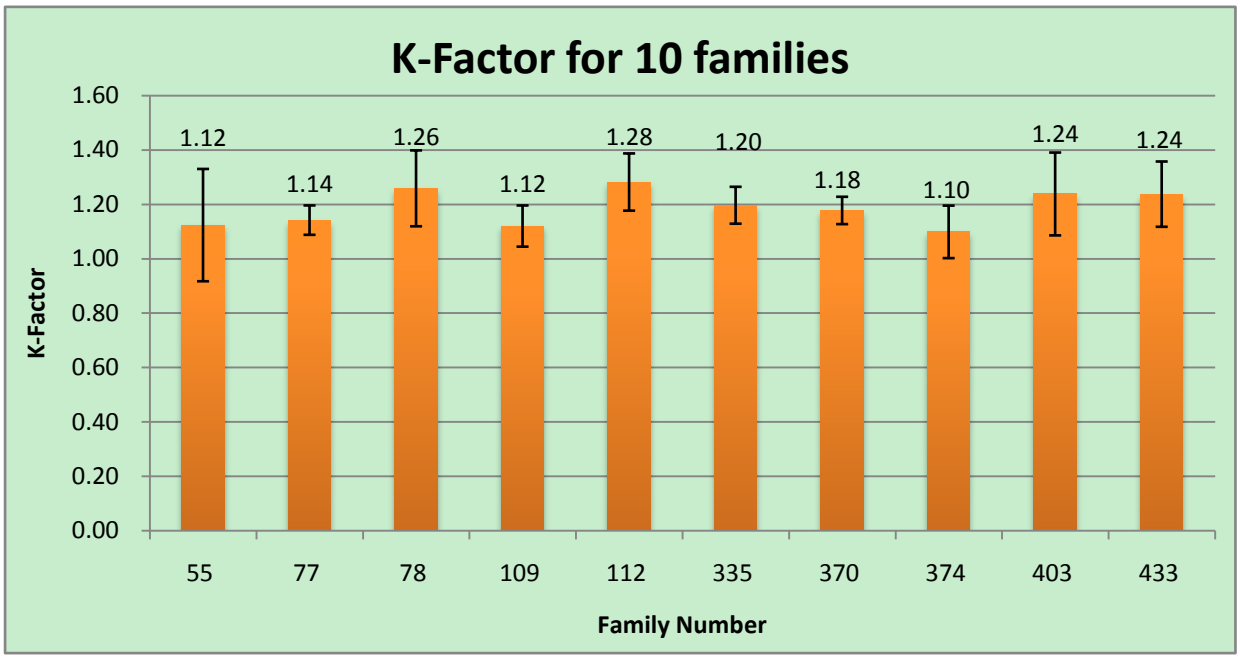

Figure 3.5：K-Factor for 10 salmon families.

The range of K-Factor form 10 salmon families were between 1.10 to 1.28，which means those 10 families of salmon fish were very good quality and fat, contained much fish oil.

3.2 Break force comparison for 98 fish and 10 families.

Break force of muscle from 98 fish was measured instrumentally at 5 days post-mortem and illustrates as below.

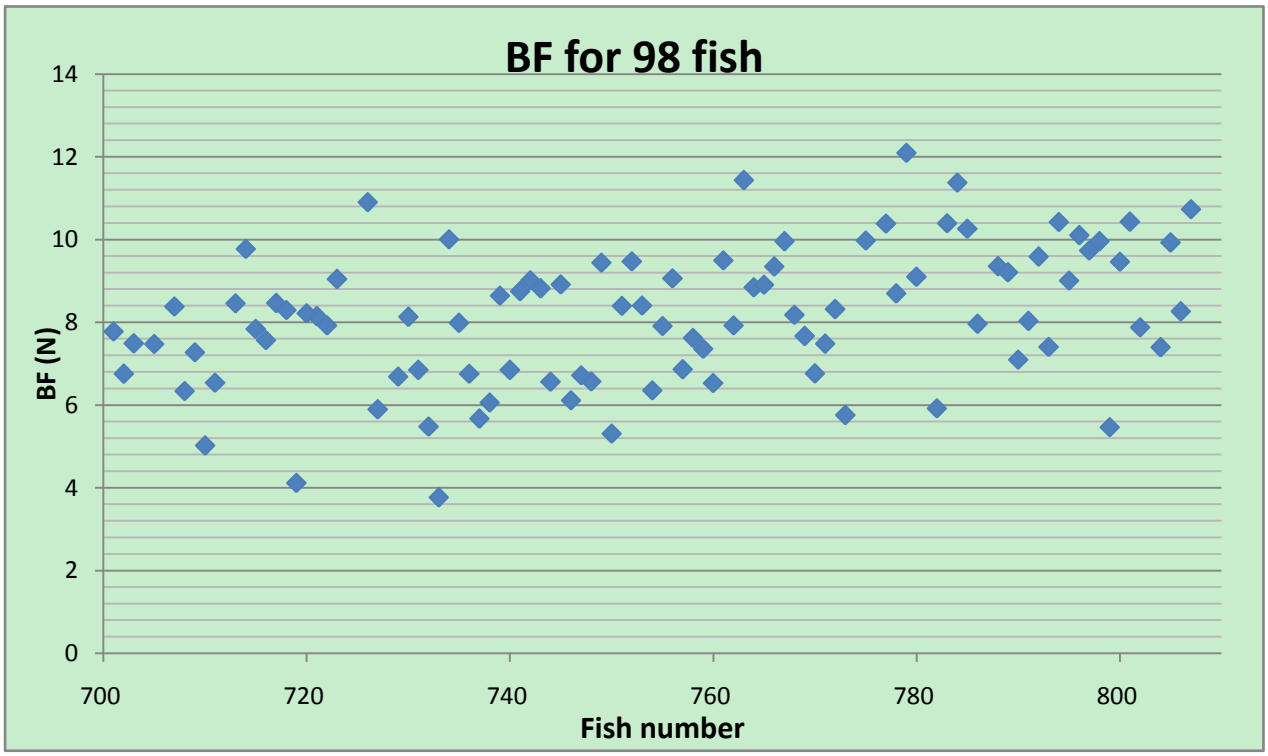

Figure 3.6 BF for 98 fish.

Only few fish had break force lower than 6. This means that most fish had good texture.

Break force from 10 salmon families shows the salmon muscle texture tenderness in figure 3.7.

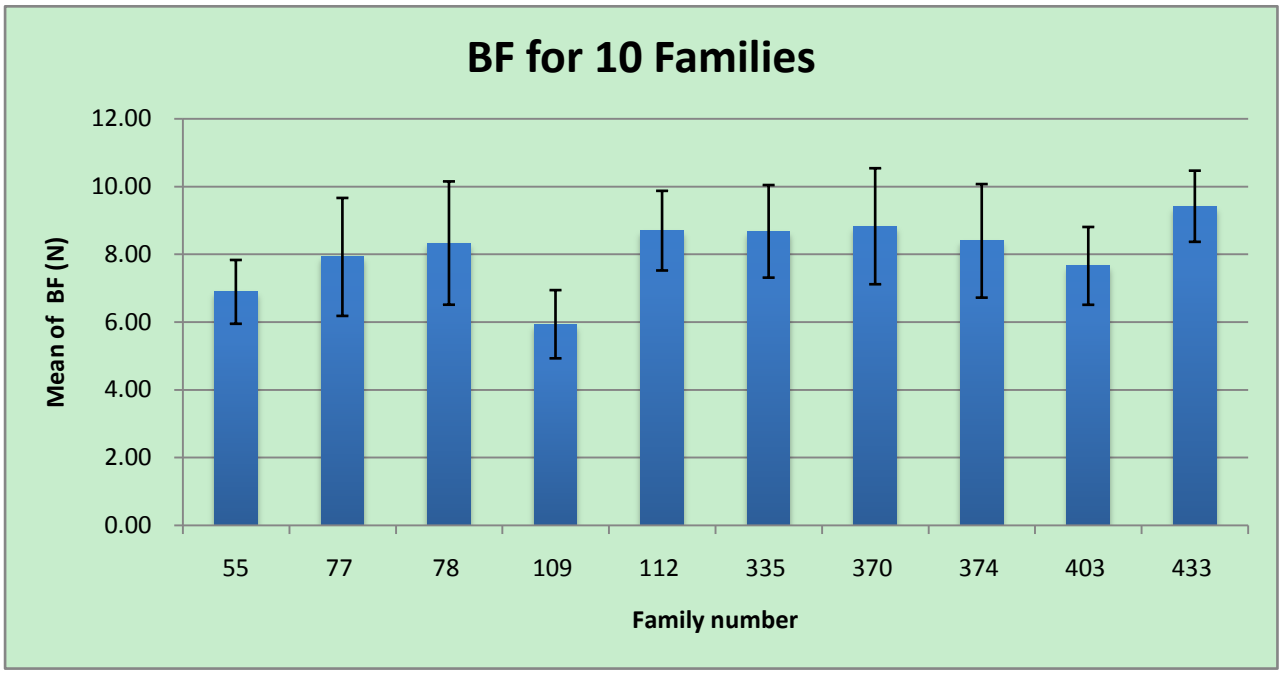

Figure 3.7 BF for 10 Families.

Figure 3.7 presents the differences of break force between 10 salmon families .The highest mean value of break force is found in family 433 with 9.41N,while the significant lowest is in family 109 with mean 5.93N.

The mean values of break force from another 8 salmon families are between 6.89 N to 8.82 N.

Here presented the statistic analysis of break force from 10 salmon families in figure 3.8.

Means with the same letter are not significantly different.

Duncan Grouping			Mean	N	FAMILY
	A		9.4144	9	433
	A				
B	A		8.8255	11	370
B	A				
B	A		8.6933	9	112
B	A				
B	A		8.6758	12	335
B	A				
B	A		8.3929	7	374
B	A				
B	A	C	8.3293	14	78
B	A	C			
B	A	C	7.9200	9	77
B		C			
B		C	7.6567	12	403
		C			
	D	C	6.8880	5	55
	D				
	D		5.9356	9	109

Figure 3.8: Statistic analysis of break force from 10 salmon families.

Based on the results (P = 0.0059, R^2 = 21) from SAS analysis, figure 3.8presented the break force of 10 salmon families were statistic significantly difference.

3.3 Cathepsin B+L activity comparison for 98fish and 10 families

The activity of Cathepsin B+L from 98 fish was measured fluorimetrically at excitation 355 nm and emission wavelength360 nm, as presented below.

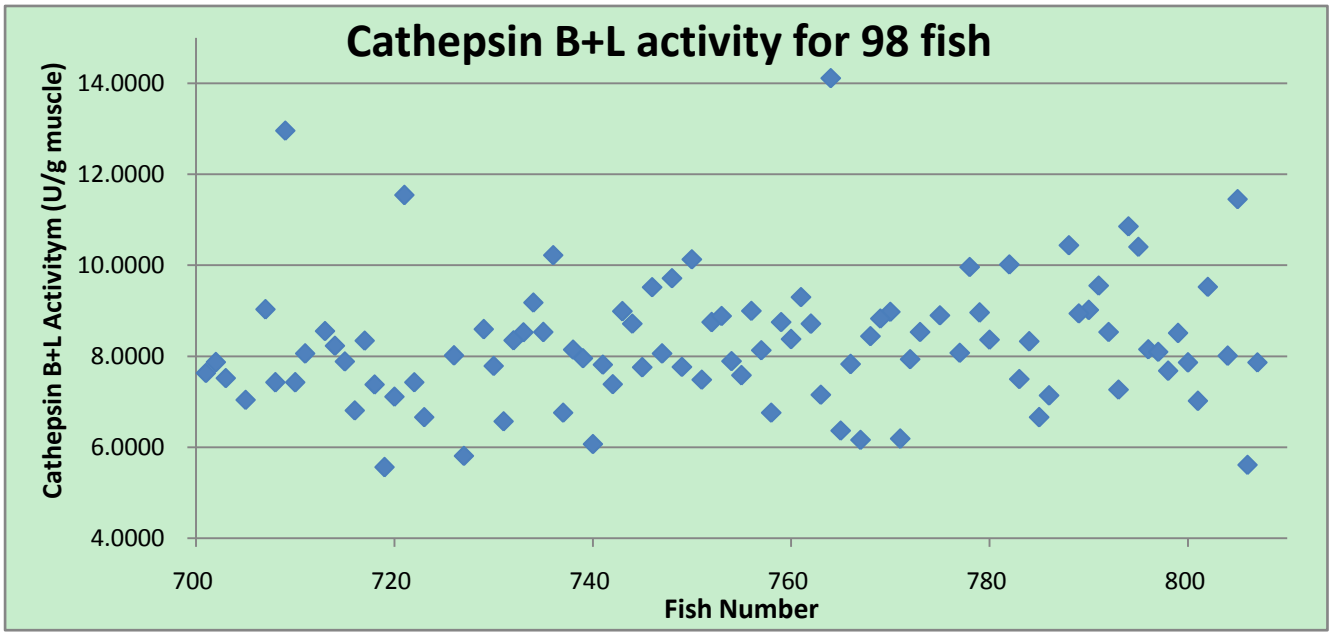

Figure 3.9 Cathepsin B+L activity for 98 fish.

Here shows the mean value of cathepsin B+L activity comparison for 10 salmon families in figure 3.10.

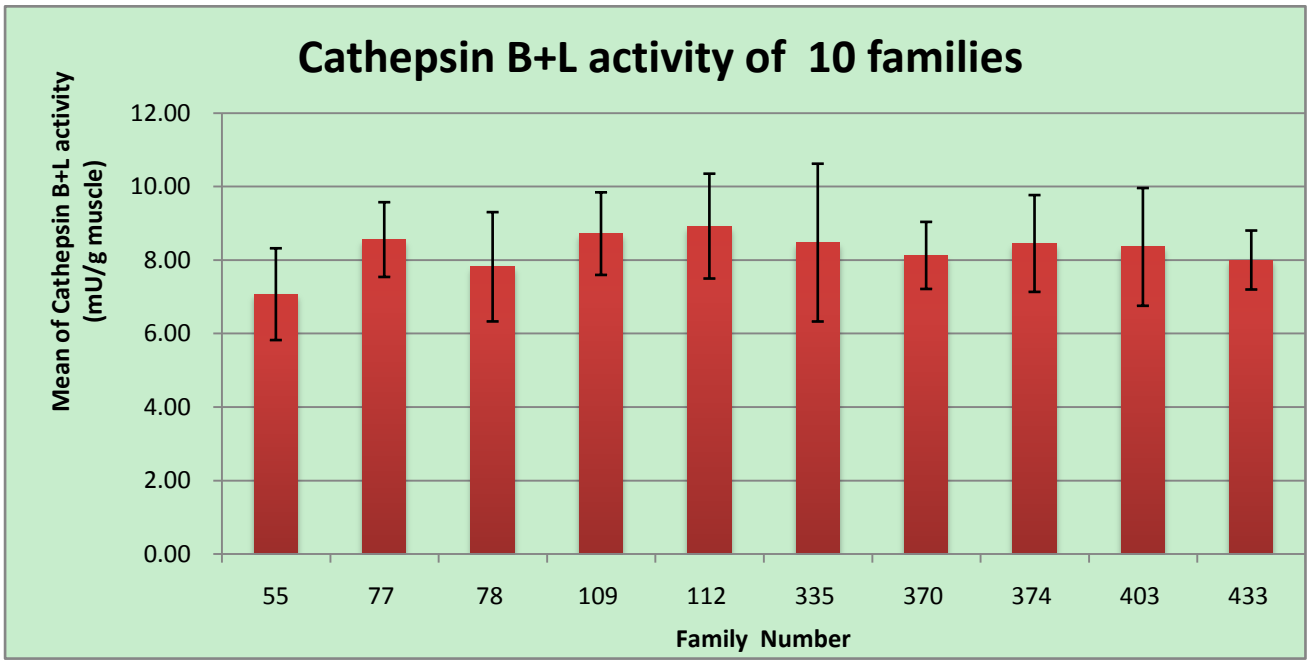

Figure 3.10 Cathepsin B+L activity of 10 families

Figure 3.10 presents the differences of mean value cathepsinB+L activity between 10 fish families .The highest mean value of cathepsin B+L activity is found in family 112 with 8.93mU/g muscle, while the lowest is in family 55 with mean 6.98mU/g muscle.

The mean value of cathepsin B+L activity from another 9 salmon families are between 7.82mU/g muscle to 8.72 mU/g muscle.

Statistic analysis of the cathepsin B+L activity for 10 salmon families was illustrated as below in figure 3.11.

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	FAMILY
	A	9.6700	1	555
	A			
B	A	8.9289	9	112
B	A			
B	A	8.7222	9	109
B	A			
B	A	8.5611	9	77
B	A			
B	A	8.4792	12	335
B	A			
B	A	8.4543	7	374
B	A			
B	A	8.3633	12	403
B	A			
B	A	8.1309	11	370
B	A			
B	A	8.0067	9	433
B	A			
B	A	7.8229	14	78
B				
B		7.0760	5	55

Figure 3.11：Statistic analysis of the cathepsin B+L activity for 10 salmon families

According to the results ($P = 0.44$, $R^2 = 10$) from SAS analysis, Figure 3.11 presented no statistic significantly difference between the cathepsin B+L

activity for 10 salmon families (Except family 555 which had only one fish).

3.4 Correlation between BF and Cathepsin B+L activity

The mean value of break force and cathepsin B+L of 10 salmon families is illustrated in table 3.2.

Family	Mean Break force (N)	Mean Cathepsin B+L （mU/g muscle）
55	6.89	6.98
77	7.92	8.56
78	8.33	7.82
109	5.93	8.72
112	8.69	8.93
335	8.67	8.48
370	8.82	8.13
374	8.39	8.46
403	7.66	8.36
433	9.41	8.01

Table3.2. Mean BF and mean Cathepsin B+L activity for 10 families

Here presents the correlation between mean BF and mean Cathepsin B+L activity of 98 fish in figure 3.12.

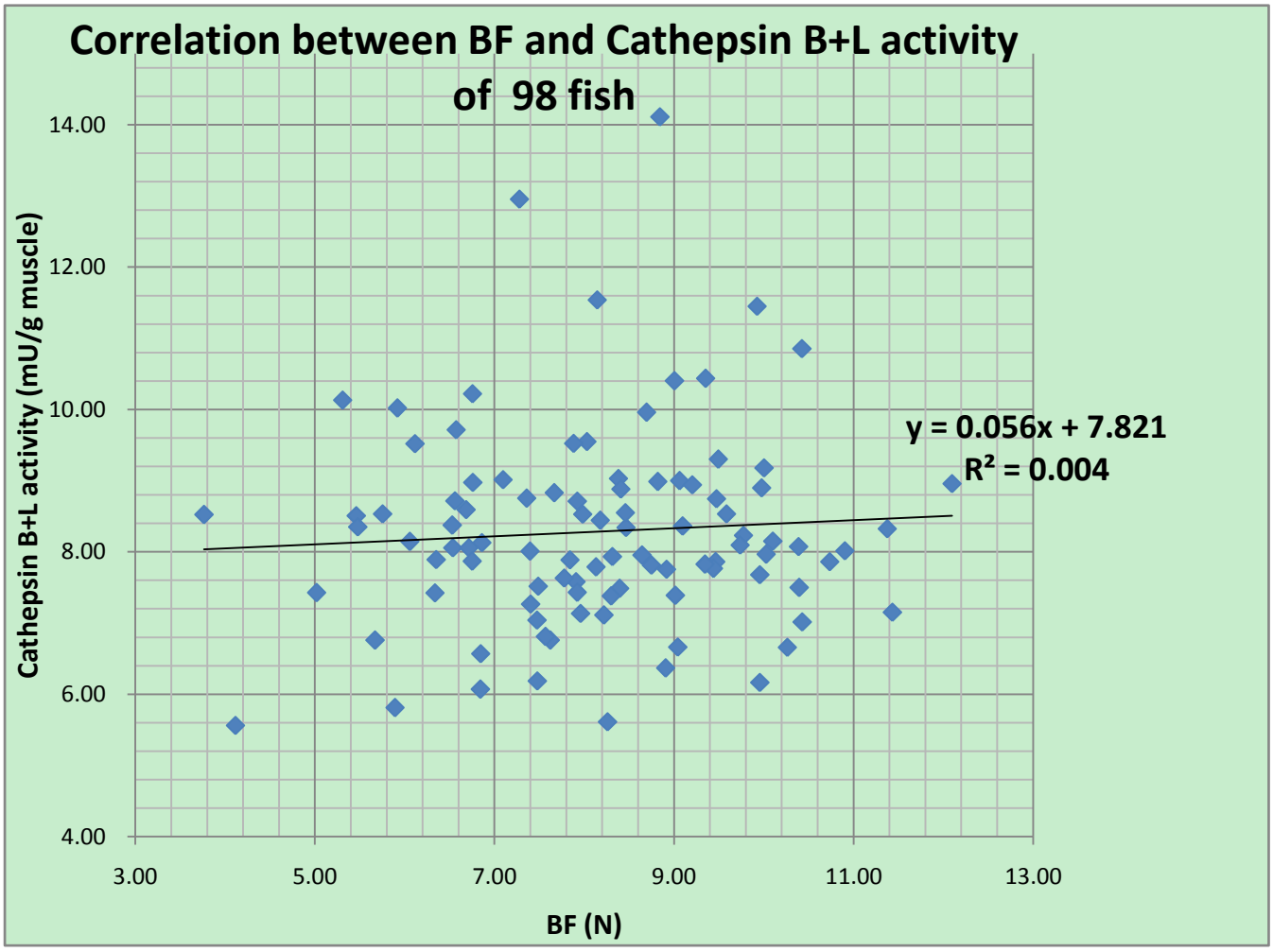

Figure3.12Correlation between BF and Cathepsin B+L activity for 98 fish

Correlation: **R ^{2}= 0.004**

According correlation **R** ^{2}is close to 0， this means there is also no significant correlation between mean break force and mean Cathepsin B+L activity for all 98 salmon samples.

The correlation between mean BF and mean Cathepsin B+L activity for 10 families illustrates as below in figure 3.13.

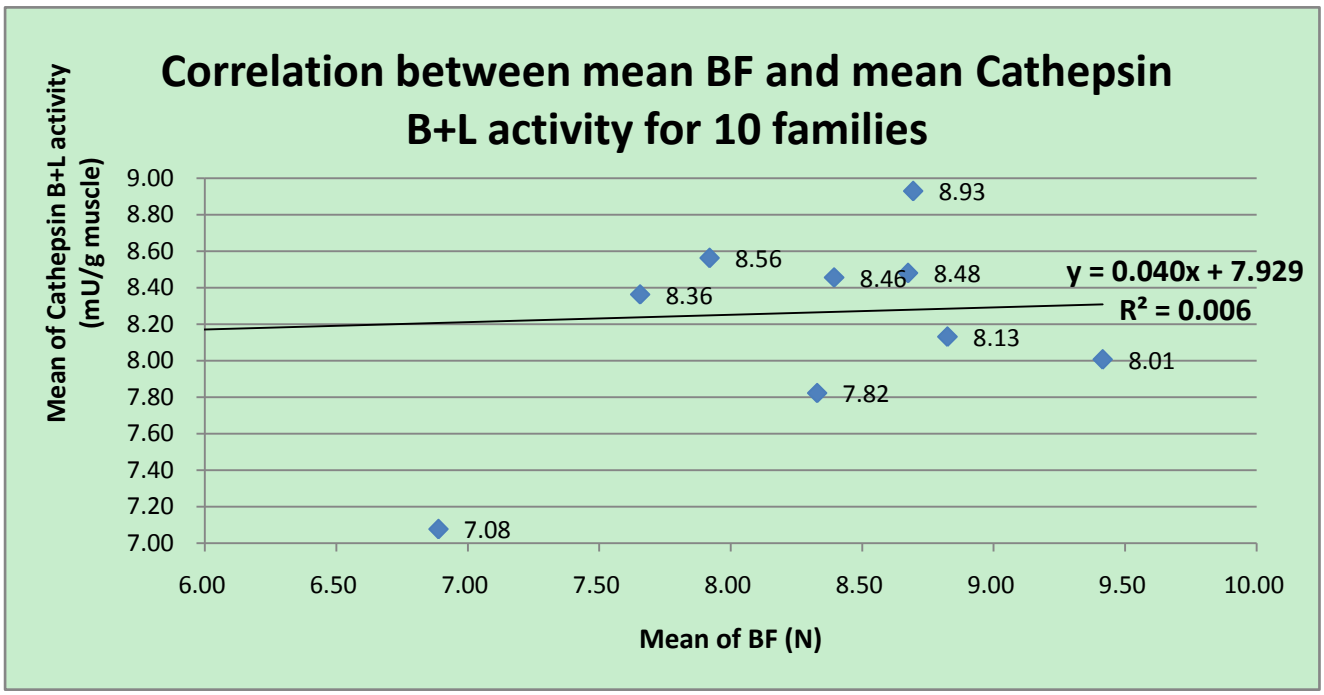

Figure 3.13Correlation between mean BF and mean Cathepsin B+L activity for 10 families

Correlation: **R^2= 0.006**

According correlation **R^2** is close to 0， this means there is no significant correlation between mean Break force and mean Cathepsin B+L activity for 10 salmon families.

4. Discussion

4.1 Texture instrumental measurements

In the present study, instrumental measurements of 98 individual Atlantic salmon from 10 different families showed statistic significant difference*(P = 0.0059, R^2 = 21)*in mean value of break force between families at 5 days post-mortem. In Salemet al. (2005) research also showed that the significant different of fillet shear force in rainbow trout was found between strains.

During the study, the mean values of break force among 10 families were ranged from 5.93 N to 9.41 N at 5 days post-mortem. In Mørkøre research (2008), the range of Atlantic salmon fillet with break force between 8 N to 11 N was acceptable texture ranging, and the break force below 6 N could be considered as too soft. Therefore, the break force of 5.93 N in salmon family 109 presented very soft muscle texture because the different genetic background, belonging to different families.

4.2 CathepsinB+L activity

Cathepsin B+L are lysosomal cysteine proteases to degrade fish and

mammalian muscle post-mortem. In research of Yamashita and Konagaya(1990 and 1991) and Godikesn et al (2009), cathepisnB+L was the most active enzymes for muscle softening in salmonids.

In the present study, showed the different mean value of cathepsin activity between 10 Atlantic salmon families but not statistic significant difference(P = 0.44, R^2 = 10), which from 7.82 mU/g muscle to 8.93 mU/g muscle, while family 55 with 6.98 mU/g muscle. However, family 55 presented the extremely lowest among 10 families, its maybe due to the significant lowest fish weight(1032±296.5g) and body length (23±5 cm). It's also occurred in Jian Gu(2013) research, which studied the same fish resources as in the present study, showed that no significant correlation could be found between calpain activity and muscle texture.

4.3 Correlation between break force and cathepsin B+L activity

In the present study, we found no significant difference mean value between break force and cathepsin B+L activity of 10 Atlantic salmon families(**R^2 = 0.006**). This is in accordance with the results on the same fish resources between calpain and texture by Jian Gu(2013).One possible explanation could be to consider that cathepsin B+L increase during the

period after slaughter. In accordance with Mari Øvrum Gaarder (2011) research, presented cathepsin B+L were proteolytic active in the storage period, which had first increased from 6 hours and then stabilized to 24hours postmortem. Our samples were frozen very shortly after slaughter, The similar experiment could be conducted after few hours or a couple of days to check the correlation between break force and cathepsin B+L activity. This hypothesis however needs further investigation.

During the study, the mean value of break force among 10 families ranged from 5.93 N to 9.41 N at 5 days post-mortem, while 4 families among them were under 8 N which means under the range of acceptable texture ranging (Mørkøre, 2008).

5. Conclusion

According to the results from the present study, revealed the analysis significant difference in muscle texture between 10 Atlantic salmon families, but no significant difference in cathepsin B+L activity mean values. And no significant correction was found between Atlantic salmon muscle texture and cathepsin B+L activity.

Based on this, it is difficult to conclude that cathepsin B+L have significance affects on Atlantic salmon muscle texture, although it was found in salmon by Bahuaud et al (2010) and also other fish species (Sherekar et al., 1988; Toyohara,1993; An H. et al., 1994; Jiang et al.1996; Jiang et, 1997; al., 1994;). Further studies on a bigger number of families are perhaps necessary.

6. Reference

An H; Seymour TA; Wu JW; Morrissey MT. 1994. Assay systems and char-acterization of Pacific whiting (Merluccius productus) protease. J FoodSci. 59: 277-281.

Aoki, T. and Ueno, R. 1997. Involvement of cathepsins B and L in the post-mortem autolysis of mackerel muscle. Food Research International,30:585-591.doi: 10.1016/S0963-9969(98)00014-3..

Aranishi, F., Ogata, H., Hara, K., Osatomi, K., and Ishihara, T. (1997). Purification andcharacterization of cathepsin L from hepatopancreas of carp (Cyprinus carpio). Comp.Biochem. Physiol. 118B: 531-537

Bahuaud , D., Østbye, T. K., Torstensen, B. E., Rørå, M. B., Ofstad, R., Veiseth, E., et al(2009). Atlantic salmon (salmo salar) muscle structure integrity and lysosomal cathepsin B and L influenced by dietary n-6 and n-3 fatty acids. Food Chemistry, 101, 1474-1432.

Barrett, A.J. and KIRSCHKE, H. 1981. Cathepsin B, cathepsin H, andcathepsin L. Methods Enzymol. 80,535-561.

Bahuaud , D., Gaarder M., Veiseth-kent E., Thomassen M. Texture and protease activities in different genetic families of Atlantic salmon (Salmosalar L). Aquaculture, 310， 213-220.

Bahuaud, D., Mørkøre, T., Langsrud, Ø., Sinnes, K., Veiseth, E., Ofstad, R., et al(2008)/ Effects of -1.5℃ Super-chilling on quality of

Atlantic salmon (salmo salar) pre-rigor Fillerts: cathepsin activity, muscle histology, texture and liquid leakage. Food Chemistry, 111(2), 329-339.

Cheret, R., Delbarre-Ladrat, C., de Lamballerie-Anton, M. &Verrez-Bagnis, V. (2007).Calpain and cathepsin activities in post mortem fish and meat muscles. Food Chemistry, 101,1474-1479.

D.Bahuaud, M.Garrder, E.Veiseth-Kent, M.Thomassen., Fillet texture and protease activities in different families of farmed Atlantic salmon(salmo salsr L.) Aquaculture 310 (2010)213-220.

De Ceuninck, F., Poiraudeau, S., Pagano, M., Tsagris, L., Blanchard, O., Willeput, J., et al. (1995). Inhibition of chondrocyte cathepsin B and L activities by insulin- like growth factor-Ⅱ(IGF-Ⅱ) and its Ser29 variant in vitro : possible role of the mannose 6-phosohate/IGF-Ⅱ receptor. Molecular and Cellular Endocrinology, 113,205.

Diane Sophie O Bahuaud, Atlantic salmon (Salmosalar L.) flesh quality – Role of lysosomes and cathepsins in muscle degradation(2009).

Gjedrem,T., 2000. Genetic improvement of cold-water fish species. Aquaculture Research 31,25-33.

Godiksen, H., Morzel M, Hyldig G, &Jessen F.(2009) . Contribution of cathepsin B, L and D to muscle protein profiles correlated with texture in rainbow trout (Oncorhynchus mykiss). Food Chemistry, 113(4), 889-896.

Hindar K, Garcia de Leaniz C, Koljonen M-L, Tufto J, Youngson AF(2007) Fisheries exploitation. In: Verspoor E, StradmeyerL,Nielsen J (eds) The Atlantic Salmon: Genetics, conservation and management. Blackwell, Oxford, pp 306–330.

Jian Gu, Calpain activity and texture in Atlantic salmon muscle(2013).

Jiang ST; Lee JJ. 1992. Purification, characterization and utilization of pigplasma factor XIIIa. J Agric Food Chem. 40: 1101-1107.

Jiang, ST, LEE, JJ and CHEN, HC 1994. Purification and characterization of cathepsin B from ordinary muscle of mackerel (Scomberaustralasicus). J.Agric. Food Chem. 42, 1073-1079

Jiang ST; Lee JJ; Chen HC. 1996. Proteolysis of actomyosin by cathepsinsB, L, L-like and X from mackerel (Scomber australasicus). J Agric FoodChem. 44: 769-773.

Jiang ST; Lee BL; Tsao CY; Lee JJ. 1997. Mackerel cathepsin B and L effectson thermal degradation of surimi. J Food Sci.

Johnston, I.A., Li, X., Vieira, V.L.A., Nickell, D., Dingwall, A., Alderson, R., Campbell, P., Bickerdike, R., 2006. Muscle and flesh quality traits in wild and farmed Atlantic salmon. Aquaculture 256, 323-349.

Kirschke, H., Wood, L., Roisen, F J., & Bird, J. W. C, (1983). Activity of lysosomal cysteine proteinase during differentiation of rat skeletal-muscle. Biochemical Journal, 214,871-877.

Lee JJ; Chen HC; Jiang ST. 1993. Purification and characterization of

pro-teinases identified as cathepsin L and L-like (58 kDa) from mackerel(Scomber australasics). Biosci Biotech Biochem. 57: 1470-1476.

Lee, JJ, CHEN, HC and JIANG, ST 1996. Comparison of the kinetics ofcathepsins B, L, L-like, and X from the dorsal muscle of mackerel on thehydrolysis of methylcoumarylamide substrates. J. Agric. Food Chem. 44, 774-778

Makinodan Y; Toyohara H; Ikeda S. 1984. Comparison of muscle pro-teinase activity among fish species. Comp Biochem Physiol. 79: 129-134

Mari Øvrum Gaarder, D. Bahuaud, E. Veiseth-kent, T. Mørkøre, M.S.Thomassen., Relevance of calpain and calpastatin activity for texture in super-chilled and ice stored Atlantic salmon (Salmon salar L) fillets.

Mari Øvrum Gaarder, The importance of the calpain system in post mortem Atlantic salmon(Salmo salar L.) muscle in relation to texture(2011).

Masaki T; Shimomukai M; Miyauchi Y; Ono S; Tuchiya T; Mastuda T;Akazawa H; Soejima M. 1993. Isolation and characterization of theprotease responsible for jellification of pacific hake muscle.NipponSuisan Gakkaishi 59: 683-690.

Mørkøre, T., 2008. Tkstur i oppdrettslaks-Kunnskapsstatus og forhold

som bidrar til fastere fillet. Norske Sjømatbedrifters landsforening. www.nsl.no/filer/fou/loe/loe5.pdf2008Last access 01.04.29

Ogata, H., Aranishi, F., Hara, K., Osatomi, K., & Ishihara, T. (1998). Proteolytic degradationof myofibrillar components by carp cathepsin L. Journal of the Science of Food andAgriculture, 76(4), 499-504.

Pangkey, H., Hara, K., Tachibana, K., Cao, M. J. Osatomi, K. and Ishihara, T. 2000. Purification and characterization ofcathepsin S from hepatopancreas of carp Cyprinus carpio. Fisheries Science 66(6): 1130-1137.

Rasmussen, R. S(2001). Quality of farmed salmonids with emphasis on proximate composition, yield and sensory characteristics. Aquaculture Research 32(10), 767-786.

Shann-Tzong Jiang and Li-Jung Yin. 2004. Surimi enzymology and biotechnology. Proceedings of the Annual Meeting of American Meat Science Association, Presented at "Reciprocal Meat Conference" of the Annual Meeting of American Meat Science Association, Kentucky, USA.

Sherekar, SV, Gore, MS, Ninjoor, B (1988) Purification and characterization of Cathepsin B from the skeletal muscle of fresh water fish. J. Food Sci. 53: pp. 1018-23

Toyohara H; Kinoshita M; Kimura I; Satake M; Sakaguchi M. 1993.Cathepsin L-like protease in Pacific hake muscle infected by

myxo-sporidian parasites. Nippon Suisan Gakkaishi 59: l101-1107.

World Wild life Fund (2001)， The status of wild Atlantic salmon: arriver by river assessment. assests.panda.org/downloads/salmon2.pdf

Yamashita M; Konagaya S. 1990a. High activities of cathepsins B, D, Hand L in the white muscle of chum salmon in spawning migration.Comp Biochem Physiol. 95: 149-152.

Yamashita M; Konagaya S. 1990b. Participation of cathepsin L into exten-sive softening of the muscle of chum salmon caught during spawningmigration. Nippon Suisan Gakkaishi 56: 1271-1277.

Yamashita M; Konagaya S. 1990c. Purification and characterization ofcathepsin L from the white muscle of chum salmon, Oncorhynchusketa. Comp Biochem Physiol. 96: 247-252.

Yamashita M; Konagaya S. 1991a. Hydrolytic action of salmon cathepsinsB and L to muscle structural proteins in respect of muscle softening.Nippon Suisan Gakkaishi 57: 1917-1922.

Zhao, G. M., Zhou, G. H., Wang, Y . L., Xu, X, L., Huan, Y. J., &Wu,J.Q.(2005). Time-related changes in cathepsin B and L activities during processing of Jinhua ham as a function of pH, salt and temperature. Meat Science, 70, 381-388.

Website 1: http://www.globefish.org/salmon-june-2013.html

Website 2:http://www.villmarken.net/k-faktor.12159-2689.html

Website3:http://www.hooked.no/artikler/k-faktor-beregn-fiskens-kondisjo

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