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Computer Vision for Quality Grading in Fish Processing

Thesis for the degree of philosophiae doctor

Trondheim, November 2007

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ISBN 978-82-471-5434-2 (printed ver.)
ISBN 978-82-471-5448-9 (electronic ver.)
ISSN 1503-8181
ITK report number 2007-7-W

Theses at NTNU, 2007:244

Printed by Tapir Uttrykk

ABSTRACT

High labour costs, due to the existing technology that still involves a high degree of manually based processing, incur overall high production costs in the fish processing industry. Therefore, a higher degree of automation of processing lines is often desirable, and this strategy has been adopted by the Norwegian fish processing industry to cut-down production costs. In fish processing, despite a slower uptake than in other domains of industry, the use of computer vision as a strategy for automation is beginning to gain the necessary maturity for online grading and evaluation of various attributes related to fish quality. This can enable lower production costs and simultaneously increase quality through more consistent and non-destructive evaluation of the fish products.

This thesis investigates the possibility for automation of fish processing operations by the application of computer vision. The thesis summarises research conducted towards the development of computer vision-based methods for evaluation of various attributes related to whole fish and flesh quality. A brief summary of the main findings is presented here.

By application of computer vision, a method for the inspection of the presence of residual blood in the body cavity of whole Atlantic salmon was developed to determine the adequacy of washing. Inadequate washing of fish after bleeding is quite common in commercial processing plants. By segmenting the body cavity and performing a colour analysis, it was shown that the degree of bleeding correlated well with colour parameters, resulting in correct classification of the fish with residual blood. The developed computer vision-based classifier showed a good agreement with the manual classification of the fish that needed re-washing. The proposed method has potential to automate this type of inspection in fish processing lines.

In addition, a computer vision-based classifier for quality grading of whole Atlantic salmon in different grading classes, as specified by the industrial standard, was developed. In the proposed solution, after segmentation of the salmon from the image scene, with the use of the computer vision techniques, it was possible to extract non-redundant geometrical features describing the size and shape of fish. Based on these features, a classifier was developed for classification of fish into respective grading classes. The average correct rate

of classification was in good agreement with the manual labelling, and the method has a potential for grading of Atlantic salmon in fish processing lines.

Regarding fillet grading, a computer vision-based sorting method for Atlantic salmon fillets according to their colour score was developed. The method and classifier/matching algorithm was based on the present industrial standard NS 9402 for evaluation of fillets by colour according to Roche Cards. As a result, fillets or parts of fillets, could be classified into different colour grades. This is important for the industry since different markets tend to have different preferences for fillet colour. This classification method is suitable for on-line industrial purposes. In addition, the method gives colour evaluation of fresh and smoked fillets in the CIELab space, similar to the L, a, and b values generated by a Minolta Chromameter, for different parts of fillets as well as for the entire fillet. The advantage of the computer vision-based method derives from the flexibility in the choice of the size of the region of interest of the fillet for colour measurement, as opposed to the Chromameter, where the Minolta generated values are obtained by interrogating a very small area of the fillet (8 mm). The method can also be used for detection of colour non-uniformities (discoloration) in both fresh and smoked fillets.

A method for computer vision-based measurements and monitoring of transient 2D and 3D changes in the size and shape of fillets during the rigor process and ice storage was developed. The method successfully measured the size (length, width, area) and shape (roundness) of Atlantic salmon and cod fillets, and monitored changes to these during ice storage with high precision. This was demonstrated by comparison of the exhausted and anesthetized fillets. By laser scanning of the fillet, it was possible to obtain size changes in the height (mm) and area of the fillet in cross-section. The method can be used not only for size and shape analysis of fillets but also for other fish products, both in on-line, as well as off-line conditions as a tool for monitoring 2D/3D size and shape changes. The method can also be used for determination of fillet yield measured in thickness, which is an important parameter for the industry. Together with the colour grading ability, this method can also be used for full feature evaluation and classification of any fish or food product from a single image (colour, size and shape in 2D/3D).

If filleting of fish is done pre-rigor, care should be exercised during colour grading since transient colour changes occur in the post-mortem period. As these changes are more pronounced than those that occur during ice storage, incorrect colour grading can occur. The computer vision method developed for evaluation of colour changes in fillets during rigor, ice storage, and due to effects of perimortem handling stress was considered as the most suitable method for industrial purposes when compared to both the Minolta Chromamater and sensory analysis by a panel.

A computer vision-based method for evaluation of fresh and smoked fillets with respect to bleeding was developed. This form of evaluation is important for the industry as residual blood in fillets may lead to reduced visual acceptance of the product. The method was considered suitable for the purpose of this type of evaluation.

The developed computer vision methods have potential for automation of the mentioned grading operations in the commercial fish processing lines. Application of the proposed solutions would lower the production costs, while simultaneously increasing the quality of the products through a more consistent and non-destructive evaluation of these products.

PREFACE

The research presented in this thesis is the result of my PhD studies at the Norwegian University of Science and Technology (NTNU), under the guidance of Associate Professor Amund Skavhaug, at the Department of Engineering Cybernetics, and Senior Scientist Ulf Erikson at the SINTEF Fisheries and Aquaculture.

The research presented in this thesis has been carried out at the SINTEF Fisheries and Aquaculture laboratories in Trondheim.

The work in this thesis has been funded by the Norwegian Research Council grant 145634/140 through SINTEF Fisheries and Aquaculture in the Strategic Institute Programme “Efficient and economic sustainable fish processing industry”, headed by Research Director Marit Aursand at the SINTEF Fisheries and Aquaculture. The work in the thesis was partly financed from the Department of Engineering Cybernetics at the NTNU.

ACKNOWLEDGMENTS

During the PhD studies, collaboration and communication with other people is a natural part of the work. For their valuable advices, supervision, and feedback, I would first of all like to thank my advisors: Associate Professor Amund Skavhaug, from the Department of Engineering Cybernetics at NTNU, for accepting me as a PhD student, for his exceptional supervision and motivation, and for support throughout the work in this thesis. His support and advice, especially in the final period of my PhD studies, has been of tremendous importance.

My deepest thanks go also to my co-advisor Ulf Erikson, Senior Scientist at SINTEF, who has been a great support. His inspiration, his vast knowledge in the field of fisheries has made the work in this thesis so much easier. He was an excellent coordinator of the work that we carried out at the laboratories, nonetheless an excellent advisor when it comes to scientific writing.

I would like to thank Marit Aursand, the research director at the SINTEF Fisheries and Aquaculture, for believing in me, for support, and for solving financial related issues concerning the part of this project where I was involved. My gratitude goes also to research scientist John Reidar Mathiassen, with whom I have collaborated in a number of papers in this thesis. Working together with him has made many computer vision challenges seem much easier. He has also been a great support during the work in this thesis. I appreciate the discussions that I had with him through which I have gained much more understanding. I would also like to express my gratitude to research scientist Hanne Digre, with whom I have collaborated in the paper concerning the monitoring of rigor process in Atlantic cod fillets.

I would like to thank Professor Rolf Henriksen and especially Professor Morten Hovd, head of the Department of Engineering Cybernetics at the NTNU, for financing the final year of my PhD studies in collaboration with SINTEF.

I thank my friends and colleagues for a very pleasant and motivating working environment. At SINTEF: Ida, for always making me laugh with her wittiness and for being my best office-neighbour, Rasa and Egis for our recent discussions on baby related

topics, Tord Hanssen for his friendship from the first day, Tim Dempster for his friendship and proof-reading of my introductory chapter, Pascal Klebert for our discussions on wine and our disagreements on that, Emil Veliyulin, Inger-Beate Standal and to many more. At NTNU: Thanks go to Åsmund Tjora, with whom I shared the office during my last period as a PhD student, to Trygve Lunheim, Stefano Bertelli, Knut Reklev and many more.

I would also like to thank my friend Skender Kosumi from the Vienna University of Technology for help with some of the figures presented in this thesis.

Finally, I would like to thank my family. I would like to thank my parents for their love and unconditional support during my education. I am grateful to my brother and sisters for supporting and encouraging me during the work in this thesis.

I would like to express my deepest gratitude to my wife Florentina, for being the ultimate supporter throughout the work in this thesis. Her love and support despite difficult periods has meant a great deal to me. Our little son Bentian can surely not be thanked for making the work in this thesis easier. His contribution would rather be in giving me something else to think about during my occasional “escapes” from working on this thesis. I would partly like to dedicate this thesis to my family as an appreciation for their endless support and love.

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List of Papers

1. Misimi, E., Mathiassen, J.R., Erikson, U., Skavhaug, A. 2006. *Computer vision based sorting of Atlantic salmon (Salmo salar) according to shape and size*. Proceedings of International Conference on Computer Vision Theory and Applications, VISAPP 2006, Vol. 1. pp. 265-270.

2. Misimi, E., Mathiassen, J.R., Erikson, U. 2007a. Computer vision based sorting of Atlantic salmon *(Salmo salar) according* to their colour level. Journal of Food Science. 72(1):S30-S35.

3. Erikson, U., Misimi, E., 2007b. *Atlantic salmon skin and fillet colour as affected by peri-mortem handling stress, rigor mortis, and ice storage*. Journal of Food Science, in press.

4. Misimi, E., Erikson, U., Digre, H., Skavhaug, A, Mathiassen, J.R. 2007c. *Computer vision based evaluation of pre- and postrigor changes in size and shape of Atlantic salmon (Salmo salar) and Atlantic cod (Ghadus morhua) fillets during rigor mortis and ice storage: Effects of perimortem handling stress*. Journal of Food Science, in press.

5. Misimi, E., Erikson, U., Skavhaug, A. 2007d. *Quality grading of Atlantic salmon (Salmo salar) by computer vision*. Journal of Food Science, submitted.

6. Erikson, U., Misimi, E., Fismen, B. 2007e. *Bleeding of anesthetized and exhausted Atlantic salmon – Residual blood in pre-rigor and smoked fillets as determined by various analytical methods*. Journal of Food Science, submitted.

Outline of the thesis

This thesis is organized in two parts. Part 1 is organized in five chapters, while Part 2 consists of the papers that represent the central work in this thesis.

Part 1 of the thesis is meant as an extended introduction and summary. We start with the introductory chapter, where it is discussed about some of the problem issues in today's fish industry. This chapter also includes a review of the sensor technologies in use in the food and fish industry. At the end of the chapter, shortly are listed the main contributions of the thesis. Chapter 2 provides with the background knowledge regarding the fish processing industry as well as computer vision fundamentals. This chapter gives an understanding on the structure of a typical fish processing line, explaining some of the unit operations along the line which are related to this study. The computer vision part provides an understanding on the application of computer vision in an industrial setting related to fish processing. It covers specific issues of computer vision that are critical for a successful implementation, such as illumination, shape recognition, image segmentation, colour analysis, segmentation as well as pattern recognition concepts.

Chapter 3 has a typical "methods and results" outline and it covers the common thread of the study, consisting of the problems/operations that were chosen to deal with during the research for the thesis, and the proposed computer vision-based solutions. The results are the individual contributions of the thesis. Here, the proposed solutions for the operation of sorting/grading of whole salmon into different grading classes using the size/shape recognition are presented, as well as the non-destructive quality evaluation of fillets based on colour level, influence of handling stress, rigor mortis, ice storage, and bleeding method.

Chapter 4 consists of discussions and reflections over the work. Here, it will also be discussed about the implications of the combined results of the research in the thesis.

Chapter 5 contains the conclusions drawn from the work in the thesis, a summary of contributions, and suggestions for future work.

Part 2 of the thesis consists of the papers that have resulted from the research. They represent the central work in this thesis.

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PART I

CHAPTER 1

Introduction

"With the level of prosperity in our society, it is more necessary than ever to reduce the labour cost in production and transport. This demands development of machinery ***and automation of work process****."*

Grønnevet Committee Report, 15 June 2004[1]

"The potential for increasing the value generation and the degree of processing in Norway lies in market oriented work ***…..and further automation****."*

Fisheries Ministry's White Paper Nr. 19.[2]

1.1 Background

The Norwegian fisheries and aquaculture industry presently faces a number of challenges. One of the most important challenges involves creating a market-based profitable refinement of marine raw materials. There are several obstacles to this. The major obstacle, however, is that Norway has high production costs and is experiencing increasingly stronger competition within the international market from a number of producers from countries with low production costs such as Chile, regarding the marine raw materials, and Poland and China, when it comes to fish processing. The high production costs in Norway together with the existing processing technology (still high degree of manually based processing) make the processing of fish products in Norway in average 6-10 NOK/kg more

[1] The strategy for higher value generation in fish industry is outlined in the Report "Økt verdiskapning i fiskeindustrien" edited by the Grønnevet committee in 2004, p. 51., at http://odin.dep.no/filarkiv/214606/Sluttrapport_Gronnevet_trykk.pdf

[2] The report "Den blå åker" outlines the marine market development and the important challenges ahead. The report can be found at http://odin.dep.no/fkd/norsk/dok/regpubl/stmeld/047001-040003/dok-bn.html

expensive than it is in some competitor countries. This situation has given negative effects in several domains. It has reduced the amount of processing of fish products in Norway creating such conditions where roughly 90% of the entire Norwegian fish product export consists of export of whole gutted fish (fresh and frozen), while the export of the processed fish products in the form of fillets is about 10-13% (Andahl and Kristiansen, 2005). It has also made fish processing plants move their facilities abroad where lower production costs make their production more profitable, and in general it has reduced the competitiveness and market position of the Norwegian fish industry.

To create an economically robust business environment, to increase the profitability through a competitive level of production costs, and to improve its market position, the Norwegian fish industry has adopted the automation of fish processing as a strategy for achieving these goals. The benefits from automation are manifold. Firstly, automation can reduce the production costs, which mainly consist of high labour costs, thus achieving a long term level of competitiveness of Norwegian fish industry against the low-cost production countries. Secondly, automation can increase the overall quality of the fish products at the end of the processing chain.

An overview of the production cost level in Norway compared to other countries, such as Poland and China, reveals a huge gap (Table 1.1). The production cost per kilogram is highest in Norway, while it is lowest in China. In Norway, the labour costs make up more than 6 NOK/kg (1$/kg) from the total production cost per kilogram. Low cost of labour force, in an industry that is manually based, make China have the lowest production/labour cost in the fish industry.

Table 1.1 Comparison of production costs for gutted weight as well as bone and skinless products between Norway, Poland and China (Ostvik and Jansson, 2004)

Production/labour costs (NOK/kg)	Gutted weight	Bone and skinless	Labour costs
Norway	21,00	57,69	6,69
Poland	22,10	49,07	1,00
China	22,00	47,63	0,40

In Norway, despite the use of some technology, production in the fish industry still requires a substantial manual labour because many operations remain non-automated. Manual labour is traditionally used for fine trimming and quality sorting of fillets (Figure 1.1). Operations to sort/grade whole fish into different quality grading classes are also performed manually by human inspectors.

Table 1.2 Estimated gain with a fully automated processing (Ostvik and Jansson, 2004)

	Norway, 1 shift Existing techn.	Poland	China	Norway, 3 shift New techn.
Product volume (kg)	10200	10200	10200	10200
Persons per shift	50			6
Labour costs, (NOK/kg)	6,69	1,00	0,40	0,74
Difference: NOK/kg	0,00	-6,69	-6,92	-6,73

Table 1.2 shows the difference between the production costs in Norway with the existing technology and the predicted labour costs with the use of a new technology. According to this estimate, the labour costs of 6.69 (NOK/kg) could almost be eliminated if the new technology can fully automate the operations which today are manually based.

Figure 1.1 Extensive use of manual labour in performing different operations along the processing line (Courtesy of Salmar AS).

With automation of fish operations, the Norwegian fish processing industry would lower its production costs. Automation would provide for a better utilization of machines by having a continuous production during three shifts and would reduce the amount of manual labour from 50 persons per line/shift, as it is today, to approximately 6. Furthermore, an increase of product quality is to be expected as a result of automated fish processing.

1.2 Present situation and potential benefits of fish industry from automation

The existing technology in fish processing industry in Norway involves mechanical machinery available for the majority of individual operations such as killing, bleeding, gutting, de-heading, filleting with removal of the backbone and belly bones, removal of pin-bones, trimming and skinning. For whole fish, control and grading with respect to size and quality aspects are normally included in the manufacture of fish consumer products. The size-grading machinery in use comes from various producers[3]. This means that the machinery has a limited degree of compatibility. Transfer and capacity adaptation for fish material flow is done by the use of conveyors, although other systems for supportive transport during processing are available[4].

Today, the quality grading of whole salmon is done manually. This includes interior and exterior assessment of the fish based on standardized quality parameters. Weight grading is done in integrated systems for packaging (Marel, Scanvaegt, Seafood automation).

The manual labour involved in fish line operations has several other drawbacks, except the one of incurring high production costs. Workers along the line, who are involved in manual operations, usually work in the upright standing position. The highly repetitive nature of their work causes stress and boredom because simple manual operations are repeated hundreds of times every day. These operations are also characterized by a high demand for concentration all day. Altogether, these factors contribute to a considerable human fatigue while performing operations in the line, and human inspection becomes too

[3] Nordischer Maschinenbau Rud.Baader GmbH+Co.KG, Trio Fish Processing Machinery AS, Marel hf, Uni-Food Technic A/S, Carnitech A/S, Scanvaegt International A/S

slow and error prone with respect to quality evaluation. This leads to a decrease of the product quality and thereby lessens the profit (Pau and Olafsson, 1991).

1.2.1 Quality enhancement through automation

In parallel to the demand for a more efficient production in the fish processing industry, the demand for quality assurance is just as important. Quality assurance of fish products implies a continuous evaluation of these products. In the fish processing industry, this has traditionally been done visually. Human evaluation of fish products, as in other domains of the food industry, is inherently limited by human inability to objectively, consistently and accurately (Panigrahi and Gunasekaran, 2001) evaluate quality by means of sight and contact. The inability to do so affects the sorting/grading operations according to relevant quality parameters of fish such as the colour level and intensity, length, external blemishes, shape defects, and degree of bleeding. For example if human vision is compared to computer vision, the human based vision is very strong in recognition of objects (for instance in grading of whole Atlantic salmon) but is less powerful for accurate measurements of colour level, gray level, length or area (Jahne 2002).

Benefits regarding the quality of the products would be considerable if the technology aiming the automation of these operations would employ some kind of non-contact quality evaluation of fish products (whole fish, fillets). Today, due to the manual quality grading, evaluation, and processing there is a frequent contact of human operators with the products and this lessens the quality and quality assurance of the products. Although fish plants have strict regulations on the hygiene, it is noted that humans are a factor in product contamination. Therefore, due to the costs for preserving hygiene in fish plant with a large number of staff, the overall production costs are additionally increased (Purnell 1998). Automation could, on the other hand, result in the improvement of fish products hygiene. It would also reduce the need for lighting and heating of the production premises. This would allow processing in environments beneficial to the quality of fish products, for instance, sustained low temperatures.

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[4] Baader, patents US5413525, US4084294, GB2061854 and GB2103920

Another issue where automation would contribute to a considerable decrease in the production costs are the training costs for human inspectors involved in the quality grading and evaluation. For a long time, the tradition in the fish industry has been to base the quality sorting/grading on human inspectors with many years of experience. During the last decade, the situation has changed as in the fish processing industry new staff and young people with little experience are mainly in charge of evaluation of quality (Olafsdottir et al. 2004). In addition, the trend is that young people work in the plants for short periods before leaving, meaning employment gaps often occur. This adds a higher cost to the entire production and lessens the quality assurance.

Therefore, automation of the processing industry not only would reduce production costs and ensure a long-term competitiveness of the Norwegian fish industry in the world market, but would also provide a basis for a faster processing of fish products and consistent non-destructive quality determination of these products.

What is, then, the right strategy to follow when talking about automation, and which technology should be pursued for automation of the quality evaluation and grading of fish products? For this purpose a short review of the state of the art in the sensor technology, that has potential to be used for automation, will follow. Regarding the issue of the right strategy, we see that there are a number of sensor technologies available. Nevertheless, any technology that is aimed to be used in the automation of fish operations should be able to satisfy the criteria of being rapid, non-destructive/non-contact and, most importantly, have on-line capabilities of use.

1.3 Sensor technology in Food and Fish Industry

Sensor technology is having a rapid development and has been implemented in various degrees in areas such as medicine, pharmaceutical industry, and food industry. Many of these sensors are based on spectroscopic techniques (fluorescence, measurements in the Near Infra Red part of the spectrum), microwaves, NMR (MRI), ultrasound, electronic nose, optical techniques, computer vision, and electrochemical methods. Typical product

features one is out to evaluate and control in the food industry are eventual defects, colour, texture, freshness, taste and composition.

Despite the fact that the aforementioned sensors assist in replacing human evaluation when it comes to the important quality parameters of food products, the on-line evaluation and control of food products and processes remains a major challenge. In order to be able to successfully replace the human factor, the sensor technologies should have on-line capabilities of operation. Therefore, as above mentioned, rapid, non-destructive, and on-line food/fish quality evaluation is necessary in order to ensure high quality of products, but also to improve plant productivity and cost-effectiveness.

There are a number of technologies that all have, to various extents, potential of being implemented on-line for control of different quality parameters. The technologies to be mentioned here are planar X-ray, CT, ultrasound, MRI, TD NMR, NIR and computer vision. Some of the techniques like CT, MRI and computer vision are essentially imaging methods, which can produce images of internal slices (CT, MRI, and ultrasound), object's surface (computer vision) or planar projections (planar X-ray).

Near Infrared Spectroscopy (NIR) is a promising technique for detection of the nematodes in fish fillets and can be fit for on-line implementation (1 fillet per sec). In combination with tailored statistical software near infrared reflectance (NIR) and near infrared transmittance (NIT) spectroscopy can be successfully used to determine fat contents in fish giving high correlations with chemical analysis, provided that specific calibrations are made for each sample type. Such non-destructive measurements of fat content in Atlantic salmon have been performed by Wold et al. (1996) and, in-vivo, by Solberg et al. (2003). Using non-contact reflectance diode array NIR measurement time of 3 s was achieved. On-line measurement of the fat content in salmon fillets using NIR reflection spectroscopy and diode array sensors is commercially available (Qvision AS). Similar measurements on whole salmon and pelagic fish (herring, mackerel) are under development with promising

results (www.matforsk.no). Same technology can be optimized for on-line measurements of the water content in clip-fish, colour, and pigment in fillets.

MRI is a technique that offers a unique opportunity to produce high quality cross-section images of intact organisms and thus to obtain basic insight into a number of issues related to anatomical studies, composition and structure of tissues, distribution maps of fat, water and salt as well as temperature mapping. In fish processing, MRI can be used as a tool for the optimization of various unit operations such as salting, freezing and thawing, some of which are described by Hills (1998). The main disadvantages of the MRI technology when it comes to the use as an on-line scanning technique are:

- long imaging time
- often too small magnet bore opening
- shear size and infrastructure requirements
- high costs
- requirement of trained personnel

MRI instruments can also measure NMR spectra from the specimen, which requires less scanning time. Such spectroscopic NMR instrument is implemented on-line in baby-food production for quality control of the final production (Hills, 1998).

Planar X-ray technique is a simple imaging where the object is irradiated by an X-ray source and a 2D projection image is recorded on the other side. The image intensity is proportional to the local density of the object. The technique has been successfully implemented for on-line detection of the spine deformities in live salmon of up to 16 cm length (Spectral Fusion Technologies Ltd., UK). Examination throughput was between 700 and 1200 fish per minute. Another example of planar X-ray on-line application is the fish bone detection by the “SensorX” system (Marel, Island) and a similar system BoneScan (Spectral Fusion Technologies Ltd.). The system detects fish bones down to 0.3 mm at full processing speed, scanning the fillets with low-energy X-rays and analyzing the images continuously.

The computer tomography (CT) is another imaging modality based on X-rays. CT can be used for non-destructive determination of the fat content and fat distribution in tissue. The method has been successfully used for quantitative determination of sodium chloride in ground pork and dry-cured hams (Håseth et al. 2007), but due to high investment costs, size and complexity, the instrument is not readily suitable for on-line measurements.

Applied to fish, the low field (LF) NMR has been proven to be a versatile analytical method for studying various topics such as effect of processing (Steen et al. 1997, Erikson et al. 2004), muscle fat and water content (Toussaint et al. 2001, Sørland et al. 2004). Unfortunately, the traditional LF NMR can not be a true non-destructive technique when studying whole fish because of the restricted magnet bore size (typically 10 - 40 mm in diameter). The mobile NMR instrumentation which is a low field NMR analyser for measurements in the near surface volume overcomes the object size restriction and can be used to whole fish. Recently the mobile NMR technique was demonstrated to be a potential on-site analytical method for *in-vivo* assessment of fat content in salmon (Veliyulin et al. 2005). On-line implementation can be feasible with a new magnet design based on unilateral magnet array allowing short measurement time (Marble et al. 2007).

Ultrasound is another measurement modality that has been successfully applied to quality control of foods with typical frequencies of about 10^6 Hz (wavelength $\approx$ 1 mm). The detection principle is based on the fact that at phase separation borders within the sample there are often significant physical differences in the parameters like density, elasticity and viscoelastisity, and the ultrasound waves are reflected, diffracted and scattered from such structures. The most well-known application of the ultrasound technology is *in-vivo* imaging for embryo studies under gravidity, which can also be used for muscle food investigation. Using high frequency ultrasound (*acoustic microscopy*) one can achieve a resolution close to that of the optic microscopy. This technique has been successfully used for detection of nematodes and bones in cod fillets (Hafsteinsson and Rizvi, 1987).

Freese and Makow (1968) have performed a number of basic experiments on '*whitefish*' and found that the measured at 6.9 MHz absorption coefficient of the ultrasound waves has decreased from 4.2 down to 3.6 dB/cm when fat content in the muscle increased from 7.5 to 11.5 %. Response from the low-frequency vibration (1-200 Hz) can be used to measure stiffness of the fish. In combination with the '*neural networks*' approach, this method was successfully applied for automatic classification of salmon regarding the rigor-mortis development (Berg et.al. 1997). The main disadvantage of the conventional ultrasound instrumentation is requirements of the physical contact between the detector and the object or contact through a layer of water

Computer vision is a relatively young discipline and its origins can be traced back to the 1960s (Baxes, 1994). Previous work in this research field includes many examples of using computer vision for inspection and grading of agricultural and food products, evaluation of different parameters in food and meat products, grading of fish according to species, and evaluation of different parameters of quality in fish and fish products.

1.4 Choice of the suitable sensor technology

From the previous discussion, it is seen that the operations of fish grading and determination of quality parameters in fish and fish fillets are done by human inspectors in two manners. They use both their own sense of vision and the knowledge base that originates from training and experience (grading, blemishes), or some kind of instrumentation that helps them measure the quality parameters manually, such is the case with the colour of fillets. In addition, the standards for quality in fish processing operations demand that automation would have to be compatible with the speed of the other machinery that is used in operations like gutting and de-heading of whole fish. With respect to the quality assurance, automation must also be non-destructive and must ensure the sheer speed at which the fish can be quality evaluated and sorted.

Out of the all aforementioned sensor technologies, it seems that, presently, NIR and computer vision fulfil most of the requirements when it comes to rapid, non-destructive and on-line evaluation. While NIR is mainly used for compositional analysis (Gunasekaran and Irudayaraj, 2001), such as fat (colour) and water content, computer vision has a broader spectrum of applications ranging from size/shape/colour grading to compositional analysis. In addition, cost, versatility, maintainability, ease of operation and setup (Chan and Palmer 1995; Zuech 2004) are factors that seem to favor computer vision in an on-line context.

Therefore, when evaluating the new technology, implementation of which could be used for automation of fish processing line operations, while at the same time fulfilling the above conditions, as the work progressed, it became clear that it was more efficient to focus on the computer vision as a single strategy for automation.

1.5 Previous work in Computer Vision related to Food/Fish Industry

Computer vision has been widely used for the inspection and grading of fruits. Kanali et al. (1998) reported that automated inspection of products not only results in labour savings, but it can also improve inspection objectivity. Paulus and Schrevens (1999) developed a computer vision algorithm to characterize objectively the apple shape. Computer vision has also been used to classification of oranges. Ruiz et al. (1996) studied three image analysis methods to solve the problem of long stems attached to mechanically harvested oranges. Nagata et al. (1997) investigated the use of computer vision to sort fresh strawberries according to size and shape. The experimental results show that the developed system had 94-98% of classification accuracy into three grades based on shape and three based on size. Nielsen et al. (1998) developed a technique, based on the applied fuzzy sets, to correlate attributes of size, colour, shape and abnormalities, obtained from tomato images with the inner quality of tomato samples.

Computer vision has been shown to be a viable inspection approach for grading of vegetables (Shearer and Payne, 1990). Heinemann et al. (1994) assessed the quality of mushrooms using a computer vision algorithm to grade the mushrooms by an automated system. Consequently, computer vision has been used to automate different operations

regarding mushroom grading and production automation (Reed et al., 1995). Some other earlier studies of applications of computer vision for inspection and grading of vegetables include grading of potatoes according to shape in order to ensure the sale of uniform potatoes classes for different markets (Tao et al., 1995) and grading an inspection of peppers according to colour and defects (Shearer and Payne, 1990).

Visual features are also in extensive use when performing quality evaluation of meat. McDonald and Chen (1990) pioneered early work in the area of computer vision based beef grading. Based on the reflectance characteristics, they discriminated between fat and lean muscle. Gerrard et al. (1996) examined the degree of marbling and colour in 60 steaks. Li et al. (1999) used computer vision to characterize beef meat regarding colour, fat marbling (based on the area) and texture characteristics. Methods based on processing of digital colour images have also been used for detecting defects in chicken meat (Barni et al,1997), quality grading of beef (Shiranita et al, 2000), grading of beef with respect to fat marbling (Yoshikawa et al, 2000), and crack detection in eggs (Patel et al, 1998).

The vast amount of work done in applications similar to those described appear to suggest that the field of computer vision in recent years has reached a level of maturity that is necessary in order to solve grading, inspection and processing tasks in the fish processing industry.

When it comes to the use of computer vision in fish, Tou et al. (1982) showed that different fish species could be discriminated from one another by using computer vision. In order to be able to discriminate sea fish species, Strachan (1993) developed algorithms to generate descriptors for shape and colour. The descriptors were functional even if the fish was taken picture in a deformed position. With this computer vision algorithm, 18 species of demersal and five species of pelagic fish could be sorted with 98-100% reliability. Jia et al. (1996) developed computer vision algorithms for automated processing of ‘channel catfish’ (*Ictalurus punctatus*) including the detection for fish orientation, identification of head and different fins. So and Wheaton (1996) used computer vision regarding automated opening of oysters. Gunnlaugsson (1997) made a review on how computer vision technology could be used in fish processing plants.

For automated classification of fresh water fish, Zion et al. (1999) developed a computer vision algorithm based on the moment invariants and geometrical parameters. This algorithm could sort three different species from one another. Computer vision in fish has also been used for grading herring row (Hu et al., 1998) and determination of the fat and connective tissue amounts in salmon fillets (Borderias et al, 1999). Although Borderias et al. did not achieve a good correlation in their work (R=0,44), the authors concluded that this method could be applied for on-line quality control of salmon (at least rough grading in different fat classes). Recently Marty-Mahé et al. (2004) have estimated the brown trout cutlet fat contents by automated colour image analysis in the CIELab colour space, while Stien et al. (2005, 2006) have used image analysis to study the colour composition as well as the rigor development of rainbow trout fillet.

1.6 Main contributions and other research

Despite the above mentioned reported work in the field of computer vision, there is still a lack of work which could be beneficial for employing this knowledge in automation of fish processing plants. Nonetheless, the uptake of the computer vision in fish industry has been slow. Although sorting of fish according to species (Strachan et al. 1990; Strachan 1993; Strachan 1994; Zion et al 1999; White et al. 2006; Zion et al. 2007) has been reported, there has been no work dealing with the quality grading of Atlantic salmon into different grading classes. There is also a lack of work on how computer vision could be used for quality evaluation of fish and fillets along the processing line, according to the existing industrial standards that describe important quality parameters, with the exception of few recent studies reported in the field (Marty-Mahe et al. 2004; Stien et al. 2005; Stien et al. 2006).

Therefore, in this thesis, the research has been focused on finding computer vision based solutions for the automation of several quality evaluation operations along the fish processing line. These include automation of grading/sorting of whole salmon according to quality grade, and presence of residual blood in the body cavity. In addition, the focus was also on developing computer vision methods for the quality evaluation of salmon fillets according to parameters of colour, the effect of perimortem handling stress during rigor and

ice storage on both colour, 2D and 3D geometry (salmon and cod), and the effect of bleeding. Parallel to developing solutions for automation of the above mentioned operations in the fish processing industry, showing that computer vision is well-suited for automation of fish grading and non-destructive evaluation of the quality of fillets, has also been one of the goals of the thesis. The review of the technologies, in the beginning of research, to find out which technology was best suited for use in automation of fish processing, can also be viewed as a separate contribution.

Main contributions in this thesis are:

- Design of a computer vision algorithm for sorting/grading of whole Atlantic salmon into quality grading classes.
- Computer vision algorithm for inspection of the body cavity of Atlantic salmon for the residual blood.
- Computer vision sorting of Atlantic salmon fillets according to their colour level based on the industrial Roche colour card standard for sorting/grading of fillets.
- Computer vision evaluation of colour changes of Atlantic salmon fillets during rigor and ice storage, and effect of bleeding.
- Computer vision identification of transient changes in 2D size and shape (length, area, width, roundness) and 3D (height, area of cross-section) of Atlantic salmon (*Salmo salar*) and cod (*Ghadus morhua*) fillets due to rigor contractions, during ice storage; effects of perimortem handling stress.

CHAPTER 2

Computer Vision in Fish Processing

2.1 Introduction

This chapter is intended to give an introduction of the basic concepts in computer vision and fish processing. This background information is beneficial for both communities and it is believed to be necessary to be able to follow the remaining of the material in this thesis. In this chapter, people involved in fisheries will gain the necessary knowledge of what computer vision is about and how it can be used for solving different tasks in the fish processing. In this context, the computer vision topics which will be covered in this chapter include operations such as image acquisition, illumination, camera selection, optics, calibration issues, image processing, segmentation, morphological operations, colour, as well as basic concepts from pattern recognition such as feature extraction and classifier design.

On the other hand, computer vision community will gain the insight on the fish and the fish products which are processed in the processing line. This will be helpful to understand certain operations along the line. For this community, it is important to gain basic knowledge about fish, mainly Atlantic salmon, and their visual appearance. In addition, topics like physiology, size, geometrical and optical properties of Atlantic salmon will be described. Fillet colour and other important properties of fillets will also be a part of this description.

2.2 Physiology of Atlantic salmon

Atlantic salmon (*Salmo salar*) is a cold water fish belonging to the family of salmonids (*salmonidae*) and is found in the northern part of the Atlantic Ocean. The Atlantic salmon available in the world markets is predominantly farmed. The salmon, which is the subject in this work, is present in Norwegian waters along the Atlantic Ocean up to the Arctic Ocean.

The Atlantic salmon (Figure 2.1) has a spool-shaped form and can be up to 150 cm long and 40 kg in weight (Pethon, 1994). The streamlined shape makes it easier for salmon to move through water. Including the tail, Atlantic salmon have eight fines and each of these fines has a different function. The caudal fin or tail is the largest and most powerful and it is used to push the fish forward. The other fins are used for steering or balancing. Fins of the farmed salmon are heavily reduced and crippled compared to the wild salmon.

The skin has also a layer of scales. These are small, hard plates that cover the body and gives it the necessary stiffness for protection. Scales (Figure 2.1) overlap to form a kind of armour plate to protect from predators and bruising. The scales are covered by a slime which can vary in thickness. The slime helps the salmon decrease the water resistance during swimming (Salte and Åsgård, 1986).

The lateral line functions like an ear. It can detect the pressure and vibrations in water. It consists of several liquid-filled canals, below the skin, along the entire length of the fish. This combines the aspects of touch, hearing and seeing.

During the life period, salmon change their appearance and the colour changes with age, resulting in very few black spots along the lateral line. In addition, sexual maturity of salmon can be estimated from the external colour appearance. In the case of a moderate sexual maturity, the head of the salmon is black (Figure 2.1) but in the case of a high degree of sexual maturity the colour of the entire salmon is predominantly heavy green.

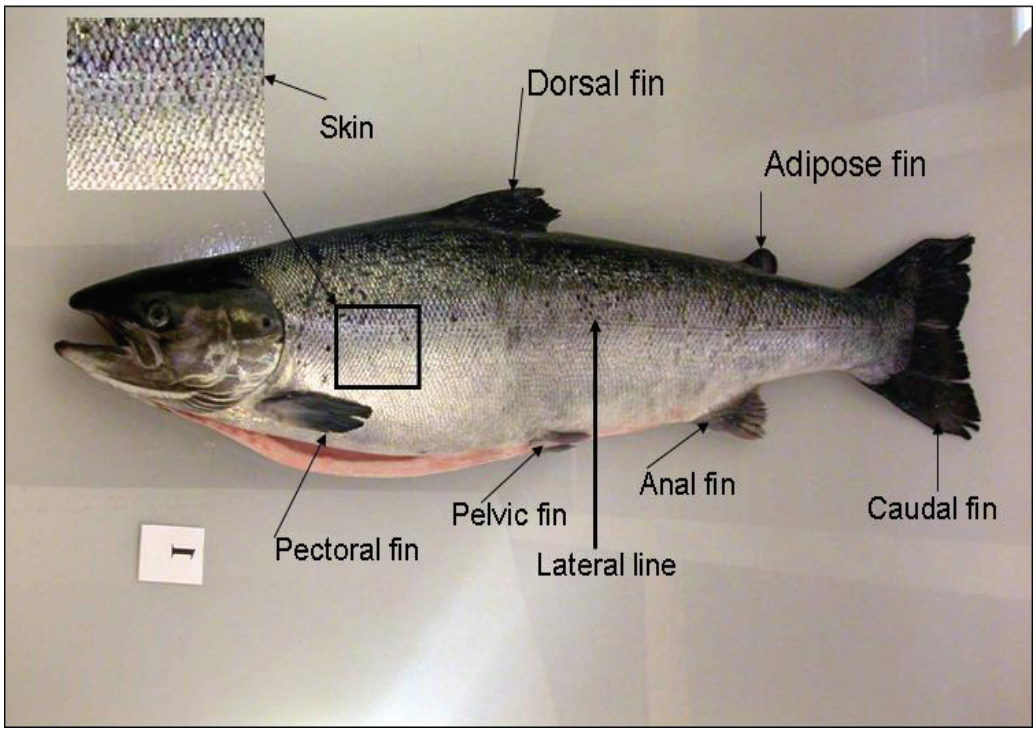


Figure 2.1 The physiological and morphological properties of Atlantic salmon

2.3 Processing of Atlantic Salmon

After gutting and deheading, the salmon are filleted. Salmon is a white-flesh fish and the colour of the flesh depends from the salmon diet, and usually varies from red to pink (Figure 2.2). The natural colour of salmon results from carotenoids (astaxanthin) (Sigurgisladottir et al. 1997). Astaxanthin is the chemical that gives krills, lobsters, shrimp, and to some crabs their red colour when they are cooked. Wild salmon get carotenoids from eating krill and other tiny shellfish. Farmed salmon have a diet with astaxanthin, along with the other essential nutrients.

Figure 2.2 Fillet after filleting of whole salmon. The flesh colour is reddish. The fillet is fully trimmed.

After filleting, fillets undergo the bone removal process and trimming. Trimming of fillets consists on removing peripheral fat along both sides. Depending on the requirements, there are different grades of trimming denoted with labels A, B and C. Fillet in Figure 2.2 has the C trim grade.

2.4 Optical Properties of Atlantic salmon

From a computer vision perspective, the knowledge about the optical properties of skin and flesh of Atlantic salmon is important. As mentioned, the skin is covered by scales and slime. From the optical point of view, the scales can be viewed as small mirrors (Bengoetxea, 1991). Because of the slime and the scale layer, Atlantic salmon is considered a shiny object in the computer vision. In addition, in processing line the fish are wet and this makes them even shinier. Flesh can be reddish for salmon or white for cod and it is not as shiny as the skin because flesh has a non-flat surface/structure.

2.5 Computer Vision

Every computer vision application is characterized by a set of operations such as image acquisition, image processing, segmentation and morphological operations. Though

necessary, these operations alone can not be used for fulfilling a computer vision task. At this point, the result of these operations can be a good segmented silhouette of a whole salmon or cod, or even a fine color-balanced fillet image. To be able to tell or classify which fish or fillet has a higher quality grade, application of concepts such as feature extraction, classifier and knowledge base is necessary. Therefore, a computer vision application involves not only image acquisition and image processing operations but also image analysis and image understanding as well as classifier algorithms which behave as a knowledge base (Figure 2.3).

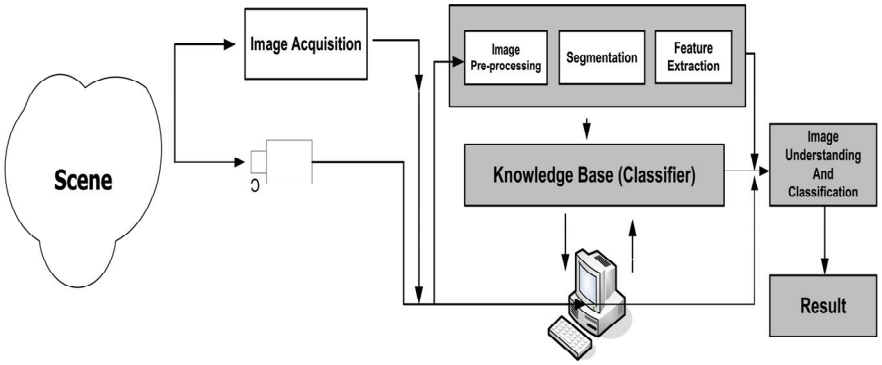


Figure 2.3 The structure of a typical computer vision application.

2.5.1 Image acquisition

Image acquisition step is critical. From this step depends the design of a successful computer vision application. If acquisition results in a poor quality image, then no matter how processing resources one can use, the possibility of enhancing the quality of the image is limited. On the other hand, images with little noise and with a uniform illumination can make the extraction of the necessary information from images much easier. High quality images can also save a lot of computational power since images need less pre-processing operations prior to the feature extraction. Important elements to take into consideration during the design of the image acquisition step are illumination, choice of a camera, optics and calibration of images.

2.5.1.1 Illumination

If image acquisition is a critical step for the computer vision application, illumination is critical for the image acquisition. In fact, a prerequisite for any computer vision application is that the features which are examined can be seen in the image (Pironen 1991; Panigrahi and Gunasekaran 2001). If the light is of the wrong type, installed incorrectly, housed inefficiently, or mismanaged in some other way, then the computer vision application is doomed to failure (Hardin 2004).

In the age of rapid advances in the processor speed and in camera production, one has tendency not to focus on the illumination (Novini 1993), relying too much on the computation power of hardware, high resolution images and on the complexity of image processing algorithms. This does not guarantee the highest quality of images and the best results. In fact, the focus should be the other way around. High quality images can be obtained if the illumination is improved and if it is designed in such a way that it enhances the image features for the subsequent analysis. In addition, proper illumination always makes the application cheaper since it reduces the processing time and hardware requirements.

Improper illumination may cause different problems to appear in images of the scene object. Blooming or hot spots, shadowing, interference of ambient light, non-uniform illumination and poor light are only some of the most typical effects of incorrect illumination. Hot spots and shadowing can make difficult the extraction of important features on the object such as colour and texture because these features are obscured. Non-uniform illumination and shadowing almost always cause problems during the segmentation of the objects from the background because the tresholding is difficult.

Important considerations during the illumination design are the type of light, illumination technique, control of illumination, and geometry of propagation (Zuech 2000). The type of light should be chosen so that it makes possible the best feature discrimination in the object. This can be achieved by using a light source which emits light in different wavelengths such as incandescent light, fluorescent, laser or light emitting diodes (LED). Control of illumination may include the blocking of light from the ambient.

Depending on the nature of features for subsequent analysis there are three main illumination techniques which can be used in the design. These are front lighting, backlighting and structured lighting (Bengoetxea 1991; Awcock 2000). In front lighting, the camera and the light source are on the same side of the object (Figure 2.4a) and this type is best suited when the surface of the object is the feature of interest. Backlighting is best suited for silhouette extraction of the object and for subsurface features. It does not allow extraction of any surface information about the object (Figure 2.4b). Structured lighting is a light source with the shape and form of its projected beam (Novini 1993) and it is mainly used for showing 3-D information about the object.

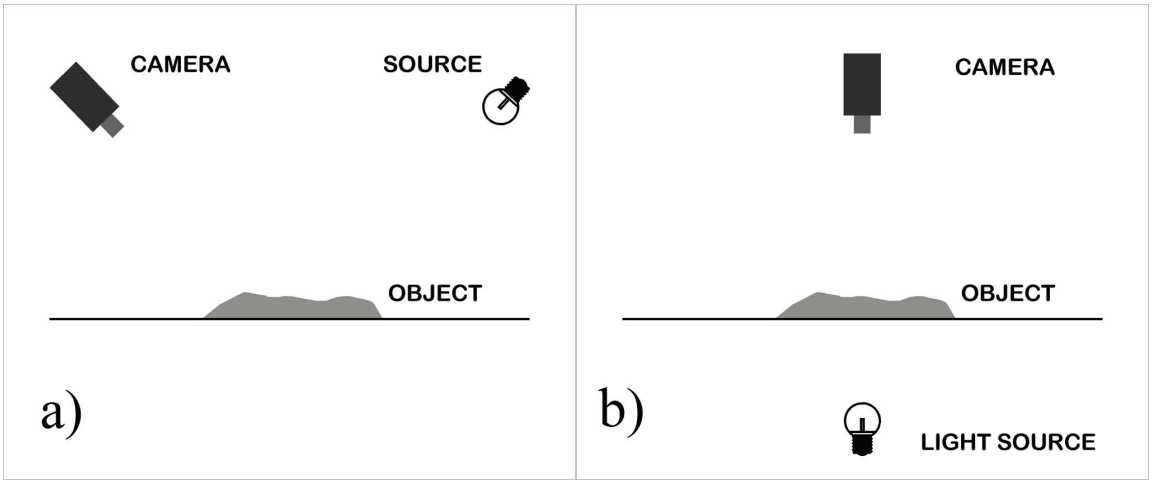


Figure 2.4. a) Front lighting arrangement; b) Backlighting arrangement

Geometry of light propagation is also an important illumination consideration. The geometry of light may be direct or diffuse. If the object is lit under even illumination conditions then direct light may be used for a computer vision inspection of any type of object. In case of shiny objects such as fish, in order to reduce the specular reflections from the surface of fish, diffuse light is preferred. This type of lighting enhances the surface features such as colour, texture or eventual damages while avoiding the generation of specular reflection which would obscure such information.

2.5.1.2 Camera and Optics

Camera and optics are other key considerations during the design of image acquisition stage. Camera is a sensor which is used to capture the image of the object of interest. The function of a camera is similar to the function of eye in human vision. Cameras should be selected based on resolution, geometrical precision, stability, spectral response, automatic gain control, signal-to-noise ratio and response time (Guda et al. 2000). Cameras can be of charged coupled device (CCD) type and of CMOS type. CCD cameras have nearly been used for all computer vision applications since their introduction almost 25 ago (Wilson 1998). CCD cameras offer superior image quality and flexibility to the expense of the system size and are therefore suitable for industrial applications. CMOS cameras offer superior integration, power dissipation and system size at the expense of image quality and flexibility (Litwiller 2001).

Cameras come in different resolutions and their choice is application dependent. If inspection of surface is needed, where colour and texture are the features of interest, then usually cameras with higher resolution are chosen. However, if analysis of shape of an object is needed it can be carried out with a lower resolution camera. Cameras are commercially available in many varieties of black-and-white and colour types. Here too, the choice is application dependent. To evaluate only shape and other geometrical attributes of objects, a black-and white camera could be appropriate. If colour of objects or other colour-related parameters are to be evaluated then a colour camera should be selected.

The four most basic parameters concerning optics in a computer vision system are the field of view, resolution, working distance and the depth of the field (Figure 2.5) (Fales 2003). The field of view is the size of the object to be inspected, while the working distance is the distance from the front of the lens to the object under inspection. The depth of field is the measure of the range of object distances within which the image appears to be sharp and in focus. The resolution is the minimum distinguishable feature size of the object under inspection. Numerical calculations of these parameters can be found in Zuech (2000).

Lens is another key element of camera optics. The function of lens is to project the image onto the camera sensor. Lenses have different optical qualities and not all of them

are suitable for computer vision applications. The quality becomes crucial when high quality images are required such as industrial or scientific applications. Lenses are usually categorized on bases of focal length and aperture. The focus is adjusted with ring adjuster which is integrated in the lens, in order to bring the certain region of object or entire object under focus. From the aperture of the lens depends the maximum amount that can pass through the lens. A small aperture makes everything in focus, while a wide aperture can make either background or foreground of the object come to focus.

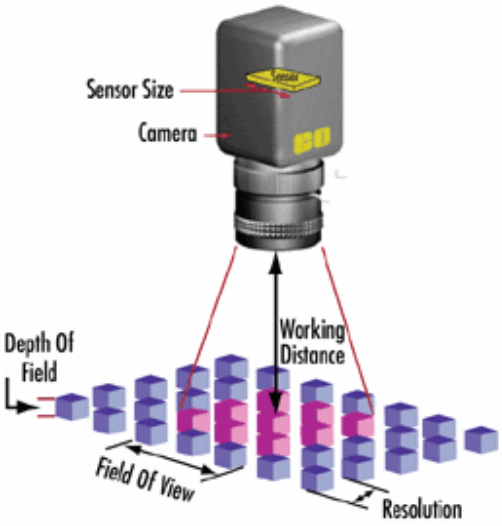


Figure 2.5. Definition of the most important parameters of camera optics (Courtesy of Edmund Optics).

2.5.1.3 Calibration

The obtained images may not always preserve with fidelity the true geometrical parameters and geometrical representation of the object under inspection on the scene. The images may simply be geometrically distorted. The distortions may be such that the images appear 'pin cushioned' or with a barrel (fish eye) shape (Figure 2.6) (Busch 2005). These distortions are caused by camera lens imperfections such as optical aberrations. Perspective distortion of images is another geometrical distortion caused by using a wide-angle lens (Lowrie 2005). Image distortions like these can cause problems in computer vision applications because it is necessary that in such applications images are true representations of the

object. Regarding imperfections and quality, during a design of computer vision application, one has to choose quality lenses.

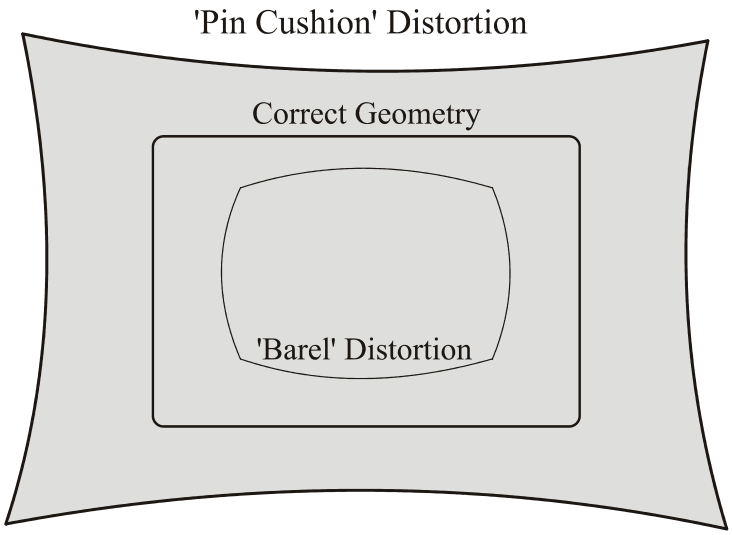


Figure 2.6 Geometric and perspective distortions of the image caused by lens imperfections or use of a wide-angle lens.

2.5.2 Image processing

Image Processing involves steps such as image preprocessing and segmentation. The purpose of pre-processing is to enhance the quality of images obtained by the image acquisition step. Images after acquisition are often degraded because of distortion and noise in the camera and optical system. Image preprocessing steps involves operations of noise reduction, contrast enhancement and smoothing, image sharpening and gray level correction and transformation (Sonka et al. 1999; Panigrahi et al. 2001; Gonzales et al. 2004).

2.5.2.1 Segmentation

Segmentation is the process of separating objects of interest from the rest of the scene or background (Zeuch 2000). Segmentation is usually performed based on two properties of image intensity values: discontinuity and similarity. The first approach segments the image

based on the abrupt changes of intensity such as edges (Gonzales et al. 2004). Two of most fundamental approaches used for segmentation are thresholding and region-based segmentation. Segmentation based on thresholding consists in choosing a value-threshold which separates the object from the background. In the simplest case, intensity values of pixels of an object and a background are in two different modes. By selecting a threshold that separates these two modes, it is possible to extract the object from the scene. In region-based segmentation, the aim is to partition an image into regions. This is done by using the approach of region growing and region splitting.

2.5.2.2 Morphological Operations

Morphology is a word that deals with forms and structures. In image processing, the mathematical morphology is used as a tool to extract image components that are useful in representation and description of region shapes (Gonzales et al. 2004). Two most fundamental morphological operations are *dilation* and *erosion*. Dilation is an operation that grows or thickens the objects in a binary image, while erosion shrinks or thins the objects in a binary image. The manner and the grade of thickening and thinning are controlled by a structuring element.

Opening and closing as more complex morphological operations are obtained as a combination of dilation and erosion. Opening is erosion followed by dilation, while closing is a dilation followed by erosion. Opening is used to remove completely the regions of an object that can not contain the structuring element, to smooth object contours and to break thin connections. Closing joins narrow breaks, fills long thin gulfs and fills holes that are smaller than a structuring element.

Morphological operations are used in pre or post-segmentation stage of the objects in a scene. Segmentation alone might not solely isolate the object of interest from a background. During segmentation and binary conversion of a gray scale image, it is usual that small imperfections appear in the image in the form of isles that do not belong to the objects. Morphological operations are used to remove these imperfections resulting in the isolated

object of interest. More on morphological operations the reader can find in Sonka et al. (1999) and Gonzales et al. (2004).

2.5.2 Feature extraction

After images are processed and segmented they have certain features or properties. These images are sent to a feature extractor, whose purpose is to reduce the image data by measuring specific image features or properties (Duda et al. 2000). In plain words, this means that the image of the object of interest has to be quantified with some feature values before it is processed further and before the decision making process takes place. Interesting features can be the length or the area of the object or any other size/shape parameters. After the relevant, non-redundant features (statistically independent) are selected and extracted, these are passed to a classifier (knowledge base) (Figure 2.3) for decision making.

2.5.3 Colour

Colour is an important property of objects in general. In food industry, colour is an important quality parameter. Colour is our perception, our response to the combination of light, object and human observer (Levkowitz 1997). Colour perception depends upon physics of light and complex processing by the eye-brain system. Regarding physics of colour, every electromagnetic radiation with a wavelength (λ) between 400 and 700 nm stimulates the human neurosensors and results in sensation of light. This band is the so called visible band of the electromagnetic spectrum.

Human receptors react only to some wavelengths and are more sensitive to some wavelengths than other. Human eye has three different types of cone receptors containing different chemical pigments sensitive to certain wavelengths (Shapiro et al. 2000). One type of cone is sensitive to blue light at 400 to 500 nm, the other type is sensitive to green light and the third type of cones is sensitive to the red light. This makes it possible that every colour can be represented as a sum of three independent stimuli: red (R), green (G) and blue (B) (Trussell et al. 2005), which are called colour coordinates. Based on this, a colour

imaging system represents colour information in terms of colour coordinates. The RGB (Red, Green, Blue) colour space is the most known system, but there are also other spaces based on specific colour coordinates such as CIELab (L-lightness, a-redness, b-yellowness) and HSV (H-hue, S-saturation, V-value).

2.5.4 Pattern recognition

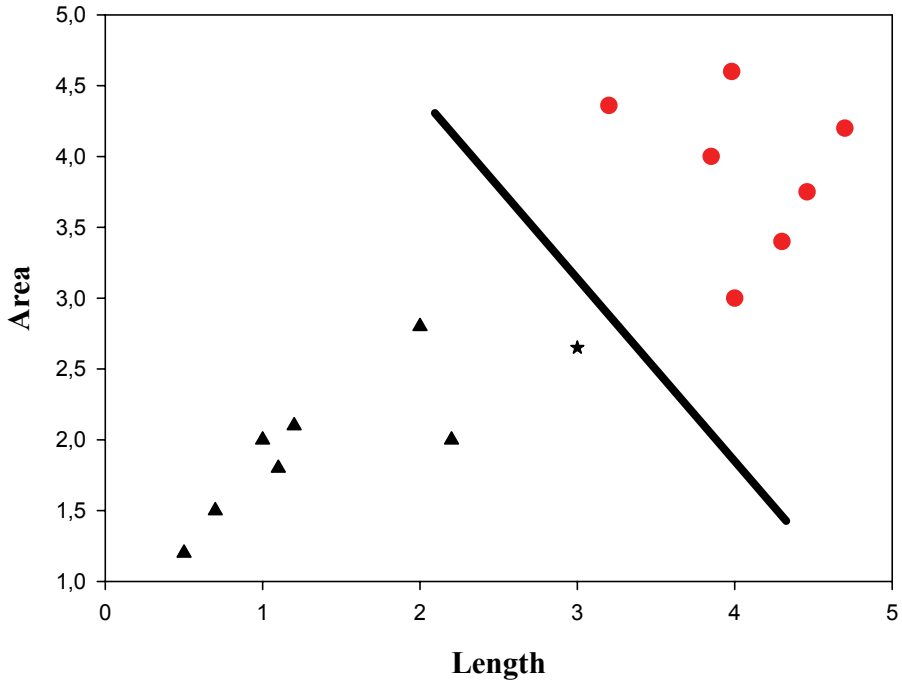
From the discussion above, it can be seen that image acquisition, image processing and feature extraction are not sufficient for deciding whether an object, for example, has deformities or not. None of these stages and operations is able to decide if a fish is qualitative enough or if it belongs to certain species or a class. These parts if computer vision helps only on capturing images and their analysis in order to produce the descriptors (features) of the objects that are imaged, without the ability to classify these in classes. In order to perform this, a decision logic or model is required. The decision making comprises of what is known as pattern recognition, which is "*the act of taking raw data and taking an action based on the category of a pattern*" (Duda et al. 2000). Thus, pattern recognition is a scientific discipline whose goal is classification of objects into a number of categories or classes. The objects, which are referred to as patterns, can be images or signals (Theodoridis et al. 2003).

The part that does the decision making and classifies objects into classes is simply called a classifier. The classifier operates in such a manner that it takes the feature vector consisting of object features, generated by a computer vision system, and on the bases of these classifies the objects into classes.

To illustrate the operation of a classifier let us assume that a plant wants grading of fish in salmon and cod. Prior to the design of a classifier, one looks at the features that might give a good separability between these two classes. If at the plant the production chief tells that cod is shorter in length than salmon and smaller in general than salmon, then two features which can be used for discrimination might be the length and the area of the fish. If the plot of area vs. length is made for both species, it is shown (Figure 2.7) that the class A

of cod spreads in a different region than the class B of salmon. In Figure 2.7, each point corresponds to a different image from the available database.

If then we are given a new image of fish (*) which is an unknown pattern, the natural thing to do is to plot the area of fish versus its length. From the plot, it can be assumed that the fish is more likely to belong to class A than B. This hypothetical example only illustrates some of the concepts underlined above.



Figur 2.7 Example plot of the area versus length for a number of images corresponding to class A (triangles) of cod and class B (circles) of salmon. In this case, a straight line separates both classes.

The measurements (area, length), which are used in the classification, are the features. These features are extracted after the processing of fish images and segmentation of only fish silhouettes. The straight line which is driven in the plot is what is known as a decision line and it actually constitutes the classifier itself. In order to 'enable' the classifier to classify unknown patterns, the classifier is trained with patterns whose class is already known. These training patterns are used to design the classifier so that when an unknown

pattern is fed, the classifier will ‘know’, from its previous knowledge, which class will this pattern to be assigned.

In the case of a good separability of classes, a linear classifier can be used for the classification. This practically means that if one plots the feature vectors of training patterns of two classes then these can be separated well enough with a simple line, as in the previous example. The reader can find more on different approaches of classifier design in Duda et al. (2000) and Theodoridis et al. (2003).

CHAPTER 3

Automation of Fish Processing Line with Computer Vision

3.1 Introduction

In this chapter, the common thread of the problems/operations, which were dealt with in the thesis, is given. The common thread is the fish processing line, mainly of Atlantic salmon but in the aspects of the quality evaluation of fillets, the same solutions could be applied also at the cod processing line. In this section, the chain of operations in a typical fish processing line will be shown followed by a brief description. The focus will be on the operations that are carried out manually by human inspectors using their sense of vision, but also on the other operations that have potential to be automated with computer vision. Therefore, in the following sections, the proposed solutions for the addressed operations of fish processing line, in form of methods and results, will be presented.

3.2 Structure of the fish processing line

The overall structure of a typical fish processing line for Atlantic salmon is shown in Figure 3.1. This represents the common thread for the research in this thesis, and the denominator of all the resulting papers.

From the Figure 3.1, it can be seen that fish undergo quite an intensive processing from their intake by pumping from the sea cage, at the fish processing plant, up to the filleting stage. A typical fish processing plant processes about 100 tons of fish (25.000 to 30.000 salmon) per shift (7h), where the Atlantic salmon has an average weight of 3-6 kg.

During transportation from the farm to the sea cage and during the pumping from the sea cage, handling has an important impact in the quality of fish products because it may stimulate undesirable effects in fish such as stress, which affects the quality of the end products (Erikson 2001). Trough pumping, the Atlantic salmon is sent to the so called live chilling tanks filled with a sea water, at a temperature of 4°C, and with a certain quantity of CO_2 in order to anesthetize the fish. The anesthetized fish is then slaughtered where the attention is focused on methods which have ethical considerations. Thereafter comes bleeding, which consists on tapping the blood from the fish body and this usually takes place in specially made tanks for this purpose. Here, the majority of blood is tapped (90%). Then, the whole fish is cut, gutted and inner parts of the fish are removed. This is done with machines which are already in use (Baader 142). Subsequently, fish are inspected for the remaining inner parts or residual blood in the cavity and, if necessary, they are sent to washing prior to undergoing the operation of grading. At this point, the fish is graded according to its quality class, where human inspectors use different visual features of fish to do this.

Grading operation is followed by the ripening of fish, a process of maturing of fish meat for easier processing. Then, the whole fish are deheaded and filleted by the existing machinery. However, there are a number of fish plants which do not deal with filleting at all, meaning they do sell only whole gutted fish. From the point of deheading and filleting begins the fish processing part, consisting of the set of operations which are carried out in fillets. In brief, in a typical Atlantic salmon fish plant, this is the point where two units of fish processing meet; the unit dealing with only slaughtering, gutting and grading of whole fish and the unit of filleting and operations performed in fillets.

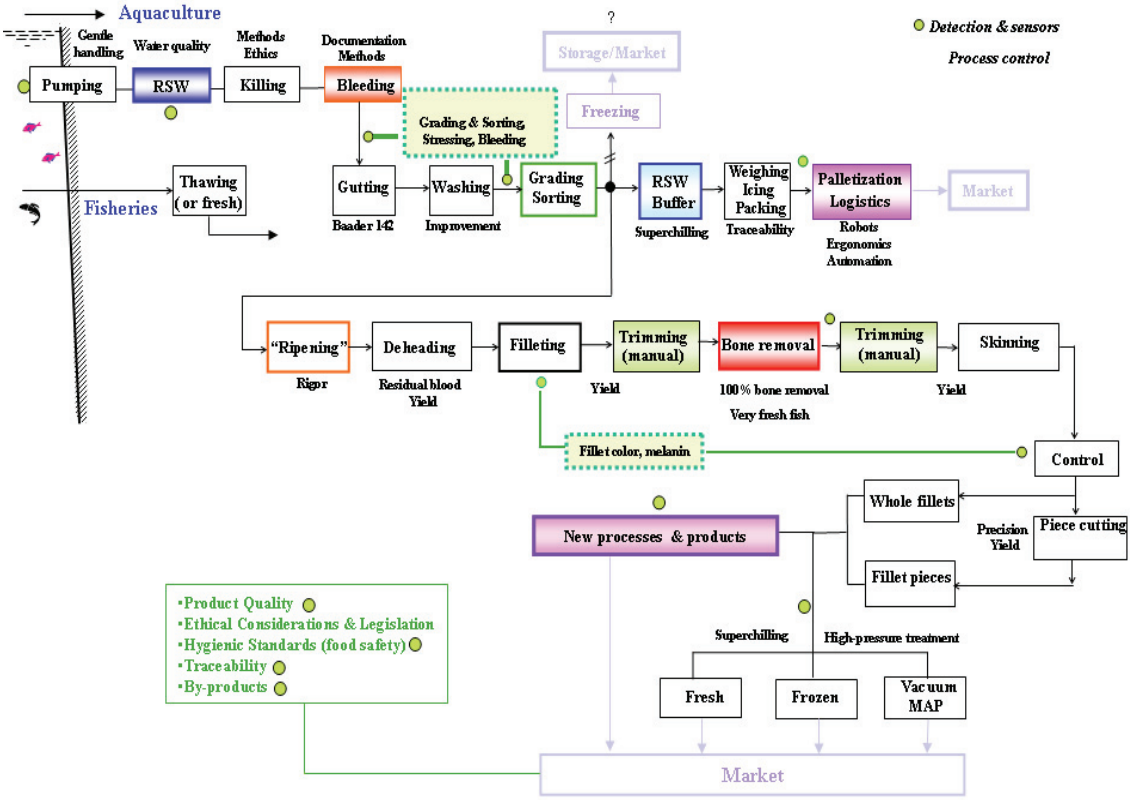


Figure 3.1 Overview of a typical Atlantic salmon processing line. Block diagrams with green colour represent points and operations in processing line where some kind of use of human sense of vision is used to perform certain operations.

Fillets are then trimmed in different grades of trimming. The most basic trim is done by the existing machinery. Then the fillets are subjected to bone removal and skinning which are also successfully performed with the existing machinery. From this point on, fillets are packed as a whole and subjected to other processing (freezing) and are ready for market delivery. Fillets can also be cut in pieces because some markets prefer fish in portion packages. Additional processing of fillets includes freezing or vacuum-packaging before the market delivery. A great deal of fillet production is also sold as fresh.

3.3 Presence of blood in the body cavity

After slaughtering, fish undergo bleeding. In the Norwegian salmon industry, the fish are typically bled for 15 - 20 min in a refrigerated seawater tank. Alternatively, the fish can be bled in air, preferably with head down. Appropriate bleeding is necessary for maintaining a good product quality at the end of the line. In the salmon industry, blood-spotting and discoloration as a result of inappropriate bleeding can cause decrease of fillet flesh quality and reduce the market value. These are some of the major causes for fillet downgrading (Michie, 2001). Therefore, good bleeding procedures must be followed.

3.3.1 Inspection of the body cavity for residual blood

After bleeding of whole fish in tanks, fish is washed and then sent to the grading/sorting operation. For quality grading, both external and internal attributes are inspected. In the latter case, possible presence of residual blood (adequacy of washing and re-washing after bleeding operation, potential blood spots or discoloured areas) is considered. This can be done by simply inspecting the body cavity of a whole fish by a human inspector. Thereafter, the need for washing can be decided depending on the presence of the residual blood in the cavity.

The proposed method for inspection of body cavity of whole fish for residual blood is given in paper 6 (Erikson et al. 2007e). Here, provided that there is a mechanism for opening of the gutted fish, computer vision can be used for evaluation of the presence of residual blood and adequacy of re-washing. According to the proposed method, by image processing techniques is segmented only the body cavity of the fish as a region of interest.

The image analysis is done by looking at the changes that blood causes into colour components of the region of interest. The analysis of images and classifier design is done in CIELab colour space, as it is known that this space is very close to the way humans perceive colour (Hunt 1991), and is considered as the best color space for quantification of colour in food products (Mendoza et al. 2006).

Presence of blood in the cavity will simply influence the colour features such as lightness *(L)*, *a* and *b* values, as well as the green (G) channel of the RGB space, for the

entire region of interest. A subset of only three features (*a*, *b*, G) was used to train the classifier, which was able to classify whole fish into "wash" and "ok" category. The "wash" category consisted of the fish with residual blood in the cavity, meaning that, prior to any further processing, such fish should be sent to re-washing, and the 'ok' category consisted of the fish with no residual blood. Such fish can be processed further without the need for re-washing.

The classifier was based on the Linear Discriminant Analysis. The predicted performance accuracy of this classifier was 92%, as cross-validated with the leave-one-out method, a well-established technique for assessing the classification performance (Ripley 1996; Theodoridis and Koutroumbas 2003).

3.3.2 Quality inspection of fillets as affected by bleeding, stress, salting and smoking

Residual blood in fillets may lead to reduced visual acceptance of the product (Kelly 1969; Huss 1995). The effects of inadequate bleeding are particularly pronounced in salted and smoked products like smoked salmon fillets (Robb et al., 2003). Although there is some disagreement as to what is the best bleeding method (Huss 1995), it seems clear that immediate bleeding of live fish is more important than the actual bleeding method (Roth et al. 2005).

Handling and peri-mortem stress is another important factor in bleeding. Bleeding delays the rigor onset in fish. When rested fish are exposed stressors and they show escape behaviour (white muscle work), the blood flow is gradually redistributed from the viscera to the locomotory muscles to meet the increased oxygen demand (Thorarensen et al. 1993).

In our study, various analytical methods were used to evaluate the quality of fresh and smoked fillets with respect to bleeding procedure and perimortem handling stress. No significant difference was registered between the bleeding methods. Salting and smoking, rather than handling stress and bleeding, induced the main effect on fillet color in our study. The smoked salmon fillets, exhibited a more yellowish appearance than the fresh ones, by having a larger Hue and *b* colour parameter. This was significantly different for all the

fillets regardless of the bleeding method. The proposed method for this type of fillet evaluation by computer vision is described in paper 6 (Erikson et al., 2007e).

3.4 Quality grading of farmed whole Atlantic salmon

Grading of whole salmon according to external attributes is based on shape parameters and blemishes. In the salmon industry in Norway, commonly two to four workers are necessary for manual fish grading and sorting (Figure 3.2), i.e. when biomasses between 80 -120 tons are processed per shift (7 h).

3.4.1 Sensory evaluation into grading classes

According to the Norwegian industry standard for quality grading of farmed salmon (NBS 10-01), the whole salmon can be classified into three classes: production, ordinary and superior.

Production grade salmon can be characterized by sexual maturity, sores, damages, bleeding fins, deformities, deformed jaw, crooked backbone, shortened tail, serious handling defects, scales scrapped off, and internal quality faults with significant amounts of melanin in muscles. However, the most significant parameters which characterize the production class and which appear most often are the crooked backbone, non-streamlined shape, and short tail (Figure 2.1).

Figure 3.2 Manual grading and sorting of whole salmon in quality classes by human inspectors. Two to four persons per shift are necessary to carry out the task (Courtesy of Salmar AS).

Ordinary grade salmon has a limited number of external or internal faults and is without substantial faults. However, it can be unsymmetrical, thick/broad in the posterior part, have sometimes a shorter tail and moderate external blemishes (loss of scale, minor damages of skin). Superior grade salmon is without external or internal faults. It is characterized mainly by a natural symmetrical streamline shape and skin without a loss of scales or open sores. In a typical farmed Atlantic salmon processing plant, if there are no specific problems with the fish, the Superior grade constitutes approximately 90-97% of the biomasses of fish which are processed.

3.4.2 Computer vision based grading of whole Atlantic salmon

From a computer vision perspective, some of the above mentioned characteristics can be quantified into shape parameters and, if necessary, colour descriptors. Size and shape of the Atlantic salmon can be extracted by segmenting the fish silhouette from the background.

Once having the silhouette, one can easily extract parameters such as, for example, length, area, width, and roundness.

Size and shape analysis is the essence of the proposed methods (Misimi et al. 2006, Misimi et al. 2007d) for quality grading of the farmed Atlantic salmon. In paper 1 (Misimi et al. 2006), size and shape analysis was used for extraction of parameters such as length of fish and tail, width, area, and aspect ratio. These parameters were used for designing a linear classifier based on linear discriminant function analysis-LDA which was able to grade between production and superior/ordinary class with a reliability of 87%, for the given data set. In paper 5 (Misimi et al. 2007d), a LDA-based classifier was designed in order to perform quality grading, between the ordinary and superior class, with a predicted performance accuracy of 91% for the given data set. The algorithm can be extended also for the case of the multiple classes, if such classification would be necessary. In such a case, instead of generating a threshold $y=t$ which would divide class C_1 and C_2, the focus would be on designing a single K-class discriminant y_k, where the sample x would be assigned to class C_k if $y_k(x)>y_j(x)$ for all $j\neq k$.

In general, when it comes to geometry of the fish, we found that the production grade salmon has usually a deformed back, as the most often deformity, while the ordinary grade salmon differs from the superior in having a broader back (posterior) part and/or shorter tail (Misimi et al. 2007d). These seem to be the most important geometric features to be considered when classifying the farmed Atlantic salmon into respective quality grades.

3.5 Quality assessment of fillets

After filleting of salmon, the fillets undergo the operations of trimming, bone removal and skinning. In this chain of operations, a quality evaluation of fillets is necessary. Before defining the quality parameters of attributes by which the quality is measured or quantified, it is necessary to define what is usually meant with the quality of fillets.

Quality is defined as a description of the product that meets the needs or requirements of consumers (Sigurgisladottir, 2001). This description is assisted by taking into consideration different characteristics of the products. The most important parameters of fresh Atlantic

salmon are colour, fat, texture and freshness. Freshness is a more complex and not a standalone attribute as it is usually determined on the basis of colour and texture. Actually, in this way the freshness of salmon is perceived by consumers. Other important quality parameters of salmon are the presence of discoloration, in form of either bloodspots or huge colour variations in fillets, white stripes as well as defects such as melanin spots (Koteng, 1992).

3.5.1 Colour evaluation of fillets in fish industry

It is generally accepted that colour of salmon fillets is one of the most important quality parameters (Anderson, 2000). Consumers usually perceive the colour of the farmed Atlantic salmon to be related

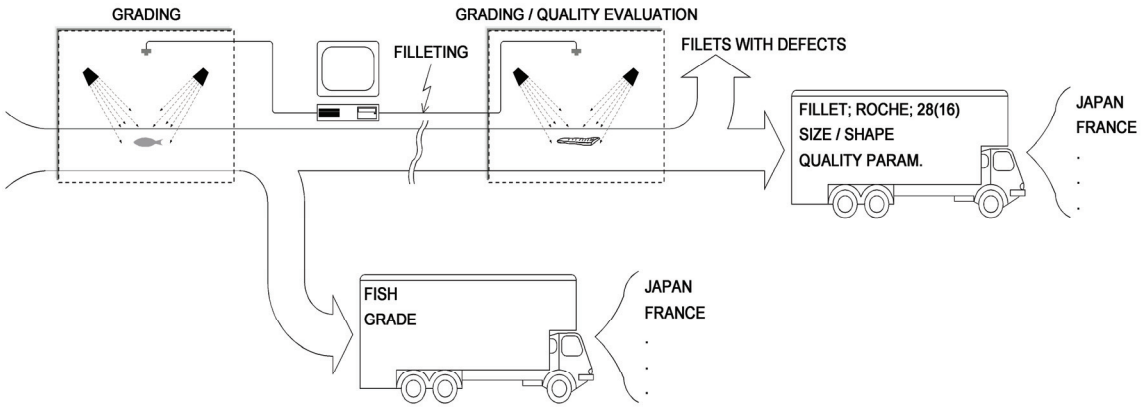


Figure 3.3 The description of the computer vision methods developed in this thesis and flow chart of the fish processing operations that these methods have potential to automate.

with such characteristics as freshness, flavour, quality and price. While colour is important for quality perception and evaluation, consumer taste is different when it comes to what colour is "best", or which colour "ensures" better quality. Consumers, in different markets in the world, have different expectations about the salmon colour. While consumers in Japan prefer a more reddish salmon colour (Osland, 2001), consumers in Norway or France

most certainly have other colour preferences (Figure 3.3). This makes the colour play an important role when evaluating the quality of the product at the ready-for-sale point. It also introduces the need for sorting/grading of fillets according to colour in order to categorize shipments on the basis of the markets they are intended.

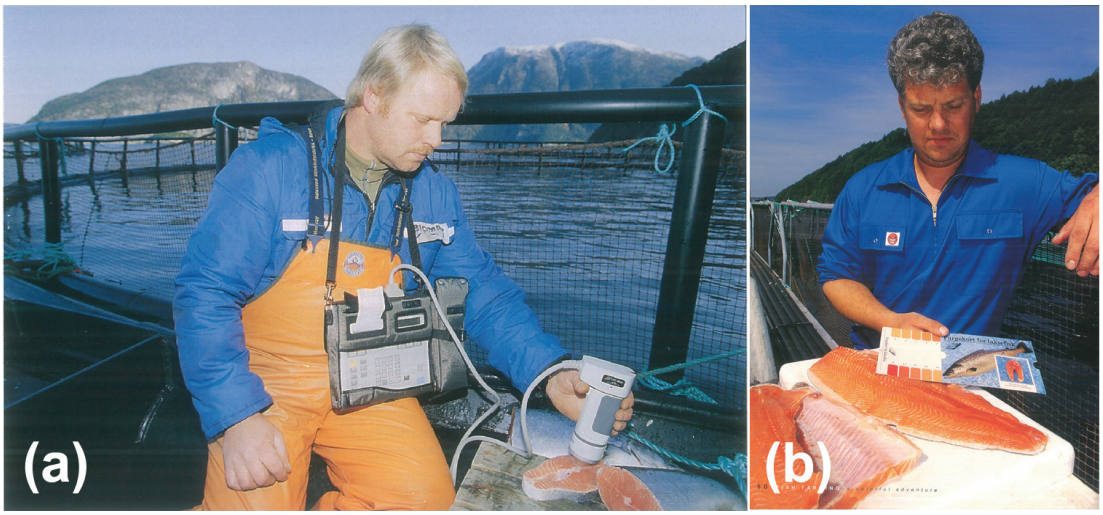


Figure 3.4 a) Use of Minolta chromameter to measure colour b) Use of human vision for comparison of salmon flesh with the color card scores. (Both figures adopted from *Fish Farming-a colorful adventure*, with the permission of Vidar Vassvik).

Today, the colour evaluation of fillets is done in several different manners, including the chemical analysis of some few batch samples of fillets. According to the Norwegian industrial standard (NS 9402, 1994), the colour of Atlantic salmon is measured by using the Roche colour card for salmonids or Roche SalmoFan™ (Hoffman-La Roche, Switzerland). This can be done manually, by using the human sense of vision (Figure 3.4b). Human inspectors can perform colour matching of some samples of salmon fillets against the mentioned Roche cards and obtain the average colour for the given batch. Alternatively, the colour of salmon fillets, for a limited number of samples, is performed using some kind of spectrometric equipment such as Minolta chromameter CR200/300/400 (Figure 3.4a), some other spectroscopic instruments, or the samples are sent to laboratory for analysis. The measurement with chromameter, for example, is done for a few fillets, so that an average colour for the batch of fish, which is processed, is acquired. The measurement head of the

instrument has a measuring area of only 8mm, which raises the issue of how representative the measurement is for the entire fillet. Measurements in the fillet, when using Minolta chromameter, are usually taken in more than one place in the fillet. Three such measurements can be taken on the back region of the fillet, over the entire length, and two other on the belly region. The average colour for the fillet can then be obtained as the mean value of the N measurements:

$$M_{Col} = \frac{1}{N}\sum_{i=1}^{N}\mu_c \tag{3.1}$$

For a given batch of K samples of fillets, the average colour of the sampled fillets is calculated as the mean value of fillet average colours (3.1):

$$Batch_{Col} = \frac{1}{K}\sum_{j=1}^{K}M_{Colj} \tag{3.2}$$

Although this may sound as an objective colour measurement method, this manner of quantification of colour of fillets has several disadvantages. Not only it does not give the best representation of colour of sampled fillets, due to measurement in a very tiny area of 8mm, but it also gives an unreliable measure for the colour of the processed batch. Colour of salmon, even from the same batch, varies. In the same batch, variations of colour from one fillet to another may be large. In addition, when we performed measurements of fillet colour, the low Minolta *a* and *b* values suggested the fillets had a somewhat grayish appearance, when in fact the Atlantic salmon fillets are of orange/red appearance (Erikson et al. 2007b). This can be explained by the fact that the Minolta Chroma Meter is primarily designed for flat, nontranslucent and diffuse surfaces, opposite to what a fillet is.

3.5.2 Computer vision based sorting of fillets according to colour

The proposed method for automation of the fillet colour evaluation based on computer vision is presented in papers 2 and 3 (Misimi et al. 2007a; Erikson et al. 2007b). This method has three fundamental characteristics which make it the natural solution choice when considering online automation of this operation in a fish processing line. It has the

necessary speed to cope with time requirements of a processing line, the necessary accuracy for colour evaluation, due to the colour calibration, and *all-in-one* concept of the colour evaluation.

The *all-in-one* concept means that from a single fillet image, one can get all the necessary data about the fillet colour in the form of its 1). class colour category according to Roche colour cards and in the form of 2). *Lab* values in the CIELab colour space. Additionally, the flexibility for integration and compatibility with the other quality evaluation units based on computer vision along the processing line is another advantage.

The algorithm for colour matching, described in paper 2 (Misimi et al. 2007a) and paper 3 (Erikson and Misimi 2007b, in press), was based on the nearest neighbor principle. From the Roche *SalmoFan*™ ruler and Roche colour card were created the look-up tables with the colour values, which the algorithm uses for the colour matching. The colour score of the fillet is then set by finding the closest match from the look-up table for the given pixels of the chosen fillet region of interest, or for the average value of a small region.

The results from the computer vision colour evaluation correspond well with the sensory evaluation of colour by human inspectors. The generated computer vision colour scores according to both Roche cards are, in average, for one unit higher than those perceived in sensory evaluation. Nevertheless, the standard deviation of the measurements with the computer vision method is smaller, indicating a more consistent measurement. In addition, the better side of the computer vision method of colour evaluation is that it is faster, robust, consistent and cheaper than the traditional methods.

3.5.3 Effects of ice storage and stress on colour

Filleting of whole fish is not done immediately after the fish are gutted and deheaded as pre-rigor, despite that there is a chain of connected operations and tasks throughout the line, as shown in Figure 3.1, between the slaughtering and grading unit and filleting and quality evaluation unit. Usually there has to pass some time so that the bones are easily removed from the fillets and whole salmon are put in tanks for some hours prior to the start of the filleting stage. This is the so-called post-rigor processing of fish.

In the meantime, even before the killing and gutting, there are some processes that influence the overall quality of fillets at the ready-to-sell point. The way the salmon is handled, the rigor process that starts after killing and ice storage of fillets may significantly change the appearance and quality of the product. In fact, Robb and Harris (1997) have shown that the fillet colour perception depends on peri-mortem handling stress. They found that fish that were stressed during the handling stage, showed one unit of Roche colour card readings more than those of unstressed fish as determined 24 h post mortem. This difference persisted when measurements were repeated four days later, in post-rigor stage.

Rigor mortis process in salmon may also have an impact in quality parameters of fillet. It has been shown previously (Sørensen et al. 1997, Skjervold et al. 2001) that during the rigor process, the geometry of fillets changes. Fillets during rigor shrink and this shrinkage is irreversible. Since fillets or whole salmon/fish usually are stored in ice, when they are not sold as fresh products, it was interesting to see if ice storage, in addition to handling stress and rigor mortis process, has any influence in quality parameters of fillet such as colour or geometry.

Development of a computer vision method that would be able to perform such evaluations has been a problem of investigation in papers 3 and 4. With the proposed method, from a single fillet image, we were able to quantify the colour as well as the 2D geometry of the fillet. Additionally, by the use of the 3D scanning with the 3D camera we were also able to obtain information on the 3D structure of the fillet and its changes during the rigor and the ice storage.

The main result from the development of the computer vision method for a colour evaluation of Atlantic salmon fillets (Erikson and Misimi 2007b, in press) is related to the change of colour of fillets during the ice storage. As the rigor progresses, the colour of the fillets changed. Therefore, any colour grading of fillets in pre-rigor would not correspond to the actual colour of fillets in post-rigor, when they reach the market, resulting in erroneous colour labelling of fillets. While the sensory panel was not able to detect any significant differences in the colour between the groups of stressed and unstressed fillets, after a week of ice storage, the computer vision method exhibited significantly different colour scores

($p<0.05$) between the two groups. It was concluded that, the evaluation of the colour of the fillets by either sensory analysis or manually based instrumental methods has disadvantages compared to the computer vision method. The drawbacks of the sensory analysis are that the method is slow, costly and subjective. The subjectivity and inconsistency of the sensory evaluation probably derived from the physical limitations of the human eye to adequately perceive colour (eye fatigue and lack of colour memory). Manually-based instrumental colour analysis, on the other hand, is labour intensive and not fast enough to cope with the required processing speed. Another typical limitation of such instruments is that they are based on direct contact with the fillet and the obtained colour values result from a small sample surface, and are usually meant for use in measurement of the colour of flat and non-translucent surfaces. On the other hand, computer vision method allows fast, nondestructive and contact free colour assessment. Therefore, the computer vision method evaluation of colour of fish or fillets was considered a method of choice for an on-line colour grading/classification.

3.5.4 Effects of ice storage and stress on 2D and 3D size and shape of fillets

The transient changes of 2D and 3D geometry of the fillets were easily quantified with the proposed computer vision method described in paper 4 (Misimi et al. 2007c). During the rigor and ice storage, fillets underwent changes in length, area, and width as well as height and cross-section area. Pre-rigor fillets and post-rigor fillets of Atlantic salmon and cod after ice storage of seven and three days, respectively, significantly differed in a number of these parameters. The proposed computer vision method was able to quantify these changes.

For Atlantic salmon, we found that pre-rigor filleting resulted in average final contraction in length of 10.0% and 7.3% ($p<0.05$) for unstressed and stressed fillets, respectively. The mean fillet areas had contracted in average for 6.4 and 3.0% ($p>0.05$), respectively, compared to their original pre-rigor area. For Atlantic cod fillets, the respective contractions in length and area, at the end of the ice storage, were at 14.5 ($p<0.05$) and 11% ($p>0.05$).

Perimortem stress did not affect the maximal width changes in salmon fillets and roundness of the cod fillets, only a phase displacement did occur. However, the perimortem stress affected the roundness of salmon fillets and the width of cod fillets. The maximum width increase for salmon and cod were 4 ($p<0.05$) and 6% ($p<0.05$), respectively. When it comes to roundness, the average final change due to contractions was 11.5 and 6.7% in salmon (unstressed vs. stressed), and 16.6 and 18.0% in cod (unstressed vs. stressed).

As for the cross-section area and height of salmon fillets, both of these parameters at the end of ice storage had decreased significantly compared to their initial values (height 5mm in average, cross-section area 15% smaller in average, $p<0.05$). Regarding the effect of perimortem stress on the fillet height and cross-section area, ate the end of the ice storage, the stressed fillets were significantly thinner (height:16% or 4mm thinner, area 20% smaller). In cod fillets, in contrast, despite the relative increase in cross-section area and height, at the end of the ice storage only the stressed fillets (at profile A) were significantly thicker compared to their initial values. When it comes to the effect of the treatment on the fillet height and thickness, no significant differences were observed between the unstressed and stressed cod fillets.

While the changes in Atlantic salmon fillets were more emphasized between the unstressed and stressed groups, in Atlantic cod fillets no significant difference between the groups was registered except for the width. In the Atlantic cod fillets, the stressed fillets showed also a more rapid rate of contractions compared to the unstressed fillets, but not to the degree that was observed in the Atlantic salmon fillets.

To sum up, the differences in geometrical features of unstressed and stressed fillets during the rigor contractions and ice storage indicated that the fillets changed size and shape and ended up with a different size and shape compared with their initial geometries. Perimortem stress resulted in a different ultimate size and shape of the Atlantic salmon fillets after ice storage. The industrial relevance of the method is that is quantifies the transient changes of standard parameters of the fillets as a result of biochemical changes during rigor and ice storage.

CHAPTER 4

Discussion and Reflections

Although a discussion is included in each paper, the aim with this section is to discuss and reflect on some aspects of the research in the thesis. Specifically, the focus will be on those aspects which have been important for the experimental design and the consequent processing of the resulting data. This discussion will complement the discussions already found in the papers. A natural start is to summarize the discussion regarding the overall contribution with this thesis. In the previous chapters, it was shown which tasks of the fish processing line were chosen to be addressed. The proposed computer vision based solutions for these tasks contained the individual contributions of the research. The combination and integration of these individual contributions can be used for automation of the addressed operations in a typical fish processing line with the use of computer vision.

4.1 Review of technologies

In the introduction chapter, a review of the available sensor technologies was made and the choice of the computer vision as a single strategy for automation was elaborated. The advantages of computer vision regarding cost, on-line use, maintenance, non-destructive/non-contact evaluation, and versatility influenced this choice. In addition, the

possibility for simultaneous grading of fish and/or fillets, according to a number of different criteria, was pointed out as one of the primary advantages of the computer vision against the other technologies. Therefore, since the computer vision was chosen as the single strategy for automation, this excluded, at the time, a number of other sensor technologies that may otherwise be suitable for use in some of the tasks, mainly for a compositional analysis, such as Near Infra-Red (NIR) (Heia et al. 2007), or Magnetic Resonance Imaging (MRI) (Veliyulin et al. 2007).

These conclusions were preceded by a careful analysis of a number of sensors and technologies that may have potential for use in automation of fish processing operations. This review was done at the start of this research. Despite good on-line response, the dependence on calibration procedures prior to use (Wiedemann et al. 1998), presence of broad-superimposed bands and low absorption intensities (Zanola et al. 2005), expensive instrumentation as well as more difficult maintenance, compared to computer vision, were some of the disadvantages of the NIR technology. In addition, NIR is mostly suitable for compositional analysis such as, for example, fat and water content (Wold et al. 1996, Solberg et al. 2003).

Ultrasound and MRI have limitations in coping with the real-time requirements of fish processing line. Although MRI possibilities for exploiting it as an on-line sensor in the future are great, there are actually no possibilities to do this at the present (Hills 1998), because the measurement time (speed) is too long. In addition, the equipment is very expensive and there are a number of factors and physical constraints that make this technology not suitable for industrial environments such as the delicacy of the equipment, as well as highly demanding and expensive maintenance.

Restrictions of the fish processing line regarding speed are that a fish/fillet should be evaluated or assessed within maximum 2 seconds, preferably within 1 second. Hence, computer vision was considered suitable for this speed requirement as it offers the

advantage of the accurate quantification and rapid image and data handling (Panigrahi et al. 2001). In addition, the flexibility of computer vision when it comes to the possibility of eventual integration/fusion of other sensor technologies in a fully automated fish processing line make computer vision as a natural choice for fish/fillet grading according to a number of quality parameters.

This flexibility for fusion of sensor technologies becomes even more realistic since computer vision is about finding methods to process and analyze images of fish products. Therefore, the integration of other technologies which have images as an output would not be a major challenge, as soon as the sensor technology becomes available for on-line use. In this regard, particularly MRI and ultrasound have prospects to be a part of such fusion of technologies. Furthermore, computer vision systems show a high flexibility when it comes to object type which is being inspected (whole fish or fillets of different fish arts), which could be seen as another advantage (Low et al. 2001).

Perhaps the biggest advantage of the computer vision over the other technologies is its versatility; the ability of computer vision to identify the individual fish/food products and to evaluate and grade them according to several different criteria such as size, shape, colour, and texture. This means that a computer vision-based system which is grading whole fish can very easily be reprogrammed to grade fillets or other fish products, or it can even perform grading at two points in line of different products. These aspects contribute to the high degree of flexibility.

Therefore, the aforementioned arguments favored the choice of computer vision as a single strategy for automation, while not excluding the other technologies that might become available in the future. With the benefit of the hindsight, it is easy to see that we could have spared an entire year if we had focused on computer vision alone from the outset of the research. Nevertheless, the review and analysis of all the available technologies were necessary so that we could choose the optimal technology for us to study, which can be used for automation of the fish line operations.

One thing that needs to be emphasized is that despite the advances in the recent period, there is still no computer vision technology available that can fully match the qualitative

and interpretative ability of human vision (Chan and Palmer 1995; Jahne 2002). But in a situation when quality grading of, for example, whole Atlantic salmon is very repetitive and error prone task, the computer vision has the potential to perform this task just as good, or even better than human inspectors, and with undoubtedly higher consistency. A computer vision based system would not be influenced from the human fatigue or boredom and can be very consistent once the grading parameters are set (Zuech 2004; Misimi et al. 2007a).

4.2 General remarks

During the work in this thesis, it has been noted that planning of experiments ahead in time had a valuable positive impact on the flow of the experiments that were carried out. Nevertheless, some of the problems which came up during the experiments were of such a nature that we were unable to affect in advance. Some of the noted difficulties were related to the fish/fillet delivery from the processing plant and fish/fillet sampling at the site. In addition, some practical problems that were encountered during the image acquisition and processing stage will be pointed out.

4.2.1 Fish delivery and sampling

Fish delivery and fish sampling was highly dependent on the daily catch. Boat-loads of fish, in variation to season, day, and farm, can be different when it comes to fish size and weight, fish quality, class membership and other external and internal parameters as elaborated previously. In this regard, we found out that it is not always possible to acquire a uniform distribution of fish with respect to the desired features. In addition, fish processors tend to classify most of the fish as “Superior”, in order to maximize the profit.

Our observation is in line to what Sørensen (2003) has reported earlier. He points out that the fish industry and various fish producers have the tendency to interpret the industrial standards in a voluntary manner when performing the quality grade labelling. Moreover, they tend to adopt relative sorting/grading criteria depending from the quality of the

incoming batch of fish. For example, we experienced that manual labelling of fish with regard to their quality class membership was done a bit differently when comparing two leading fish processing plants (samplings at Marine Harvest in 2003 and samplings at Salmar AS in April 2006), especially when it comes to the grading into "Ordinary" and "Superior". There could be a number of reasons for that, since the manual grading process depends from factors such as the season, batch of the day, and subjectivity of the different human inspectors who perform labelling at different shifts. These factors contribute to an inconsistent manual grading of fish regarding quality, which is another disadvantage of this process apart from incurring higher production costs. Computer vision grading as a technology for automation will therefore introduce a more consistent way of grading, since it will avoid the classification of fish whose appearance and quality is inconsistent with the industrial standard. Once the grading standards have been established, they will be consistently applied in grading, unlike human inspectors who tend to be subjective when doing so.

Regarding the classifier, one should investigate the possibility for such a classifier design which is able to be tuned for the choice of certain quality parameters from the group. This would practically mean that, after a large enough database of images for the training set is obtained, the operator could be able to tune the classification according to the desired parameters. In this way, it can be possible to reduce the variability of the cost of classification (Misimi et al. 2007e), which also depends from the ability and readiness of fish processor to accept that cost. Although the possibility for tuning of classification according to the classification cost and according to certain quality parameters can be seen as arguable, one has to take into consideration the fact that one should be able to offer solutions for which the fish industry would show readiness to apply in their sorting/grading operation.

Sample sizes used for the purpose of the research in this thesis could arguably have been larger. A larger sample size would be preferable but it is not always practical, and

economical. Limitation to the reported sample sizes is partly due to the high cost of the experiments of this nature in the industry, and limitations that the industry sets for the number of the extracted fish/fillet samples from a single batch. The main idea was to develop methods that can be used for automation of fish/fillet quality grading. On the other hand, the used sample sizes were within the standard size reported in other research experiments of this nature with fish/fillets or food samples which do range from 15 to 60 individual samples (Jerret et al. 1998; Skjervold et al. 2001; Marty-Mahe et al. 2004; Mendoza et al. 2004; Panigrahi et al. 2006; Stien et al. 2006; Heia et al. 2007). Therefore, a proper utilization of the images from the existing data sets was important, especially where the development of the classification algorithms was involved. To avoid overtraining and overfitting, we used a minimal number of the relevant features as well as we avoided the overly complexity of our classification model. While a complex classification model, and with a large number of features, may result in perfect classification performance of the training samples, it is unlikely to perform well on new patterns. This is known as overfitting (Duda et al. 2001). In addition, we applied a full cross-validation in the form of the leave-one-out method (Ripley, 1996). With this method, each fish sample in the data set was left out in turn as a test sample, while the remaining (N-1) samples were used as a training data. This was repeated for each fish sample in the dataset.

4.2.2 Image acquisition

The image acquisition has been one of the most challenging steps during the experiments, and especially the choice and the design of illumination has been critical for the quality of the obtained images. From the beginning, we experimented with different setups of illumination, in order to be able to obtain the most optimal quality of images. Therefore, as the work progressed, we attained better illumination solutions for quality evaluation of fish and fillets by computer vision. As elaborated in chapter 2, fish and fillets are shiny objects from the optical point of view. Because of this, the presence of specular reflections and hot points made the image processing difficult when using particular illumination setups.

The illumination setup, presented in paper 2, was adapted from Papadakis et al. (2000). This setup used 4 fluorescent lamps as light sources and gave satisfactorily results for a number of investigations. However, to ensure a more uniform and diffuse illumination it was concluded that this could be best achieved by using a light-box in which the light was confined in a limited space. The light-box consisted from two fluorescent tubes as light sources, had one camera opening on the top, and another opening for placing of fish/fillets to be photographed. The structure of this light-box is depicted and explained in paper 4. The opening of light-box was closed during the acquisition of fish/fillet images. In this way, the light-box cut out the interference of the ambient illumination which can introduce illumination non-uniformities. In all of the experiments that were carried out, to achieve stable illumination and camera conditions, the light-box and camera were switched on at least one hour before the experiment and were not switched off until the experiment was over. It is known that fluorescent tubes take some time to warm-up and to reach their full output, and stabilize their colour temperature (McDermott et al. 2007).

Further improvements in the illumination setup can be achieved if LED arrays would be used as a light source. LEDs would provide for a more even illumination. In addition, the output, size, low power consumption, lifetime, and stability make them the light source of choice in computer vision applications (Telljohann 2006, McDermott et al. 2007). Although relatively low-cost, the price of LED arrays is still higher than of fluorescent tubes and presently this may affect the availability to industry or research communities.

Type of illumination can be application-dependent. The more detailed the investigation is, the higher the requirements for the illumination will be. Colour inspection, for example, requires more uniform and diffuse illumination, contrary to the requirements when only isolation of an object from the background, by segmentation, is needed. In applications involving both of these operations, such is the inspection of fillets, the illumination must be as much uniform and diffuse as possible. In this research, confining the light into the light-box and avoiding interference with the ambient light helped in achieving the uniformity of illumination. The diffuse lighting was achieved using grey colour paint on the walls of the light-box to ensure the appropriate reflectance and to control the colour effect of eventual

multiple reflections (Connolly and Palus 1998). These illumination characteristics resulted in a satisfactory image quality and facilitated extraction of the investigated features.

4.2.3 Image processing

The quality of images deriving from the image acquisition stage was satisfactory with respect to the feature extraction. Two aspects which are especially worth for discussion are segmentation of fish/fillets and extraction of colour.

Segmentation

For isolation of fish or fillets, as a region of interest, from their backgrounds there were several factors which had to be taken into consideration. Apart from the proper illumination conditions, the type of the chosen background can be just as important. Therefore, the background of the scene where the image acquisition took place was chosen to be such that its spectral characteristics were different from the spectral characteristics of fish and fillets. From experience, failing to do so can make the segmentation of fish or fillets a challenging task as it can be difficult to find the real boundary which separates the fillet/fish and the background because of the irregular shape of the fillet and possible reflections. This was especially important in cases when quantification of the accurate size of fillets or fish was necessary. As a result, a suitable background was chosen, which made the segmentation operation easier.

In our research experiments, for the Atlantic salmon fillets, a gray-white as well as a light-blue background was used, aiming to choose backgrounds with different spectral characteristics than the fillet. In this way, maximal contrast between the red fillet and the background was achieved. For the cod fillets, the use of the gray-white background was not appropriate because of the white colour of the cod fillets. Thereby, the maximal contrast between the cod fillet and the background of the scene was achieved with a light-blue background. The same is valid for the whole fish. In all cases, we found that the automatic segmentation of a fish silhouette was easier with the use of the light-blue background, because of different spectral characteristics compared to the colour of fish skin or fillet.

When the gray-white background was used, we experienced the segmentation as a more challenging task and additional image processing operations were necessary for extraction of the exact silhouette of fish. This was mainly because of non-uniform color distribution of the skin. The skin of the belly part of Atlantic salmon is white; hence finding the exact boundary between the fish and such a background can be difficult no matter how good the illumination is. Therefore, we adopted the light-blue colour as a background colour for our research experiments.

For segmentation, the global thresholding algorithm was used, which is based on the method reported by Otsu (1979). Although trivial, it was sufficient for the purpose of isolating the fish/fillet from the background. Because of optimal illumination conditions, there was no need for the use of any other more complex algorithm for thresholding. More complex schemes of thresholding, would probably add some computational load. Therefore, the choice of the global thresholding, as a simple thresholding algorithm, in this study, has been optimal because of the illumination conditions. Fast thresholding computation and segmentation is known to play an important role on the reduction of the overall processing time and achieve improvement of the total performance (Lin 2005).

Colour extraction and grading issues

In chapter 3, it was shown that the colour of fillets is one of the most important quality properties for Atlantic salmon fillets (Anderson 2000; Olafsdottir et al. 2004). Out of all sensory properties, colour significantly influences the customer acceptance of the product as it is associated with quality, safety and value (Nieto-Sandoval et al. 1999). Therefore, it was important to ensure that the colour extracted from the images related directly to the colour properties (Finlayson and others 2005) of fillets or any other fish product. This was done by taking into consideration a number of aspects such is providing with uniform and diffuse illumination under controlled conditions, and eventually, colour calibration of images. However, there are some aspects which deserve special attention.

As for the effect of illumination in the perceived colour, it is known that the colour appearance depends from the colour temperature of the illumination source (Ayama et al. 2003) and rendering index. As mentioned above, different types of illumination setups were used as the work progressed. Initially, a source with colour temperature of approximately 2800-2900K was used (Misimi et al. 2007a), which is similar to the illumination type used by Strachan et al. (1990). In the light-box, the fluorescent tubes had a colour temperature of 5000K, which is similar to the colour temperature of the daylight (Sandor and Schanda 2006). In addition, colour calibration of images was performed in order to provide as good colour grading as possible. As long as the colour targets for calibration and grading were illuminated with the same respective illumination, we experienced satisfactory colour grading results of the fillets, which were in good correlation with the subjective perception of colour by human inspectors.

The main objective with the colour grading is to automatically grade fillets according to the existing industrial colour standard for Atlantic salmon (Roche Standard). The grading of fillets by colour according to this standard has a high relevance to consumers (Robb 2001). The colour grading is done to generate different classes of fillets regarding their colour scores so that fillets with a certain colour grade are, then, shipped to different markets. It was previously shown that certain markets prefer different colour of fillet flesh (Misimi et al. 2007a).

However, in this research, the illumination type that is used in the premises where the graded fillets are sold, and how this illumination affects consumers in their colour perception has not been taken into consideration. It is known that the type of illumination that is used in the retail displays, where the food and fish products are sold, differs from the illumination that is used for grading (Saenz et al. 2005). Therefore, the retail display illumination can make the fillets appear more yellowish or reddish, depending on the colour temperature of the illumination and interference of the ambient illumination around. In this situation, the consumers may not have the same perception of the colour label of the fillet, as graded in the plant under a different illumination source. This is one aspect of illumination which may be addressed in the future, if found relevant.

During the research, regarding colour, there were also discovered new aspects about colour grading which were not the initial aim. It was previously shown that colour of the pre-rigor fillets may change due to rigor contractions during the ice storage (Robb and Warris 1997). By using computer vision, we found that the colour of post-rigor Atlantic salmon fillets is significantly different from the colour of the same fillets in pre-rigor (Erikson and Misimi 2007b, in press). Therefore, any colour grading of fillets in pre-rigor would not correspond to the actual colour of fillets in post-rigor, resulting in erroneous colour labelling of fillets.

4.3 Implications of the research

4.3.1 Availability

The results of the research can be implemented industrially since the computer vision is an available technology which makes possible the low cost implementation of the proposed solutions. This plays an important role since, as previously shown in sections 1.3 and 4.1, there are sensor technologies which, due to the cost, unavailability, physical constraints and speed limitations, can not deliver equipments for on-line use, although the application of such a technology may be suitable for the evaluation of a given quality parameter. For example, it was shown that MRI, despite good capabilities in the compositional analysis of fillets (Veliyulin et al. 2007), presently has not the type of the availability that the fish processing industry needs.

Computer vision, on the other hand, has none of these limitations. Not only it is fast, consistent, and robust enough, but also the size of the instruments is such that they can be housed in a relatively small space, above the conveyor belts. The easy access and low-cost of cameras, lenses, illumination, fast PCs, and relatively easy maintenance, altogether, make the computer vision-based solutions affordable for the industrial use.

4.3.2 Automation

The proposed solutions can be used for automation of the above mentioned quality evaluation operations in a typical farmed fish plant line. The most important benefits from

automation of fish operations are: 1) increase of profitability by low cost production; 2) automated quality grading; 3) higher percentage of processed products; and 4) a more rapid processing and quality evaluation of fish products resulting in the increased production volume. Altogether, these benefits can result in a higher degree of automation of the Norwegian fish industry in general. This can strengthen the position of the Norwegian fish industry in the world market against the low-cost production and processing countries such as Chile, China, and Poland. As a result, it can also prevent the flagging out of the domestic fish processing plants abroad. In addition, Norway may arise as a global supplier of automated processing technology. Last but not least, all these factors can also make the fish industry as an attractive working place.

Therefore, a higher degree, preferably full, of automation of the fish industry will make the productions costs lower, while at the same time increase the quality of the products and their quality assurance, especially with the use of computer vision as a non-destructive/non-contact, and rapid sensor technology. This can have a manifold reflection in the value generation over the entire fish production chain.

In addition to automation, the closeness of Norwegian fish industry to fish resources will affect the quality of fish products, measured in freshness, which is an important advantage compared to the low-cost processing countries. This implies also that, with automation, the utilization of the raw by-products such as heads of fish, fish entrails, and filet cut-offs will remain in the country, since the Norwegian fish industry will be in a position to export more bone and skinless products and less unprocessed whole gutted fresh or frozen fish. From utilization of by-products, useful products can derive through processing and value-adding. Apart from the nutritional use, proteins, fatty acids, and minerals of the by-products can be used for production of the ingredients for health, pharmaceutical, or cosmetic products. Altogether, these factors will provide with the possibility to develop new fish processing products, something that is possible only for the countries with a direct access to fresh fishery products, such as Norway.

CHAPTER 5

Conclusions

In this thesis, computer vision based solutions for a number of fish operations along the fish processing line were developed. In general, we have demonstrated that computer vision methods can be successfully applied in automation of fish processing line.

5.1 Contributions

The main contributions of this thesis are as follows:

In chapter 1, after a brief description of the situation in Norwegian fish processing plants, a review of the available technologies which can have a potential for use in automation of fish processing was given. The review of technologies resulted in selecting computer vision as the technology which seemed to be best suited for automation of a number of operations in a typical fish processing line.

In the beginning of chapter 3, the proposed solution for the inspection of the bleeding degree in the body cavity of the whole Atlantic salmon prior to quality-class grading was presented. This is a part of the contribution described in Paper 6. By segmentation of the body cavity of the whole Atlantic salmon and by performing a colour based analysis, it was shown that the degree of bleeding correlated well with colour parameters both in the CIE Lab as well as in the RGB normalized colour space. This colour analysis was used for

designing a classifier with two classes labelled as “OK” and “Wash”, based on the Linear Discriminant Analysis. The classifier was able to grade fish that needed re-washing of the body cavity due to the presence of blood, labelled as “Wash”, from those which did not have any residual blood in the cavity, labelled as ‘OK’, and showed a good agreement with the ground truth labelling. It was shown how this type of quality grading can be solved with a design of a relatively simple classifier.

Further in this chapter, a design of a classifier for quality grading of whole Atlantic salmon in different quality classes was presented. The results of this work are a part of contribution described in Paper 1 and Paper 5. In the proposed solution, by segmentation of the silhouette of the Atlantic salmon from the scene, the algorithm is able to extract non-redundant geometrical parameters describing the shape of each individual salmon. On basis of these features a classifier was designed. The classifier employed the linear discriminant analysis-LDA to separate fish between the classes. For the given dataset, it was shown that the average correct rate of classification was in a good agreement with the manual labelling. In general, it was shown that by a simple LDA based classifier it was possible to simulate the human ability for quality grading.

Design of the computer vision-based sorting system for Atlantic salmon fillets according to their colour score was, then, presented. The design of the system and classifier/matching algorithm was explained in detail in Paper 2 and 3. Starting from the present industrial standard (NS 9402) for sorting of fillets by colour according to Roche Cards, initially a Look-up table from these cards was created. These colour scores were used by the algorithm in a colour-matching procedure with the flesh of the fillets. The matching was performed using the nearest-neighbour principle. As a result, fillets could be classified into different colour grades. This classification was not significantly different from the sensory evaluation performed by humans and is feasible for on-line industrial purposes.

Computer vision system for the evaluation of colour of fillets in CIELab space was presented in Paper 2, 3 and Paper 6. This system can be used for evaluation of colour, as a quality parameter of fillets, in both fresh and smoked Atlantic salmon fillets. The measurements of the colour by this method are in the form of CIELab values, similar to the ones generated by Minolta Chromamater or any other colorimetric/spectrometric measuring instrument. The advantage of the method by computer vision is viewed on the ability of the algorithm to generate Lab values for different parts of the fillet as well as for the entire fillet. This is contrary to the Minolta measurements, where the generated values are obtained by interrogating a very small area of the fillet (8mm). As Minolta chromameter is primarily designed for flat, nontranslucent and diffuse surfaces, the colour *a* and *b* values generated by Minolta are very low, indicating a greyish colour while the salmon fillet is orange/read, which makes this device an inappropriate for measurement of colour of fillets. Our computer vision method can also be used for detection of colour non-uniformities (discoloration) in both fresh and smoked fillets. Because of the optimal illumination conditions, the method can be used for any other fish product for the purpose of colour evaluation in on-line conditions.

Development of the computer vision method for measurements of transient 2D and 3D changes in size and shape of fillets during the rigor process and ice storage was presented in Paper 4. The method was capable to measure the size and shape of the Atlantic salmon and cod fillets, and their changes during the ice storage, with a high precision. This was demonstrated by comparison of the exhausted and anesthetized fillets. From a single fillet image 2D size and shape of the fillets was extracted in the form of length, area, width and roundness. Regarding 3D size, by laser scanning of the fillet, it was possible to obtain size in the form of combination of the height and the area of the cross-section. Integration of these profiles along the entire fillet length can result in the 3D volume images of fillets, suitable for volume change study and measurement. This computer vision method can be used not only for size and shape analysis of the fillets but also of fish and other fish products, both in on-line and off-line conditions. Together with the colour

measurement/matching ability, this method can also be used for fully feature evaluation and classification of any fish or food product from a single image (colour, size and shape in 2D and 3D).

The findings in this thesis are original work and are published in the international conferences/journals with peer review. The list of the papers was given in the introductory part and they are listed in the Part 2 of the thesis, which begins after this chapter.

Looking back at what has been done in this thesis, it is a general impression that the main objectives were met. Although the work in this thesis is not a complete recipe on how to fully automate a typical fish processing line, it is an important step towards this goal, since it proposes solutions to numerous quality grading/assessment operations along a fish processing line.

The main conclusion that can be drawn from this work is that computer vision is a sensor technology that has reached the theoretical and practical level of maturity, and industrialization capability, where it can be utilized industrially in fish processing plants, something the author recommends to be done in the Norwegian fish industry. The versatility, cost, on-line use, maintainability, ease of operation, consistency in evaluation as well as contact-free feature of use suggests that computer vision is, presently, a natural choice when automation of fish processing plants is to be considered.

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PART 2

PAPER I

COMPUTER VISION BASED SORTING OF ATLANTIC SALMON (*SALMO SALAR*) ACCORDING TO SIZE AND SHAPE

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Keywords: Computer vision, feature extraction, fish grading, processing line, Atlantic salmon.

Abstract: Intensive use of manual labour is necessary in the majority of operations in today's fish processing plants, incurring high labour costs, and human mistakes in processing, evaluation and assessment. Automatization of processing line operations is therefore a necessity for faster, low-cost processing. In this paper, we present a computer vision system for sorting Atlantic salmon according to size and shape. Sorting is done into two grading classes of salmon: "Production Grade" and "Superior/Ordinary Grade". Images of salmon were segmented into binary images, and then feature extraction was performed on the geometrical parameters to ensure separability between the two grading classes. The classification algorithm was a threshold type classifier. We show that our computer vision system can be used to evaluate and sort salmon by shape and deformities in a fast and non-destructive manner. Today, the low-cost of implementing advanced computer vision solutions makes this a real possibility for replacing manual labour in fish processing plants.

1 INTRODUCTION

During the last few decades, the number of whitefish processing plants in Norway has diminished considerably for several reasons. In aquaculture, although the production volume of salmonids has increased tremendously over the same period of time, most of the fish are currently exported as raw material, i.e. gutted fresh or frozen. In both sectors, particularly due to the high labour costs, fish processing is often unprofitable. For instance, for slaughtering of farmed salmonids, the needed manpower is typically 25-40 persons per shift to process 40-100 tons of bled, gutted fish packed in ice. Therefore, greater automatization of various unit operations, preferably at low investment costs, represents a common strategy within the fish processing industry today. A fish processing line consists of several separate unit operations. Arnarson et al. (1988) reviewed and outlined a number of possibilities for implementing computer vision for automation and improving product quality in the fish processing sector. However, several unit operations in a fish processing line still rely on, at least in part, repetitive manual labour. Manual processing and grading has several drawbacks. It is influenced by human factors such as mistakes, occasional omissions in processing and fatigue. These factors may result in imperfections that decrease product quality and thereby reduce profit (Pau and Olafsson, 1991). Therefore, there is a need for automation of basic processing operations to obtain faster processing and a more objective and consistent quality determination (Strachan and Murray, 1991; Gunnlaugsson, 1997; Brosnan and Sun, 2002). Here computer vision can contribute to further improving the quality of fish products. With the latest developments in camera technology and the continuous increases in CPU speed, computer vision technology has become increasingly more relevant.

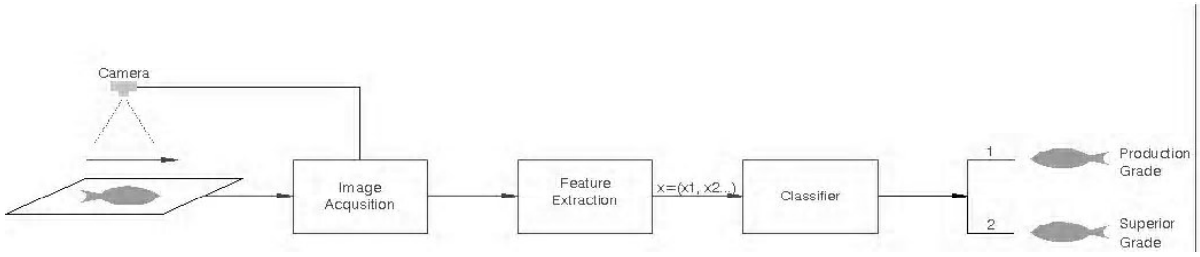


Figure 1: Stages of classifier design.

Today computer vision solutions are easy to implement with high flexibility and low cost. Until recently, the cost of high-resolution, high-speed cameras has been comparatively high. These factors imply that computer vision can be used effectively for online processing of fish (Arnason et al., 1988). The non-destructive nature and the sheer speed at which the quality of fish can be evaluated and sorted are other important factors that encourage the use of computer vision based solutions.

Computer vision has proven successful for online process control and inspection of food and agricultural products with applications ranging from simple automatic visual inspection to more complex vision control (Gunasekaran, 2001). Strachan and Murray (1991) describe how they developed a machine, based on image analysis, for discriminating mature herring by sex using infrared light.

Computer vision algorithms for automated processing of channel catfish (*Ictalurus punctatus)* have been developed to detect fish orientation, identify the head, tail and fins, and to determine cutting lines for deheading, detailing, and defining (Jia et al., 1996). Moreover, automated separation has been developed for several marine fish species (Wagner et al., 1987; Strachan and Murray, 1991; Strachan, 1993) and for freshwater species such as carp *(Cyprinus carpio),* St. Peter's fish (*Oreochromis sp.)* and grey mullet *(Mugil cephalus)* (Zion et al., 1999). Walkott (1996) gives examples on how shape region features can be used for object recognition.

When farmed salmonids are slaughtered, the fish size distribution approximately follows a Gaussian distribution curve. From a processing point of view, a uniform fish size is much favored. This has to do with production planning including issues such as the correct adjustment of gutting machines, possible further processing to a certain uniform product (e.g. fillet) and delivery of chilled gutted fish of a given weight class to a specific costumer. Another factor is that a certain fraction of the fish carries different kinds of blemishes that originate from the farming period. Sexually mature fish, fish with different body deformities ('short tails' and 'humpbacks')

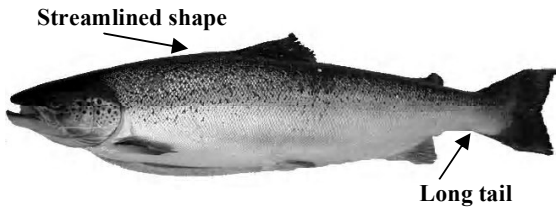


Figure 2: Superior class salmon.

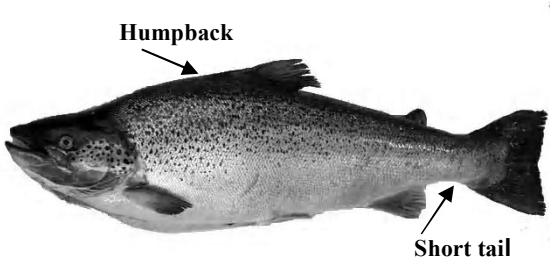


Figure 3: Production class salmon.

(fig. 3) and skin defects (excessive loss of scales, wounds, etc) all occur. Accordingly, our goals were to develop computer vision based methods able to (fig. 1):

(i) reject sexually mature fish and sort/grade fish with deformities in shape. Such a sensor system should be placed prior to fish processing since such fish are not worth processing.

(ii) grade fish according to these shape parameters.

Today, salmonids in Norway are graded according to external quality as follows: 'Superior' (no blemishes), 'Ordinary' (minor degree of blemishes), (fig. 2), 'Production' (part of the fish may be used for human consumption) (fig. 3) and 'Rejected' (not for human consumption, see (i)).

2 MATERIALS AND METHODS

2.1 Fish and fish sampling

Atlantic salmon (*Salmo salar*) from one fish processing plant were used. *Group I*: Nine 'Production Grade' (weight: 3.58 ± 0.23 kg; length: 50 ± 2 cm; were selected from the slaughter line on 12 Oct 2003. The fish were bled and gutted at the plant.

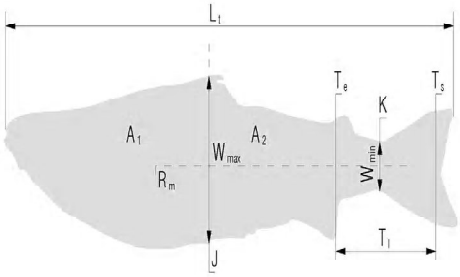


Figure 4: Shape parameters for feature extraction.

Group II: Fourteen "Superior/Ordinary Grade" fish were collected from the same commercial processing line on 12 Oct 2003. Thus, the fish ('Superior Grade') had been bled and gutted at the plant. The mean fish weight and length were 4.60 ± 0.4 kg and 59 ± 3 cm, respectively.

2.2 Image Acquisition

The images, intended for feature extraction, were captured using an image acquisition system for a digital colour camera (Nikon Coolpix 5000, Japan) at a resolution of 1600 x 1200 pixels and acquired in the JPEG format. These were still images. However, ccommercial industrial full frame digital cameras with comparable resolution are available at near real-time speeds (HVDUO-5M, HanVision Co, Korea). The use of line-scan colour cameras is most likely preferable in an industrial setting, due to their high-speed and the fact that fish in most cases are transported on conveyor belts. The white balance of the camera was set using the camera's automatic white balance. The fish were illuminated using only one illumination setup. This setup used two parallel halogen lamps under a white glass board to provide the necessary illumination, with colour temperature 2900 K. The lamps were placed 30 cm below the fillet. The images were acquired with a camera mounted in a stand on a 90˚ angle, 100 cm above the fillet. Images were processed with Adobe Photoshop prior to processing with Matlab Development Environment 7.01 (Mathworks, Natick, Massachusetts, USA). Images were filtered, scaled and rotated appropriately in the Matlab Image Processing toolbox. Images originally had random orientation, with a different angle to the horizontal axis. Some images were in the flipped orientation. By using and writing Matlab functions, all these images were oriented in the same direction prior to the feature extraction procedure in Matlab.

2.3 Feature Extraction

The features are derived from the geometry of the salmon's shape. Standards that the fish processing industry uses for classification of "Production Grade" and "Superior/Ordinary Grade" are also based on the geometrical parameters of salmon shape. An inspector at the processing line usually looks after parameters such as 'humpback', 'short tail' and 'sexual maturity' when he wants to detect and grade a "Production Grade" salmon. "Superior/Ordinary Grade" salmon has a 'streamlined' shape and with a 'long tail' and reduced 'roundness' compared to "Production Grade" salmon.

Based on the industrial standards and the geometrical parameters defining the shape of salmon (fig. 4), four features were chosen for extraction, which would allow us to classify the salmon. The first parameter was the ratio (R_{lw}):

$$R_{lw} = \frac{L_t}{W_{\max}} \tag{1}$$

where L_t is the total length of fish from its nose to the end of the tail, and $W_{\max}$ is the maximum width of fish.

The second parameter was the area ratio (A_r):

$$A_r = \frac{A_1}{A_2} \tag{2}$$

where A_1 is the area on the front half of the fish and A_2 is the area on the back.

The third parameter was the ratio (W_r):

$$W_r = \frac{W_{\max}}{W_{\min}} \tag{3}$$

where $W_{\max}$ is the maximum width of fish and $W_{\min}$ is the minimum length of the fish.
The final parameter was the ratio (R_{tl}):

$$R_{tl} = \frac{T_l}{L_t} \tag{4}$$

where T_l is the tail length, and L_t is the total length of the fish.

In this way we used a total of four features x_i, $i = 1,2,3,4$:

$$x_1 = R_{lw} \tag{5}$$
$$x_2 = A_r \tag{6}$$
$$x_3 = W_r \tag{7}$$
$$x_4 = R_{tl} \tag{8}$$

creating the (1x4) feature vector:

$$x = \left[x_1, x_2, x_3, x_4\right] \tag{9}$$

Figure 5: Segmented binary image.

The geometrical parameters in figure 4, which are used in the feature's definition, were derived in Matlab from the segmented binary image of the salmon (fig. 5). The size of the image was defined with the pair (r,c), where r is the total number of rows, and c is the total number of columns. The images were cropped and scaled in Matlab in such a manner that the first column is the start point of the nose of the fish, and the last column corresponds to the end of the tail. Consequently the total length L_t, which is the length from the nose to the end of the tail, was defined as equal to the total number of columns in the image:

$$L_t = c \tag{10}$$

The width W of fish is the width of the fish at any point. In Matlab it was calculated as the number of pixels equal to one (=1) in the row direction at the given column position. The maximum width of fish, and the appropriate column position, where the maximum width occurred, was defined as:

$$\left[W_{\max}, J\right] = \arg\max_j \left[W\right] \tag{11}$$

The maximum width occurred at the column position located between the dorsal fin (fig. 4) and the belly. The minimum width of the fish was defined in the same fashion, where we ensured that the searching was done on the back side of the fish, from column J to the end of the tail. The minimum width of a fish, together with the position where it occurred, was:

$$\left[W_{\max}, K\right] = \arg\min_j \left[W\right] \tag{12}$$

In the $x_2 = A_r$ feature, A_1 in figure 4 was defined as the area of the front half of the fish, from the head of the fish until the midpoint J at the dorsal fin, where the maximum width occurred. A_2, on the other hand, is the area portion of the back half of the fish from the midpoint position J from the dorsal fin to the end of the tail. The reason why the area ratio was recorded as a feature was that the ratio aspect analysis indicated that the "Production Grade" fish was rounder than the "Superior/Ordinary Grade". The mean area ratio for "Production Grade" fish was 1.3 ± 0.183, while for "Superior/Ordinary" fish the mean area ratio was 0.9 ± 0.15.

Tail length T_l (fig. 4), was defined as the difference:

$$T_l = T_s - T_e \tag{13}$$

where, T_s was the position calculated as the beginning of the tail, seen from the tail side of the fish, and which was calculated as the difference between the total length of the fish L_t and the value which was 10% of the L_t .

$$T_s = L_t - \frac{L_t}{10} \tag{14}$$

The point position T_e was designated as the end of the tail and was located at the ventral fin. Calculating this involved using more parameters. The ventral fin of salmon served as the boundary for the tail length. After localizing the point K , where the minimum width occurred, the middle position R_m was found, which was the row point at half of the $W_{\min}$. By scanning the binary image from the midpoint J to the point K we found the point T_e where the width of the fish was 50% bigger than $W_{half} = \frac{W_{\min}}{2}$:

$$T_e = \arg_j \left[W; W \geq \frac{3}{2} W_{half}, J \leq j \leq K \right] \quad (15)$$

2.4 Training of the Classifier

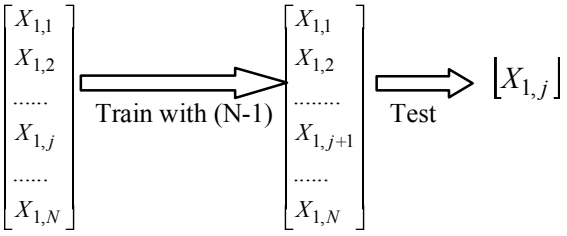
A dataset consisting of 23 labeled binary images of salmon was used to train the classifier. Nine images of "Production Grade" label salmon and fourteen "Superior/Ordinary Grade" label salmon were used for this purpose. Prior to training we had to decide what type of classifier was most suitable for this case. By analyzing the adopted criteria for feature extractions one by one, we determined how good these criteria were if used as a single classification criterion.

Using only a single criterion for classification was ineffective. We could not reliably separate the "Production Grade" from the "Superior Grade" salmon. By combining two or more criteria, the separability between classes was more reliable. By applying aspect ratio R_{lw} in combination with the area ratio A_r, the separability of classes improved (fig. 6). Similar results were obtained with the other combinations of features.

The decision boundary in figure 6 implied that a linear classifier might perform the classification quite well. Therefore, we applied Linear Discriminant Analysis – LDA to train the classifier and took into consideration all four features. The function written in Matlab was based on the Fisher's linear discriminant (Theodoridis and Koutroumbas, 2003):

$$FDF = \frac{(\mu_1 - \mu_2)^2}{\sigma_1^2 + \sigma_2^2} \quad (16)$$

Testing of the classifier's performance was done with the Leave One Out (LOO) method (Theodoridis and Koutroumbas, 2003). Training of the algorithm was done with N-1 samples and the test was carried out using the excluded sample. If X_1 and X_2 were the respective data for classes 1-"Production Grade" and 2-"Superior Grade", then the training was done using $[X_1 - X(j)]$ and $[X_2 - X(j)]$ samples respectively and the test was carried out with the excluded sample $X(j)$.



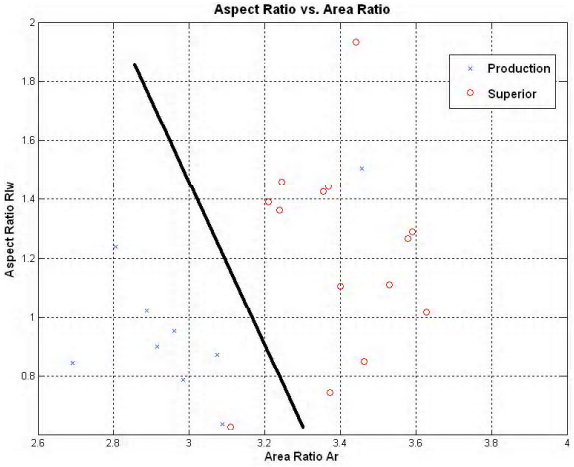


Figure 6: Features of aspect ratio R_{lw} and area ratio A_r. The dark line could serve as a decision boundary for our classifier. Classification error was lower than when we used only one feature.

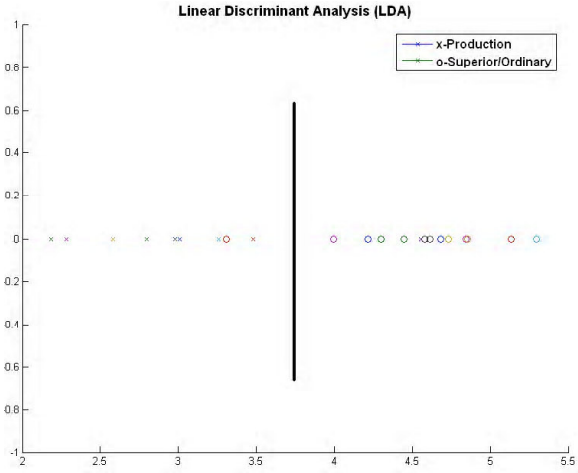


Figure 7: Linear discriminant analysis for training the algorithm with all four features used.

3 RESULTS AND DISCUSSION

Twenty three salmon of "Production Grade" and "Superior/Ordinary Grade" were sorted according to four features. The classification error was three, two from "Production Grade" and one from "Superior/Ordinary Grade". In percent this classifier has an 87% (20 out of 23) sorting reliability as estimated using the Leave One Out method.

One of the two "Production Grade" salmons which are not correctly classified lies further to the right (fig. 7). From the data log we have from the day we picked the fish at the processing plant, the existing 'outlier' has neither 'humpback' nor 'short tail'. It was classified as "Production Grade" salmon

from the production chief because it had a 'black head'. The work presented, carried out in laboratory conditions, with this classification reliability has to be repeated with a bigger dataset and repeated in the working conditions in the fish processing plant. The work shows a feasibility of sorting one type of fish into different grading classes based on the standards specified by the fish processing industry. There are several problems on which one must focus attention when doing image acquisition of salmon and labelling them into grading classes:

1. The illumination/backlighting system has to be carefully set in order to provide easy thresholding and segmentation of fish images.
2. Labelling of salmon, for the training phase, into grading classes has to be carried out by experts; otherwise one might end up with fish having, for instance, a wrong class label without satisfying any of the parameters defining that class.

4 CONCLUSION

A computer vision system and algorithm for sorting Atlantic salmon into two grading classes is described. This classification algorithm works with an estimated sorting reliability of 87%. An improved version of this system can potentially be used to substitute manual inspectors in the fish processing line. Further work is required in acquiring a bigger dataset and expert help on the correct, unmistakable labelling of grading classes, before building a prototype.

ACKNOWLEDGEMENTS

The project was funded by the Research Council of Norway (*NFR project No. 145634/140 –'Efficient and economic sustainable fish processing industry').*

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PAPER II

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PAPER III

Atlantic Salmon Skin and Fillet Color Changes Effected by Perimortem Handling Stress, Rigor Mortis, and Ice Storage

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Abstract: The changes in skin and fillet color of anesthetized and exhausted Atlantic salmon were determined immediately after killing, during rigor mortis and after ice storage for 7 days. Skin color (CIE L*, a*, b*, and related values) was determined by a Minolta Chroma Meter. Roche *Salmo*Fan^{TM} Lineal and Roche Color Card values were determined by a computer vision method and a sensory panel. Before color assessment, the stress levels of the 2 fish groups were characterized in terms of white muscle parameters (pH, twitches, rigor mortis and core temperature). The results showed that perimortem handling stress initially significantly affected several color parameters of skin and fillets. Significant transient fillet color changes also occurred in the prerigor phase and during the development of rigor mortis. Our results suggested that fillet color was affected by postmortem glycolysis (pH drop, particularly in anesthetized fillets), then by onset and development of rigor mortis. The color change patterns during storage were different for the 2 groups of fish. The computer vision method was considered suitable for automated (on-line) quality control and grading of salmonid fillets according to color.

Keywords: Computer vision, skin color, fillet color, handling stress, rigor mortis, Atlantic salmon

Introduction

The red/orange coloration of salmon and rainbow trout fillets is an important product property appreciated by consumers. The flesh color should be deep red/orange and evenly distributed along the fillet. Proper coloration is dependent on the astaxanthin and cantaxanthin contents in the flesh which are affected by feed composition and feeding regimens before harvesting (Nickell and Springate 2001). The high fat content in farmed salmonids causes dilution of astaxanthin and interferes with color perception (Christiansen and others 1995). The color is considerably paler in high fat regions, such as the belly flap, compared with, for example, muscular tissues above the sideline. This is because fat is not evenly distributed along the fillet (Aursand and others 1994). Moreover, Robb and Warriss (1997) have shown that fillet color perception also depends on perimortem handling stress. They found that fish exhibiting high muscle activity at slaughter had about one unit lower Roche Color Card readings than those of unstressed fish when determined 24 h post mortem. This difference persisted when readings were repeated post rigor, after 4 days. Likewise, electro-stimulation of rainbow trout (*Oncorhynchus mykiss*) muscles immediately after death to simulate premortem muscle activity resulted in significantly higher flesh L* and hue (H_{ab}^{o}) values, as well as lower chroma (C^{*}_{ab}) values compared with anesthetised fish. After 3 d of storage, the differences in color persisted, but the chroma values of the electro-stimulated fish increased. Roche Color Card scores were also lower for electro-stimulated muscles (Robb and others 2000). In the case of Arctic char (*Salvelinus alpinus),* stress reduced the muscle a* value and affected L* values depending on the hatching temperature (Jittinandana and others 2003). On the contrary, by comparing Atlantic salmon (*Salmo salar*) subjected to carbon dioxide (high stress) or iso-eugenol (low stress) anesthesia, Kiessling and others (2004) concluded that the flesh from the carbon dioxide-exposed fish had slightly higher a* and b* values than the fish exposed to iso-eugenol. The fish were filleted and evaluated after ice storage for 5 days. After frozen storage for 12 months, the Roche *Salmo*Fan^{TM} color scores of cutlets were about one unit higher for the carbon dioxide-exposed fish.

Iger and others (2001) suggested that alterations in skin color may be used as a stress indicator for fish reared in aquaculture. Stress induces skin structural changes in several species and the fish darken with the onset of the stress incident. Subsequently, the fish turned paler than the control fish. In aquaculture, skin coloration patterns can be regarded as an index of animal welfare and as an important quality factor (market value) for some fish species (Pavlidis and others 2006). Postmortem measurements of skin color during storage have been suggested as a method to monitor quality changes, such as in cod (*Gadus morhua*) where skin L* and a* values show a fairly good linear relationship with both QIM values and changes in skin appearance (Schubring 2003). Significant changes in skin coloration patterns during ice storage for up to 7 days post mortem have also been observed for farmed red porgies (*Pagrus pagrus*) (Pavlidis and others 2006).

Several analytical methods can be employed for assessment of fillet color. Roche Color Card and *Salmo*Fan™ are well established concepts for quality control in the salmonid industry. Relationships between astaxanthin concentrations and Roche Color Card and Roche *Salmo*Fan™ scores have been established (Christiansen and others 1995; Johnston and others 2000).

CIE L*a*b* values are determined instrumentally. The intensity of redness (a*) increases with increasing carotenoid contents in raw flesh of Atlantic salmon, while lightness (L*) decreases and yellowness (b*) is not affected (Skrede and Storebakken 1986). Higher a* values are observed with increasing Roche Color Card scores (Christiansen and others 1995). Increasing fat content in raw fillets has been shown to coincide with increasing L* and b* values (Mørkøre and others 2001). Furthermore, the thickness of the flesh is another factor affecting color values (Stien and others 2006).

Computer vision has previously been used for sorting different fish species where the color was obtained from the red, green and blue (RGB) outputs from a video camera (Strachan 1993). After color calibration, L*, a*, and b* values were determined in brown trout (*Salmo trutta*) cutlets by computer vision and image analysis. It was found that the L* values significantly decreased from the head to the tail and that higher muscle fat

contents resulted in higher L* values (Marty-Mahé 2004). Automated image analysis has also been suggested as a rapid method to quantify composition and color of rainbow trout cutlets (Stien and others 2006).

Based on sampling of Atlantic salmon with different stress levels (as defined by white muscle pH) at several commercial processing plants, we have never been able to confirm that differences in perimortem stress affect postrigor fillet color. This suggests that color differences can only be observed under controlled conditions when extreme cases of stress, such as with anesthetized and exhausted fish groups, are compared. Another factor might be the time post mortem at which the color is assessed. In the present experiment, our goals were to study potential skin and fillet color differences at the point of slaughter, during rigor mortis, and after ice storage for 7 days, at a time when the fish are typically available to consumers. The extremes of premortem muscle activity (anesthetized and exhausted salmon) were used to elucidate whether (1) perimortem handling stress affected initial skin and fillet color, (2) fillet color changed during the course of rigor mortis, (3) skin and fillet color differences were discernible after ice storage for a week, and (4) a computer vision-based method was suitable for on-line measurements of skin and fillet color.

Materials and Methods

Fish

Commercially farmed Atlantic salmon weighing 3.8 ± 1.1 kg with fork lengths of 66 ± 6 cm (n=40) were netted from the sea cage and transferred to a 1000-L tub. The fish had been fasted for 8 days. The tub was transported (< 5 min) by boat to the quay where the fish were netted and divided equally into 3 transport tanks on a truck. The tanks had just been filled with fresh seawater. The fish were transported under constant oxygenation for 2 h to our laboratory at a fish density of 17 kg m^{-3}. By arrival, the fish were calm; however, some individuals were observed gulping air near the water surface. Foaming was observed in all tanks. The salinity was 35 ppt and the water temperature, pH, dissolved oxygen (DO), TA-N, and alkalinity in the 3 tanks ranged from 6.7 – 6.8 ^{o}C, 6.3

- 7.1, and 114 -310 % saturation, 0.38-0.84 mg L^{-1}, and 113 - 121 mg L^{-1}, respectively. The low pH indicated elevated levels of carbon dioxide. The fish were netted from the truck and transferred (< 5 min) by means of seawater-filled 200-L tubs to 2 holding tanks (4000-L) in our laboratory. The inside color of the holding tanks was green and the photoperiod was 8L:16D. The light source in the room consisted of 8 Osram warm white L58W/830 fluorescent tubes. Twenty fish were kept in each tank at 22 kg m^{-3}. Fresh seawater was pumped, sand-filtered and circulated to the tanks at a rate of 5 m^3 h^{-1}. After about 2 h, the fish exhibited normal behavior and distributed themselves evenly in the water column. The water temperature, pH and DO in both tanks were 8.8 - 9.0 ^oC, 7.83, and 80 - 90% saturation, respectively, throughout the 5 days that the fish were kept in the tanks. They were not fed during this period.

Fish sampling – defining stress

The day before slaughter, one tank was covered with black plastic to reduce possible fish stress. On the next morning, the water supply to this tank was closed and oxygen gas was distributed to the tank using a diffuser (Point Four Systems Inc, Richmond, Canada). A predetermined amount of AQUI-S^{TM} (AQUI-S Ltd., Lower Hutt, New Zealand), 17 mg L^{-1}, was added to the tank to anesthetize the fish. During this process, no fish struggled and the DO levels were kept within 97 – 104% saturation. The first fish was then netted from the tank and killed by iki jime using an 'Iki Jime Tool' (AQUI-S Ltd.). Each fish was subjected to various measurements before the next one was sampled. Immediately after brain destruction by iki jime, a blood sample (3-5 mL) was withdrawn from the ventral aorta region (behind the operculum) using a heparinized syringe. The blood samples were subjected to determination of hematocrit, plasma chloride, and glucose. After making a cut with a scalpel between the side line and the dorsal fin, the initial white muscle pH and body temperature were measured. Finally, length and weight were recorded. The skin color was immediately determined. The fish were sampled over a period of 2 h. All fish were alive when sampled except 2 individuals that were sampled after about 1.5 h. They were declared dead as they did not respond to stimuli.

In the other tank, the fish were chased to exhaustion while the water level was gradually reduced to about 15 cm height. In sum, the fish were stressed for 30 min before the first fish was netted and killed by iki jime. All 20 fish were sampled and analyzed as with the anesthetized group within 2 h. The fish were not allowed to recover from stress during this period. No fish were dead by the time of sampling and no fish (both groups) were bled.

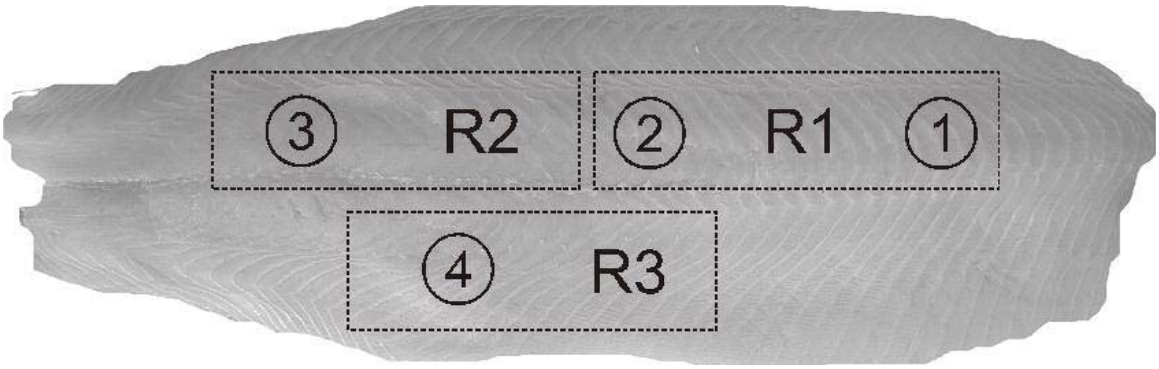

Figure 1-Atlantic salmon fillet color (CIE L*a*b*) was determined at 4 locations L1-L4 (Minolta Chroma Meter), as the average of 3 regions R1-R3, or as the average of the whole fillet (computer vision and image processing). The measurements were carried out immediately after slaughter and filleting, during the course of rigor mortis, and after ice storage for 7 days. The total fat content of a slice (10 x 5 cm) within region R1 was 11.5 ± 3.1 % (n = 10).

Postmortem assessment of color

Immediately after killing and assessments of stress, the skin color was measured at 6 different locations (Figure 1) on each side of the fish (n = 40). Since there were no differences ($p>0.05$) between the 2 sides, only data from the left side of the fish, which was not in direct contact with ice during subsequent storage, are reported here. Seven fish in each group were gutted, washed and placed in styrofoam boxes. The rigor mortis development in these fish was assessed along with core temperature, muscle pH and muscle twitches in restricted zones (few cm^2). After 7 days of ice storage, the skin color was assessed once more at the same locations as before.

The remaining 13 fish in each group were filleted immediately after killing. The surfaces of the fillets (skin on) were washed briefly (blood removal) in running tap water before they were labeled. All fillets (52 in total) were subjected to initial color measurements (t

= 0 h) at different locations (Figure 1). Subsequently, the color changes during the course of rigor mortis (t = 0, 11, 22, 27, 33, 45, and 70 h) and after 7 days (t = 168 h) of ice storage were recorded. Between each measurement, the fillets were stored in styrofoam boxes on ice (skin side down) in a cold room (5 ^{o}C).

Analytical methods

Body and core temperature. The fish body temperature was measured in the epaxial muscle between the sideline and the dorsal fin immediately after killing. In processed fish, the core temperature was measured near the backbone in the thickest part of the fish. A Testo 110 thermometer (Testo AG, Lenzkirch, Germany) was used.

Muscle pH. The pH was measured directly in the white muscle in the same location as the temperature was determined. After death, the pH was measured during the course of rigor mortis and after ice storage for 7 days using a shielded glass electrode (WTW SenTix 41) connected to a portable pH meter (model WTW 315i; WTW, Weilheim, Germany). During the measurements, the instrument was frequently calibrated using pH 4.01 and pH 7.00 buffers. Frequent cleaning of the electrodes was needed to obtain consistent results.

Skin and fillet color by computer vision. Color analysis was done in CIELab color space. Images were captured using an image acquisition system for digital cameras. Whole fish or fillets were illuminated in a light box (Waagan AS, Skodje, Norway) equipped with two fluorescent tubes (18 W, 5000K). A color digital camera, PixeLINK (PixeLink, Ottawa, Canada), was mounted on the top of the illumination box at a vertical distance of 60 cm from the fillet sample and images were captured at the maximum (1280 x 1024) resolution. Images were stored in the computer for later evaluation without compression in a bitmap file format (.bmp) in three-dimensional RGB (red, green and blue) color space, and processing was carried out in the captured images. The images were filtered from noise with a median filter and color was calibrated using a GretagMacbeth ColorChecker chart as a color target with 24 color patches (Colour-Science AG, Hinwil, Switzerland). The device-independent CIE 1931 XYZ values of the

colour patches were used for this calibration. Consequently, the calibrated color data were converted into CIELab color space using the expressions defined in Wyszecki and Stiles (2000), resulting in 3 gray-scale color maps of the L*, a*, and b* components. This method determined fillet color in the CIELAB color space by calculating the mean L* a* b* values for the entire fillet. Each gray-scale color map of the fillet, prior to calculation of mean L*, a*, and b*, was segmented by generating a binary mask (Gonzales and others 2004). Then, CIE L*a*b* values for the entire fillet were obtained by calculating the average value for CIE L*, a* and b* over the pixels of the fillet region of interest.

Fillet color was also measured over regions (R1-R3, Figure 1) by applying the color matching computer vision algorithm (Misimi and others, 2007) according to Roche *Salmo*Fan^{TM} and Roche Color Card scales (regions R1 = 5 x 16 cm; R2 and R3 = 3 x 13 cm). The Roche color scores were used to create a look-up table which was subsequently used by the computer vision algorithm for fillet color matching.

Roche Color **-** Fillet color was assessed visually by using both Roche *Salmo*Fan^{TM} Lineal and Color Card (Hoffman-La Roche, Basel, Switzerland). The color scales ranged from 20 (pink) to 34 (dark red) and from 11 (light orange) to 18 (dark red), respectively. At Day 7, 3 people at our laboratory evaluated overall color using both Roche color scales ('sensory panel'). All readings were performed in the light box mentioned above.

Minolta Chroma Meter. CIE L*a*b* values were determined on skin (t = 0 h and 168 h) and fillets during the course of rigor mortis by using the Minolta Chroma Meter CR-200 (Minolta, Osaka, Japan). The instrument readings cover an area of 8 mm in diameter. The hue angle (0^o = red hue; 90^o = yellow hue) and color saturation (higher values mean more intense color perception) were calculated as H^o_{ab} = arctan (b*/a*) for a* > 0 or H^o_{ab} = 180^o + arctan (b*/a*) for a* < 0, and as $C^*_{ab} = (a^{*\,2} + b^{*\,2})^{1/2}$, respectively. The Entire Color Index (ECI) for color assessments was also used (Pavlidis and others, 2006).

Rigor mortis. The course of rigor mortis during ice storage was determined using the Rigor Status Method [0 = pre- or postrigor; 1 = rigor onset (first sign of stiffness, for

instance. in the neck or tail region); 2 = rigor (a larger area is clearly in rigor); 3 = whole fish in rigor; 4 = stronger rigor; 5 = very strong rigor (the fish is extremely stiff, rod-like)] (Erikson 2001). Our goal here was to study whether muscle structure (shrinkage during rigor) could explain color changes induced by changes in light refraction. The fillet color (Minolta Chroma Meter) measurements during rigor mortis were carried out simultaneously with the rigor assessments of whole fish.

Statistical analysis

Unpaired Student's t-tests (assuming equal variances) were used to compare the levels of plasma chloride, hematocrit, blood glucose, NMR relaxation times, and populations, as well as fillet Roche color values of anesthetized and exhausted fish. The effect of treatment (anesthetized as compared to exhausted fish) and postmortem storage time on CIE L*a*b* and related color values were analyzed using a two-way ANOVA. Where significance ($p<0.05$) was indicated, a Tukey post hoc test was run. All data are presented as mean values ± standard error of means (SEM).

Results and Discussion

Blood chemistry (plasma chloride, hematocrit and glucose) of both fish groups showed clear stress-related changes related to antemortem muscle activity (exhausted fish) and some extent, due to the effect of the anesthetic per se (data not shown).

White muscle biochemistry and early postmortem changes

The initial white muscle pH of the 2 fish groups and the subsequent drop due to postmortem degradation of glycogen is shown in Figure 2. Since the AQUI-S^{TM} anesthetized fish did not struggle, our initial pH values of 7.5 ± 0.1 were typical of rested fish. Chasing the fish to exhaustion resulted in use of muscular glycogen and the mean initial pH was reduced to 6.7 ± 0.1. The initial pH values of the 2 groups represented the *in vivo* extremes of Atlantic salmon (Booth and others 1995; Erikson and others 2006). During subsequent ice storage, the pH of both groups dropped to around pH 6.45. This occurred after about 55 h for the anesthetized fish and after 3 h for the exhausted fish. For both groups, rigor mortis started when the muscle pH was reduced to 6.6 - 6.7. Thus,

perimortem exercise did not seem to affect muscle pH at the onset of rigor. For chinook salmon (*O. tshawytscha)*, Jerrett and Holland (1998) arrived at a similar conclusion. They also found that onset of rigor contractions coincided with a muscle pH of 6.6. Nakayama and others (1992) reported similar results for carp (*Cyprinus carpio*).

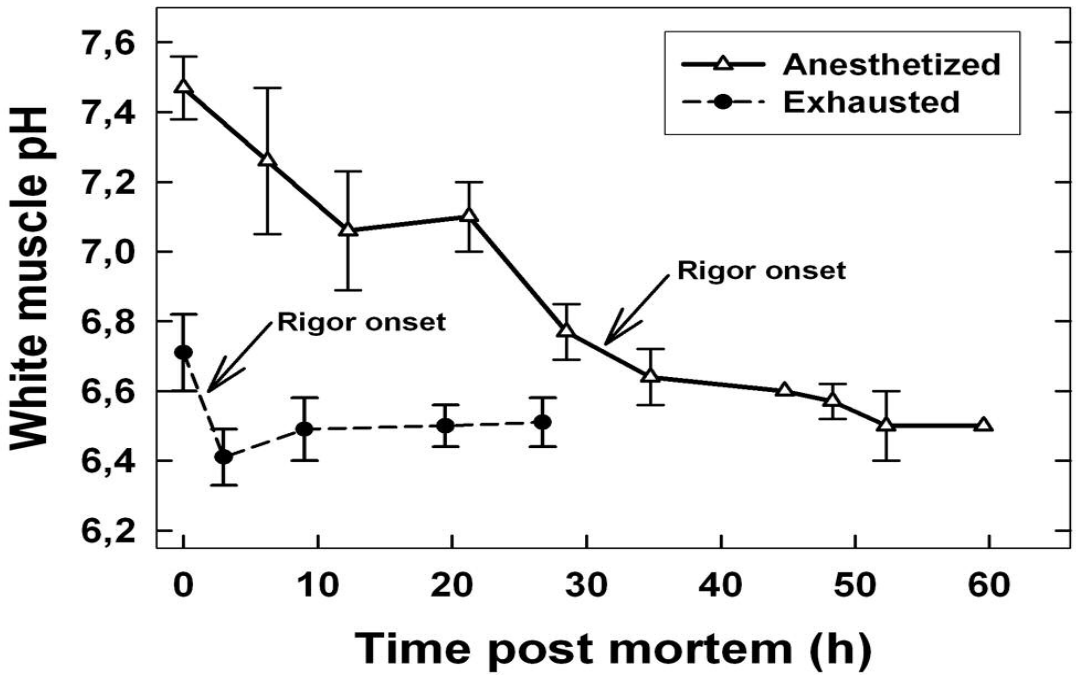

Figure 2-Initial white muscle pH (t = 0 h) and subsequent postmortem decay during ice storage of anesthetized and exhausted Atlantic salmon. Onset of rigor mortis occurred at a pH of 6.6 – 6.7. Mean ± SD (n = 7).

The onset of rigor mortis occurred after about 30 h for anesthetized fish. For exhausted fish, this happened after less than 3 h (Figure 3). Maximum rigor strength (at 10 h) was higher in exhausted fish and these fish had passed through rigor after 30 h. For the anesthetized fish, rigor strength peaked at about 48 h and the fish were in the post-rigor state after 60 h. The temperature of the fish during the course of rigor mortis might have had some influence on rigor strength and fillet color. As both fish groups were iced immediately after slaughter, this meant that the stress effect caused rigor in exhausted fish to begin to develop with increasing strength at higher body temperatures than with

the anesthetized ones. In fact, for exhausted fish, the rigor peaked roughly around the time that the core temperature had leveled out at about 1 ^{o}C, the same temperature to which the entire rigor course of anesthetized fish were exposed. Even though the temperature difference was relatively modest (ΔT decreasing from 8 ^{o}C), this might, in addition to the effect of stress, have had an influence on rigor development during the first few hours (< 10 h). It is well established that higher storage temperatures promote faster rigor development as well as stronger rigor contractions (Burt and others 1970; Erikson 2001).

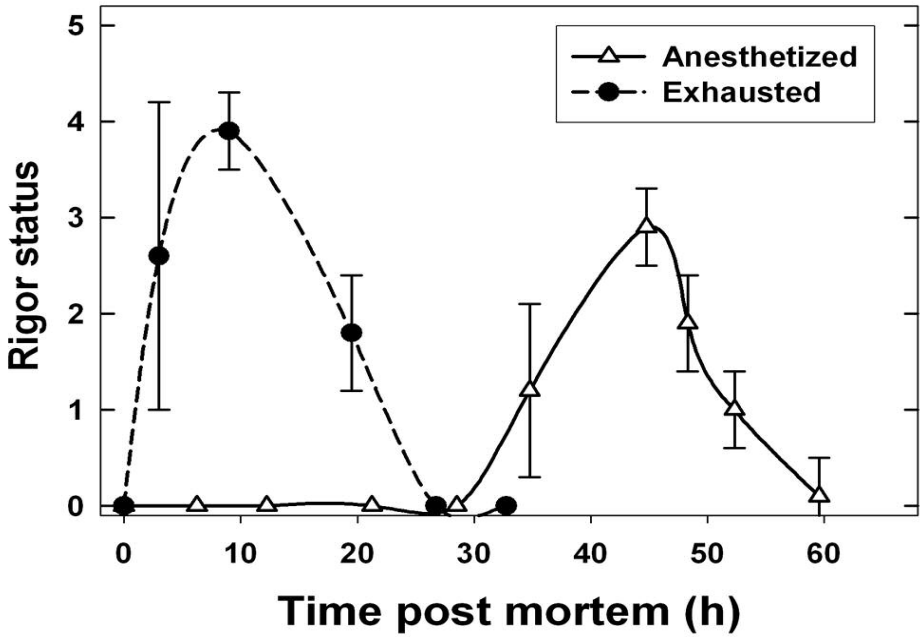

Figure 3-Development of rigor mortis during ice storage of whole gutted Atlantic salmon. Note differences between anesthetized and exhausted fish regarding peak rigor values (rigor strength). Mean ± SD (n = 7).

Skin color

As the fish were kept alive for 5 days in our green-walled laboratory tanks, the skin color may have been altered as compared with the situation in sea cages. It has been shown that some fish species, such as red porgy, alter their skin color within minutes after the fish are transferred to a new tank with a background color that is different than that of the original environment (van der Salm and others 2006). Just after slaughter (t = 0 h), some

of the mean skin color values (Minolta Chroma Meter) on the back (average of L1-L3, Figure 1) differed ($p < 0.05$) between treatments. Different values were obtained in the case of a*, b* and H^{o}_{ab} (Table 1). Thus, the effect of treatments were related to small changes in a* and b* values. In the belly region (average of L4-L6), the b*, H^{o}_{ab}, C^{*}_{ab} and ECI values differed between treatments.

Table 1 - Anesthetized and exhausted Atlantic salmon skin color as determined (Minolta Chroma Meter) immediately after killing (t = 0 h) and after ice storage for 7 days (t = 168 h). Back region: average of locations L1-L3, Belly region: average of locations L4-L6 (Figure 1).

Fish group and storage time	L*	a*	b*	H^{o}_{ab} (^{o})	C^{*}_{ab}	ECI
Anesthetized			Back region			
t = 0 h	30.0 ± 0.8^{aX}	-1.8 ± 0.1^{aX}	0.9 ± 0.1^{aX}	155 ± 2^{aX}	2.6 ± 0.1^{aX}	0.5 ± 0.4^{aX}
t = 168 h	40.8 ± 0.5^{bX}	-1.3 ± 0.1^{bX}	1.0 ± 0.4^{aX}	148 ± 10^{aX}	1.8 ± 0.3^{aX}	0.2 ± 0.5^{aX}
			Belly region			
t = 0 h	84.8 ± 0.2^{aX}	0.8 ± 0.1^{aX}	0.0 ± 0.5^{aX}	326 ± 3^{aX}	1.2 ± 0.1^{aX}	0.8 ± 0.1^{aX}
t = 168 h	85.7 ± 0.3^{aX}	0.5 ± 0.1^{aX}	1.9 ± 0.3^{bX}	72 ± 4^{aX}	2.0 ± 0.3^{aX}	1.4 ± 0.4^{ax}
Exhausted			Back region			
t = 0 h	34.1 ± 1.1^{aX}	-1.1 ± 0.0^{aY}	-1.3 ± 0.1^{aY}	311 ± 2^{aY}	1.7 ± 0.1^{aX}	-1.5 ± 0.1^{aX}
t = 168 h	43.3 ± 2.2^{bX}	-1.5 ± 0.1^{bX}	0.1 ± 0.3^{bX}	285 ± 44^{aX}	0.6 ± 0.1^{bX}	-0.5 ± 0.1^{bX}
			Belly region			
t = 0 h	85.7 ± 0.3^{aX}	1.1 ± 0.1^{aX}	3.2 ± 0.2^{aY}	71 ± 1^{aY}	3.4 ± 0.1^{aY}	3.1 ± 0.2^{aY}
t = 168 h	85.4 ± 0.5^{aX}	0.4 ± 0.1^{bX}	3.7 ± 0.3^{aY}	84 ± 1^{aY}	1.7 ± 0.5^{aX}	1.5 ± 0.4^{aX}

Mean ± SEM, n = 20 (t = 0 h) and n = 7 (t = 168 h). Different letters within each column denote significant difference ($p<0.05$); a vs b: due to ice storage for 1 week. Valid only within each group and fish region (back or belly); X vs Y: due to treatment. Valid only between groups, within similar storage time (t = 0 h or t = 168 h) and fish region.

It should be noted that when the a* and b* values are very low (as in this study), only small changes in these variables can lead to large changes in the calculated H^{o}_{ab} values, as can be seen in Table 2. Higher chroma and ECI values indicated handling stress which

induced higher color saturation and a visible color change in the belly region. After ice storage for a week, no differences were observed in the back region, whereas the b* and hue values in the belly region were still different.

When the postmortem color changes for each treatment are compared, the main effect was that both anesthetized and exhausted fish exhibited higher L* values in the back region during ice storage (t = 0 h *vs.* t = 168 h). The L* values in the belly region were unaffected over time. Other significant changes for anesthetized fish comprised a* in the back region and b* in the belly region. For the exhausted fish, storage resulted in color changes in the back (a*, b*, C^*_{ab}, and ECI) as well as in the belly (a*). As the a* and b* values were consistently low throughout the experiment, this indicated that the skin had a greyish color.

It is difficult to compare the effects of stress on Atlantic salmon skin color with data from other fish species. In addition, color changes after a stress incident seem to occur while the fish are still alive. Iger and others (2001) reported that the stressed fish first darkened and became paler afterwards. Therefore, the skin color at the time of killing would then be dependent on the time after the stressor was introduced. Depending on the species and anatomical region on the fish, there are six kinds of chromatophores with different hues. They may appear in different combinations (Fujii 2000). In the grayish skin and whitish belly of Atlantic salmon, melanophores (black or brown hue) and leucophores (whitish) might constitute a major part of the pigment cells. Some types of iridophores (metallic), which are responsible for silvery glitters and whiteness on the side and belly skin, are immotile cells that are not directly involved in physiological color changes (Fujii 2000).

Fillet color

Due to the thermochromic effect, the initial differences in temperature between groups may have had a presumably minor effect on fillet color. However, it is known that the effect is observable at room temperature when temperature varies by just a few degrees. Red - and orange-colored samples are particularly sensitive (Hiltunen and others 2002).

For example, the L* values of cod fillets are considerably higher when determined (Minolta Chroma Meter) at 20 ^{o}C than at 4 ^{o}C (Stien and others 2005).

Minolta Chroma Meter

The mean color values of locations L1 - L4 (Figure 1) are presented throughout. In all cases, locations L2 - L4 showed nearly similar values whereas location L1 consistently exhibited somewhat higher values. Salmon fillet color has been shown to vary between different parts of the fillet (Skjervold and others 2001).

The mean Minolta Chroma Meter readings are shown in Figures 4 - 9 and in Table 2. At t= 0 h, the mean L* values were 40.6 (anesthetized) and 37.8 (exhausted) showed that perimortem stress produced darker fillets initially ($p<0.05$, Table 2). For both treatments, a further drop was observed that peaked at about 20 h and 10 h post mortem for anesthetized and exhausted fillets, respectively (Figure 4). After this, the L* values rose rather sharply over the next 20 – 40 h before starting to level out. For the anesthetized fillets, the L* values showed a further moderate increase during ice storage. After ice storage for one week (t = 168 h), the mean L* value of the anesthetized fillets was still significantly higher (42.1) than that of their exhausted counterparts (39.8). Both groups of fillets became lighter during ice storage. When the L* curves are compared with the rigor curves (Figure 3), we can see for the exhausted fillets that the peak region of L* curve basically resembles an inverted rigor-curve (similar time courses). This strongly suggests that muscle contractions and altered muscle light scattering properties caused changes in lightness. Another factor to consider, typical of very fresh fish, is the translucency of the muscle. After killing, the flesh gradually becomes less translucent (Stien and others 2005). When the flesh becomes opaque, the light-absorbing and light-reflecting properties will change (Ozbay and others 2006). As long as the flesh is translucent, color reading may also be affected by blood. Since our fish were not bled, it might be that the initial fillet color levels reported here would have been somewhat altered had the fish been bled. For instance, in bullfrog meat, it has been reported that the meat of bled animals was whiter and more yellow (b* value) (Ramos and others 2005).

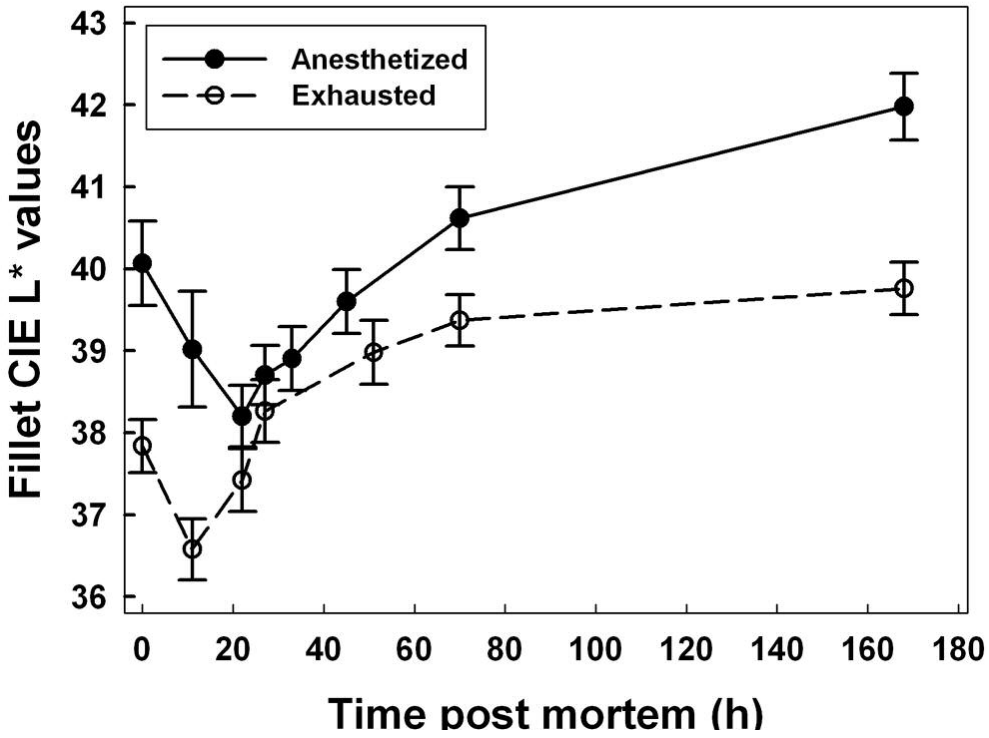

Figure 4- Anesthetized and exhausted Atlantic salmon fillet lightness (L*). Effects of perimortem handling stress (0 h), rigor mortis, and ice storage for 7 days (168 h). The mean Minolta Chroma Meter readings of locations L1-L4 (Figure 1) are shown. Mean ± SEM (n = 26 fillets).

For the anesthetized fillets, the peak of the L* curve is shifted towards longer storage times as compared with the exhausted fillets. This indicates that the magnitude and pattern of the changes were affected by perimortem stress in addition to postmortem age. No apparent difference was observed in the magnitude of the drop in L* values (2-3 units). Thus, the lightness differences observed after 1 week of storage may be ascribed to perimortem stress rather than to structural changes occurring during the different developments of rigor mortis (Figure 3). In whole fish, the rigor peak of anesthetized fish occurred between 30 - 60 h. The peak of the corresponding L* curve, however, occurred after 20 h. One explanation for this discrepancy may be that handling of fish during rigor assessments shortens the duration of rigor (Berg and others 1997). Another explanation could be that handling during pre-rigor filleting may have shortened the time to rigor onset. It is likely that the anesthetized fish would be most affected by these factors. Typical for anesthetized fish was the pre-rigor drop in white muscle pH (Figure 2). The

major changes occurred before rigor onset, namely during the first 30 h. The drop in pH would cause structural changes as the protein network shrinks (Foegeding and others 1996), whereas ATP depletion leads to altered membrane permeability and ultimately to cessation of the ATP-driven ionic pumps affecting chemical composition of the various tissue compartments. Thus, it appears that transient early postmortem color changes in salmon fillets were, in addition to the factor mentioned above, affected by at least 2 factors related to muscle structure, shrinkage due to lowered pH, and then to rigor contractions. In swollen DFD (dark, firm and dry) meat (high pH), the fibers scatter less light than normal meat. At low pH, the opposite effect occurs as the shrunken fibers of PSE (pale, soft and exudative) meat scatter more light than normal, resulting in a pale meat (Foegeding and others 1996). Thus, due to the higher pH in the anesthetized fish in the early postmortem phase, a darker fillet color might be expected for the anesthetized fish compared with the exhausted fish. However, we found that the opposite was true, the lightness of our anesthetized salmon was higher and it actually decreased during the period with a drop in pH. On the other hand, redness, yellowness, hue, as well as color saturation (C^*_{ab}), decreased over the same period for the anesthetized fish (see below). When rigor started and the pH had more or less leveled out, the lightness increased for both fish groups. For salmonids, where the carotenoids are important for fillet color, Johnston and others (2000) explained the effect of increasing light scattering by stating that light does not penetrate deeply into the fillet before being scattered, resulting in less absorption by astaxanthin and, hence, paler color can be perceived. Also, it has been hypothesized that differences in lightness, hue and chroma between electrostimulated and rested rainbow trout may be caused by differences in the levels of insoluble protein (Robb and others 2000).

The corresponding changes in redness (a* values) are shown in Figure 5 and Table 2. For the anesthetized fillets, a similar pattern as for the changes in L* values could be observed. However, the low a* peak values occurred after longer storage time, namely, after 30 h.

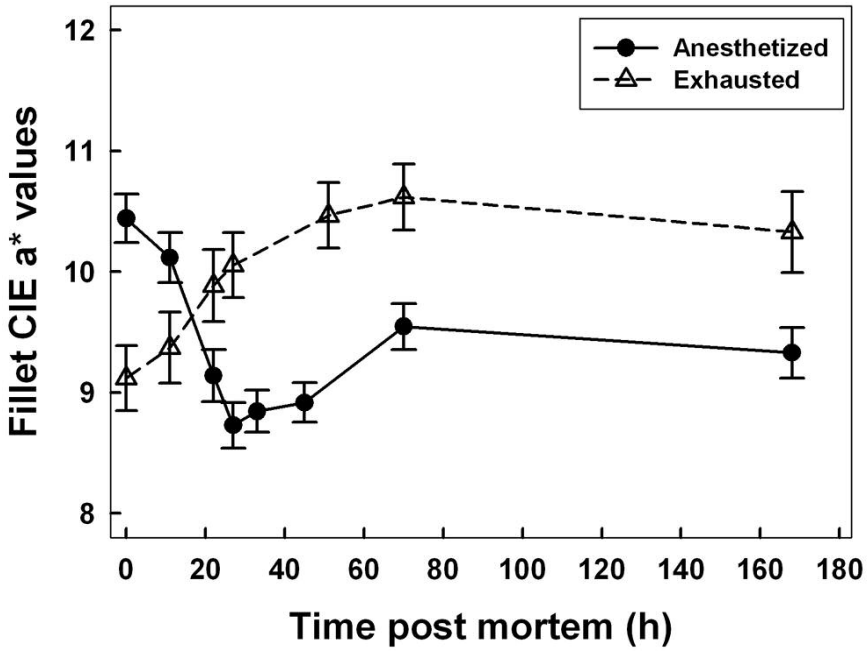

Figure 5- Anesthetized and exhausted Atlantic salmon fillet redness (a*). Effects of perimortem handling stress (0 h), rigor mortis, and ice storage for 7 days (168 h). The mean Minolta Chroma Meter readings of locations L1-L4 (Figure 1) are shown. Mean ± SEM (n=26 fillets).

Initially, the fillet a* values gradually decreased. After 30 h, when whole fish rigor started, the a* values began to increase and ended up at a slightly lower level (9.3) than the mean initial values (10.5). In the exhausted fillets, where rigor started very rapidly (Figure 3), the a* values rose and leveled out after 60 - 70 h, basically corresponding to completion of the rigor development. In contrast to the anesthetized fillets, the final a* values (10.3) were higher than the initial ones (9.1). When the groups are compared, the anesthetized fillets had higher a* values initially ($p<0.05$), but this difference evened out after ice storage for 1 week ($p>0.05$).

The patterns of the postmortem development of yellowness (b*) were basically similar to those of redness (Figure 6 and Table 2). Initially, the anesthetized fillets exhibited higher b* values than those from their exhausted counterparts ($p<0.05$).

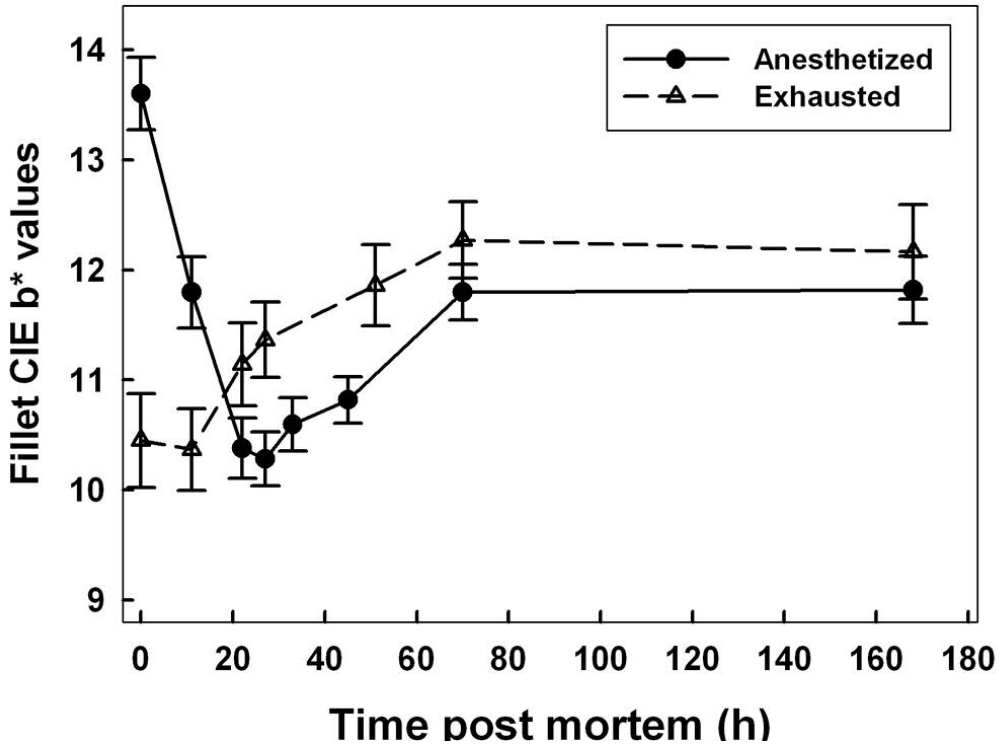

Figure 6- Anesthetized and exhausted Atlantic salmon fillet yellowness (b*). Effects of perimortem handling stress (0 h), rigor mortis, and ice storage for 7 days (168 h). The mean Minolta Chroma Meter readings of locations L1-L4 (Figure 1) are shown. Mean ± SEM (n=26 fillets).

Like redness, however, the difference in yellowness between groups did not persist ($p>0.05$) until a time when the fresh fillets would typically be available to consumers (1 week). When each group was considered separately, however, significant changes took place in both cases. For the anesthetized fillets, the mean b* values decreased from 13.6 (t = 0 h) to 11.8 (t = 168 h). In contrast, for the exhausted fillets, a significant increase from 10.5 to 12.2 was observed ($p<0.05$).

Apart from transient changes during rigor, the mean hue (H^{o}_{ab}) of both groups did not change during ice storage (Figure 7). When anesthetized and exhausted fillets are compared, the initial higher hue values of the anesthetized fillets (52.5 ^{o} *vs.* 48.9 ^{o}, Table 3) also persisted ($p<0.05$) after one week of ice storage (51.7 ^{o} *vs.* 49.7 ^{o}).

Table 2 - Comparison between anesthetized and exhausted Atlantic salmon fillets CIE L* a* b*, H^{o}_{ab}, and C^{*}_{ab} values immediately after killing (t = 0 h) and after ice storage for 1 week (t = 168 h). The average color of whole fillets was determined by the computer vision method and the mean color of locations L1 - L4 (Figure 1) was determined by the Minolta Chroma Meter.

Storage time and method	L*	a*	b*	H^{o}_{ab} (^{o})	C^{*}_{ab}
t = 0 h			*Anesthetized*		
Minolta	40.6 ± 5.6^{aX}	10.5 ± 2.6^{aX}	13.6 ± 3.1^{aX}	52.5 ± 1.5^{aX}	17.2 ± 4.1^{aX}
Computer vision	38.7 ± 0.9^{aX}	41.7 ± 1.1^{aX}	27.5 ± 1.0^{aX}	33.3 ± 0.8^{aX}	50.0 ± 2.0^{aX}
t = 168 h					
Minolta	42.1 ± 6.1^{bX}	9.3 ± 2.6^{bX}	11.8 ± 3.6^{bX}	51.7 ± 3.6^{aX}	15.1 ± 4.1^{bX}
Computer vision	39.7 ± 0.8^{bX}	38.0 ± 1.5^{bX}	24.0 ± 1.4^{bX}	32.7 ± 0.7^{bX}	44.3 ± 1.5^{bX}
t = 0 h			*Exhausted*		
Minolta	37.8 ± 3.1^{aY}	9.1 ± 3.1^{aY}	10.5 ± 3.1^{aY}	48.9 ± 3.6^{aY}	13.9 ± 4.1^{aY}
Computer vision	36.7 ± 0.7^{aY}	39.7 ± 1.1^{aY}	25.3 ± 1.0^{aY}	32.3 ± 0.8^{aY}	47.2 ± 1.3^{aY}
t = 168 h					
Minolta	39.8 ± 4.6^{bY}	10.3 ± 3.6^{bX}	12.2 ± 4.1^{bX}	49.7 ± 2.0^{aY}	15.9 ± 5.1^{bX}
Computer vision	38.9 ± 0.9^{bX}	42.3 ± 2.0^{bY}	28.4 ± 2.1^{bY}	33.9 ± 1.0^{bY}	50.8 ± 3.5^{bY}

Mean ± SD (n = 26); Different letters within each column denote significant difference (p < 0.05); a vs b: due to ice storage for 1 week within each group; X vs Y: due to treatment (between groups); The data obtained by the Minolta Chroma Meter and the computer vision method were not compared statistically;

Apart from transient changes during rigor, the mean hue (H^{o}_{ab}) of both groups did not change during ice storage (Figure 7). When anesthetized and exhausted fillets are compared, the initial higher hue values of the anesthetized fillets (52.5 ^{o} *vs.* 48.9 ^{o}, Table 3) also persisted ($p<0.05$) after one week of ice storage (51.7 ^{o} *vs.* 49.7 ^{o}).

Similarly, the mean color saturation (C^{*}_{ab}) also decreased during the pre-rigor phase of the anaesthetized fillets (Figure 8), that is, during the period with the large drop in pH (Figure 2). After this, the chroma increased and leveled out at about 70 h, when the fish were in the post-rigor state (Figure 3). Perimortem handling stress initially produced fillets with lower color saturation ($p<0.05$), since the average C^{*}_{ab} values of anesthetized and exhausted fillets were 17.2 and 13.9, respectively (Table 3). After ice storage, the mean chroma of the anesthetized fillets was reduced to 15.1 ($p<0.05$). For the exhausted fillets, the opposite trend was observed, the chroma value increased to 15.9 during the

same period ($p<0.05$). After storage for one week, the chroma was no longer different between the groups.

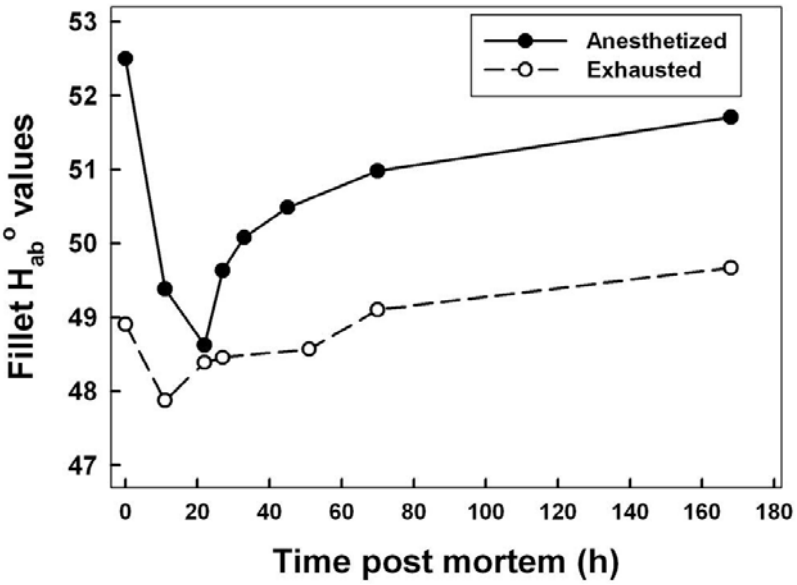

Figure 7- Anesthetized and exhausted Atlantic salmon fillet hue (Hoab). Effects of perimortem handling stress (0 h), rigor mortis, and ice storage for 7 days (168 h). The mean values based on Minolta Chroma Meter readings of locations L1-L4 (Figure 1) are shown. Mean ± SEM (n=26 fillets).

Figure 9 shows the Entire Color Index (ECI) based on the hue and chroma values. The color difference between the groups was most evident initially, i.e. the effect of perimortem handling stress seemed to be the single most important factor. The mean (locations 1-4) ECI value of anesthetized fillets was -7.0 as opposed to 12.9 for the exhausted fillets ($p<0.05$). For the anesthetized fillets, the ECI values increased during the pre-rigor period as the pH was gradually reduced. After rigor and ice storage, the effect of stress was no longer significant ($p>0.05$). The average ECI values of anesthetized and exhausted fillets were then 4.3 and 8.7, respectively.

Taken together, the color variations as a result of either stress or ice storage were comparatively modest. Chroma, hue (Robb 2001) and ECI (Pavlidis and others 2006) are considered to represent the actual color of an object more accurately that the L* a* b* values alone since they simultaneously take both a* and b* (hue and chroma) or hue and

chroma (ECI) into account. Simply speaking, perimortem stress initially produced fillets with more vivid and saturated colors (red and yellow). After storage, the fillets were lighter. To get a visual impression of how individual variation in L* a* b* values affect the overall color perception of an object, refer to Wyszecki and Stiles (1982) and Sharma (2003).

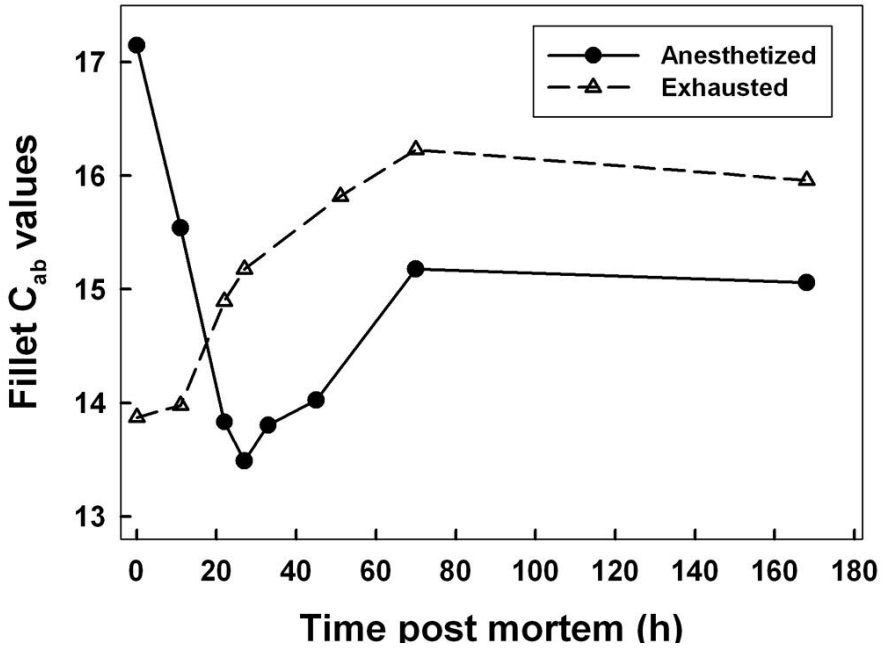

Figure 8- Anesthetized and exhausted Atlantic salmon fillet chroma (C*ab). Effects of perimortem handling stress (0 h), rigor mortis, and ice storage for 7 days (168 h). The mean values based on Minolta Chroma Meter readings of locations L1-L4 (Figure 1) are shown. Mean ± SEM (n=26 fillets).

Our L*, a*, b*, H^{o}_{ab}, and C^{*}_{ab} values were largely within the ranges typical of Atlantic salmon fillets, as reported by Skrede and Storebakken (1986), Christiansen and others (1995) and Rørå and others (1998). Comparison with other studies should, however, be done with care as Stien and others (2005, 2006) showed that determination of fish flesh color using different colorimetric instruments resulted in considerable variation in the L* a* b* values.

By using correlation plots given by Christiansen and others (1995), the astaxanthin concentration in our fillets was estimated to be about 5 - 7 mg kg^{-1}. Accordingly, based on our a* values, we could expect Roche Color Card readings between 14 to 15.

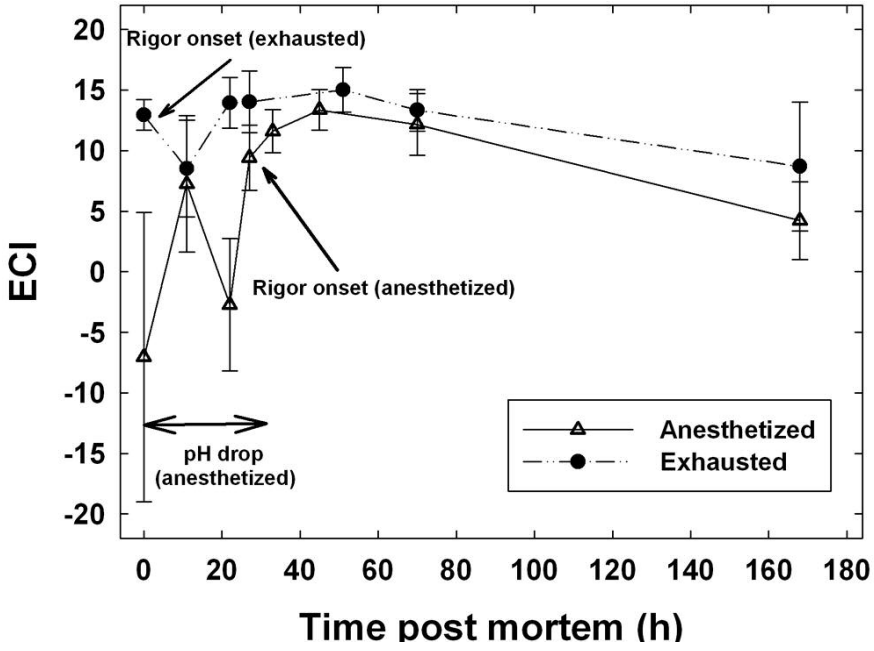

Figure 9- Anesthetized and exhausted Atlantic salmon fillet Entire Color Index (ECI). Effects of perimortem handling stress (0 h), rigor mortis, and ice storage for 7 days (168 h). The mean values based on Minolta Chroma Meter readings of locations L1-L4 (Figure 1) are shown. Mean ± SEM (n = 26 fillets). The early postmortem period where the white muscle pH in anesthetized fish dropped significantly is indicated. Onset of rigor mortis for both groups are also indicated.

Indeed, this is similar to the values we obtained with our sensory panel (Table 3). For AQUI-S^{TM} anesthetized and electrostimulated rainbow trout, Robb and others (2000) also observed an initial drop in L* values before they gradually increased up to 75 h post mortem, when the experiment was terminated. Their anesthetized fillets exhibited significantly higher L* values during this period, whereas our L* values did not differ significantly between groups during the same period (Figure 4). Our anesthetized salmon fillets had higher initial (t = 0 h) and final (t = 168 h) hue values than the exhausted fillets (Figure 7). The anesthetized rainbow trout, on the other hand, exhibited significantly lower hue values throughout storage (75 h) as compared with the electro-stimulated fish.

For both our salmon (Figure 8) and the rainbow trout, the initial chroma values were highest in the case of anesthetized fish.

Table 3 - Anesthetized and exhausted Atlantic salmon fillet color after ice storage for 7 days. Roche color was determined by averaging fillet regions R1-R3 (computer vision method). The same fillets were also assessed by a sensory panel.

Roche color	Computer vision			Sensory panel[nsd]
	R1	R2	R3	
		Anesthetized		
*Salmo*Fan™	29.7 ± 0.5[a]	30.2 ± 0.3[a]	30.1 ± 0.2[a]	27.0 ± 1.2
Color Card	16.7 ± 0.4[nsd]	16.9 ± 0.8[nsd]	16.9 ± 0.5[nsd]	15.0 ± 0.7
		Exhausted		
*Salmo*Fan™	28.0 ± 1.2[b]	29.5 ± 0.5[b]	29.4 ± 0.5[b]	26.6 ± 1.1
Color Card	16.4 ± 0.7[nsd]	16.6 ± 0.6[nsd]	16.5 ± 0.5[nsd]	14.9 ± 0.8

Mean ± SD (n = 22 - 24 fillets, sensory panel),'a' and 'b' within each column denotes significant difference ($p < 0.05$) for each Roche color scale. ' nsd' = no significant difference ($p > 0.05$).

After 70 - 80 h post mortem, this difference between groups was offset in the case of our salmon, whereas the chroma values for rainbow trout were always (> 10 h post mortem) lower than those of the electrostimulated trout. Kiessling and others (2004) compared AQUI-S™ and carbon dioxide-anesthetized salmon fillets. After 5 days of ice storage, they found no significant difference between groups in terms of L* values (around 55), but they found significantly higher a* (mean CO_2 *vs.* AQUI-S™: 27.1 *vs.* 26.4) and b* (22.6 *vs.* 22.0) values for the fish subjected to the carbon dioxide treatment. On the other hand, our corresponding values from evaluation after 7 days post mortem showed higher L* values for the AQUI-S™-treated fish. Otherwise, we found lower a* values, but no difference in b* values for such fish (Figures 4 - 6). Reduced a* values due to antemortem handling stress have also been reported for Arctic char (Jittinandana and others 2003). Skjervold and others (2001) compared the color of pre- and post rigor Atlantic salmon fillets. At Day 0, the Minolta L* values of prerigor fillets (45.7) were lower than post rigor fillets (50.2). Since the experiment was carried out at a fish processing plant, their fish most likely correspond to our exhausted fish. When evaluated at 5 - 6 days post mortem, the difference in L* values persisted and the a* and b* values of fillets cut pre rigor were significantly higher than those cut post rigor. In another

study, rested and stressed Atlantic salmon fillets were compared 4 days post mortem. Apart from a higher mean L* value for the rested fish, no significant differences were observed in the case of the a*, b*, hue and chroma values (Roth and others 2006).

Computer vision – CIE L*a*b* color space. Table 2 shows a comparison between color values obtained practically simultaneously at 0 and 168 h by the Minolta Chroma Meter and the computer vision method. The ECI values were practically identical with the chroma values and are therefore not shown in the table. While the L* values from the computer vision method were in the same range as those generated from the Minolta Chroma Meter, the a*, b*, $H^{o}_{ab,}$ and C^{*}_{ab} values from the computer vision method were very different. Nevertheless, all of the values were within the range of CIE L*a*b* values obtained by other computer vision methods (Kane and others 2003; Marty-Mahe and others 2004; Yam and Papadakis 2004; Kim and others 2005). The Minolta Chroma Meter, with the probe in direct contact with the sample, uses a pulse of xenon light to illuminate the examination area (8 mm in diameter) and measures the light reflected from the sample. For the computer vision method, with no direct contact between fish/fillet and camera, once the RGB values are calibrated into device independent color data and mapped into CIEL*a*b values, the system provides consistent color readings regardless of the input or the output device such as digital camera, monitor, or scanner provided that the illumination is controllable (Yam and Papadakis 2004).

At t = 0 h, all color parameters determined by the computer vision method were different ($p < 0.05$), that is, affected by perimortem struggling. After ice storage, all of the parameters remained different. According to the computer vision method, stress produced darker, less red and yellow fillets with lower hue and color saturation. These results are similar to those of the Minolta Chroma Meter method. After rigor and ice storage, on the other hand, the anesthetized fillets were less red and yellow, and they exhibited lower hue and chroma values than their exhausted counterparts. In both cases, the fillets exhibited higher L* values. We have no plausible explanation as to why the results from the 2 methods differed only after ice storage. Perhaps the discrepancy can be related to the

different areas of color determination (Minolta: L1-L4 *vs.* Computer vision: whole fillet, Figure 1) for the methods.

The mentioned values generated with the computer vision method differed from those obtained using the Minolta Chroma Meter. The range, low-to-high readings (Δ), of L*,a* and b* were largely similar for both methods with Δ values between 2 and 6. The higher a* and b* values obtained by the computer vision method seemed to describe the typical rather bright red-orange appearance of salmon fillets better than the Minolta instrument, since the comparatively low Minolta a* and b* values suggested that the fillets had a somewhat greyish appearance. This can probably be explained by the calibration of the computer vision system against a ColorChecker chart, while the Minolta Chroma Meter is primarily designed for flat, nontranslucent and diffuse surfaces. Altogether, we think that the computer vision system can operate in an on-line context where salmonid fillets are graded and sorted according to color.

Computer vision and sensory panel – Roche color scales

Table 3 shows a comparison between Roche color values obtained by the computer vision method and the sensory panel. The computer vision-based values (both Roche color scales) at the R1-R3 (Figure 1) regions did not differ ($p>0.05$). For both the *Salmo*Fan™ and color card, the computer vision-based method consistently gave higher values with lower standard deviations than the values obtained by the sensory panel. Due to the more detailed discrimination of color (15 shades), the Roche *Salmo*Fan Lineal™ readings were probably more precise than those determined with the color card (8 shades). Therefore, only the *Salmo*Fan™ readings determined by the computer vision method exhibited significantly different values ($p<0.05$) between anesthetized and exhausted fillets after ice storage for a week.

Since the mean Roche *Salmo*Fan™ values of exhausted fish were lower at 7 days post mortem, larger and significant differences between groups might well have occurred after a shorter storage period. Also, the color card might then have detected differences between groups. For instance, when fillet color was evaluated after 3 (Robb and others

2000) and 4 (Robb and Warriss 1997) days, AQUI-S^{TM}-anesthetized trout fillets exhibited 1 unit higher readings on the Roche Color Card scale than their counterparts that were subjected to high muscle activity before killing. Moreover, Atlantic salmon fillets cut pre-rigor exhibited significantly higher mean Roche *Salmo*Fan^{TM} values than fillets cut post-rigor at Day 0, 25.6 and 23.6, respectively. Six days later, the groups were no longer different at 24.6 and 24.3 (Skjervold and others 2001).

A summary of skin and fillet color changes – Comparison of analytical methods

On skin, none of the initial stress-related changes (redness, yellowness and hue) could be detected after ice storage. In contrast, the initial difference between groups regarding yellowness and hue in the belly section lasted throughout ice storage.

Both the Minolta Chroma Meter and computer vision method detected all stress-related changes in fillets initially (lightness, redness, yellowness, hue, chroma and ECI). After ice storage for a week, the number of significant ($p<0.05$) parameters were different between the 2 methods. The sensory panel did not detect differences in Roche color card scales when assessed after 7 days. The computer vision method detected a significant difference only in the case of the *Salmo*Fan^{TM}. Taken together, perimortem handling stress clearly affected skin and fillet color when color was assessed immediately after killing. For some color parameters, the difference in color between anesthetized and exhausted fish persisted throughout ice storage. However, since they could only be detected instrumentally, consumers would likely not be able to distinguish between the 2 groups of fish after 1 week. In other words, the stress had largely been offset by storage time.

The evaluation of fillet color by either sensory analysis (grading by trained inspectors using Roche color cards) or by manually-based instrumental methods had disadvantages compared with the computer vision method. The drawbacks of sensory analysis are that the method is slow, costly and subjective. The subjectivity and inconsistency of sensory evaluation probably derived from the physical limitations of the human eye to adequately perceive color (eye fatigue and lack of color memory). This may result in incorrect fillet

grading. Manually-based instrumental color analysis is objective, but still labor-intensive and not fast enough to cope with the required processing speed (such as 1 fillet per sec). Another typical limitation of such instruments is that they are based on direct contact with the fillet and that the obtained color values result from a small sample surface. In addition, the instrument is primarily designed for measurements of the color of flat, nontranslucent and diffuse surfaces, making it inappropriate for color measurements of fillets, since they are typically not flat or translucent. On the other hand, the computer vision method allows for fast, nondestructive and contact-free color assessments. Moreover, by using the computer vision method, we were able to evaluate the color of the entire area of the fish skin or fillet. In our study, the computer vision method generated smaller standard deviations (Table 3) than sensory evaluation, suggesting that greater robustness and consistency can be expected when using the computer vision method. Automated sorting of whole fish or fillets by color with the aid of computer vision and image processing was therefore considered as a method of choice for color grading in fish processing plants that deal with large volumes.

Conclusions

We have shown that when Atlantic salmon were exposed to two extremes of perimortem muscle activity, no exercise and exercise to exhaustion, significant changes in both initial skin and fillet color occurred. To some extent, some color parameters were detectable after ice storage for 7 days; however, these differences were relatively small and probably could not be spotted by consumers. With current commercial harvesting routines, at least some muscle activity will take place, and with less than ideal storage conditions, it seems unlikely that possible initial differences in skin and fillet color due to better harvesting routines would result in improved coloration of the product after 7 days of ice storage. However, previous studies have shown that differences can be detected after shorter storage times. Markedly transient color changes occurred during the pre-rigor phase and during the course of rigor mortis. This could be explained by the altered physical state of the myofibrils that affect light scattering properties as muscle/cell structure was affected by a drop in muscle-pH (glycolysis) and subsequently by rigor contractions. The computer vision method was considered to be at least as good as the Minolta Chroma

Meter for salmon grading and sorting purposes, and better than a sensory panel for assessing Roche color scales. For on-line purposes, the computer vision method was considered the method of choice. Notably, if filleting is done pre rigor, care should be exercised during color grading since transient color changes occur in the early postmortem period. As these changes are more pronounced than those occurring during ice storage, incorrect color grading of fillets may result.

Acknowledgements

We express our gratitude to AQUI-S Ltd. for providing AQUI-S^{TM} anesthetic and the Iki Jime Tool. Thanks also go to The Research Council of Norway for financial support of this study (*NFR projects No. 145634/I40 –'Efficient and economic sustainable fish processing industry' and No. 173530/I30 – 'Technology for efficient and profitable fish industry').*

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PAPER IV

Computer vision-based evaluation of pre- and postrigor changes in size and shape of Atlantic cod (*Gadus morhua*) and Atlantic salmon (*Salmo salar*) fillets during rigor mortis and ice storage: Effects of perimortem handling stress

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Food Engineering and Physical Properties

ABSTRACT

The present study describes the possibilities for using an on-line computer vision method for the detection of transient 2D and 3D changes in the geometry of a given product. The rigor contractions of unstressed and stressed fillets of Atlantic salmon (*Salmo salar*) and Atlantic cod (*Gadus morhua*) was used as a model system. Gradual changes in fillet shape and size (area, length, width and roundness) were recorded for 7 and 3 d, respectively. Also, changes in fillet area and height (cross-section profiles) were tracked using a laser beam and a 3D digital camera. Another goal was to compare rigor developments of the two species of farmed fish, and whether perimortem stress affected the appearance of the fillets. Some significant changes were found between unstressed and stressed fillets during the course of rigor mortis as well as after ice storage (post rigor). However, the observed irreversible stress-related changes were small and would hardly mean anything for post-rigor fish processors or consumers. The cod were less stressed (as defined by muscle biochemistry) than the salmon after the two species had been subjected to similar stress bouts. Consequently, the difference between the rigor courses of unstressed and stressed fish was more extreme in case of salmon. However, the maximal whole fish rigor strength was judged to be about the same for both species. Moreover, the reductions in fillet area and length, as well as the increases in width, were basically of similar magnitude for both species. In fact, the increases in fillet roundness and cross-section height were larger for the cod. We conclude that the computer vision method can be used effectively for automated tracking of changes in 2D and 3D shape and size of objects such as fish fillets during rigor mortis and ice storage. The method is rapid, nondestructive and contact-free and can therefore be regarded as suitable for industrial on-line purposes.

Key words: Computer vision, fillet shape, rigor mortis, salmon, cod, handling stress

Introduction

Due to the high labor costs prevailing in several countries, a higher degree of automation of processing lines is often desirable. In the fish processing industry, computer vision is beginning to gain the necessary maturity for online assessments of various attributes related to flesh quality. This can enable lower production costs through automation and higher product quality through a more consistent non-destructive evaluation of the products (Strachan and Murray 1991; Strachan 1993; Stien and others 2005, 2006; Misimi and others 2006, 2007).

A certain raw material may change in shape and size during the early postmortem phase. The development of rigor mortis in meat and fish are familiar examples of this. Early onset of rigor mortis, as a result of perimortem handling stress, can occur while fish still are in processing line (Berg and others 1997). Processing of such fish should be avoided since this may have detrimental effects on fillet quality (Stroud 1968). Prerigor fillets have several properties that differ from their postrigor counterparts (Sørensen and others 1997; Rørå and others 2004; Kristoffersen and others 2006a). Mostly, these properties are considered favourable in terms of flesh quality (Skjervold and others 2001a, b; Stien and others 2005). The concept of prerigor filleting is therefore currently a goal for several salmon processors in Norway. With this situation as a backdrop, it is relevant to establish knowledge of the extent of the changes in fillet shape taking place shortly after slaughtering.

At the time of death, the muscle is relaxed, limp and has an elastic structure. As the rigor process develops, the fish become gradually more hard and stiff. In stressed Atlantic salmon (*Salmo salar*), rigor onset occurs after only 2 - 4 h post mortem. If antemortem stress is avoided altogether, rigor onset is delayed for another 20-25 h (Erikson 2001a). Moreover, higher mechanical strength is observed in stressed fish muscle during rigor (Nakayama and others 1992). During the course of rigor, the fillets change their geometrical size and shape as they shrink in length (Connell 1990; Sørensen and others 1997). The degree of shrinkage is different depending on fish species and the way they are handled. Typical shrinkages of Atlantic cod (*Gadus morhua*) fillets range from approximately 7 % (Karl and others 1997) to 25% (Connell 1990). Skjervold and others (2001a) reported a 2.4 – 2.7 mm (8 -11 %)

reduction in fillet height after rigor was completed. Rigor contractions in excised rested and exercised chinook salmon (*Oncorhyncus tshawytscha*) muscle strips were studied by Jerrett and others (1998). They used a CCD camera connected to a time-lapse video recorder to record the changes in sample length. Rigor contractions of Atlantic cod fillets were studied by Stien and others (2005). After about 29 h post mortem at 4^{o}C, unstressed and stressed fillets had contracted 15 and 20 %, respectively. At a similar temperature, the rigor contractions in Atlantic salmon fillets were determined up to 5 d post mortem. Images were captured by a digital camera and the fillet contraction was determined by analyzing the images. The method was also employed to assess rigor contractions (shrinkage) in rainbow trout (*Oncorhynchus mykiss*) (Stien and others 2006) and Atlantic salmon Kiessling and others (2006) fillets. Furthermore, based on image analysis, rigor contractions (reduction in length) have been shown to be more severe in stressed (13.8 %) than in unstressed (5.4 - 11.2 %) salmon fillets (Veiseth and others 2006).

Rigor assessments can be carried out by using a variety of different methods based on various principles of detection (see Erikson 2001a). Computer vision and image processing has been used to study various aspects of the development of rigor mortis in fish. From images obtained during the course of rigor, the tail drop angle has been determined as the tail varies between a hanging-down (limp fish) and a horizontal (stiff fish) position (Azam and others 1990; Oliveira and others 2004). This basically represents an automation of the widely-used Rigor Index method (Bito and others 1983).

Since fillets undergo both changes in 2D size (length, width, area) (Sørensen and others 1997; Einen and others 2002) as well as in 3D (2D + height/area of cross-section) (Skjervold and others 2001a), the main goal of our study was to develop a computer vision based method for automatic online evaluation of changes in both 2 and 3 D geometry. By using the effects of extreme perimortem handling stress on muscle biochemistry as a model, similarly treated farmed unstressed and stressed Atlantic salmon and Atlantic cod were compared. Our specific goals were to assess changes in (1) fillet area, length, width and roundness, (2) area and height of different fillet cross-sections, and whether, (3) the 3D computer vision method was suited for online sorting applications according to differences in shape and size (volume), and

(4) the perimortem stress-affected differences in geometrical shape and size were irreversible and thus discernible from unstressed fillets at a time when the fillets would be available to the consumers.

2. Materials and Methods

Fish and fish sampling

Two experiments were carried out with a lean (cod) and a fatty (salmon) fish species.

Atlantic cod. Cod (weight: 2.0 ± 0.3 kg; fork length: 53 ± 3 cm, n = 61) fasted for 12 days at 9^{o}C were obtained from a fish farm located in Central Norway. The fish were netted from the cage and transferred to 2 tubs containing clean seawater (SW). A small boat transported the tubs for 5 min and the tubs were brought ashore. The fish were immediately netted into 3 transport tanks on a truck used for commercial transport of live fish. The tanks contained clean SW and they were oxygenated during the 3 h transport without water renewal to our laboratory. The fish densities were 32 - 40 kg m^{-3}. At arrival, the dissolved oxygen concentration (DO) showed that the SW (salinity 35 ppt) in all tanks was supersaturated (112, 202 and 270 %, respectively) and the pH ranged from 7.3 to 7.5 which indicated that carbon dioxide had accumulated during the transport. No mortality had occurred and the fish were calmly swimming around in the tanks. They were then transferred in SW-filled 1000-L tubs to 2 holding tanks (4000-L) where the fish, equally distributed among the tanks, were kept for 6 d without feeding at a fish density of about 18 kg m^{-3}. SW from 80 m depth was pumped, sand-filtered, and circulated to the tanks at a rate of 5 m^3 h^{-1}. During the holding period, the water temperature, pH, carbon dioxide, and DO in both tanks were within the ranges of 8.7 - 9.2 ^{o}C, 7.9 - 8.0, 1.5 - 2.0 mg L^{-1}, and 77 – 84 % saturation, respectively.

At Day 0, when the experiment started, the water supply to one of the holding tanks was stopped and oxygen gas was added. A pre-defined amount of AQUI-S^{TM} (AQUI-S Ltd, Lower Hutt, NZ) was added to give a final concentration of 30 mg L^{-1}. After 20 min, all fish were anesthetized. No vigorous muscle activity took place during the treatment. However, after 45 min all fish were declared dead as judged from cessation

of gill movements and lack of response to stimuli. The six fish, allocated to the computer vision study, were lifted individually from the tank between 4.0 and 4.5 h after the anesthetic was added to the tank, that is, about 3.5 h post mortem. The gill arches on one side of the fish were severed. With their head pointing downwards, they were then bled in air for 2 min. The glucose content was immediately determined in a sub-sample of the collected blood. Then the white muscle pH, twitches, body temperature, body weight and fork length were measured. About 2 g of epaxial muscle was excised for determination of the total water content. Subsequently, the fish were gutted, filleted and briefly washed under running tap water before the fillets were tagged and placed on ice in Styrofoam boxes. All 12 fillets were immediately subjected to analysis using the computer vision method for the next 4 days (t = 0, 5, 16, 23, 33, 53 and 72 h post mortem). After each sampling time, the fillets were kept on ice in a cold room. Only the skin side of the fillet was in direct contact with ice.

The fish in the other holding tank were stressed to exhaustion by chasing for 30 min. At the same time the water level in the tank was reduced to a height of about 10 cm. Six fish were then killed by a blow to the head within 30 min after the stress bout (the fish were not allowed to recover). Subsequently, they were treated as with the unstressed fish.

The mean round weight and fork length of the unstressed and stressed subgroups of cod that were filleted and used for the present rigor study were 2.2 ± 0.5 kg and 57 ± 4 cm (n = 6), and 2.0 ± 0.4 kg and 54 ± 3 cm (n = 6), respectively. In addition, five other fish from each group were allocated for the assessment of whole fish rigor development. They were also ice stored and used for direct comparison with rigor development of fillets. The time post mortem for assessment of whole-fish rigor was synchronized with the computer vision-based assessment of rigor development in fillets.

Atlantic salmon. Salmon (fasted for 8 d), weighing 3.8 ± 1.1 kg with fork length 66 ± 6 cm (n = 40), were commercially farmed. They were transported similarly as with the cod live by truck to our laboratory where they were kept under good water quality conditions for 5 d before they were slaughtered. As with the cod, one group was unstressed (AQUI-S^{TM}) and the other one was stressed to exhaustion before killing,

except that the fish were not bled. For more details on salmon experimental design, handling and assessments of stress, and the effects of handling stress and rigor on the skin and fillet color of the same fillets presented here, refer to Erikson and others (in press).

Thirteen unstressed (round weight: 4.1 ± 0.9 kg; fork length: 67 ± 5 cm) and 12 stressed (3.8 ± 1.3 kg; 66 ± 7 cm) salmon were used for the present study, i.e. 26 and 22 fillets respectively were subjected to assessments of total length, area, width, height and roundness during the rigor mortis using the computer vision method. The mean white muscle total fat content of 10 other fish from the same batch was 11.5 ± 3.1 % (Erikson and others, in press).

The changes during the course of rigor mortis and ice storage were recorded at t = 0, 11, 22, 27, 33, 45, 70 and 168 h post mortem using an experimental set-up as shown in Figure 1. A fillet was placed in the light-box and photographed one at a time before it was placed on ice once again. Seven other fish from the unstressed and stressed groups were, as with the cod, used for assessment of whole-fish rigor to be compared with simultaneous fillet rigor assessments using computer vision.

Analytical methods

White muscle pH, muscle twitches, body temperature and sensory assessment of rigor mortis

Refer to Erikson and others (in press).

White muscle total water content

The water content in cod muscle was calculated after drying triplicates of approximately 2 g of muscle at 105 ^{o}C for 24 h.

Development of the computer vision method

The computer vision system and the flow chart of the steps of the computer vision algorithm are shown in Figure 1.

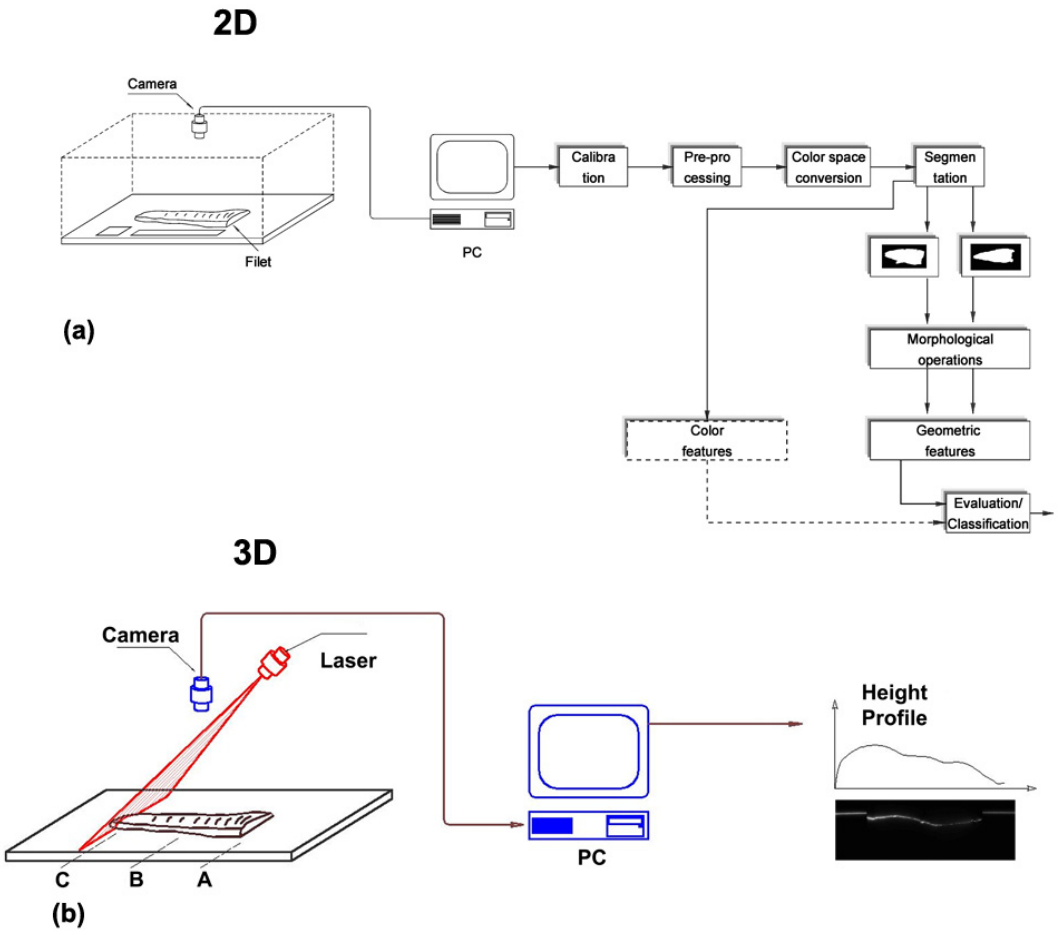

Figure 1-Block diagram of the computer vision system and image processing steps for (a) 2D-tracking of fillet changes, and, (b) 3D-tracking of changes in fillet cross- section area and height profiles. For 2D-tracking a normal color camera was used, while for 3D-tracking, a multiscan 3D camera with a laser source was used.

Image Acquisition

The images of the cod fillets were captured using an image acquisition system using a digital color camera (Pixelink PL-A776, Ottawa, Canada) with a built-in flat-field correction at the resolution of 2048 x 1536 pixels (Pixelink PL-A770) (Figure 1a). The same computer vision setup was also used for in the salmon experiment, except that the camera (Pixelink A770, Ottawa, Canada) had the resolution of 1280 x 1024 pixels. The images were stored in the computer for later evaluation without compression, in a bitmap file format (.bmp) and in three-dimensional RGB (red, green and blue) color space.

The fillets were illuminated in a light-box setup (Figure 1a). The size of the light-box (Waagan AS, Skodje, Norway) was 77 x 77 x 60 cm (length-width-height), and the light-box had an opening for placing of fillets. During the photographing of the fillets, this opening was closed to ensure uniform illumination conditions. In addition, the image acquisition took place in absence of ambient illumination (darkened laboratory). The light-box had a grey neutral color inside and used two fluorescent tubes (18W) with a color temperature greater than 5000 K and a Rendering Index greater than 95%. In this way, the illumination conditions in this box were controlled to give a uniform and diffuse illumination. A gray background was used for the salmon fillets, whereas a light blue background was used for the cod fillets. The camera was mounted and fixed on the upper part of the light-box, perpendicular to the field of view, at a vertical distance of 60 cm from the background were the fillet samples were placed during image acquisition. This distance was the same for all the photographed fillets. The camera and light-box was switched on at least 2 h before the experiment at the start day and were not switched off until the experiment was over. This was done to obtain stable camera and illumination conditions (Luo and others 2006).

After each fillet was photographed with a 2D camera, the fillets were sent to 3D scanning. This was done using a laser source (diode module, 635 nm, 5 mW, Edmund Optics, York, UK) and a high speed 3D digital camera Ranger Multiscan (Sick IVP AB, Linkøping, Sweden) (Figure 1b). The locations of the tracked cross-section profiles (A, B, C) were marked with push-pins on each side of the fillet to make it possible to scan the fillet at the exact same location at each sampling time (Figure 1b). Scanning with the laser beam was done in the absence of ambient illumination, resulting in the acquisition of thin profile lines (Figure 1b).

Calibration of images

Calibration of images was performed prior to further processing as described by Erikson and others, in press.

Pre-processing

The calibrated images were filtered for possible noise with a low pass filter and averaged with a median filter.

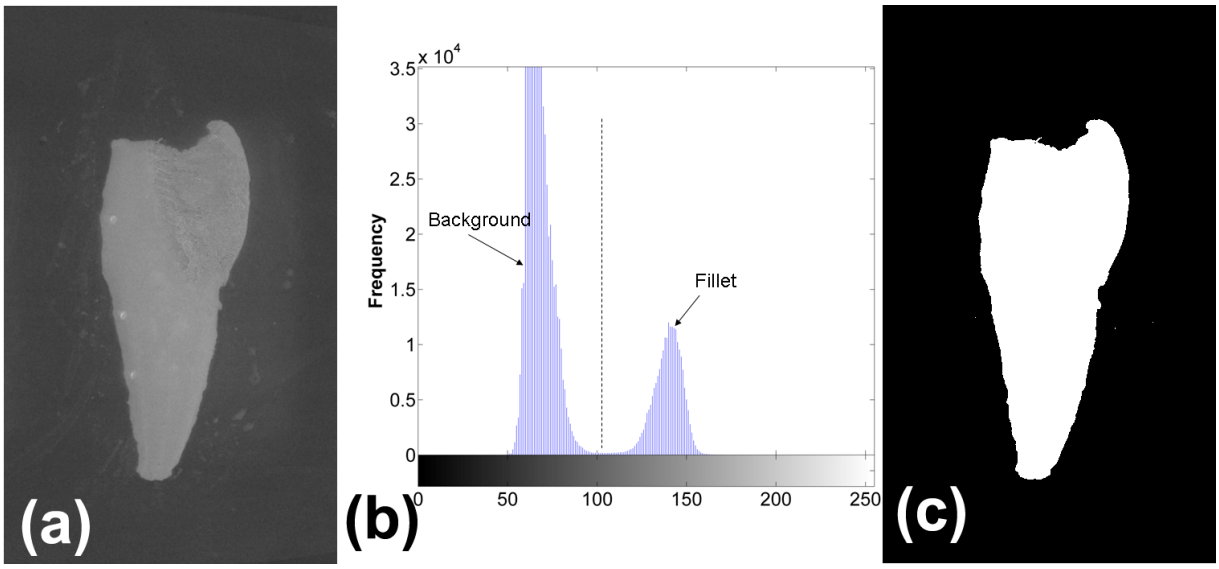

Figure 2-Gray-scale image of fish fillets; (b) Histogram; (c) Binary image after segmentation.

Segmentation

Segmentation of the fillets as a region of interest from the background was performed by global thresholding of the a*-channel for salmon and b*- channel for cod fillets (Figure 2). Since the backgrounds were of different spectral characteristics from the color of the fillets, this form of thresholding method provided an accurate segmentation of the fillets. The binary image *BW(x, y)* for both groups of fillets was defined as (Gonzales and others 2004):

$$BW(x,y) = \begin{cases} 1 & if \quad f(x,y) \geq T \\ 0 & if \quad f(x,y) < T \end{cases} \tag{1}$$

By looking at the histogram (Figure 2b) it is seen that the choice of global thresholding was justified. The histogram shows that fillet and background pixels are grouped into 2 dominant modes that can be clearly partitioned. Finding a threshold T that separated these two modes can however result in the extraction of the fillet from the background. This was done automatically for each image by using the *graythresh* function in Matlab. With this function, any image pixel $f(x,y)>T$ was classified as a

fillet pixel and was labeled as “1” (white), whereas all other pixels were classified as a background and were therefore labeled “0” (black) (Figure 2c). To ensure that only the fillet was segmented from the background, a set of morphological operations was performed consisting of *open-close filtering*. The opening operation was used to remove small details from the background, while closing was used to remove small details from the segmented fillet. For this set of operations, the *‘square’* structured element in Matlab was used. Subsequently, extraction of geometrical features was performed on the segmented fillet as a binary image. This process was mainly focused on the measurement of geometrical properties of fillets such as the size (area, length, width) and shape (roundness). All of the 2D geometric features were computed from the segmented binary images (Figure 2c). By putting an object with known dimensions (ruler) beside the fillets in the light-box, it was possible to find the relationship between the pixels and dimensions of fillets in mm. The expressions below provided these relationships for salmon (2) and cod (3). In these expressions, L_{object} is the measured real-world length of the particular ruler in mm, while L_{pixels} is the length of the same object in pixels derived from the image data.

$$1\ \ pixel = \frac{L_{object}}{L_{pixels}}[mm] = \frac{320}{824}[mm] = 0.388\ mm \tag{2}$$

$$1\ \ pixel = \frac{L_{object}}{L_{pixels}}[mm] = \frac{290}{760}[mm] = 0.381\ mm \tag{3}$$

Ground truth

To validate the accuracy of segmentation of fillets by the computer vision algorithm, a set of ground truth images was acquired by manual segmentation of fillets for the initial measurements at Day 0 and for the last measurements at Day 7. The manual segmentation was performed by using Adobe Photoshop 8.0 (Adobe, San Jose, CA, USA). In addition, a ruler was placed beside the fillet to be able to correlate the automatic computer vision measurements with those from manual segmentation.

Measurements of geometric features

Area, length, width and roundness were measured on the segmented binary images of fillets. All size and shape descriptors of the fillet can be calculated from the definitions shown in Figure 3. The area was calculated simply as the number of binary fillet pixels (Gonzales and others 2004). The expression (Ohm 2004) used to calculate area in pixels is given by

$$Area = \sum_{r}\sum_{c} b(r,c) \tag{4}$$

where *'r*' is the number of rows and '*c*' is the number of columns.

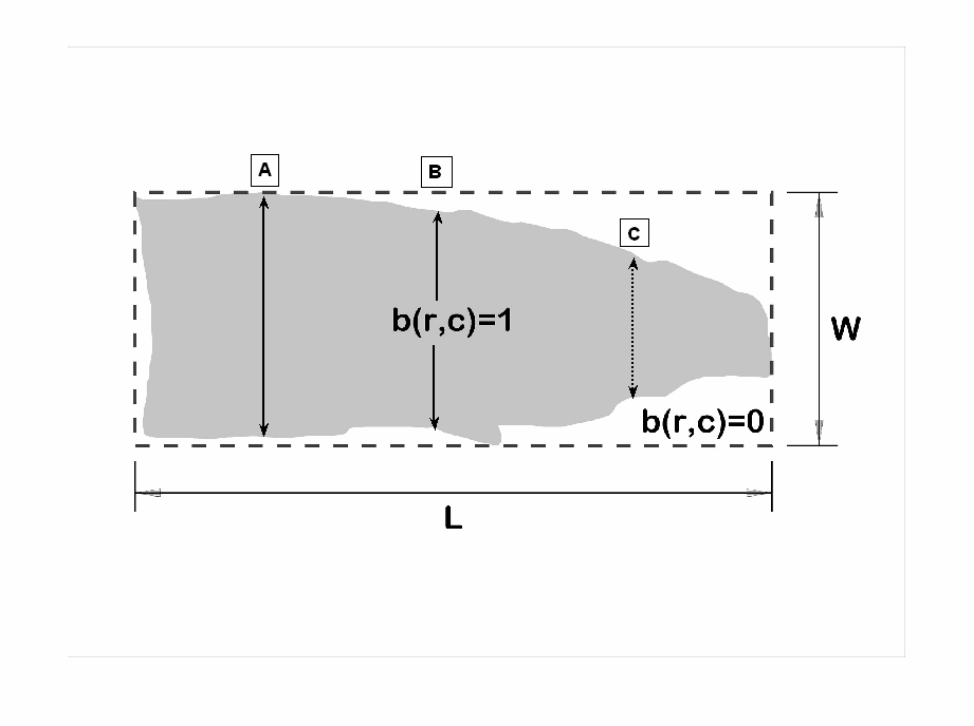

Figure 3-Definition of 2D geometrical features from the bounding rectangle for the binary images of fillets, and location of cross-sections A, B and C on the fillet used for laser scanning (fillet height profiles). For repeated scanning of the same cross-section during storage, push-pins were placed on either side of the fillet.

The length *L* of the fillet was calculated as the maximal fillet length of the bounding rectangle, while the width *W*, as the minimal length of this rectangle (Figure 3). During the experiments, we observed that the fillets were becoming slightly rounder as the rigor contractions progressed. Therefore, it was of interest to quantify the roundness of the fillets related to changes in overall shape for the different fillet species of fish and for unstressed and stressed fillets. The roundness, as a shape measure, was calculated by using the following expression (Ohm 2004; Umbaugh 2005):

$$Roundness = \frac{4\pi\ A}{P^2} \tag{5}$$

where '*A'* is the area and *'P'* is perimeter of the fillet. The maximum value for roundness is 1 (corresponding to a perfect circle). For other irregular objects such as fillets, the roundness values are in the interval (0,1). The closer the value is to 1, the rounder the object is.

Fillet cross-section area and height

The area of the cross-section and the height of the fillets were obtained by scanning the cross-section at the line locations (A, B, C, Figure 3).

If $f(x)$ is the function denoting the surface curve of the fillet cross section and *a* and *b* are the boundaries of fillet width at this cross section (Figure 7b), then the area of the cross section under the curve was calculated as:

$$A_{CS} = \sum_{i=1}^{n} f(x_i)\Delta x \tag{6}$$

where Δx is the infinitesimal width of the profile. The fillet height was determined as the height of the profile curve. The maximum fillet height $h_{\max}$ is the point where the surface curve function of the fillet attains the highest value:

$$\begin{aligned} f(x_0) &= h_{\max} \\ x_0 &= \arg\max_{x\in[a,b]} f(x) \end{aligned} \tag{7}$$

By scanning a cubical formed object of known real dimensions with a laser beam, it was possible to find the relationship between the profile height ($H_{profile}$) and the real-dimensions (H_{object}) in mm:

$$1\ unit = \frac{H_{object}}{H_{pixels}} = \frac{27}{77}\ mm = 0.35mm \tag{8}$$

Statistics

Means ± standard deviations (SD) are generally shown. The effects of treatment (unstressed *vs* stressed fish) and post mortem storage time on the geometrical features

of fillets were analyzed using a two-way analysis of variance (ANOVA). Where significance ($p<0.05$) was indicated, a Tukey post hoc test was run.

Results and Discussion

Handling stress

When early postmortem changes, such as rigor mortis, is studied and the different experimental groups have been subjected to various treatments, control and analysis of at least one parameter related to muscle energy metabolism (e.g. pH, phosphocreatine, ATP:IMP ratio) at the time of death is of paramount importance to clearly define experimental groups. This should also be done to rule out possible unintended effects of ante-mortem stress or erroneous sampling and analytical protocols. If such data are missing and a priori assumptions are made, the intended goals of the study could be masked or the wrong conclusions could be made. Unfortunately, these factors are occasionally encountered in the literature. As we wanted to make sure we actually studied the extremes of rigor developments (anesthetized and exhausted muscles), the initial white muscle pH, was assessed in all cases (Table 1). Our unstressed fish exhibited typical muscle pH values of rested salmon at pH 7.4-7.5 (Kieffer and others 1994) and cod at pH 7.3 (Stien and others, 2005), although lower than pH 7.9 reported by Kristoffersen and others (2006b) in unstressed cod. In stressed fish our pH values (Table 1) were also typical, as Kieffer and others 1994 reported values of pH 6.7-6.8 in exhausted salmon, and both Stien and others (2005) and Kristoffersen and others (2006b) reported a value of pH 7.0 in stressed cod. Our mean pH value of the stressed cod was 0.2 units higher than with our salmon. A marked difference between species with respect to behavior during the similar stress bouts was observed. Cod, being a more sedate species, did not struggle to the same extent as the salmon did. Clearly, the cod showed considerable less stamina than the salmon. Perhaps this fact was also reflected in the twitch tester values (Table 1). The cod values were not different between treatments whereas the muscle twitches of salmon were less intense after the stress bout. For both species, the ultimate pH values were around 6.4. Thus, the current study was conducted under a similar drop in muscle pH (7.4 to 6.4) for both species. Also the rearing water

temperature (body temperature at death) was nearly similar. A slight tendency for higher body temperature in stressed fish was observed (Table 1), possibly due to the muscle work during the stress bout. The mean blood glucose values for both species did not differ between treatments. The values were elevated compared with typical resting levels (Waring and others 1992). Even though no vigorous muscle activity took place for neither fishes, the AQUI-S^{TM} treatment could nevertheless be expected to result in elevated blood glucose values (Wood and Wang 1999; Erikson and others in print). The prerigor white muscle water content (about 80 % cod) was not affected by handling stress (Table 1).

Table 1 - ***Different stress parameters (blood glucose, initial pH and muscle twitches), initial body temperature, ultimate pH, and water contents in unstressed and stressed Atlantic cod and Atlantic salmon.***

Parameters	**Unstressed**		**Stressed**	
	Cod	**Salmon**	**Cod**	**Salmon**
Body temperature (^{o}C)	9.0 ± 0.3^{A}	9.7 ± 0.1	9.4 ± 0.2^{B}	9.8 ± 0.1
Blood glucose (mmol L^{-1})	5.8 ± 2.0	7.4 ± 2.5	7.3 ± 4.5	8.5 ± 2.0
Twitch tester (range: 0-3)	2.7 ± 0.5	3.0 ± 0.0^{a}	2.7 ± 0.5	2.1 ± 0.3^{b}
Initial muscle-pH (0 h)	7.4 ± 0.2^{A}	7.5 ± 0.1^{a}	6.9 ± 0.2^{B}	6.7 ± 0.1^{b}
Ultimate muscle-pH	6.4 ± 0.1 (1)	6.5 ± 0.1	6.4 ± 0.2 (1)	6.4 ± 0.1
Prerigor water content (%)	80.2 ± 2.8	NA	80.5 ± 1.4	NA

Mean ± SD (cod: n = 6; salmon: n = 12 -13) of whole fish. (1) Mean pH determined in similarly treated cod from the same batches (n=23). Means within the same row and within same fish species with different letters (cod: A, B; salmon: a, b) are significantly different ($p<0.05$). NA = not analyzed

To conclude this section, the stress levels of both fishes were clearly defined, and given the similar antemortem treatments, the near similar magnitudes of the stress parameters, body temperature and pH drop, the rigor courses of the two species were considered comparable.

Rigor mortis in whole fish

As expected from the initial pH values, the stress bouts caused significantly different rigor development from the fish not subjected to high antemortem muscle activity. Although the same basic trends were observed for the rigor development of cod and salmon, the timing was different. The time to rigor onset for unstressed and stressed whole cod was at least 12 and 6 h, respectively (Figure 4 a, b). For unstressed salmon on the other hand, the prerigor period lasted about 30 h whereas for the stressed counterparts rigor started probably around 1-2 h post mortem. These extremes are slightly higher than what we have observed before in similarly treated salmon (Erikson 2001a). The peak rigor for unstressed and stressed cod occurred after about 47 and 25 h, respectively whereas the corresponding values for salmon were 45 and 9 h. Practically identical rigor curves for farmed Atlantic cod exposed to pre-slaughter handling stress, as well as control fish, have been reported by Kristoffersen and others (2006b). They found that maximum rigor was reached after 20-24 h for stressed cod as opposed to 48 h for the control fish. Thus, when both species were subjected to AQUI-S^{TM} anesthesia, the peak rigor time was about similar even though the pre-rigor time was very different. When the fishes were subjected to a similar handling stress bout, the effect rigor development (as well as muscle pH and muscle twitches, Table 1) in case of salmon seem to be much more severe. For unstressed cod, rigor was not fully completed after 95 h. In contrast, unstressed salmon was in the postrigor state after 60 h. For the stressed groups, rigor was completed after 70-90 h and 28 h post mortem in cod and salmon, respectively. Our results show that once rigor starts, salmon pass through rigor faster than the cod do. For both species and for salmon in particular, the rigor curves (peak values) suggested that the stiffness was somewhat higher in stressed fish.

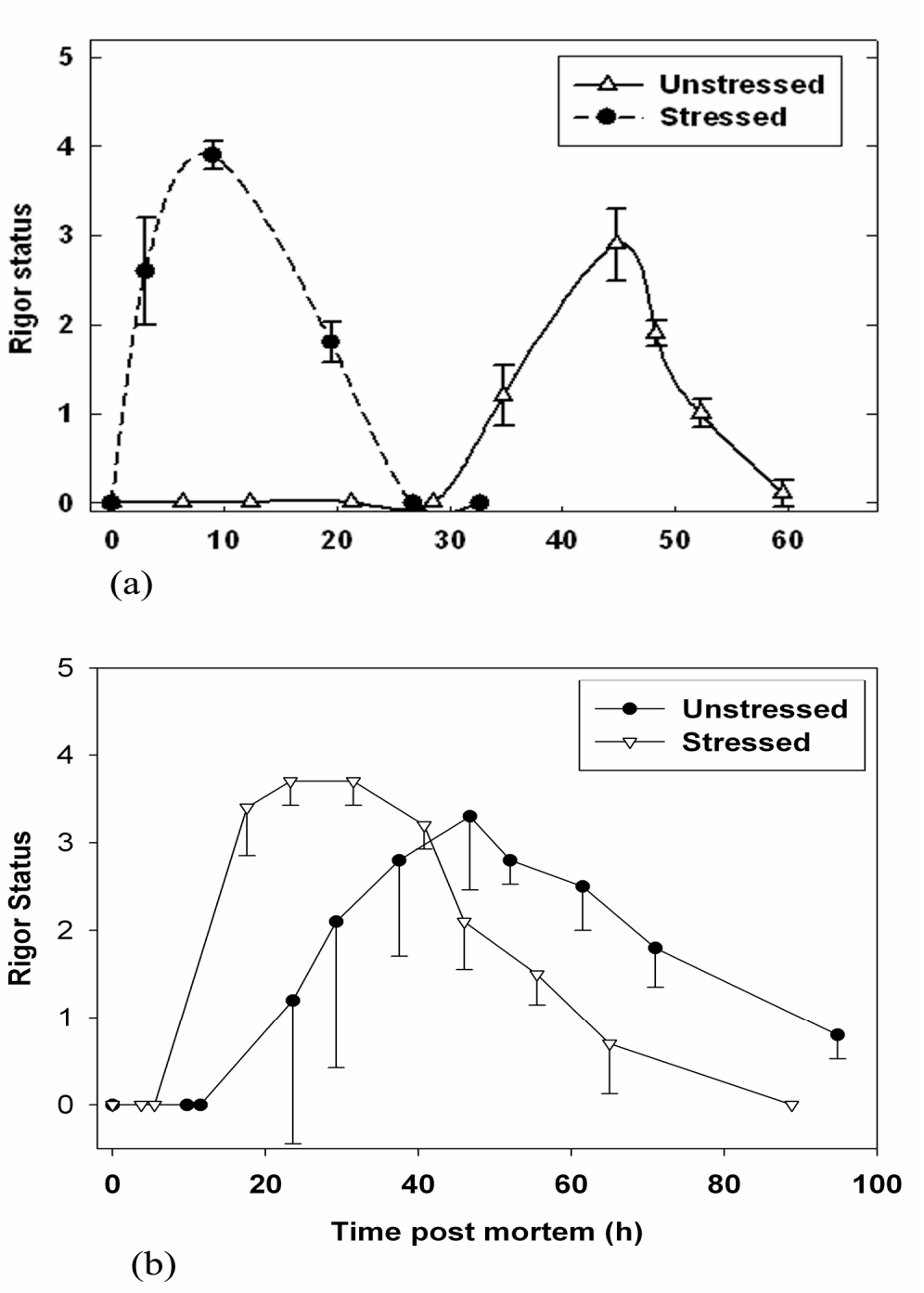

Figure 4-Development of rigor mortis during ice storage of unstressed and stressed whole gutted (a) Atlantic salmon, and, (b) Atlantic cod. Mean ± SD.

We had clearly produced and defined two fish groups representing the possible extremes in terms of handling stress for both cod and salmon and as defined by muscle biochemistry and rigor mortis. The next step was to evaluate by the computer

vision method the magnitude of possible stress-induced changes in size and shape changes of the fillets with respect to storage time on ice.

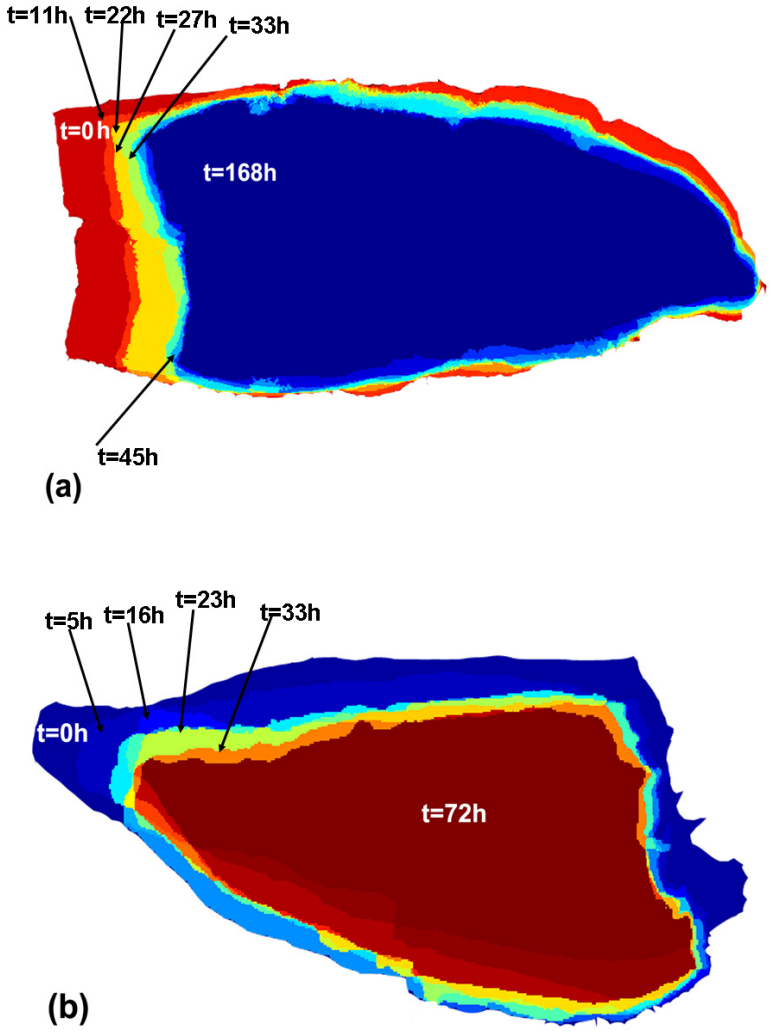

Figure 5-*(a) Visualization of changes of geometrical features of unstressed salmon fillets during rigor and ice storage for 7 d. The fillet marked t = 0 h represents initial size and shape measured immediately after slaughter (Day 0), and the fillet marked t = 168 h denotes the size and shape of the same fillet at Day 7. Maximum rigor occurred after t =45 h. (b) Visualization of changes in size and shape of stressed cod fillets during rigor and ice storage for 3 d (t=72 h. The fillet marked t = 0 h is photographed immediately after slaughtering and maximum rigor occurred after t = 23 h.*

Changes in fillet appearance during rigor mortis

The computer vision tracking of geometric features showed that area and length began to shrink immediately. The visualization of this shrinkage for unstressed salmon and stressed cod during the entire period of ice storage is depicted in Figure 5 a, b. In both

cases, the fillets shrank considerably before maximal rigor was attained. After peak rigor, only minor changes in fillet shape occurred during subsequent ice storage.

Changes in fillet length and area

Atlantic salmon - The stressed salmon fillets began to shrink earlier than their unstressed counterparts (Figure 6a), as could be expected from the biochemical measurements showing the energy status of stressed fish was more depleted (Table 1). For both levels of stress, significant shrinkage of both fillet area and length coincided with the time the whole fish were at their maximal rigor values, that is, 45-50 h (unstressed) and after 11 h (stressed) (Figure 4a). When the rigor stiffnesses decreased from their maximal values, the area and length of both unstressed and stressed fillets started to increase. However, the phenomenon was not fully reversible. At the end of ice storage (t = 168 h), significant ($p<0.05$) changes from the initial values (set to 100 %) were registered in case of length and area. Compared with initial values, the mean irreversible reduction in the length of unstressed and stressed fillets were 10 and 7 %, respectively ($p<0.05$). A significant post rigor (168 h) difference was also observed between unstressed and stressed fillets ($p<0.05$). Compared with initial values, the maximum muscle contraction was about 14 % for both salmon fillet groups. This occurred after 11 and 45 h for stressed and unstressed fish, respectively. Correspondingly, after ice storage the mean fillet length had shortened irreversibly 8 and 10 %. Sørensen and others (1997) and Einen and others (2002) also observed about 14-15 % muscle contraction at maximum rigor, while Skjervold and others (2001b) reported a final 8.6 % reduction in length.

The maximum contraction of the fillet area, occurring at maximum rigor, was approximately 9 % for both stress levels. After ice storage (168 h), the mean area of our unstressed and stressed fillets were 6 and 3 % lower than their initial values ($p<0.05$). Peri-mortem stress produced significant differences in fillet areas ($p<0.05$).

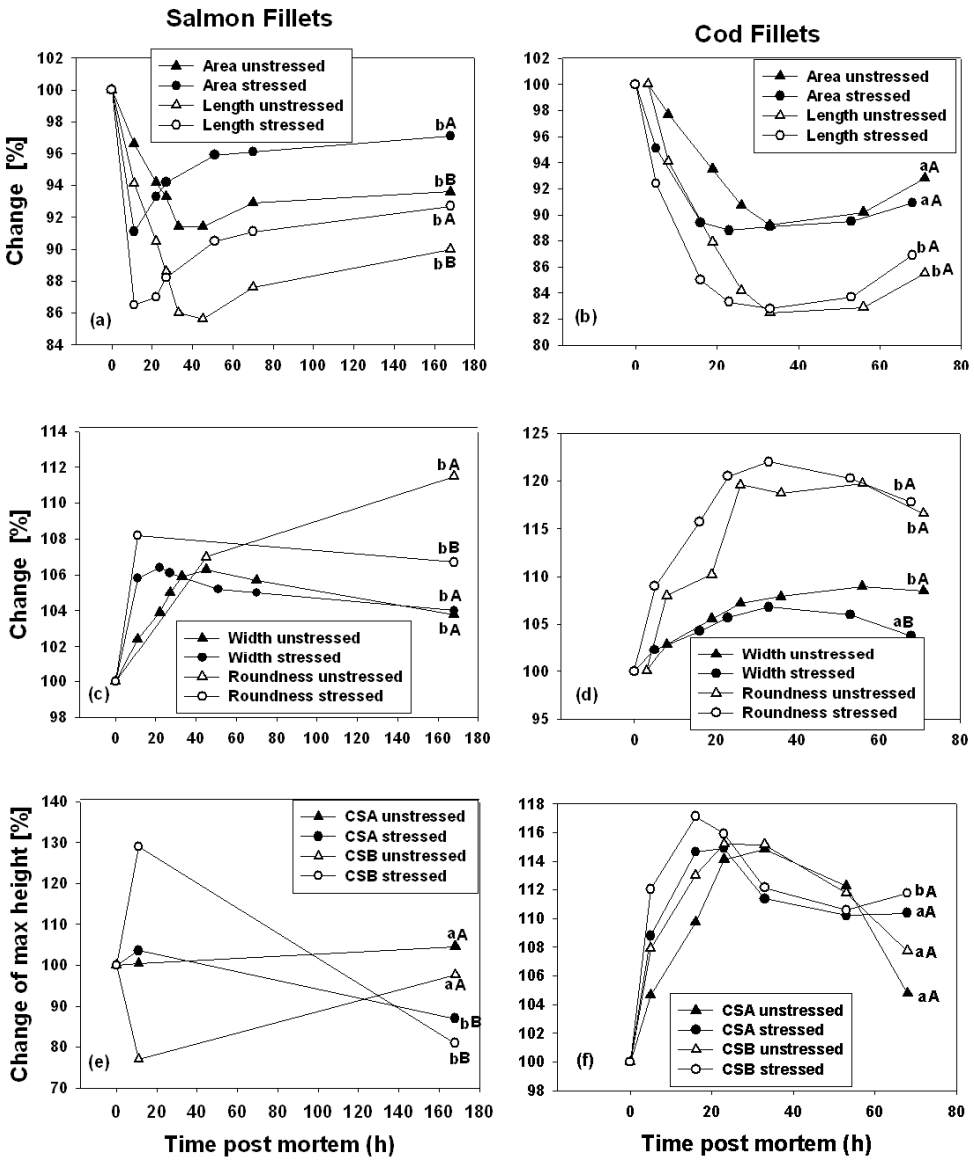

Figure 6- ***Changes in percentage of different geometrical features: Shrinkage of area and length in (a) salmon and, (b) cod fillets. Changes in width and roundness of (c) salmon and (d) cod fillets. Changes in maximal height of fillet cross-section for (e) salmon and (f) cod. By the end of ice storage, different letter A and B denotes significant (p<0.05) between unstressed and stressed fillets, whereas different letter a or b denotes not significant (a: p>0.05) or significant (b: p<0.05) difference between initial (100%) and end values of either unstressed or stressed fish. Mean values are shown (salmon: n = 22 and 26; cod: n = 12).***

Atlantic cod - In case of the cod fillets, similar post mortem reductions in area and length took place (Figure 6b). Again, due to the effect of perimortem stress, fillet shrinkage was somewhat accelerated for the stressed group. Also, shrinkage of unstressed fillets started before rigor onset. Indeed, Honikel and others (1986) have

shown that shortening of sarcomeres also takes place in the prerigor phase. The maximum rigor contractions for whole cod occurred after about 23 and 45 h for the stressed and unstressed fish, respectively (Figure 4b). For the stressed fillets, the maximal shrinkage of area and length occurred after about 25 and 35 h, respectively. The corresponding values for the unstressed cod fillets were about 35 h for both parameters. After the whole fish rigor had peaked (Figure 4b), the mean cod fillet area and length also increased somewhat as measured after 72h. The mean area shrinkage was maximally 11 % at peak rigor, whereas at 72 h shrinkage was reduced to about 7-9 % of initial values for both fillet groups. The fillet areas were then not significantly different from their initial values, nor did perimortem handling stress produce different post rigor areas ($p>0.05$).

On the other hand, reductions in mean fillet length, 18 % at full rigor, and 13 -14 % after 72 h, were significantly different from initial values in case of both fillet groups ($p<0.05$). However, perimortem stress did not have a significant effect on post rigor cod fillet length ($p>0.05$). It should be mentioned though that the post rigor changes in cod fillet geometry may not have been completed after 72 h since whole fish were not yet fully in the post-rigor state by this time (Figure 4b). Stien and others (2005) and Mørkøre (2006) also reported similar maximal length contractions of cod fillets, namely 15 - 20 % and 21 %, respectively. Moreover, Kristoffersen and others (2006 a) reported that prerigor cod fillets were 12-13 % shorter than their postrigor counterparts.

Changes in fillet width and roundness

Fillet width and roundness for both fish species are shown in Figure 6 c, d.

Atlantic salmon - As fillet length decreased during rigor, width on the contrary increased. For both unstressed and stressed salmon, the width increased gradually up to 6 % after about 45 and 10 h post mortem, respectively, corresponding with maximal rigor of whole fish (Figure 4a). After 168 h, the mean increase relative to the initial values of 100 %, had dropped to 4 % ($p< 0.05$). This value resembles width increase in rainbow trout fillets as measured 20 h post mortem by Stien and others (2006). Fillet roundness increased faster for the stressed group than with the unstressed group. Again, the increases were clearly related to the gradual increases in rigor tensions, that is, up to the points were maximal rigor were attained. The further roundness increase up to 12 % of unstressed fillets after 168 h seems peculiar. We

have no plausible explanation of this phenomenon. Conversely, stressed fillet roundness had attained a mean increase of 7 % after 168 h. Thus, salmon fillet roundness had increased significantly ($p<0.05$) both due to perimortem handling stress, rigor mortis or ice storage.

Atlantic cod - Perimortem stress did not affect the rate of increase in cod fillet width (Figure 6 d), that is, up to the point where the maximal whole fish rigor was attained (Figure 4b). When the experiment was terminated, the unstressed cod fillets had increased 8 % in width and they were significantly wider than their stressed counterparts where the relative increase was 4 %. Fillet roundness also increased according to increasing rigor tensions and as with the other geometrical parameters. Maximal increases for both fillet groups were 19-20 %, considerably higher than with the salmon. After 72 h, the relative roundness increase had been reduced to 17 %, significantly different ($p<0.05$) from initial values. However, perimortem handling stress did not induce permanent changes in cod fillet roundness ($p>0.05$).

Changes in fillet cross-section area and maximum height

While Figure 6 a-d are based on 2D assessments of geometrical features of the fillets, Figure 6 e, f are based on laser measurements of fillet height and the use of a 3D high speed camera. Examples of changes in height profiles (cross-section A) of unstressed salmon and cod fillets as a function of rigor and storage time is shown in Figure 7 a, b. For clarity, only 3 profiles are shown (pre rigor, maximal rigor, and post rigor). For both species, we can see that it was not only fillet height that was altered during rigor, but rather the overall shape of the cross-section changed their appearances. In both cases, the post rigor cross-section shapes of the fillets were intermediate between pre- and inrigor shapes. The maximum height obtained from the cross-section profiles (Figure 7 a, b) is plotted against storage time in Figure 6 e, f.

Atlantic salmon - When comparing unstressed and stressed salmon fillets, the mean change in maximal fillet height of cross-section B was considerably larger than with cross-section A (Figure 6 e). After 168 h, at both cross-sections unstressed fillets were

however not significantly different from their initial values ($p>0.05$).

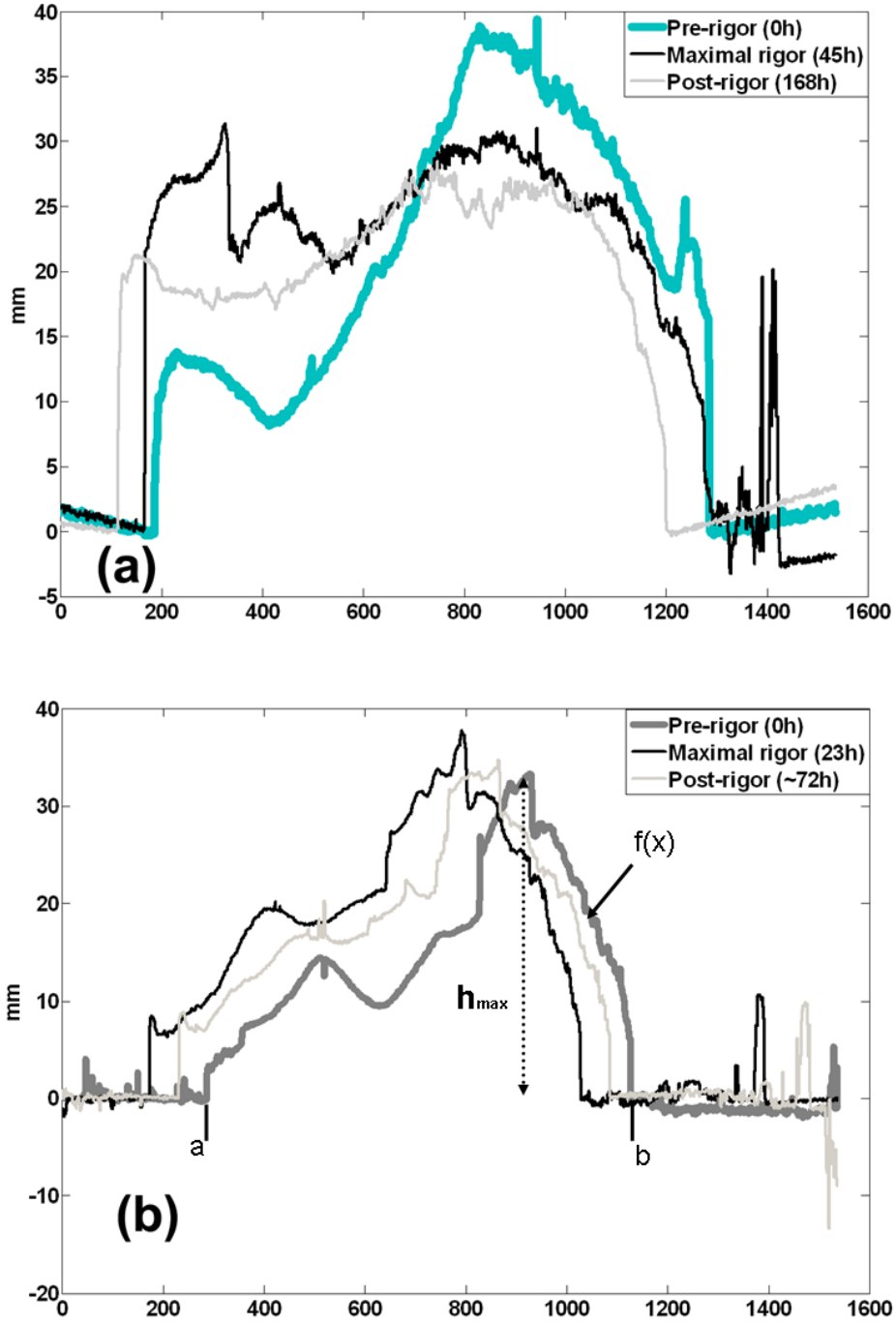

Figure 7-*(a)* Changes in unstressed salmon fillet shape and size at cross-section A, resulting in area and height changes during rigor and ice storage. The selected profiles were obtained prerigor at Day 0 (t=0 h post mortem), at maximum rigor (Day 2, t=45 h), and after ice storage (Day 7, t=168 h). (b) Visualization of similar changes in unstressed cod fillets at cross-section A: prerigor (Day 0, t= 0 h), at maximum rigor (Day 0, t=23 h), and postrigor after ice storage (Day 3, t=72 h). The parameter h_{max}, and the interval [a b] were used to calculate the area and height as defined by Equations (9) and (10). The h_{max} is plotted against storage time in Figure 6 e, f.

For the stressed fillets, the maximal area of cross-section A and B were 12 and 18 % higher than initial values, respectively. At the end of ice storage, the stressed fillets at cross-section B and C were 15 % (4 mm) thinner, in average, than their unstressed counterparts ($p<0.05$). The cross-section area A in both groups of fillets was not significantly different from the initial value ($p>0.05$) at the end of ice storage. Regarding the values in mm of maximal height of the fillets, as defined in Figure 7b, when comparing pre- and postrigor fillets, the mean unstressed and stressed fillet heights at B decreased by 1 ($p>0.05$) and 5mm ($p<0.05$), respectively. At A location the height of the unstressed fillets in post-rigor was 2mm higher ($p>0.05$), while the height of the stressed fillets was lower for 4mm ($p<0.05$) in average. By comparison, a mean decrease of 2.4 mm was measured by Skjervold and others (2001a) when salmon fillet height was determined pre- and post rigor. Results of our study from three different scanning locations show that a different trend of contraction of the fillet was observed, as shown in Figure 6e.

Atlantic cod - At cross-sections A and B, the cod fillets became about 14 -17 % thicker at maximal rigor compared with initial values ($p<0.05$). After 72 h post mortem, the mean maximal heights of both cross-sections were still greater, although not significantly so ($p>0.05$), than the corresponding initial values. Unstressed fillets were 5-8 % higher, whereas stressed fillets were 10-12 % higher. No significant differences were observed between unstressed and stressed fillet heights at none of cross-sections ($p>0.05$ %) (Table 2).

Since data acquisition (Figure 6 e, f and Table 2) of cross-sections A, B and C on the same fillet occurred practically simultaneously, and since the cross-section shapes developed differently, it may be concluded that rigor development did not proceed uniformly over the whole fillet. This observation is in line with previous studies using other techniques to study rigor development (Jeacocke 1984; Berg and others 1997; Kiessling and others 2006; Stien and others 2006). The difference in the rate of contractions between the unstressed and the stressed fillets can be explained by different depletion rates of ATP in the muscle (Berg and others 1997; Erikson and others 1999). Another factor to consider is the drop in pH occurring before and after rigor onset. In Atlantic salmon, rigor starts at about pH 6.6 (Erikson and others, in press). This means that a considerable shrinkage of the protein matrix has already

occurred before rigor onset. Possibly, this was reflected particularly in case of the unstressed fillets where they contracted considerably during the prerigor period (Figure 6 a) which lasted about 30 h (Figure 4 a).

Table 2 - ***Relative changes of cross-section (CS) area and height as measured at three Atlantic cod fillet locations (A, B and C, see Figure 3) using a laser source and a high speed 3D camera.***

Fillet	Time post mortem (h)						
CS	0	5	16	23	33	53	72
Unstressed							
Cross section area (% change)							
A	100[a]	104 ± 9	123 ± 12	124 ± 6[b]	130 ± 6[b]	127 ± 9[b]	115 ± 8
B	100[a]	106 ± 8	118 ± 10	125 ± 11[b]	125 ± 12[b]	125 ± 10[b]	113 ± 7
C	100[a]	113 ±23	123 ±17	128 ±18	128 ± 19[b]	126 ± 18	119 ±17
Maximal fillet height (mm)							
A	24 ± 5	24 ± 5	26 ± 6	27 ± 5	28 ± 6	27 ± 5	25 ± 6
B	19 ± 4	20 ±3	21 ± 3	22 ± 4	22 ± 4	21 ± 4	20 ± 4
C	15 ± 3	16 ± 3	17 ± 3	17 ± 3	17 ± 4	17 ± 3	16 ± 3
Stressed							
Cross section area (% change)							
A	100[a]	112 ± 15	122 ± 11[b]	127 ± 7[b]	124 ± 9[b]	119 ± 5[b]	126 ± 17[b]
B	100[a]	110 ± 10	119 ± 12	127 ±14[b]	131 ± 39[b]	122 ± 40	118 ± 13
C	100[a]	112 ± 13	123 ± 14	124 ± 6[b]	120 ± 9	116 ± 8	116 ± 12
Maximal fillet height (mm)							
A	24 ± 4	25 ± 4	27 ± 4	27 ± 4	26 ± 4	26 ± 4	26 ± 5
B	20 ± 2[a]	21 ± 2	22 ± 3[b]	22 ± 4	22 ± 3	21 ± 3	20 ± 3
C	14 ± 2[a]	15 ± 2	16 ± 4[b]	16 ± 3[X]	15 ± 3	15 ± 2	15 ± 3

Mean ± SD (n = 12). In each row, b denotes significant difference (p<0.05) from the initial value (a). No significant differences were observed due to treatment (stress) (p>0.05).

Table 2 summarizes the relative changes in unstressed and stressed cod fillet cross-sections A, B and C. Although significant differences were observed during rigor, a significant difference ($p<0.05$) with respect to initial value was observed only in the area of the cross section A after 72 h. Height of the fillets, as defined in Figure 7b, in the unstressed fillets was not significantly different at neither of locations during the entire rigor process. In stressed fillets, no significant difference ($p>0.05$) in height

were observed at the end of ice storage with respect to initial value. In these fillets, the height was only temporary significantly different at B and C location after t=23h, where the height had increased for 2 mm in average. In general, although there was a tendency of increase of height (2mm in average) in both groups, at the end of ice storage, none of these changes were significantly different ($p>0.05$) neither with respect to their initial value nor treatment (stress).

Comparison between fish species

The cod fillets showed largely similar contraction patterns as with those of the salmon (Figure 6 a-f). Even though the cod were apparently less stressed (Table 1) and subsequently exhibited less difference between the rigor courses of unstressed and stressed fish (Figure 4 a, b), the reductions in fillet area and length, as well as the increases in width were basically of similar magnitude. Furthermore, the increases in fillet roundness and height were in fact larger for the cod. For both species, the changes in fillet geometry occurred faster in stressed fillets and this effect of perimortem stress was more pronounced for the salmon as could be expected from the more severe struggling of salmon during the stress bout (Table 1). The maximal whole fish rigor strength was judged about the same for both species (Figure 4 a, b) even though the salmon were more severely stressed. It might be that compared with the lean cod, the high fat content in salmon white muscle, where a major fraction of the fat is located in the myosepta (Zhou and others 1995), has a dampening effect on rigor tensions. In turn, this might moderate the effects of perimortem stress on fillet shape. Perhaps this might also in part explain why gaping problems are often more severe in cod than in salmon.

Fillet shape and marketing

After ice storage, at a time the fillets would typically be available in the market, the fillets had several significantly different features regarding size and shape as compared with just after slaughtering. Although there were also some significant differences due to perimortem handling stress, we think that those would hardly be noticeable by the consumers.

Evaluation of the computer vision system

To check the fairness of the computer vision segmentation of fillets, it was necessary to validate the accuracy of the segmentation. This was performed by comparing the segmented images of the salmon fillets by the computer vision algorithm, at the beginning (t = 0 h) and the end (t = 168 h) of the ice storage, with the ground truth images of fillets segmented manually at similar sampling times. The correlations of fillet length and area as segmented with the computer vision method and as segmented manually were high. The comparison demonstrated that there were practically no deviations in the evaluation of both those two parameters ($r^2 = 0.99$). The small differences that may have existed would have only negligible effects on the geometrical features of the fillets.

As for the image acquisition, the controlled illumination conditions (uniform and diffuse) and the built-in flat field correction of the camera made the image processing and segmentation of the images much easier. It is well-known that proper illumination is important for successful computer vision applications (Whelan and Molloy 2000; Zeuch 2000, Hardin 2004). We were not confronted with reflection problems during image acquisition. In addition, a high resolution digital camera (2048 x 1536 pixels) provided accurate mapping between fillets and their real-time dimensions. Segmentation of fillets was facilitated by using a suitable scene background (gray for salmon and light-blue for cod fillets) having different spectral characteristics from the fillets. It was observed that the segmentation was easier in the case of cod fillets due to the use of light-blue background suggesting this color can be used for conveyor belts in fish processing plants transporting the fish to on-line computer vision systems. In our study, we thus found no need to apply a more complex thresholding system than the one reported by Otsu (1979). Fast thresholding computation is known to play an important role in the improvement of the total performance of computer vision systems (Lin 2005).

In addition to registration of size and shape changes in 2D and 3D, the proposed computer vision system has the ability to evaluate and classify the color of salmon skin or fillets from a single image (Misimi and others 2007, Erikson and others, in press). Therefore, the proposed computer vision system has the flexibility of choosing multiple-feature evaluation (color, size and shape, Figure 1) from a single image.

When it comes to the 3D measurements, the system determined fillet heights and provided cross section profiles of the fillets. Although the laser scanning and capturing of images using a high speed 3D multiscan camera was not performed under online conditions, there are no performance limitations for on-line usage.

Until recently, the biggest limitations for online computer vision applications have been the high costs and low processing speed (Andreadis 2001). In this regard, the proposed computer vision system uses low-cost off-the-shelf components which make it possible for many to exploit its advantages without large expenses. In addition, the rapid development and emerging of CPUs and other peripherals with high computational speed has made it possible to be able to cope with almost any real-time requirement.

Conclusions

We have shown that when salmon and cod were exposed to two extremes of peri-mortem stress and were subsequently stored on ice, significant changes in fillet length, area, roundness and cross-section height profiles were detected during storage. After ice storage of cod (72 h), only fillet width was still significantly affected by perimortem stress. In contrast, salmon fillets exhibited significantly different geometrical features after ice storage (168 h) regarding all parameters except from the width. Although there were several transient significant differences in fillet geometry due to perimortem stress, they were comparatively small and the stress effect on fillet shape would hardly be an issue for both post-rigor processors and for consumers by the time the fillets are normally marketed. If prerigor filleting and processing are attempted, it is important to realize that significant changes in fillet shape do in fact occur before a noticeable onset of rigor mortis can be spotted.

The proposed nondestructive and contact-free computer vision system was capable of detecting changes in fillet length, area, width and roundness as well as in fillet height profiles and cross-section during the course of rigor and ice storage. The method showed a high sensitivity and it detected the characteristic features of the rigor developments of unstressed and stressed fish. Furthermore, the system is inexpensive since it can be implemented with off-the-shelf components and it may be feasible for

industrial purposes to assess possible transient 2D and 3D changes in the shape and size of different products occurring during processing.

Acknowledgements

The project was funded by the Research Council of Norway (*NFR project Nos. 145634/140 –'Efficient and economic sustainable fish processing industry', and 173530/I30 'Technology for efficient and profitable fish industry').*

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PAPER V

Quality grading of Atlantic Salmon (*Salmo salar*) by Computer Vision

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Food Engineering and Physical Properties

Abstract

In this study, we present a promising method of computer vision-based quality grading of whole Atlantic salmon (*Salmo salar*). It is shown that with the use of computer vision it is possible to differentiate between different quality grades of Atlantic salmon based on the external geometrical information contained in the fish images. Initially, before the image acquisition, the fish were subjectively graded and labeled into grading classes by a qualified human inspector in the fish plant. Prior to classification, the images of Atlantic salmon were segmented into binary images, and then feature extraction was performed on the geometrical parameters of the fish from the grading classes. The classification algorithm was a threshold-based classifier, which was designed using the linear discriminant analysis. The performance of the classifier was tested by using the leave-one-out cross-validation method, and the classification results showed a good agreement between the classification done by human inspectors and by the computer vision. Overall, it is shown that computer vision can be used as a powerful tool to grade Atlantic salmon into quality grades in a fast and non-destructive manner by a relatively simple classifier algorithm. The low-cost of implementation of today's advanced computer vision solutions makes this method feasible for industrial purposes in fish plants as it can replace manual labor, on which grading tasks still rely.

Keywords: Computer vision, Atlantic salmon, grading, processing line

Introduction

During the last two decades, the number of whitefish processing plants in Norway has diminished considerably for several reasons. In aquaculture, although the production volume of salmonids has increased tremendously over the same period of time, most of the fish are currently exported as raw material, i.e. gutted fresh or frozen fish. Fish processing is often not profitable particularly because of the high labor costs. For instance, for slaughtering of farmed salmonids, the needed manpower is typically 20-25 persons per shift to process 40-100 tons of bled, gutted fish packed in ice. Strong competition from low-cost production and processing countries such as Chile, China and Poland, as well as high labor costs in Norway have forced many Norwegian fish processors to move their processing facilities to low-cost countries, such as the Baltic countries.

High labor costs are predominantly due to the extensive use of the manual labor. A number of fish quality grading and processing tasks are still performed manually by human inspectors. Quality grading (external and internal) of whole Atlantic salmon, at the present, completely relies on manual labor. This grading is based mainly on some visual properties that represent important criteria for distributors and consumers in the fish markets. In Norway, 2 to 4 persons are necessary for whole Atlantic salmon quality grading, when biomasses between 80-120 tons (25.000 fish) are processed per shift (7h). In farmed Atlantic salmon processing plants, human inspectors stand beside the conveyor belt and perform grading into mainly the following quality classes: “Superior”, “Ordinary”, and “Production”. According to the Norwegian industrial standards for quality grading of farmed Atlantic salmon (NBS 10-01, 1999), the “Superior” grade has a streamlined shape and has no external blemishes, while the “Ordinary” grade may have an unsymmetrical shape or have a limited number of blemishes. At a typical farmed Atlantic salmon processing plant, if there are no specific problems with the fish, the Superior grade constitutes approximately 90-97% of the biomasses of fish that are processed (Michie 2001). The fish that do not fit into these grades are mainly graded as “Production”; fish with body deformities, mainly with a back-humpback and a short tail (Misimi and others 2006). According to the Norwegian regulations, the export of the

"Production" grade salmon is not allowed and it is mainly used as a raw material for processed products (Sørensen 2003). Fish that have too many blemishes, severe deformities, or sexually mature signs, are completely rejected from the processing.

Reliance of quality grading of Atlantic salmon on manual labor incurs higher production costs and lower efficiency. Therefore, introduction of a higher degree of automation of various unit operations in fish plants, preferably at low investment costs, represents a common strategy within the fish industry today (Erikson and Misimi 2007, in press). With the automation of this operation, as well as other operations that still rely on manual labor, the productions costs would go down, the processing rate would be increased and a higher quality assurance of the end products would be introduced.

In 1988, Arnarson and others (1988) reviewed and outlined a number of possibilities for implementing computer vision for automation and improving product quality in the fish processing sector. Gunnlaugsson (1997) also reviewed the benefits of applying vision as an intelligent form for fish processing. Even so, several unit operations in a fish processing line still rely on, at least in part, repetitive manual labor. In the meantime, computer vision has proven successful for online process control and inspection of food and agricultural products with applications ranging from simple automatic visual inspection to more complex vision control (Gunasekaran 2001; Brosnan and Sun 2004). In fish industry, despite the slow uptake, computer vision is beginning to gain the necessary maturity for quality evaluation applications (Strachan and Murray 1991; Strachan 1993; Strachan and Kell 1995; Stien and others 2005; Stien and others 2006; Misimi and others 2007).
Although recently there have been a number of studies on fish species recognition (Strachan and Nesvadba 1990; Strachan 1994; Zion and others 1999; White and others 2006; Zion and others 2007), there is still a gap to be filled concerning the quality grading of whole Atlantic salmon.

The "Production" grade Atlantic salmon is easier to classify from the other two quality grades (Misimi and others 2006), since it comes usually with a deformed shape in the

form of mainly a humpback and a short tail. The algorithm for this classification is described by Misimi and others (2006). The distinction between the other two grades has been regarded as more difficult since between the "Superior" and "Ordinary" there is a larger similarity regarding shape and visual appearance. The "Production" grade salmon was very rare at the sampling day at the site. At the plant we were told that nowadays it occurred in negligible percentages and that most of the fish were graded as "Superior" and "Ordinary", implying an improvement of fish quality in the farming stage.

Therefore, in this work, the objective was to investigate the ability to differentiate between the "Superior" and "Ordinary" quality grading classes of Atlantic salmon by means of computer vision and pattern recognition techniques. Here, it was interesting to identify the features which can be used for external quality grade classification. The findings of this work can be important for eventual development of quality grading instrumental inspection systems for use in the fish industry. The computer vision system for quality grading consisted from an image acquisition light-box, camera, interfacing to PC and a personal computer, where the software was developed. Geometrical and dimensional properties of the fish samples were used as features during the classification. Linear discriminant analysis (LDA) was used for classification of fish samples and the performance of the classifier was investigated by comparing this classification with the one performed by a qualified human inspector.

Materials and methods

The fish

Commercially farmed Atlantic salmon with a length 69.8 ± 7.6 cm (n=60) were sampled on site, at Salmar AS (Frøya, Norway) commercial fish processing plant, in April 2006. Fish quality grading at the site was performed by a trained human inspector, and the computer vision system that was installed temporary in the premises of the processing plant was used for acquisition of fish images.

Sensory evaluation/grading

The quality grade of the Atlantic salmon was descriptively evaluated by a trained human inspector, the chief human inspector for the shift, at the fish plant. The human inspector

labeled every sample of the Atlantic salmon with the appropriate quality grade based on external appearance, and elaborated the criteria upon which the particular labeling was based. This labeling was used as a ground truth. From the grading criteria, as explained by the human inspector, the Ordinary fish were thicker/broader in the back(posterior) part of the fish from point S to T (Figure 1), had a shorter tail than the Superior grade, and were more unsymmetrical.

The computer vision system and the flow chart describing the specific functions of the image acquisition and classifier design are shown in Figure 2a. All the algorithms for image processing, color calibration, classification and testing were written in Matlab 7.3 (Mathworks, Natick, Mass., U.S.A.)

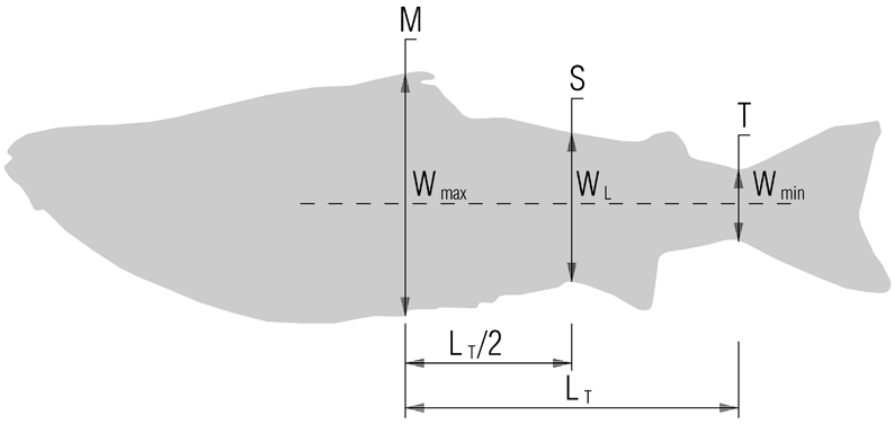

Figure 1-Definition of generated features for the subsequent feature extraction for classification. Points M and T are the points with the maximum and minimum width of fish respectively, while the point S is the width of the fish at the half of the distance from point M to T.

Image Acquisition

The images of Atlantic salmon for the computer vision grading were captured using an image acquisition system for a digital color camera (Pixelink PL-A776, Ottawa, Canada) with a built-in flat-field correction at the resolution of *2048x1536* pixels (Figure 2a).

Images were stored in the computer for later evaluation without compression, in a bitmap file format (.bmp) and in three-dimensional RGB (red, green and blue) color space. The processing was carried out in the captured images. The images were consequently downscaled to the resolution of 1024x967 for further processing.

The fish were illuminated in a specially made light-box setup (Figure 2b). The light-box had 1 opening to allow the placing of fish into the light-box. This opening was closed when fish were photographed in order to cut out the interference from the ambient illumination. The light-box (F. Waagan AS, Skodje, Norway) had a grey neutral color inside and used two fluorescent tubes (18W) with a color temperature greater than 5000K and a Rendering Index (Ra) close to 95%, as recommended from Sandor and Schanda (2006). The color rendering index is a measure of the ability of a light source to reproduce the colors of various objects being lit by the source.

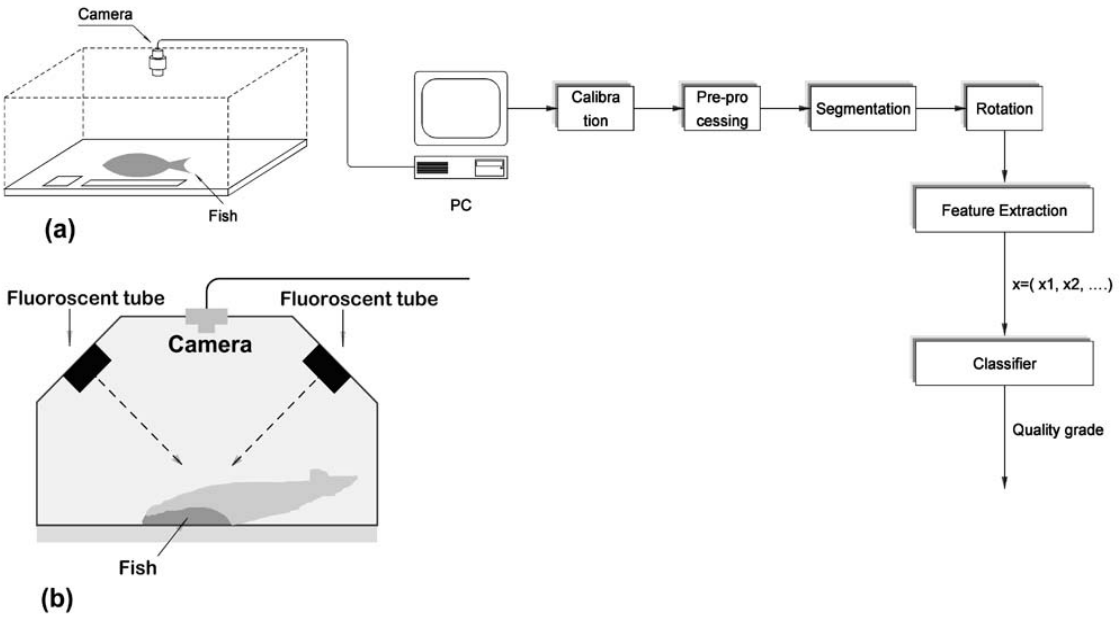

Figure 2-a) The structure of the computer vision system for an image acquisition and the flow chart of the most important computer vision stages during the image analysis and the classifier design, b) the structure of the light-box, positioning of lamps, fish, and camera.

The fluorescent tubes (60 cm) were arranged above the fish and at an angle of 45˚ with the fish (Figure 2b). In this way, the illumination conditions in this box were controlled to

give a relatively even and diffuse illumination. The built-in flat-field correction of the camera was used to compensate for eventual uneven illumination.

The background of Atlantic salmon, in which they were placed during the image acquisition, was light-blue, similar to the color of conveyor belts some time used by fish processing plants. The camera was mounted and fixed on the upper part of the light-box, perpendicular to the field of view, at a vertical distance of 60 cm from the background were the fish samples were placed. The angle between the camera and the fluorescent tubes was approximately 45˚ (Figure 2b). The camera was connected to a PC through a fire wire interface adapter. The camera and the light-box were switched on at least 1 h before the experiment and were not switched off until the experiment was over. This was done to obtain stable camera and illumination conditions (Luo and others 2006).

Calibration

Calibration of images was performed after their acquisition. The aim of the calibration was to ensure that the images taken from the digital camera were true representation of the fish in the scene both when it comes to their real-world dimensions and color. Color calibration was performed using the Macbeth ColorChecker (Gretag-Macbeth Ltd., UK) with 24 patches (color squares) as described in (Erikson and Misimi 2007, in press).

Pre-Processing and color conversion

The calibrated color images were images in CIELab color space, according to the definitions given in Wyszecki and Stiles (2000), as described in (Erikson and Misimi 2007, in press).

Segmentation

Segmentation of the fish, as a region of interest (Figure 3a, b), from the background was performed using the thresholding method. The threshold T=126 was found to be the most suitable one for the segmentation of Atlantic salmon. This threshold was the same for all the images, during the segmentation. Since the scene background of the fish was of different spectral characteristics from the color of fish, this form of thresholding provided

an accurate segmentation of the fish. The binary image *BW(x, y)* of the fish (Gonzales and others 2004) was defined as:

$$BW(x,y) = \begin{cases} 1 & if \quad f(x,y) \geq T \\ 0 & if \quad f(x,y) < T \end{cases} \tag{1}$$

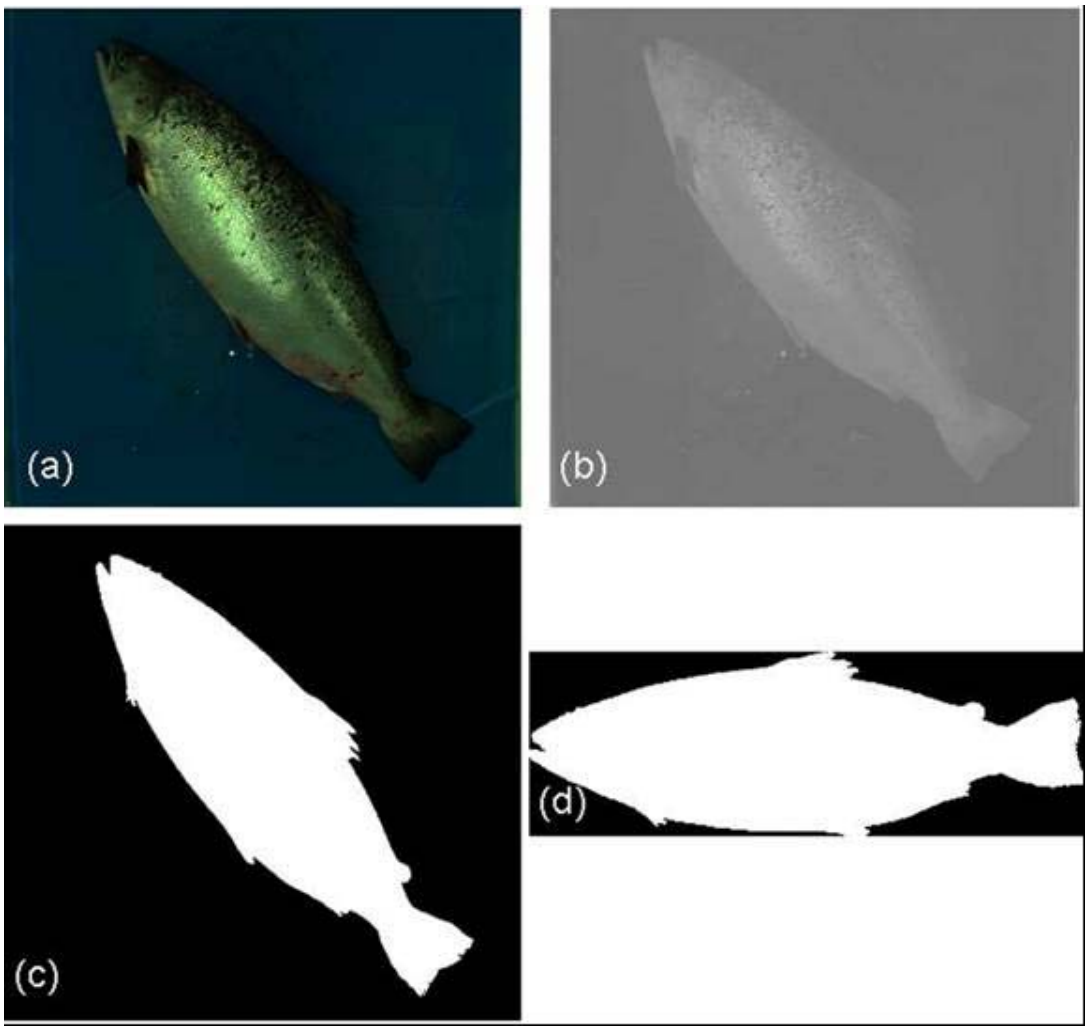

Figure 3-Image processing and segmentation of Atlantic salmon from background: a) Calibrated image of Atlantic salmon; b) Gray-level image; c) Segmentation (black and white image) from the background; d) Automatic rotation and cropping of the bw image for feature extraction.

Subsequently, any image pixel $f(x,y)>T$ was classified as a fish pixel and was labeled as "1" (white), while all the other pixels were classified as a background and were labeled "0" (black) (Figure 3c). To ensure that only the fish was segmented from the background,

a set of morphological operations was performed consisting of *open-close filtering*. Opening operation was used to remove small details from the background, while closing was used to remove small details from the segmented fish. For this set of operations, the *'square'* structured element in Matlab was used. All Atlantic salmon images were segmented from the background using this procedure, automatically as a batch of images.

Rotation

Even if the existing machinery in fish processing plants would perform the head - tail orientation of fish, the proposed method is also invariant to the orientation of fish. During the image acquisition stage, the fish was not oriented to lie along any of the *xy* axis of the background plane. Instead, the fish was placed at a random position, closing a random angle with the *y*-axis (Figure 3a). The algorithm took into account the angle of orientation and, after the image processing, all the fish were oriented in such a way that they closed a 0˚ degree angle with the *x*-axis (Figure 3d). Additionally, the algorithm was capable of recognizing the tail part from the head by comparing the area on the front part, calculated from the maximum width in each direction, and the back of the fish (area on the front is larger than in the back). This resulted in a proper head-to-tail orientation similar to the one reported in Misimi and others (2006). An alternative method for head-to-tail orientation is also reported by Strachan (1993).

Feature Extraction

Subsequently, extraction of geometrical features was performed on the binary image of the segmented fish. This process was focused on the measurement of geometrical properties of fish such as the size (area, length, width) and shape (roundness). All of the 2D-geometric features were computed from the segmented binary images (Figure 1). This process was important for identification of those geometrical properties that could be used for differentiation between the quality grading classes.

Initially, a number of features (6) were generated aiming to choose only the non-redundant and uncorrelated features. These features were further reduced to 4, consisting of the aspect ratio and the three other features as defined in equations (3), (4), (5). These features were seen as being among those possible features that do contain information

about the tail length and broadness in the posterior part of the fish. This is the justification for taking into consideration features as defined in equations (3), (4) and (5). At the end, the subset of features, consisting of only 3 features (Figure 1), was chosen for the purpose of classification. The aspect ratio was not a part of this subset, because this feature was highly correlated to the X_2 feature in equation (4), meaning it was redundant information. The final 3-dimensional feature vector *x*:

$$x = [x_1, x_2, x_3] \tag{2}$$

consisted of the following features:

$$x_1 = \frac{L_t}{W_{Max}} \tag{3}$$

$$x_2 = \frac{W_{Min} + W_L}{W_{Max}} \tag{4}$$

$$x_3 = L_t \tag{5}$$

The nature of these features can be understood by looking at the Figure 1. The length L_t is the length from the maximal width of fish W_{Max}, at location M, to the location T of the minimal width in the tail W_{Min}. The W_L is the width of the fish in the back part, at location S= L_t/2. These features describe the geometrical measures of the back part of the fish, which is crucial for quality grading according to the sensory evaluation performed by the trained human inspector.

All of the geometrical measures which are used for the extraction of features defined in (3), (4) and (5) were calculated from the binary fish images. As recommended by

Theodoridis and Koutroumbas (2003), prior to the selection of the subset of features for the use in classifier design, we tested the discriminatory capability of all the generated features. The features that are not significantly different should be discarded, as they would only constitute an unnecessary computational burden. Therefore, for the generated features, we investigated whether the values they took for the different classes were significantly different ($p<0.05$). At the end, only those features with the richest discriminatory information between classes (lowest p-value), and were not correlated to each other were chosen.

Classifier design

The subset of the selected features was fed into the classifier and a classifier based on the LDA was chosen for the purpose of classification.

LDA searches for those vectors in the underlying feature space that best discriminate among classes. Therefore, LDA seeks directions that are efficient for discrimination (Duda and others 2000). For all samples of the available classes are defined two measures in form of scatter matrices. The first one is the *within-class scatter matrix*, given by

$$S_w = \sum_{j=1}^{c} \sum_{i=1}^{Nj} (x_i^j - \mu_j)(x_i^j - \mu_j)^T \tag{6}$$

where x_i^j represents the *i*th sample of class *j*, μ_j is the mean of class *j*, *c* is the number of classes, while Nj is the number of samples in each class *j*. The other scatter matrix is called *between-class matrix* given by

$$S_b = \sum_{j=1}^{c} (\mu_j - \mu)(\mu_j - \mu)^T \tag{7}$$

where μ represents the mean of all classes. The goal with LDA is to maximize the between-class measure while minimizing the within-class measure. This can be done by maximizing the function *J* given by

$$J = \frac{w^t S_b w}{w^t S_w w} \tag{8}$$

The function W which optimizes the J is given by

$$w = S_w^{-1}(\mu_1 - \mu_2) \tag{9}$$

With this transformation, the classification is converted from an n-dimensional problem to 1-dimensional one. The optimal decision boundary for separation of classes is then:

$$w^t x + w_0 = 0 \tag{10}$$

where

$$w = \sum^{-1}(\mu_1 - \mu_2) \tag{11}$$

Practically, this means that by training, from the set of 3-dimensional (3-features) samples $x = [x_1, x_2, x_3]$, the classification problem was converted to 1-dimensional one by obtaining a scalar y which projects the samples into one line

$$y = w^T x \tag{12}$$

The purpose of training with LDA was to find and select the threshold ***t=y*** that maximizes the separability between classes, which was subsequently chosen as the decision class boundary. The classifier's output were numbers 1 or 2, which are the class labels. This means that, upon the testing, the sample **x** from equation (2) was allocated to class 1-'Ordinary' if it was to the left of the threshold (y(x)<t), otherwise it was allocated to class 2-'Superior' (y(x)≥t).

Performance evaluation

To evaluate the performance of the linear classifier based on the LDA, the leave-one-out cross-validation was used, a well-established technique for assessing the classification performance (Ripley 1996; Theodoridis and Koutroumbas 2003). Each fish sample in the dataset was left out in turn as a test sample, while the remaining (N-1) samples were used as the training data. This was repeated for each fish sample, meaning that the technique requires N repetitions of classifier trainings, for a dataset with a sample size of N. The total predicted accuracy of the classifier, used to measure the classification performance was defined as:

$$Total\ accuracy = \frac{Ncc}{N} \tag{4}$$

where *Ncc* is the number of correct classifications while N is the total number of samples.

Results and discussion

Figure 4 is a graphical representation of the discrimination between classes as generated by LDA. Here, it is shown that classes are in different sides of the generated class decision boundary from training the classification algorithm with the LDA. The values of features for the respective salmon grading classes are shown in Table 1.

Table 1- Feature values for different quality grading classes of Atlantic salmon.

Feature	Ordinary	Superior
Aspect Ratio*	2.4 ± 0.2^{a}	3.2 ± 0.3^{b}
X1	1.0 ± 0.1^{a}	1.13 ± 0.00^{b}
X2	1.2 ± 0.1^{a}	1.6 ± 0.1^{b}
X3	334.5 ± 47^{a}	466.6 ± 35.2^{b}

**This feature is not taken into consideration in the final classifier design due to being highly correlated with feature X2. Values are shown as Mean ±SD (n=60). Different letter a, and b within rows indicates significant difference (p<0.05).*

The classification results by LDA and cross validated by leave-one-out method are shown in Table 2. The classification accuracy obtained in this case was 91% for the used data set. By checking the labels from the sensory evaluation for the misclassified samples, we saw that the human inspector had graded two of the three misclassified "Ordinary" samples into their respective quality grade because they had a minor scale loss area. The differences in the geometry of these samples and the "Superior" grade were minor and our classifier was not able to quantify and differentiate them. The other fish, misclassified as "Superior" from our classifier, was classified as "Ordinary" from the human inspector because it was too long and had low condition factor (Misimi and others 2007) (large aspect ratio), even though it was rather symmetrical and not broad in the posterior part, which are normal characteristics of a "Superior" fish. Increasing the size of the dataset to include more fish with a large aspect ratio could solve this problem.

When it comes to the feature selection, we found that the use of additional features (roundness, aspect ratio, area ratio) did not improve the classification accuracy. Therefore, the initial choice of the subset consisting only of three features was proven to be sufficient, because it contained the best discriminatory information for the given quality classes. Adding the aspect ratio (Table 1), for example, as the fourth feature did not improve the classification accuracy. In addition, this particular feature was highly correlated to the feature X_2 and therefore it was left out.

In our study, we did not use any color feature for discrimination, since no such feature was used in discrimination of grading classes from the qualified human inspector. However, the system has the ability to include color features if there is a need to do grading according to other aspects of fish quality such as the skin color (Schubring 2003, Erikson and others 2007)

Table 2 - Classification results as evaluated by the leave-one-out cross-validation for Atlantic salmon.

Dataset	Nr. of samples (Ground truth as labeled from human inspector)		LDA Cross validation accuracy (%)		
	Ordinary	Superior	Ordinary	Superior	TOTAL
Atlantic salmon	26	34	23	32	91

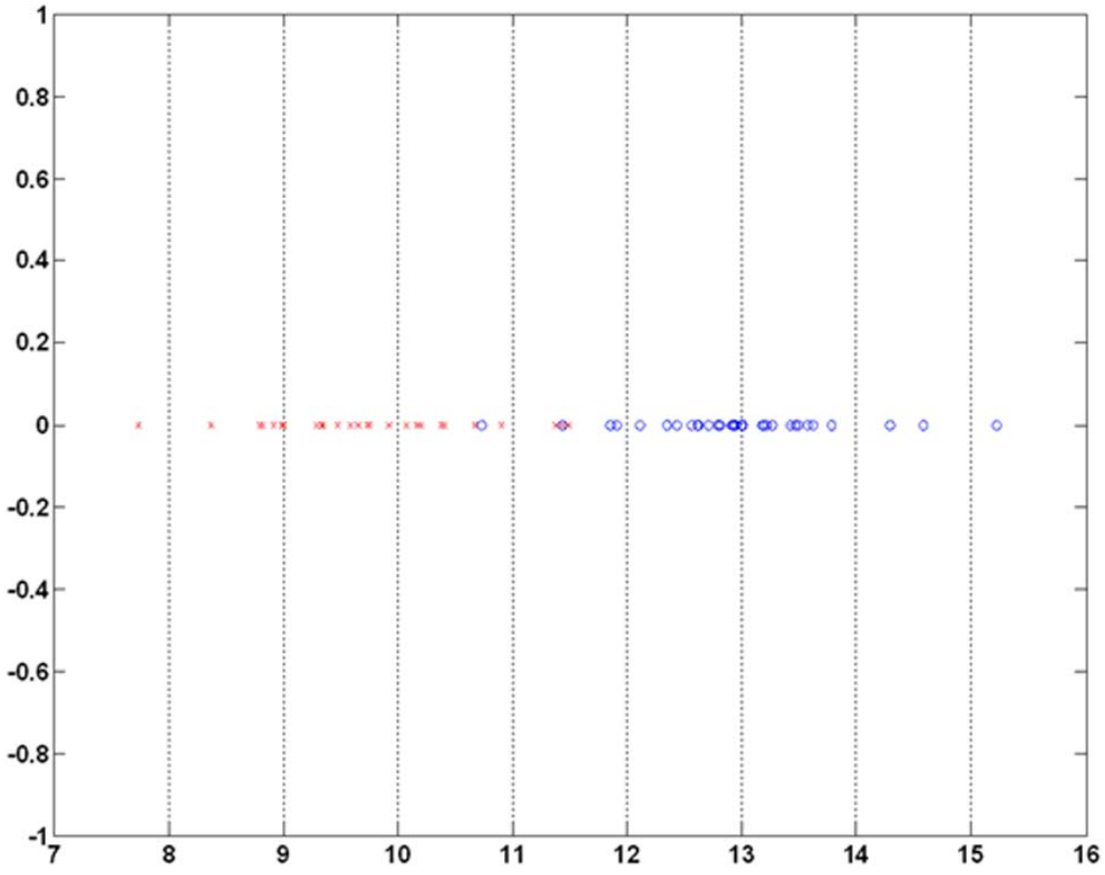

Figure 4-Discrimination between two grading classes as generated from the Linear Discriminant Analysis based classifier. Red pixels are the Ordinary grade samples; Blue pixels are the Superior grade samples.

Image pre-processing and segmentation of Atlantic salmon, as a region of interest, from the images was optimal due to the acquisition of images under controlled illumination conditions. The segmentation was facilitated from using a background with different spectral characteristics (light-blue) compared to the spectral characteristics of the fish. This is in line with the recommendations in Mendoza and others (2006), since the segmentation of the region of the interest (Atlantic salmon) depends from the contrast between the fish sample and the background. Since the necessary contrast was provided by using a background with different spectral characteristics, there was no need for use of a more complex scheme of thresholding than the one we used in this study. As long as the desired features are easily extracted, it is always more favorable to use a simpler algorithm for segmentation, as it is known that a faster and simpler segmentation by thresholding can play an important role in improvement of the total performance of computer vision systems (Lin 2005).

During the classifier design, the aim was to avoid the use of too many features, as well as proper utilization of image data. Using too many features, when having a limited data set available, can cause overfitting (Duda and others 2001). Overfitting means that, for a given sample size, the increase of the number of features would virtually improve the performance accuracy when designing the classifier, but practically would only degrade the classifier performance accuracy when dealing with newly unknown patterns. Nevertheless, in our study, adding 2 or 3 more features would still be optimal for our classifier, provided that the additional features improve the classification of accuracy (Heijden and others 2004). This is line with the previous studies regarding the maximal reasonable number of features when classifying food products. For instance, Mendoza and others (2004) report the use of 9 features for designing a classifier of bananas with an existing dataset of 49 samples. Panigrahi and others (2006) report the use of 6 to 10 features for classification of beef spoilage over an average dataset of 58 beef samples. In our study, additional statistically significant features were left out as they did not greatly improve the classification accuracy. Their extraction and inclusion in the subset of features can, however, not be excluded if, with a larger dataset, it is shown that they improve the classification accuracy.

As for the performance evaluation of the classifier using the leave-one-out cross validation, for a dataset of the similar size which we have used in our experiment, the leave one out (LOO) cross validation is the most optimal form of performance evaluation (Ripley 1996; Rosemary Tate and others 2003). Although computationally complex, the LOO method gives almost an unbiased estimation of the classifier's performance accuracy (Vapnik 1998; Theodoridis and Koutroumbas 2003).

As for the dataset in this study, a larger sample size would be preferable but not always practical, and economical. Limitation to this sample size is partly due to the high cost of the experiments of this nature in the industry, and limitations that the industry sets for the number of the extracted fish samples from a batch. The main idea in the study was to prove if there can be made class discriminations between quality grades of Atlantic salmon by the means of computer vision. Since this is a supervised classification, the increase of sample size is dependent from labeling of fish samples by a qualified human inspector, in order to be able to take these samples into the training set. This can be done in the way it is described in the Figure 5. The knowledge database of labeled fish images, which are used for training of the classification algorithm, can be gradually increased if a human inspector manually labels/classifies the actual images of fish, acquired by the camera. As a result, occasional periodical training of the classifier using the renewed knowledge database can be done, resulting in the adjustment of the classification threshold.

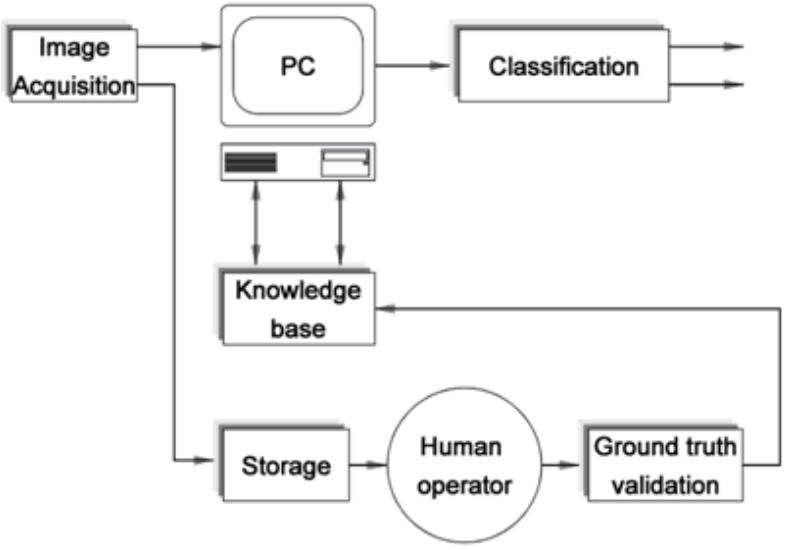

Figure 5-Increase of the knowledge image database by manual grading/labeling of fish images from human inspectors for training the classification algorithm.

Nevertheless, only a larger sample size does not necessary mean an improvement in classification accuracy. White and others (2006), for instance, found that increase of the size in the training sets did not lead to better classification scores for the different fish species. They report that, including an even distribution of the samples which belong to a certain class was an important factor in improving classification accuracy. In this regard, we have tried to have an even distribution of samples belonging to classes as much as possible.

In this study, we only used fish slaughtered at the same processing plant, evaluated by the same inspector. The fish were farmed at the site, and sampled on the same day. Therefore, potential influence in the classification accuracy of fish farmed at different sites, and the effect of season has yet to be explored. White and others (2006), for example, conclude that it is to be expected a decrease of the classification accuracy if fish samples from more than one location and from different time of the year are included. In

this respect, it is realistic to expect that the increase of the dataset and inclusion of fish from different seasons, different fish farms, and graded by different quality inspectors may decrease the classification accuracy. It is however important to observe that a whole Atlantic salmon classification does not need to have a near-perfect classification, in order to be useful for the salmon processing industry (Mathiassen and others 2007).

Since our classification was of a supervised type, it was observed that the consistency of the sensory evaluation was crucial for designing a good classifier. This becomes even more important, since human inspectors, despite some existing industrial standards for grading, may grade and label products in a way that is not according to the descriptions of industry standards (Sørensen 2003). Sørensen (2003) points out the tendency of the fish processors for a voluntary use of the industry standards for grading. Relying only on the existing industry standards, may also not be so reliable since these standards have not been revised since 1999, meaning there is a need for systematization and optimization of the grading criteria. The present situation opens up for abuse during manual grading as the majority of the salmon exporters seek to label their fish into the "Superior" class (Sørensen 2003), to maximize the profit. Computer vision as an automation technology for quality grading will therefore introduce a consistent way of quality grading.

In classification issues, the issue of cost of misclassification that occurs must also be taken into consideration: 1) "Superior" salmon can be misclassified as an "Ordinary" grade or 2) the "Ordinary" salmon as a "Superior" grade. In the first case, the customers would, most probably, not mind the misclassification but it is the producer who will loose in value (costly type of classification error). In this regard, the designed algorithm may have flexibility for the decision boundary adjustment. It is to be expected that the automated quality grading will introduce a more consistent and objective grading vs manual grading.

Conclusions

Automation of manual grading of Atlantic salmon in processing plants has become a central issue in recent years, because of the continuous diminishing of the number of

processing plants in Norway due to high labor costs and increased production volume. The findings of this study show the potential of computer vision pattern recognition in grading whole Atlantic salmon. The method is able to grade with 91% accuracy the "Ordinary" and "Superior" grade, with the existing data set. Manual sensory grading done by a qualified human inspector was used as a reference data (ground truth). By the LDA computer vision algorithm it was possible to simulate the quality grading performed by human inspectors. Therefore, computer vision has the potential to enable fully automated quality grading of Atlantic salmon in processing plants. Further large scale tests and optimization of software/hardware, and an eventual systematization/optimization of quality grading criteria are necessary before the method can become viable for industrial and commercial use.

Acknowledgements

We would like to thank The Research Council of Norway for financial support through *NFR project No. 145634/140 –'Efficient and economic sustainable fish processing industry' and No. 173530/I30 – 'Technology for efficient and profitable fish industry'*.

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PAPER VI

Bleeding of anesthetized and exhausted Atlantic salmon: Residual blood in prerigor and smoked fillets as determined by various analytical methods

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Keywords: Bleeding, residual blood, fillet color, handling stress, analytical methods, computer vision, fish processing, Atlantic salmon

ABSTRACT

The bleeding efficiency of anesthetized and exhausted Atlantic salmon was studied. Unbled fish were used as control groups for both treatments. Several analytical methods were used to evaluate color or residual blood contents of prerigor and smoked fillets. In all cases, the amount residual blood in the fillets was modest and blood was not considered a quality problem in terms of fillet appearance. Perimortem stress did not affect residual blood contents of the fillets. Only salting and smoking had a significant effect on filet color. The high bleeding efficiency for all groups was attributed to prerigor filleting allowing washing of the fillets before the blood had time to coagulate. In addition, a computer vision method was developed for automated blood inspection of the body cavity after gutting and washing. A classifier (‘no blood’ or ‘blood present’) based on linear discriminant analysis was tested and the classification accuracy was over 90% as evaluated with the leave-one-out method.

Introduction

For large fish, adequate bleeding is considered necessary for good product quality. Otherwise, residual blood in fillets may lead to reduced visual acceptance of the product (Kelly, 1969; Tretsven and Patten, 1981; Huss, 1995; Connell, 1995). For instance, uniform white fillets are commonly desirable for whitefish. To avoid brown discoloration of pre rigor cut cod (*Gadus morhua*) fillets, appropriate bleeding procedures must be followed (Kelly and White, 1966). Residual blood (heme iron) can catalyse lipid oxidation during storage of fatty fish (Richards and Hultin, 2002). Iron degraded from the heme iron complex acts as a catalyst for the oxidized flavours in cooked meats (see Turhan and others, 2004). Accordingly, Tretsven and Patten (1981) demonstrated that bleeding of rainbow trout (*Salmo gairdneri*) lead to reduced rancidity as well as better appearance and odor when evaluated after storage for 8 mo at -18 ^{o}C. On the contrary, Porter and others (1992) did not find any differences in rancidity between unbled and bled sockeye salmon (*Oncorhynchus nerka)* after storage for up to 12 mo at –20 ^{O}C. Notably, they also found no difference between white muscle hemoglobin levels of unbled and bled fish.

In the salmon industry, bloodspotting represents one of the major causes for fillet downgrading (Michie, 2001). Although bleeding affects the number of blood spots in smoked salmon, Robb and others (2003) concluded that other, unknown factors play a more important role. The visual effects of inadequate bleeding are particularly pronounced in salted and smoked products such as in smoked Atlantic salmon (*Salmo salar*) fillets (Robb and others 2003).

Exsanguination of Atlantic salmon by severing gill arches followed by bleeding in a tank filled with seawater do not represent a major stress factor (as defined by muscle biochemistry) compared with substantial antemortem handling stress. In fact, the energy status of the muscle seemed to increase as a result of bleeding (Erikson and others 1999). Similarly, from an in vitro ^{31}P NMR study of early post-mortem changes (up to 5 h post

mortem), bled loach (*Cobitis biswae*) maintained intramuscular levels of PCr, ATP and pH, whereas a decrease was observed for unbled fish (Chiba and others 1991) suggesting bleeding may delay rigor onset. This was indeed documented by Mochizuki and others (1998) who found that bleeding of unstressed horse mackerel (*Trachrus japonicus*) delayed the rate of the rigor mortis progress. Furthermore, bleeding of three pelagic species delayed, by slower degradation of pericellular collagen fibrils, muscle softening during chilled storage. On the other hand, bleeding did not effect muscle firmness of three species of demersal fish (Ando and others, 1996,1999).

The total blood volume of different fishes has been stated as ranging from 1.5 - 3.0 % (Huss 1995) up to 5 - 7 % (Itazawa and others 1983) of the body weight. The latter range also covers the blood volumes of three salmonids (Smith, 1966). Only 20 % of the blood is located in muscular tissues and the rest is located in internal organs. Since the white muscle is rather poorly vascularized, it has been assumed that blood distribution is not much affected by exercise (Huss, 1995). However, when rested fish are exposed stressors and they exhibit escape behavior, blood flow is gradually redistributed from the viscera to the locomotory muscles to meet the increased oxygen demand of the white muscles (Thorarensen and others 1993). Moreover, when fish are subjected to handling stress, the plasma clotting times is reduced, presumably due to the response of thrombocytes resulting from increased levels of blood catecholamines (Fujikata and Ikeda, 1985; Smit and Schoonbee, 1988; Ruis and Bayne, 1997). A 43 % decline in blood clotting times has been observed 10 to 60 min after a stress incident. Intravascular coagulation could be a consequence of this (Cassilas and Smith,1977).This finding alone suggests that perimortem stress might lead to poorer blood drainage. On the other hand, (Warriss and Wilkins, 1987) reported that stress during stunning normally promotes peripheral vasoconstriction through the action of catecholamines resulting in a minimum of blood amounts in muscular tissues. A well-established fact is that a beating heart does not play a significant role for effective blood drainage (Huss and Asenjo, 1976; Warriss and Wilkins, 1987; Robb and others 2003; Roth and others 2005). Furthermore, it has been speculated that rigor contractions may force some blood in tissues back into large blood vessels in the backbone. In turn, this may produce less residual blood in muscle

tissues (Kelly, 1969; Huss 1995). For the salmon industry where the current goal is to promote good welfare and minimize handling stress, the latter factor would hardly be an issue since the prerigor period for rested salmon is at least 24 h (Erikson, 2001). By then, possible residual blood would have coagulated (Botta and others, 1986) making it difficult to remove.

Fish can be bled in different ways, by severing gill arches, throat or caudal peduncle. In the Norwegian salmon industry, all gill arches on one side of the fish are normally cut. Commonly, the fish are then bled for 15 - 30 min in a refrigerated seawater (RSW) tank (0 – 5 ^{o}C) where they eventually die due to loss of blood. Alternatively, a method based on bleeding in air (head down) immediately after the fish are electrically or percussion stunned (killed) has been introduced in the industry. Yet another option is simply to send the fish directly after killing to the gutting machines to remove the viscera (containing most of the blood). Roth and others (2005) considered this method ('direct gutting') an adequate bleeding method based on sufficient removal of blood from the white muscle. However, in the salmon industry today, there is no consensus as to which is the optimal bleeding method and what are the optimal process variables (e.g. temperature) for efficient blood drainage. Jerrett and others (2000) estimated the mean freezing points of chinook salmon (*O. tshawytscha*) blood and white muscle as -0.8 ^{o}C, and -1.0 to -1.1 ^{o}C, respectively. This suggests that the water temperature in bleeding tanks should be higher than this to prevent the risk of freezing (immobilization of blood) in muscle tissues (in case of extended fish holding times in RSW bleeding tanks). The viscosity of blood is greatest as the temperature approaches the freezing point. However, rainbow trout blood experiences relative small changes in the viscosity component of the vascular resistance as water temperatures change (Fletcher and Haedrich, 1987). Thus, it seems unlikely that blood viscosity might have a major effect on blood drainage. At low water temperatures, blood coagulation time is prolonged and the blood remains fluid up to about 30 min post mortem. After this, or even before, at higher storage temperatures, clotting takes place rapidly (Connell, 1995). In salmon however, it has been observed that blood can be fluid even longer, up to 1 h post mortem (Olsen and others, 2006).

We have over the years observed at most processing plants that the carbon dioxide stunned fish exhibit little swimming activity in the RSW bleeding tank before they eventually die. According to Robb and others (2003) and Roth and others (2005), muscle activity during bleeding is not important to facilitate adequate drainage of blood. Although there is some disagreement as to what is the best bleeding method (see Huss, 1995), it seems clear that immediate bleeding of live fish after capture or stunning is more important than the actual bleeding method (Kelly, 1969; Huss and Asenjo, 1976; Valdimarsson and others, 1984; Botta and others, 1986; Warriss and Wilkins, 1987; Roth and others, 2005). Summing up, there are number of factors that may contribute to the efficiency of the bleeding as a unit operation. Some of these may be thought to increase the efficiency of blood drainage, whereas other factors may have the opposite effect. In the present study, we therefore wanted to elucidate the effect of perimortem handling stress where the fish were bled and not bled. We chose to do the experiment in a prerigor filleting context since this concept is currently a goal for several salmon processing companies.

To reduce the high costs of labor, automation of fish processing lines is an issue in several high-cost countries. In the salmon industry in Norway, 2-3 workers are commonly necessary for manual quality grading and sorting of farmed salmonids, i.e. when biomasses between for example 80 -150 tons are processed per shift (7 h). For the quality grading, both external and internal attributes are inspected. Automated external grading of whole fish (weight class, skin blemishes, condition factor, sexual maturity, and backbone-related defects) after gutting and washing has been reported elsewhere (Misimi and others, 2006). Quality inspectors are also checking the body cavity for residual blood (particularly along the backbone) as well as melanin discolored areas. Large melanin flecks can cause downgrading, whereas residual blood in body cavity would normally imply re-washing.

Since no standard method exists for assessment of residual blood in muscle-based foods, a common option for researchers has been to evaluate salmon fillets after they have been

salted and smoked. If present, residual blood will then be more clearly visible as dark blood spots or elongated vessels.

Based on the issues reviewed above, our main goals in this study were to assess: (1) whether perimortem handling stress affected residual blood contents in salmon prerigor fillets, (2) the possibility of automated quality control of the body cavity for residual blood, and (3) the suitability of several analytical methods for determining residual blood in white muscle.

Materials and Methods

Fish

Commercially farmed Atlantic salmon weighing 4.74 ± 0.71 kg with fork length 72 ± 3 cm (n = 44) were netted from the sea cage (SW temperature 8 ^{o}C) and transferred to a 1000-L tub. The fish had been fasted for 22 days. The tub was transported (< 5 min) by boat to the quay where the fish were netted and divided equally in three transport tanks on a truck. The tanks had just been filled with fresh SW. The fish were transported under constant oxygenation for 2.5 h to our laboratory at a fish density of about 52 kg m^{-3}. Although the fish were calm by arrival, they seemed somewhat uneasy. Foaming was observed in all tanks. SW temperature, pH and dissolved oxygen (DO) in the tanks were 5.6 ^{o}C, 6.7-6.8, and 360 % saturation, respectively. The low pH indicated elevated levels of carbon dioxide. This, together with the fact that the SW was heavily oxygen supersaturated, suggested that the fish had developed hypercapnia (Erikson and others, 2006). The fish were netted individually from the truck and transferred in SW-filled 200-L tubs (< 5 min) to two holding tanks (4000-L each). Twenty-two fish were kept in each tank corresponding to a fish density of 35 kg m^{-3}. Fresh SW was pumped, sand-filtrated and circulated to the tanks at a rate of 5 m^3 h^{-1}. After 1 - 2 h, the fish regained normal swimming behavior and distributed themselves evenly in the water column. The SW temperature, pH and DO levels in both tanks were 9.2 ^{o}C, 7.93, and 80-90 % saturation throughout the 7 d the fish were kept the tanks. No food was offered during this period.

Experimental protocol and sampling of fish

At the start of the experiment, the SW supply to one of the tanks was closed and oxygen gas was distributed to the tank using a diffuser. A predetermined amount of AQUI-S^{TM} was added to the tank corresponding to the recommended concentration of 17 ml L^{-1} for salmonids (AQUI-S Ltd., Lower Hutt, New Zealand). After 8-10 min, some fish were seeking to the SW surface whereas others were swimming calmly upside down (Stage 3 anesthesia - partial loss of equilibrium, Jolly and others, 1972). After 16 min, most fish were lying on the bottom of the tank, belly up (Stage 4 to 5 – Total or complete loss of equilibrium and reflex activity). No vigorous muscle activity took place. The DO levels increased from 90 to 121 % saturation over the same period. The first individual fish was then netted from the tank and subjected to bleeding and various assessments before the next fish was sampled. The last fish was sampled 2 h 23 min after sampling of the first one. Thirteen fish were bled by cutting all gill arches on one side of the head (Anesthetized and Bled group – **AB**). They were sampled between 51 and 116 min after all fish in the tank were regarded as fully anesthetized. As the gill arches on one side of the fish were severed and the fish were held head down over a bowl and the blood drained off was collected for the next 2 min before the blood was weighed (the blood flow practically ceased after < 1 min). Afterwards, the fish were immediately transferred and kept at 1^{o}C in a tub containing stagnant SW and ice (CSW). Apparently, little blood was drained off in this tub. After 20 min, the dead fish were lifted out and a cut was then made with a scalpel between the sideline and the dorsal fin, where the initial white muscle-pH and body temperature were measured. Initial muscle twitches, fork length and weight were also recorded. Then the fish were gutted and the body cavity was washed for about 10 sec in running tap water (6 ^{o}C). Possible presence of residual blood in the body cavity was evaluated using computer vision. The fish were filleted pre rigor (skin on) and tagged. The fillets were washed again for 10 – 15 sec under running tap water to clean both surfaces (exposed muscle and skin side) from blood and various debris from gutting and filleting operations. We chose to leave the peritoneum on (Zone 3, Figure 1) since potential residual blood in vessels behind it would then become more clearly visible. Immediately after washing, the fillets were subjected to residual blood analysis using the CIELab color system by the Minolta Chroma Meter and computer vision. In addition,

transflectance spectral measurements of the fillet were carried out with a fiber-based grating spectrometer. The latter two methods measured color at the same 5 fillet locations (Figure 1) on both fillet sides After the measurements were completed, a muscle sample (about 5 g), corresponding to location L2 (Figure 1), was excised, frozen and stored at -20 ^{o}C before chemical analysis of hemoglobin. The fillets were then placed on ice in styrofoam boxes for storage and transport to a commercial smokehouse.

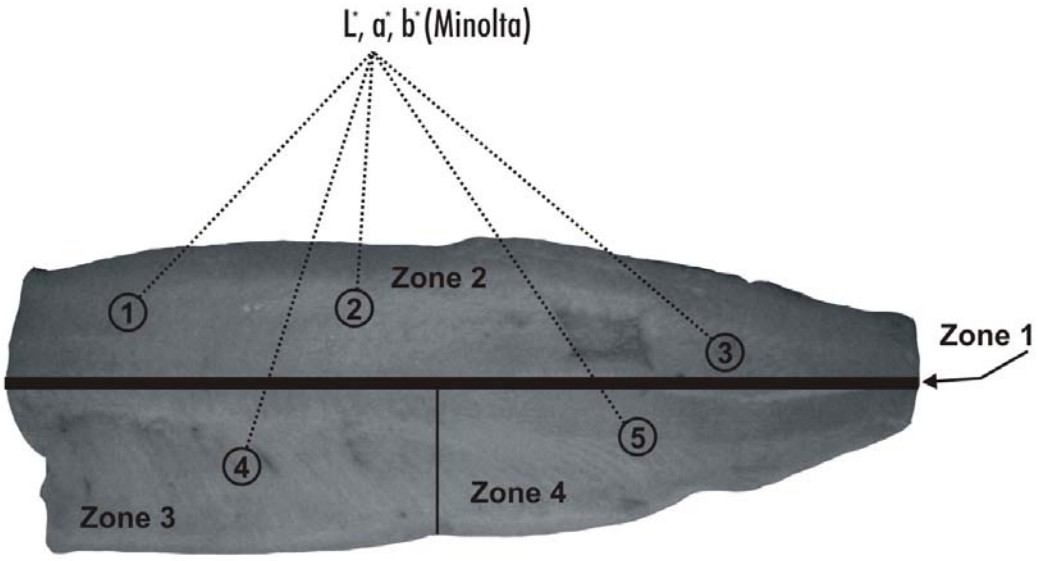

Figure 1- Color and residual blood in unbled and bled Atlantic salmon fillets were measured at different locations. Color was measured using the Minolta Chroma Meter at fillet locations L1-L5 whereas the computer vision method determined whole fillet color. Transflectance spectroscopy and chemical analysis of hemoglobin iron were carried out at location L2. The measurements were carried out immediately after killing (prerigor) of anesthetized and exhausted fish. After smoking, occurrence of blood spots and elongated blood vessels were examined at zones Z1 - Z4 on the fillet surface and subsequently on horizontally sliced fillets. The peritoneum located in Zone 4 (including location L4) was not removed.

Six fish were not bled (Anesthetized and UnBled group - **AUB**). They were sampled between 0 - 60 min, as well as between 123 – 148 min, after full anesthesia was achieved. Afterwards, the fish were netted individually from the tank and killed by a blow to the head. Subsequently, they were treated as the AB group, except that they were not bled.

In the other holding tank, the fish were chased to exhaustion for 30 min as the water level was gradually reduced to a height of about 15 cm. Thirteen fish assigned for bleeding were then transferred to a 300-L tank containing 34 ml L^{-1} AQUI-S^{TM} for rapid anesthesia (according to legislation in Norway, fish must be anesthetized before gill cutting takes place). To minimize fish holding times and potential death in the anesthetic bath, batches of three and three fish were transferred and sampled within the next 15 min. The fish (Exhausted and Bled, **EB** group) were then bled and treated like the AB group. Their Exhausted UnBled (**EUB** group) counterparts were post mortem treated as with the AUB group. The nine fish in this group were killed between 45 and 80 min after the initial stress bout. However, they were not allowed to recover during this period. No exhausted fish died before they were intentionally killed.

Salting and smoking

After ice storage for 6 days, the fillets were delivered to a commercial smokehouse where they were pickle salted for 24 h. A mixture of common household qualities of fine salt (72 % NaCl) and sugar (28 %) was lightly rubbed into the skin side of the fillet. Subsequently, some of the mixture was distributed over the flesh side before the fillets were placed (skin side down) in a tub. The fillets were piled up to a height of about 0.5 m. No drainage of liquid took place. After salting, the fillets were suspended in a smoking oven and dried for 24 h in circulating air at 10 ^{o}C. Finally, the fillets were cold smoked for 4 h at about 28 ^{o}C in the fumes from beech chips. The smoked fillets were placed on ice in styrofoam boxes and brought back to our laboratory for evaluation of appearance (residual blood) and color (Minolta and computer vision) the next day. Four fillets were evaluated using the transflectance spectra method 3 d later.

Analytical methods

All fish were subjected to similar analyses. Body and core temperatures, white muscle pH, muscle twitches and CIELab values by Minolta Chroma Meter CR-200 (Minolta, Osaka, Japan) were measured as described before (Erikson and Misimi 2007, in press)

Computer vision, image processing and segmentation

Image acquisition

To ensure uniform illumination and avoid intrusion from ambient light, the fish or fillets were put in a lightbox (Waagan AS, Skodje, Norway). Avoidance of ambient illumination is critical for reproducible imaging (Shahin and Symons 2001). The light-box (Figure 2) had a grey neutral color inside and used two fluorescent tubes (18W) with a colour temperature of 5000K and Rendering Index greater than 95%. The color rendering index is a measure of the ability of a light source to reproduce the colors of various objects being lit by the source. For good color discrimination and an overall good quality of images, rendering index greater than 90 and color temperature of 5000K is preferred (Sandor and Schanda, 2006, Erikson and others in press).

Images of the fish and fillets were captured using an image acquisition system for a digital camera (Figure 2). The fillet images were captured in the BMP file format with a digital camera (Pixelink A776, Ottawa, Canada) connected directly to the PC through a firewire interface with a resolution of 1280 x 1024 pixels. The fish body cavity images were obtained using a Nikon D70 digital camera (Nikon, Tokyo, Japan) in the JPG file format with a resolution of 3000 x 2000 pixels. The camera was mounted on the upper part of the light box, perpendicular to the field of view, at a vertical distance of 60 cm from the belly cavity of the fish or the fillet. To achieve stable illumination and camera conditions the light-box and camera were switched on at least one hour before the experiment and were not switched off until the experiment was over.

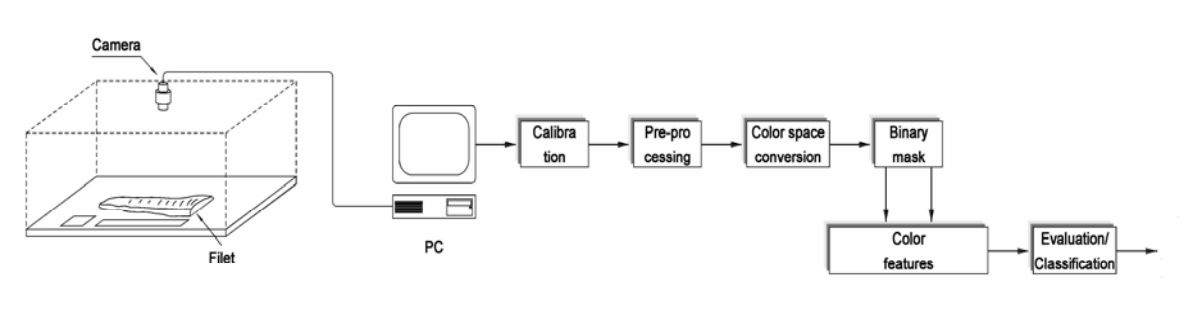

Figure 2- The computer vision system and the flow chart of computer vision operations based on images of salmon fillets/body cavities.

Initially, the images of both fillets and belly cavities were color calibrated using the Macbeth ColorChecker with 24 color patches as described in Erikson and others (in press) and the CIEXYZ values of these patches. The calibration process and the CIELab colour space of the images is described in Erikson and Misimi (2007, in press).

The segmentation of the fish body cavity (Figure 3a) as a region of interest (ROI) was performed in Matlab (2007) Image Processing Toolbox 5.4 (Mathworks, Natick, MA, USA). The procedure was fully automatic. The ROI segmentation was done by generating a binary mask (Figure 3b) of the body cavity using thresholding in channel *a*. Since illumination was controlled and there was no interference from ambient light, global thresholding was used for segmentation of the body cavity from the background. This means that if *f(x,y)* is the original image from the scene, the binary image *BW(x,y)* was defined as

$$BW(x,y) = \begin{cases} 1 & if \quad f(x,y) \geq T \\ 0 & if \quad f(x,y) < T \end{cases} \qquad (1)$$

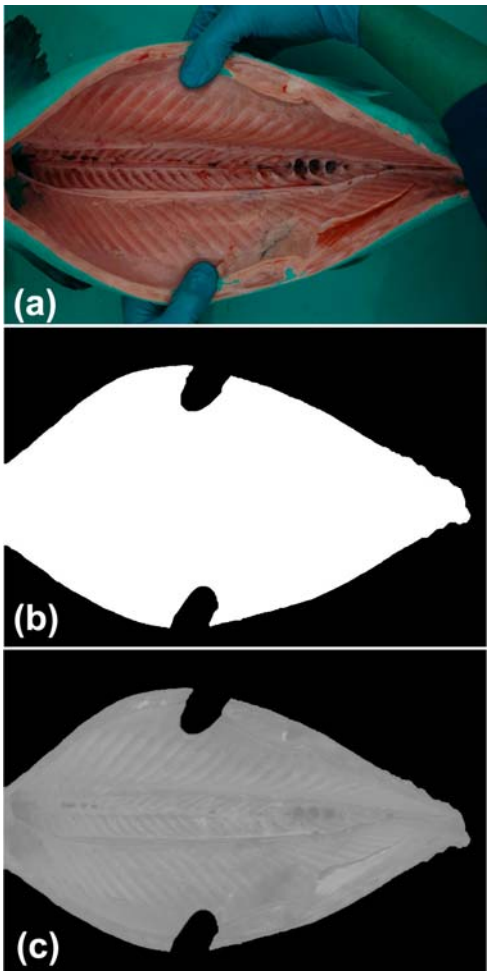

Figure 3- (a) A captured image of the body cavity of a bled fish, (b) mask generation, and (c) segmentation of the region of interest (body cavity).

Pixels labelled 1 (white) corresponded to the body cavity, while pixels labelled 0 (black) corresponded to the background. T is the threshold which separates the body cavity from the background. Initially, the generated mask from the thresholding had some small holes, but by applying the morphological operations of dilation and erosion, the mask coincided with the ROI of the body cavity (Figure 3c). By masking the images in each gray-scale channel with the generated binary mask, the body cavity ROI was segmented. The same procedure was repeated for segmentation of fillets from their background.

In the present study, we did not develop a device for holding the belly open during image acquisition. Instead, we simply held the body cavity open manually. Such a device would of course be necessary for automated inspection of the body cavity.

The color analysis of the fillets by computer vision consisted of calculating the average CIELab values for the entire fillet area (Zone 1-4, Figure 1).

Classification of fish for automated inspection of body cavity

Sensory evaluation of the body cavity with respect to presence of blood was done manually by labelling each fish either as *'OK'* (no blood present) or *'Wash'* (blood present). This labelling was used as a ground truth. Since the fish was washed manually, the badly washed fish had a considerable presence of blood in the body cavity. The fish that were properly washed had practically no blood in the body cavity, except for some very little spots of blood. In this way, the fish in the later group were assigned to the 'OK' class, while the improperly washed fish in the '*Wash*' class. Inadequate washing of fish is quite common in a real fish processing line, and we wanted to simulate these conditions in the experiment.

In order to select the appropriate features for classification, a number of color-related features were initially extracted. They were the *L* (lightness), *a* (redness) and *b* (yellowness) values from the CIELab space, as well as the green (G) value from the RGB space. Color and intensity features in different color spaces are commonly used in classification applications of food products (Pedreschi and others 2004). As recommended by Theodoridis and Koutroumbas (2003), before selection of the best subset of features for use in the design of the classifier, we tested the discriminatory capability of the generated features. The features with less discriminatory capability were discarded, as they would only constitute an unnecessary computational burden (Pedreschi and others 2004). Therefore, for the generated features, we investigated whether the values they assigned the different classes were significantly different (Theodoridis and Koutroumbas 2003). After completing this procedure, 3 final features were selected: the

average G value, *a* and *b* values of the segmented body cavity region. This subset of the features was fed into the classifier.

Classifier design was based on the Linear Discriminant Analysis (LDA) and was similar to the one reported by Misimi and others (2006). LDA searched for the vectors in the underlying feature space that best discriminated between the two selected classes (‘*OK’* or ‘*Wash’*). Therefore, LDA seeks directions that are efficient for discrimination (Duda and others 2000). From the set of 3-dimensional (3-features) samples $x = [x_1, x_2, x_3]$, the classification problem was converted to 1-dimensional one by obtaining a scalar y which projected the samples into one line.

$$y = w^T x \tag{2}$$

where

$$w = \sum^{-1} (\mu_1 - \mu_2) \tag{3}$$

Here, μ_j is the mean of class *j,* while Σ is the covariance matrix.

The purpose of LDA training was to find and select the threshold ***t* = *y*** that maximized the separability between classes. This was subsequently chosen as the decision class boundary. The classifier’s output were numbered 1 or 2 representing the class labels. This means that, upon testing, the test sample **x** in Equation (2) was allocated to Class 1 - *‘OK’* if it was to the left of the threshold (y (x) < t). Otherwise, it was allocated to Class 2- *‘Wash’* (y (x) ≥ t).

To evaluate the performance of the linear classifier based on the LDA, the leave-one-out cross-validation was used. This is a well-established technique for assessing the performance of a classifier (Ripley 1996; Theodoridis and Koutroumbas 2003). Each fish

sample in the data set was left out in turn as a test sample, while the remaining (N-1) samples were used as the training data. This was repeated for each fish sample, meaning that the technique required N repetitions of classifier trainings for a data set with a sample size of N. The total predicted accuracy of the classifier used to measure the classification performance was defined as:

$$Performance\ accuracy = \frac{Ncc}{N} \tag{4}$$

where *Ncc* is the number of correct classifications while N is the total number of samples.

Transflectance spectroscopy

The objective was to detect and possibly quantify residual blood *inside* the fillets. Blood stains and melanin on the fillet surface can easily be detected with computer vision or reflectance spectroscopy, but detection of residual blood inside the fillet is not as straightforward. In the present study, a transflectance probe was tested. Light from a 100 W halogen light source was coupled into a fiber bundle. A multimode optical fiber with diameter 200 μm was placed in contact with the fillet and a collection fibre probe was placed in proximity to the bundle. The distance between the bundle's rim and collection fibre was 10 mm. Two collection fibres were used, each coupled into a spectrometer of slightly different ranges. Since we did not find a difference between the spectrometers, data from only one of them are reported here. Light from the source was absorbed, transmitted and scattered inside the fillet, and some of the light was coupled into the optical fiber. The fiber was coupled to a grating spectrometer (Ocean Optics S2000-UV-VIS, Dunedin, FL, USA) with wavelength range 350 -1000 nm and a resolution of about 1 nm. Since the detected light had interacted with the effective fillet volume (instead of mainly the fillet surface as would be the case with reflectance spectroscopy), presence of residual blood inside the fillet would be detected if the probe was placed directly above the fillet volume under study. The reference spectrum was a reflectance spectrum of a plate painted with barium. This basic method has been further developed to a non-contact NIR imaging scanner by Wold and others (2006) where the water content of cod was measured. The spectra acquired at fillet location L2 (Figure 1) together with the hemoglobin iron reference measurement described below were inputs to a partial least

squares regression (PLS), to find a possible relationship between spectra and hemoglobin content. Full cross-validation (leave-one-out) was used. The standard deviation, the correlation coefficient R^2 of the models was calculated to evaluate the model.

Hemoglobin iron

Fillet location L2 (Figure 1) was excised and weighed (about 5 g) accurately after all other color-related measurements had been carried out. After frozen storage at –20 ^{o}C for 28 d, heme iron was determined in acidified acetone muscle homogenates as described by Hornsey (1956) and (Clark and others, 1997).

Visual assessment of residual blood in smoked fillets

After the fillets had been salted and smoked at the smokehouse, the fillets were assessed visually, that is, whether residual blood was present. Basically, our assessments were carried out according to the salting, smoking and slicing method proposed by Robb and others (2003). Firstly, the fillet surface was evaluated by dividing the fillets in different zones (Z1-Z4, Figure 1). As already mentioned, the whitish peritoneum covering the anterior lower part of the fillet (Zone 3) was not removed during filleting. The following criteria for residual blood, either present as *spots* (circular) or *threadlike vessels,* were devised (length x vessel diameter): (a) *Small: 1-4 x 1 mm, (b) Medium: 5-20 x 1-2 mm* and, (c) *Large: > 20 x 2 mm*. In addition, we looked for large discolored areas (blood or melanin). The fillets were re-evaluated similarly after they were cut horizontally. Both fillet sides of each fish were assessed by four persons at our laboratory. The mean values are reported here. The fillet pH after smoking was 6.15 ± 0.07 (Mean ± SD; n = 13).

Statistics

Effect of treatments (perimortem stress, bleeding and smoking) on the measured variables were analyzed using a two-way ANOVA. Where significance ($p<0.05$) were indicated, a Tukey post hoc test was run. All results are reported as means ± SEM.

Results and Discussion

Effect of stress on bleeding

The initial muscle-pH in the AUB and AB groups were, as expected, considerably higher ($p<0.0001$) than in the EUB and EB groups (Table 1). The stress-related values are typical for salmon after similar treatments (Erikson and Misimi 2007, in press). Bleeding did not have an effect on white muscle-pH ($p>0.05$), as could be expected since the fish hardly moved at all in the CSW bleeding tub. The muscle twitches (tail deflection) were clearly higher ($p<0.0001$) in the rested fish (AUB and AB groups) than in the exhausted fish (EUB, EU; Table 1) and as with the muscle-pH, bleeding did not have a significant effect on this parameter. Due to the low temperature in the bleeding tub, the body temperature immediately after bleeding was reduced by about 4^{o}C (Table 1). Three fish in each group were used to verify the typical development of rigor mortis (Erikson, 2001; Erikson and Misimi 2007, in press) of anesthetized and exhausted fish (data not shown). As expected, the rigor onset of rested fish occurred after about 25 h post mortem, whereas rigor started after only 2 h in exhausted fish.

Table 1 – The postharvest condition of the four experimental groups, anesthetized and exhausted Atlantic salmon, unbled and bled fish.

Parameter	Anesthetized		Exhausted	
	Unbled	Bled	Unbled	Bled
White muscle-pH	7.5 ± 0.0^{a}	7.4 ± 0.0^{a}	6.9 ± 0.1^{b}	6.7 ± 0.0^{b}
Muscle twitches	3.0 ± 0.0^{a}	2.7 ± 0.2^{a}	1.4 ± 0.2^{b}	0.9 ± 0.2^{b}
Body temperature (^{o}C)	9.8 ± 0.1^{a}	$5.8 \pm 0.4^{1,b}$	10.0 ± 0.1^{a}	$5.7 \pm 0.2^{1,b}$

Mean ± SEM; bled fish: n = 13, unbled fish: n = 9. In each row, different letter (a,b) indicates significant difference between treatments. [1]After blood drainage for 2 min in air, the fish were subsequently bled in CSW (1 °C) for 20 min.

Blood drainage and residual hem iron in prerigor fillets

Table 2 shows the collected blood volumes from the AB and EB groups as well as the residual iron contents of the white muscle (location L2). The blood pressure in rested fish seemed initially much higher than in exhausted fish because just as the gill arches were severed, the blood squirted out of the anesthetized fish as opposed to the passive flowing

of blood from the exhausted fish. However, the collected blood volumes were not different ($p>0.05$). In both cases, the blood flow more or less ceased altogether after 1 - 2 min (bleeding in air). Similar results, where most of the blood was drained off after 1 - 2 min, have been observed during exsanguination of pigs, calves, sheep and chickens (Warriss and Wilkins, 1987).

The weight of the blood drained off was 1.9 – 2.0 % of body weight and represented therefore a major fraction the total blood contents in fish (1.5 - 3.0 %) according to Huss (1995).

Table 2 – The amount of blood drained from rested and exhausted Atlantic salmon during bleeding in air for 2 min (head down), and white muscle iron contents of unbled and bled prerigor fillets (fillet location L2, Figure 1, was analyzed).

Parameter	Anesthetized		Exhausted	
	Unbled	Bled	Unbled	Bled
Blood weight (% of body weight)^{ns}	NA	1.86 ± 0.07	NA	1.99 ± 0.12
Residual iron (µg iron [g muscle]$^{-1}$)^{ns}	1.51 ± 0.22	1.55 ± 0.08[1]	1.73 ± 0.13	1.76 ± 0.14[1]

Mean ± SEM (Blood weight: n = 13; iron content: n = 6 - 13); [1]After blood drainage in air for 2min, the fish were subsequently transferred to CSW (1 °C) for 20 min; ns: No significant differences between groups (p>0.05); NA = not analyzed.

Our mean residual heme iron (hemoglobin) values were not significantly different between treatments ($p>0.05$). This is in line with data presented by Porter and others (1992) as they did not find different levels of hemoglobin in unbled and bled salmon white muscle. Our mean heme iron values, ranging from 1.5 to 1.8 µg g^{-1}, were largely similar to those determined in Atlantic cod (*Gadus morhua)* and mackerel (*Scomber scombrus*) white muscles (Gomez-Basauri and Regenstein, 1992), although they were lower than those reported with raw anchovies (*Engraulis encrasicholus*), 6.5 µg g^{-1}(Turhan and others, 2004). Olsen and others (2006) compared different killing methods and resulting residual blood levels in Atlantic salmon fillets. Their lowest hemoglobin value was 0.3 mg g^{-1} for fish killed by a blow to the head and gutted directly. Our corresponding values calculated as hemoglobin contents were similar at 0.2 - 0.3 mg g^{-1}.

Figure 4 shows typical transflectance spectra of the white muscle (fillet location L2). Neither perimortem handling stress nor drainage of blood from the fish significantly affected the shape of the spectra. However, as shown in Figure 4, a consistent shift towards shorter wavelengths was observed after smoking. The fillet color of the smoked fillets had a more yellowish appearance (see below).

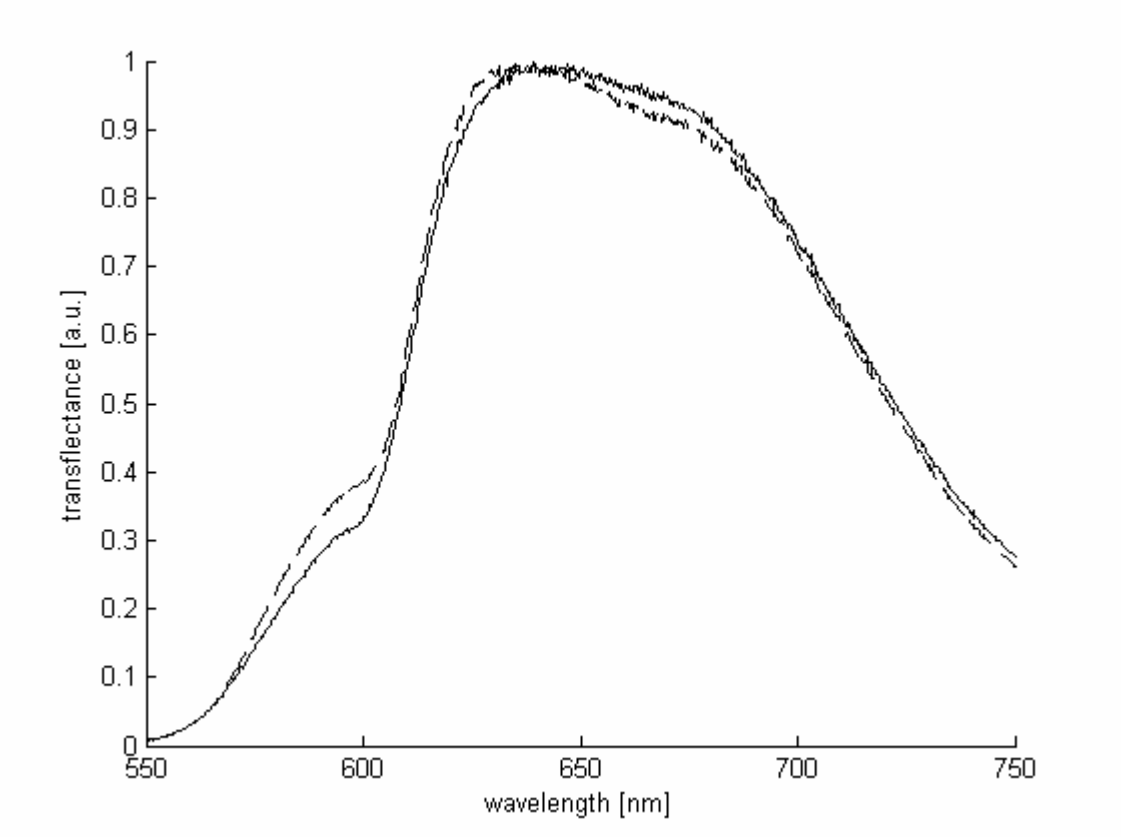

Figure 4- Typical transflectance spectroscopy spectra of fresh unbled and bled Atlantic salmon (solid line), and after smoking (dashed line) of the same filet (location L2, Figure 1). Only smoking affected the shape of the spectra as can be seen as a shift towards shorter wavelengths.

The PLS model of transflectance spectra predicted residual heme iron using three latent variables with a SD of 0.32, a correlation coefficient of 0.27 and an R^2 of 0.06. For a model that constantly predicted the amount of heme iron as the mean value of the training data set, the SD was 0.33. Thus, the correlation between residual heme iron and transflectance spectra was poor. A better correlation may be achieved by reducing the distance between the fiber and the bundle from 10 to for example 5 mm, as light with wavelength shorter than 570 nm did not reach the fibers, either due to absorption or

scattering. Therefore, the absorption peaks of hemoglobin at 560 nm and of oxyhemoglobin at 540 nm were not visible. The second absorption peak of oxyhemoglobin at 580 nm was barely visible. It has been reported that blood hemoglobin levels as measured before slaughter correlated well with absorbance in the range of 500 - 580 nm as determined in muscle 1 h post mortem. After 24 h though, the significant relationship no longer persisted. It was concluded that the post mortem changes in pH should be taken into account (Swatland 1985). Kelly and Little (1966) showed that the oxyhemoglobin content of cod is very dependent on pH as a sharp increase in the oxyhemoglobin fraction occurs from about pH 6.6 and above. Thus, the intensity of two hemoglobin peaks at about 540 and 580 nm were showed to be dependent on post mortem pH. By comparison, our measurements were carried out immediately after killing in the pH range of 7.5 - 6.7 (Table 1), and at pH 6.15 after smoking. Taken together, if we had been able to detect light with shorter wavelengths than 570 nm, the timing post mortem as well as the prevailing pH range (oxyhemoglobin content) of the fresh fillets, would theoretically have made it possible to detect potential differences in blood contents. On the other hand, when considering the overlapping spectra (at higher wavelengths) of unbled and bled fish, it should be kept in mind that the blood contents of unbled and bled muscles did not in fact differ (Table 2). Another factor to consider might be that after slaughter, the fish muscle gradually becomes less translucent (Stien and others 2005). It may be that blood inside the muscle will be more easily detectable shortly after killing, since the light-absorbing and light-reflecting properties change as the muscle becomes opaque (Ozbay and others 2006).

Body cavity inspection

Presence of blood in the body cavity produced significantly ($p<0.05$) lower *L* values (lightness), as well as higher *a* (redness) and *b* (yellowness) values (Table 3). In addition, the G (green) channel of the RGB color space was also a signature feature since presence of blood contributed to lower G values ($p<0.05$). This is in accordance with data obtained from the detection of blood in cod fillets (Bengoetxea 1991).

We trained the LDA-based classification algorithm for the classification of salmon according to presence of blood in body cavities. The classification results by the LDA and cross-validation by leave-one-out methods are summarized in Table 4. The highest classification accuracy obtained was 92 % with the reported data set. Two out of 25 fish, one from each class, were misclassified. The misclassified samples were close to the decision boundary between the two classes, generated by the LDA classifier. Comparing the misclassified samples with the ground truth, the *'OK'* fish classified as *'Wash'* from our classifier in addition to having some small amount of blood, it also had a melanin fleck in the vicinity. We can not rule out the possibility that the melanin fleck has influenced misclassification. In the visible spectrum, blood spots and melanin flecks have similar appearance.

Table 3 – CIELab and green (G) values of the RGB color space for two classes of Atlantic salmon: (i) without, or (ii) with blood in the body cavity (inspection by computer vision after gutting and washing). Features a, b and G were used for classification purposes.

Feature	Cavity without blood (*'OK')*	Cavity with blood *('Wash')*
L	42.05 ± 0.77^{b}	36.3 ± 0.78^{a}
a	13.15 ± 0.97^{b}	24.6 ± 0.98^{a}
b	8.6 ± 0.69^{b}	18.0 ± 1.06^{a}
G	91.4 ± 2.4^{b}	68.3 ± 2.13^{a}

Mean ± SEM; fish with blood: n = 12, fish with no blood: n = 13. In each row, different letter (a,b) indicates significant difference between the classes.

We originally aimed at developing a classifier also able to detect occasional melanin spots in the body cavity. However, it was not straightforward to make a clear distinction between melanin flecks and residual blood. Therefore, we recommend that sorting of flesh with melanin flecks is carried out after the fish have been filleted and washed (Mathiassen and others 2007) although this strategy would of course not be feasible when the fish are to be traded as a 'whole and gutted' product. Melanin absorbs over a broad spectral range. The absorption smoothly drops from < 200 nm until it is almost completely attenuated for wavelengths > 700 nm. Thus, distinguishing between of blood and melanin might be difficult since their spectral ranges partly overlap. The redish color

of salmon fillets is due to the presence of the caroteinoids astaxanthin or canthastaxanthin. Carotenoids have generally a strong light absorption between 400-550 nm. In particular, different astaxanthin isomers typically have absorbance maxima between 372 and 479 nm (Yuan and Chen 2004), that is, at a considerably lower absorption range than we have studied here.

Table 4 – Classification performance for the classification of Atlantic salmon body cavities as evaluated by leave-one-out cross-validation method.

Data set	No of samples as labeled manually		LDA Cross-validation accuracy		
	Ok	*Wash*	*Ok*	*Wash*	TOTAL (%)
Atlantic salmon body cavity	13	12	12	11	92

Results of the classification with computer vision (LDA) as compared with the manual classification (labeling).

During the design of the classifier, one aim was to avoid using of too many features as a basis for the decision-making. Using too many features can cause overfitting (Duda and others 2001). Overfitting means that, for a given sample size, the increase of the number of features would virtually improve the performance accuracy when designing the classifier, but in practice, the classifier's performance accuracy would in fact become lower when dealing with newly unknown samples.

In the present study, we show that classification of the body cavity using a simple classifier and a subset of only 3 features was sufficient to obtain correct classifications. The results (Table 4) showed that there was a good agreement between classification done by human inspectors and by the computer vision algorithm. The method has the potential for usage in fish processing plants in connection with sorting and grading. However, to be able to replace quality inspectors, the current method must be combined with automated inspection of external features (Misimi and others, 2006) and detection of possible melanin flecks (Mathiassen and others 2007). In addition, a device for keeping the belly of gutted and washed fish open during image acquisition would be necessary.

Notably, since the blood contents inside the fillet seemed to be little influenced by most of the potential factors outlined in the Introduction part, this suggests that the determination of residual blood in salmonids can be narrowed down to a simple detection of blood present on the surface of belly cavity only.

Color assessment of fresh and smoked fillets

Fillet color (*L*, *a*, *b*, chroma and hue) values as assessed pre rigor and after smoking are shown in Table 5. Unbled and bled anesthetized and exhausted fillets were compared using mean Minolta Chroma Meter readings and the computer vision method. The tabulated values of the two methods cannot be directly compared since the Minolta readings represent the average of locations L1 - L3 and L5 (Figure 1), whereas the computer vision-based values refers to whole fillets (including the whitish peritoneum in Zone 3, Figure 1). The Minolta values at each location were largely similar except for at location L4 (data not included in Table 5) where the lightness (*L*) values were generally higher in the fresh fillets than after smoking. Moreover, at location L4, redness (*a*), yellowness (*b*) and chroma (C^*_{ab}) were generally lower in fresh than in smoked fillets.

After smoking, lightness, as measured with Minolta, increased (higher *L* values). Minolta generated values of redness (*a*), yellowness (*b*) and chroma (C^*_{ab}) were generally higher in fresh than in smoked fillets (Table 3). A tendency ($p<0.05$ for AUB, AB, and EUB) for a decrease in hue (H^o_{ab}) was observed after smoking. The effect can be attributed to the presence of the peritoneum in Zone 3 (including location L4). Accordingly, this must be kept in mind when the computer vision data are considered.

Except for the hue of fresh fillets, the computer vision color values were generally higher than those obtained with the Minolta Chroma Meter. With both methods, no significant effects of either perimortem handling stress or bleeding were observed, since the majority of color parameters were not significantly different.

Table 5 – Color characteristics of anesthetized and exhausted Atlantic salmon fillets. Comparison between fresh and smoked unbled and bled fillets. Minolta Chroma Meter data are presented as the average of fillet locations L1-3 and L5 (Figure 1) whereas computer vision data are based on assessments of whole fillets.

Parameter	Anesthetized		Exhausted	
	Unbled	Bled[1]	Unbled	Bled[1]
Lightness (L*)	Fresh		Fresh	
Minolta	38.7 ± 1.8^{X}	38.7 ± 0.8^{X}	37.2 ± 0.6^{X}	37.1 ± 0.9^{X}
Computer vision	42.8 ± 2.3^{a}	44.7 ± 0.8^{a}	41.1 ± 2.2^{a}	41.9 ± 1.9^{a}
	Smoked		Smoked	
Minolta	40.0 ± 1.1^{X}	40.3 ± 1.0^{Y}	41.4 ± 1.0^{Y}	41.4 ± 1.0^{Y}
Computer vision	34.6 ± 2.85^{b}	37.2 ± 2.7^{b}	35.9 ± 1.8^{b}	35.6 ± 2.0^{b}
Redness (a*)	Fresh		Fresh	
Minolta	15.5 ± 0.5^{X}	15.4 ± 0.4^{X}	14.6 ± 0.5^{X}	13.4 ± 0.3^{X}
Computer vision	37.7 ± 1.4^{a}	36.5 ± 1.3^{a}	36.2 ± 1.6^{a}	34.1 ± 0.9^{a}
	Smoked		Smoked	
Minolta	11.0 ± 0.5^{Y}	11.5 ± 0.4^{Y}	12.0 ± 0.4^{Y}	11.7 ± 0.3^{Y}
Computer vision	33.3 ± 1.6^{b}	34.7 ± 1.3^{b}	34.2 ± 0.9^{b}	33.8 ± 1.4^{a}
Yellowness (b*)	Fresh		Fresh	
Minolta	10.9 ± 0.7^{X}	11.7 ± 0.7^{X}	9.9 ± 0.6^{X}	7.9 ± 0.6^{X}
Computer vision	26.05 ± 1.1^{a}	25.75 ± 1.3^{a}	25.2 ± 1.4^{a}	23.5 ± 0.9^{a}
	Smoked		Smoked	
Minolta	6.6 ± 0.6^{Y}	6.9 ± 0.6^{Y}	7.2 ± 1.3^{Y}	7.0 ± 0.5^{Y}
Computer vision	25.9 ± 1.0^{a}	27.3 ± 1.2^{b}	26.6 ± 0.8^{a}	26.3 ± 1.2^{b}
Color saturation (C^*_{ab})	Fresh		Fresh	
Minolta	19.0 ± 0.8^{X}	19.4 ± 0.7^{X}	17.7 ± 0.7^{X}	15.6 ± 0.6^{X}
Computer vision	45.8 ± 1.7^{a}	44.7 ± 1.7^{a}	44.2 ± 2.1^{a}	41.4 ± 1.2^{a}
	Smoked		Smoked	
Minolta	12.9 ± 0.6^{Y}	13.5 ± 0.6^{Y}	14.2 ± 0.7^{Y}	13.7 ± 0.4^{Y}
Computer vision	42.2 ± 1.8^{b}	44.2 ± 1.7^{a}	43.3 ± 1.2^{a}	42.8 ± 1.8^{a}
Hue (H^{o}_{ab})	Fresh		Fresh	
Minolta	34.8 ± 3.5^{X}	36.9 ± 1.3^{X}	33.7 ± 1.6^{X}	30.0 ± 0.3^{X}
Computer vision	32.9 ± 1.1^{a}	33.6 ± 1.3^{a}	33.5 ± 1.6^{a}	33.3 ± 1.1^{a}
	Smoked		Smoked	
Minolta	30.4 ± 6.9^{Y}	30.1 ± 2.0^{Y}	30.2 ± 2.9^{Y}	30.9 ± 1.6^{X}
Computer vision	35.4 ± 4.2^{b}	36.3 ± 1.2^{b}	36.5 ± 0.8^{b}	36.01 ± 1.3^{b}

Mean ± SEM; Bled fish: n = 13, Unbled fish: n = 9. [1] *After blood drainage for 2 min in air, the fish were subsequently transferred to CSW (1 °C) for 20 min. Subscripts X,Y are reserved for the Minolta values, while a, b for computer vision values.*

When it comes to bleeding effect, no effect in the hue values in the anesthetized fresh fillets was observed, while in the smoked fillets the bleeding affects hue value. In the

exhausted fillets, bleeding didn't not influence color neither in fresh nor smoked fillets. Only a significant effect in the chroma of fresh fillets was observed. Perimortem handling stress didn't affect significantly the unbled groups of both fresh and smoked fillets. In the bled groups, hue values were not significantly affected neither in fresh or smoked fillets, only the chroma and lightness of fresh fillets was significantly different.

On the other hand, when comparing fresh and smoked fillets, it is clear that salting and smoking produced lower *a* and C^*_{ab} values as assessed with both methods. Computer vision generated *b* values showed a tendency of increase (more yellowish) and were significantly affected ($p<0.05$) by smoking in the bled groups. The hue (H^o_{ab}) values were also significantly affected by smoking ($p <0.05$). Smoked fillets showed higher hue values than their fresh counterparts, which is in contrast to Minolta hue values for the smoked fillets. Opposite effects of smoking on *L* values were observed when the two analytical methods are compared (Computer vision vs Minolta). This was probably due to the relative effect of darkening effect that smoking had on the peritoneum (Zone 3).

The general differences between the two methods CIELab values can partly be related to the fact that the Minolta readings were obtained in direct contact with the flesh, the measurements were done in small area of 8mm, whereas with the computer vision method, the entire fillet area was subjected to color analysis (Erikson and Misimi 2007, in press).

We did not find significant differences in hue, *a* or *b* values between unbled and bled fresh fillets. Only *L* and chroma C was affected significantly from the bleeding method. Presence of blood reduce yellowness in flesh and processed meats as have been observed as lower *b* and *L* values, as well as higher *a* values in unbled bullfrogs (*Rana catesbeiana*). However, the unbled frogs exhibited significantly higher hemoglobin levels than their bled counterparts (Ramos and others 2005). By comparison, in our study of blood on the surface of the body cavity, we obtained considerably lower *L* values as well as higher *a* and *b* values in presence of blood (Table 3).

Salting and smoking, rather than handling stress and bleeding, induced the main effect on fillet color in our study. Our Minolta Chroma Meter assessments of color showed that smoked fillets were lighter ($p<0.05$). Only the bled anesthetized group was not affected significantly when it comes to lightness. Similar effects of smoking on salmon fillet color have been reported by Skrede and Storebakken, (1986), and Rørå and others (1998) with the exception of lower *L* values, whereas Mørkøre and others (2001) found lower *L* and *a* values, but higher *b* values in smoked salmon fillets.

The computer vision generated color values for the smoked fillets show the opposite trend compared to Minolta values. While Minolta values suggest that fillets after smoking had lower *b* and hue values ($p<0.05$, more red), the computer color values show the opposite. The higher *b* and hue values after smoking show that, according to computer vision method, fillets became more yellowish. The latter is also supported by the spectrosopic measurements.

Visual assessment of smoked fillets

The smoked fillets generally had a good appearance with no large discolored areas. Only at Zone 1 (lateral line), a limited number of small (< 1 mm in diameter) and rather pale blood spots were visible on the fillet surface of all groups, with mean values < 3 and < 1 for each unbled and bled fish (two fillets), respectively (Figure 5). Thus, perimortem handling stress did not affect the number of blood spots in the fillets. Correspondingly, in sliced fillets the pattern was the same although the mean numbers of blood spots were somewhat higher at ≤ 10 and < 4. The lower numbers observed on the surface were probably due to the effect of washing just after the fillets were cut pre rigor. The blood spots, located along the lateral line, are actually vessels filled with blood as shown in detail in photographs by Robb and others (2003). In our case, these vessels were predominantly empty. Also, the sliced smoked fillets were generally considered of good quality, with only minor traces of residual blood elsewhere (Zone 2 and 4) on the fillets.

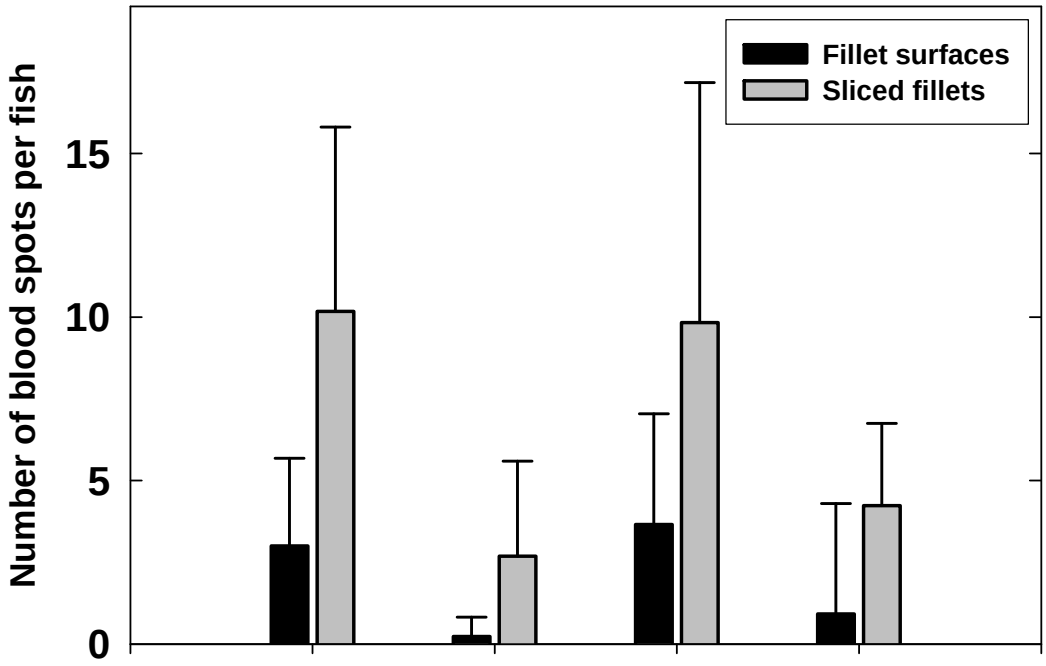

Figure 5- Blood spot counts on smoked salmon fillet surfaces and in the same fillets after horizontal slicing. The effects of perimortem stress and bleeding were studied (AUB: anesthetized unbled; AB: anesthetized bled; EUB: exhausted unbled; EB: exhausted bled). Mean + SD (bled fish: n = 13 [26 fillets], unbled fish: n = 6 [12 fillets]). The values represent the average number of small blood spots per fish (2 fillets) along the lateral line (Zone 1, Figure 1).

For Zone 2 and 4, the mean number of visible blood vessels was < 1 per fish for all treatments, with no particular pattern regarding size (small, medium or large vessels/spots). For Zone 3 though, the corresponding number ranged from 3 to 9 per fish. These were both small spots as well as elongated blood-filled vessels. The reason for the higher numbers in this zone was most likely that the peritoneum was not removed during filleting. If it had been removed, the subsequent washing of the fillet surface would probably have washed away this blood easily and the result would probably have been similar to those at Zone 2 and 4.

Similar to our findings, Robb and others (2003) did not observe bloodspots in both unbled and bled fresh fillets. However, large numbers of bloodspots were observed after frozen storage or salting in different regions of the fillets. In the salted fillets, the mean number of blood spots along the midline were 2.1 of bled and 12.9 of unbled fish. After slicing, the corresponding numbers were 8.2 and 21.5 spots per fish. In both cases their values were somewhat higher than ours. When they evaluated different bleeding methods, the mean number of bloodspots were however considerably higher ranging from 12.4 (four gills cut) to 34.7 (not bled) and 35.8 (throat cut). For fish bled immediately after killing, Roth and others (2005) also counted a mean blod spot number of about 1 per fish along the midline of smoked Atlantic salmon. This number increased significantly with an increasing delay between killing and bleeding. Similarly, the blod spot count of bled and smoked rainbow trout fillets was about 1. For unbled fish, number of blod spots ranged from 15 to 20.

Conclusions

Perimortem handling stress did not imply poor bleeding and more residual blood in prerigor-cut fillets. The slaughter procedure of unbled fish basically resembled what is referred to as 'the direct gutting method', that is, in commercial processing the fish are transferred to the gutting machines immediately after killing (without the bleeding step). This represents a quicker and less laborious alternative for blood removal. In accordance with other salmon data presented by Roth and others (2005) and Olsen and others (2006), our results also suggested that direct gutting was an adequate bleeding. It is important to realize that our fillets were cut pre rigor and that they were washed before the blood had time to coagulate. Had the fish been stored on ice and filleted post rigor, we cannot rule out that results might have been inferior to those presented here. On the other hand, Robb and others (2003) showed that when salmon were filleted immediately after slaughter, the fillets in their case had a substantial number of blood spots and the fillets were in fact not significantly different from unbled ones.

Another interpretation of our data might be that the bleeding method is not important as long as a prerigor filleting strategy is highlighted. Prerigor filleting might therefore prove

an effective remedy for inadequate bleeding routines and inferior smoked fillet quality. To confirm this, more research is needed where pre and postrigor filleting strategies are compared.

None of the analytical methods used to assess residual blood in white muscle could detect significant differences between groups. If we assume this reflected
the real situation, our data imply, at least for fish filleted pre rigor, that automated assessment of potentially poorly bled fish seems to be of little relevancy. Instead, the problem to be addressed for on-line purposes can then be simplified to merely detect residual blood on the surface of the flesh or in the body cavity. Our data suggest that the number of blod spots frequently encountered in processed fish can be reduced if fillets are cut pre rigor.

Acknowledgements

The present study was supported by the Research Council of Norway (*NFR project Nos. 145634/140 –'Efficient and economic sustainable fish processing industry',* and *173530/I30 'Technology for efficient and profitable fish industry').*

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APPENDIX

The paper is published in the Proceedings of the International Machine Vision and Image Processing Conference (IMVIP), 5-7 September, Maynooth, Ireland, pp. 192-197.

A Simple Computer Vision Method for Automatic Detection of Melanin Spots in Atlantic Salmon Fillets

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Abstract

In this paper, we describe a simple method for automatic detection of melanin spots in Atlantic salmon fillets. Melanin spots are visible dark spots that reduce the quality grade of the fillets. Atlantic salmon processing lines have several operations that involve manual quality evaluation of fillets. One such operation is the inspection of fillets to detect melanin spots. This inspection is labor intensive, and therefore desirable to automate. Two simple computer vision algorithms for melanin spot detection are presented. One algorithm operates on the red channel of RGB images and the second algorithm uses linear discriminant analysis (LDA) on all three RGB channels. A comparison between these two algorithms shows that, for most detection rates, using LDA gives a lower number of false-detections per fillet. We show that the melanin spot detection task can potentially be automated using computer vision.

1 Introduction

As a part of a long-term strategy, the Norwegian fish processing industry has set a goal of automating the fish processing industry [13]. This is necessary in order to be able to maintain competition, in the world market, with countries that have low manual labor costs. Several operations in Atlantic salmon processing lines involve quality evaluation of fillets and many of these operations are carried out by human inspectors using their sense of vision [9]. One such operation is the detection of melanin spots in fillets. The grading of 'good' fillets without melanin spots and of 'defective' fillets with melanin spots is done manually in today's processing plants. This incurres high labor costs and occasional mistakes in grading due to the repetitive nature of the work. Therefore, the automation of this grading operation is needed. Since the grading of fillets with melanin spots is done visually by inspectors, a natural approach for automation of this operation is to use computer vision.

Melanin spots are dark spots that reduce the quality grade of the fillet and are the result of an inflammatory condition most often induced by vaccination [4], [5]. Vaccination is necessary in order to protect fish in the growing phase from certain diseases. Nevertheless, melanin spots occur as a negative bi-effect of this vaccination.

Consumers associate any discoloration of fillets, in the form of melanin spots, with lower quality. Therefore, the presence of melanin spots in fillets reduces their quality and consequently the fillet price. Fillets with melanin spots are downgraded in the production line and such fillets are sent to portion cutting as they can not be sold as whole fillets.

Melanin spots are present in a substantial percentage of fillets. In typical Norwegian processing plants, 8-20% of all fillets have melanin spots, and this result in 4% of the entire production being discarded. It has also been reported [14] that the presence of melanin spots in the muscle tissue of Atlantic salmon can cause up to 30% loss in some processing plants as the part of the muscle containing melanin spot is discarded.

Computer vision has recently achieved the necessary level of maturity for being used in food and fish processing applications [2], [7], [9], [8], [11]. The advances in camera technology and the continuous increase in CPU speed over the past years have made computer vision a natural choice when designing automated quality evaluation applications.

A simple computer vision method for automatic detection of melanin spots in Atlantic salmon fillets is presented. We have compared two algorithms, one using the R-channel of RGB images and a second using the LDA-transformed RGB images. For most detection rates, using LDA gives a lower number of falsely detected melanin spots per fillet. The falsely detected melanin spots appear to be caused by two factors: 1. uneven illumination, 2. blood, viscera and fin remnants near the belly flap. The first factor can be reme-

0-7695-2887-2/07 $25.00 © 2007 IEEE
DOI 10.1109/IMVIP.2007.28

died by improved illumination, and the second by trimming the belly flap of the fillets before melanin spot inspection. This work shows that the melanin spot detection task can potentially be automated using computer vision.

2 Materials and Methods

Atlantic salmon fillets (n=37) were collected from the processing line of Salmar AS fish processing plant in Hitra, Norway, in April 2006. From the overall number of fillets, 16 fillets were 'good' and melanin-free, while 21 had melanin spots and were classified by human inspectors as 'defective'.

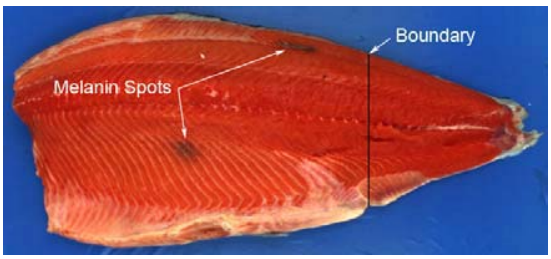

Figure 1. Atlantic salmon fillet with melanin spot defect. The melanin spot occurs due to vaccination of the salmon in its anterior part (left in this image). Melanin spots do not occur to the right of the indicated boundary.

Due to vaccination of the fish in a certain body region (close to the neck), melanin spots appear mainly on the front part of the fillet, from the dorsal fin towards the head. The region where melanin spots appear is usually in the anterior part of fillet, to the left of the boundary in figure 1.

2.1 Image Acquisition

Images of fillets were captured using a digital color camera (figure 2). In order to ensure a uniform illumination, without intrusion from ambient light, the fillets were put in a light box. The light box had a gray neutral color inside and two fluorescent tubes (18W) with a color temperature of 5000K and color rendering index greater than 95%. The exposure time of the camera was long enough to avoid the variation in light intensity due to the mains frequency. For a good color discrimination, a color rendering index greater than 90 and color temperature of 5000K is preferred [12]. The built-in flat-field correction of the camera (PixeLink A776) was used to compensate for uneven illumination.

In order to accurately represent the color of the fillets, a color calibration of the images was performed. This was done using a Macbeth ColorChecker with 24 patches.

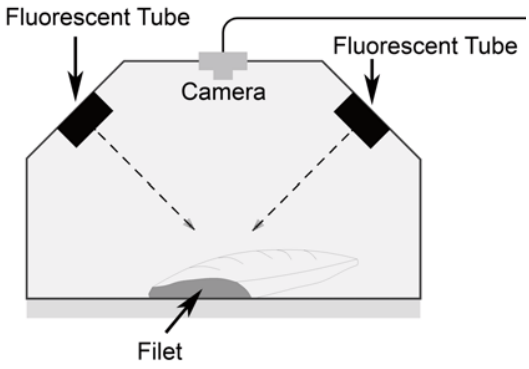

Figure 2. A cross-section view of the light box that was used for image acquisition.

2.2 Feature Extraction

Firstly, the ground truth set of images was generated by inspecting the images from the dataset. For this purpose, all images were manually segmented into regions belonging to three categories: background, fillet and melanin spots. Segmentation of the melanin spots in the ground truth set of images was done such that the boundaries between fillet and melanin spots were estimated as precisely as it was possible by manual inspection. Nevertheless, the boundary marking should be regarded as approximate, since we were more interested in region spot detection than in accurately locating the boundary between the melanin spot and the fillet muscle.

By separating the fillet RGB image into R, G and B channels, it was apparent that the single channel that best enhances the melanin spots is the R channel (figure 3). Melanin spots were only barely visible in the G or B channel. Using one color channel is required if using a monochrome camera, and thus we investigated using the R-channel as a feature to determine whether it is sufficient to use a monochrome camera in an automatic melanin spot detector.

In addition to using the R-channel, we investigated applying, to the RGB images, the optimal linear transform that separates melanin spots from spotless areas of the fillet. Linear discriminant analysis (LDA) searches for those vectors in the underlying space that best discriminate among classes. It is generally believed that LDA outperforms PCA [1], [6], [10], because while PCA seeks directions that are efficient for data representation, LDA seeks directions that are efficient for discrimination [3].

In the two class case, with an assumed equal a priori probability of each class occurring, the linear transform that

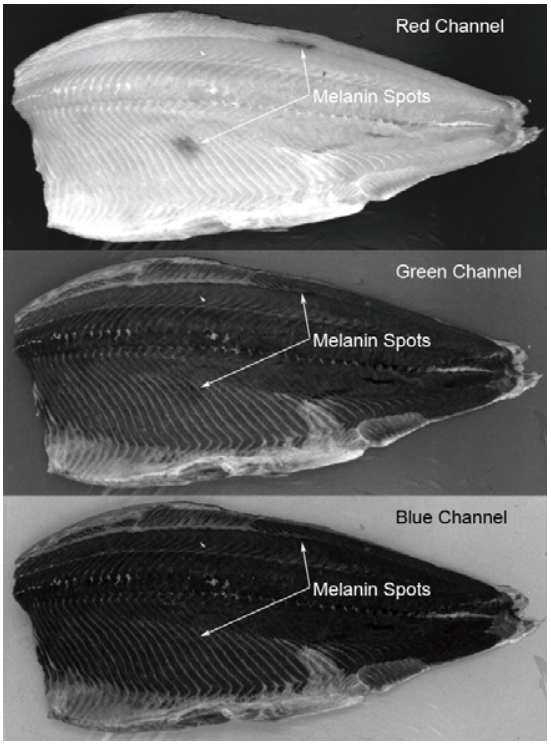

Figure 3. The red, green and blue channels of the color image. Melanin spots are most visible in the red channel.

optimally separates two classes is

$$y = \mathbf{w}^{\mathrm{T}}\mathbf{x}, \tag{1}$$

where

$$\mathbf{w} = (\boldsymbol{\Sigma}_1 + \boldsymbol{\Sigma}_2)^{-1} (\boldsymbol{\mu}_1 - \boldsymbol{\mu}_2)^{\mathrm{T}},$$

and where $\boldsymbol{\Sigma}_1$ and $\boldsymbol{\Sigma}_2$ are the class covariance matrices and $\boldsymbol{\mu}_1$ and $\boldsymbol{\mu}_2$ the class mean vectors.

2.3 Melanin Spot Detector

We developed two melanin spot detectors. They differ only in the feature images used. The two feature images are:

1. The R-channel
2. The two-class LDA-transformed RGB

Both feature images have only one channel, and the same melanin spot detection algorithm was applied to both feature images.

The algorithm is illustrated in figure 4. The first step of the algorithm was to threshold the feature image with a threshold y_0. In section 3 we describe how the threshold is varied in order to evaluate the detector performance of a large range of detection rates and number of falsely detected melanin spots per fillet.

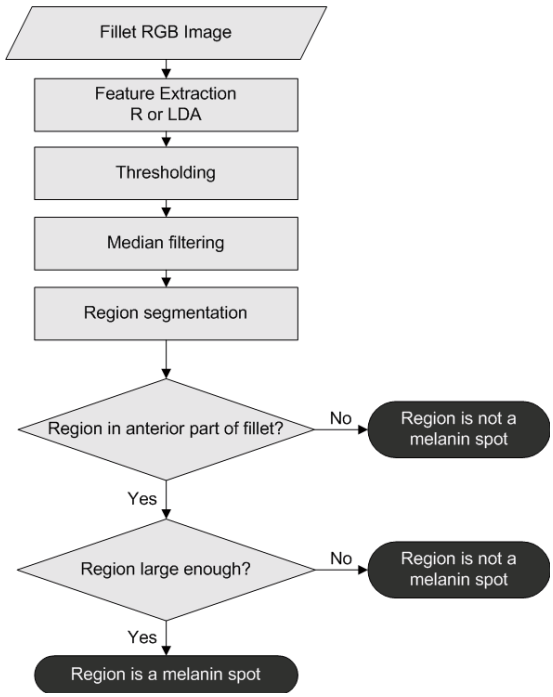

Figure 4. Flow chart describing the different stages in the melanin spot detector.

After thresholding, the binary image was filtered with a median filter that has a radius equivalent to 3 mm on the fillet. This median filter removes noise and merges smaller regions to simplify the subsequent detection of melanin spots. Next, the median filtered images were segmented into connected regions. Regions in the 25% posterior part (tail) were removed for two reasons: 1. Melanin spots do not occur in the tail part, 2. the tail contains brown fat that is similar to melanin in color, which might confuse the detector. In addition to this, we removed regions with an area less than that of a disk with radius 5 mm. Any remaining regions after this processing were classified as being melanin spots.

3 Results and Discussion

We compared the performance of the two detectors presented in this paper. For each detector we varied the threshold and computed the melanin spot detection rate and the average number of falsely detected melanin spots per fillet, which can be seen in figure 5. The detection rate was

computed by applying the detector to the 21 images of 'defective' fillets, and the number of false detections per fillet was computed by applying the detector to the 16 images of 'good' fillets.

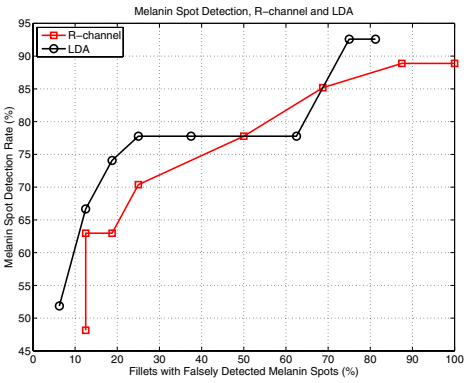

Figure 5. Percentage of melanin spots that are correctly detected, versus percentage of non-defective fillets with falsely detected melanin spots.

For most detection rates, using LDA gives a lower number of falsely detected melanin spots per fillet. At a detection rate of 93%, using the LDA features, the correctly detected melanin spots and falsely detected melanin spots are illustrated in figure 6 and figure 7, respectively. The algorithm detects most melanin spots, and clearly locates them. Spots that are not detected, such as the spot in the upper left corner of Fig. 6, are small and weak. At a detection rate of 93%, there are a large number of falsely detected melanin spots. Such falsely detected spots are found in approximately 75% of the spotless fillets. This high number seems at first disconcerting. However, the falsely detected melanin spots appear to be caused by two major factors: 1. uneven illumination, 2. blood, viscera and fin remnants near the belly flap. The first factor can be remedied by improved illumination, and the second by trimming the belly flap of the fillets before melanin spot inspection. We suggest that these two factors be addressed in future work.

Apparently, judging from the images in figure 1 and figure 3, detecting melanin spots is a trivial and simple problem. Studying a larger number of melanin spots, we see that they have varying size, shape, location and intensity. This can be seen in figure 6. This variation makes the detection of these spots a challenging problem to solve. Further challenges are posed by the need for even illumination and removal of objects that have a similar appearance to melanin spots, such as seen in figure 7.

The fact that we have chosen simplistic detectors could

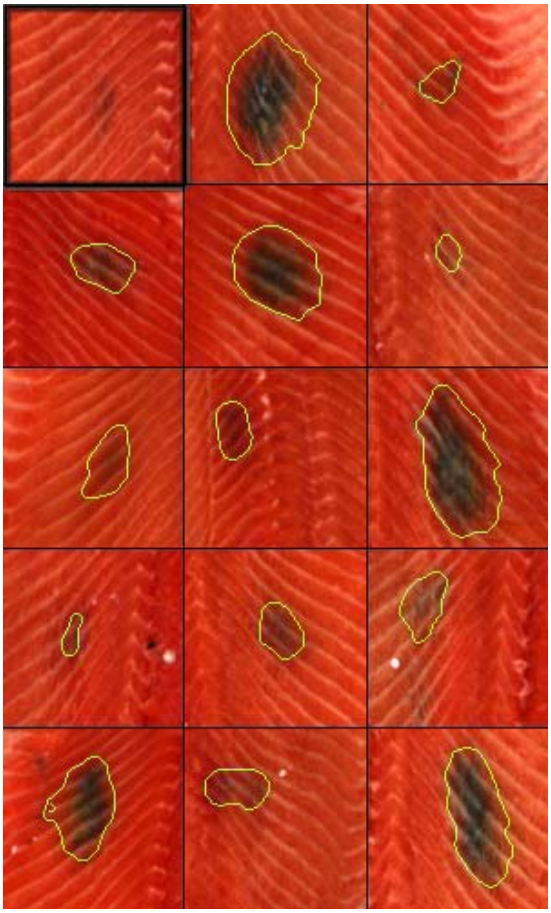

Figure 6. Detected melanin spots using the LDA features, at a detection rate of 93%. The upper left image shows an example of a melanin spot that was not detected.

be criticized. On the other hand, simple detectors are more robust with respect to overtraining. Overtraining can be a problem when designing complex classifiers or detectors and evaluating their performance on small data sets. In our case, the data set consisted of 21 images of 'defective' fillets and 16 images of 'good' fillets. Ideally, the data set should have been much larger in order to improve the detector evaluation. With such a small data set, it was important to properly utilize the images during detector development and evaluation. While developing the detectors we tested them on two images, one of a 'good' fillet and one of a 'defective' fillet. These two images were also included in the data set used for evaluating the detectors. It is possible that better detectors could have been developed if we had repeatedly tested and modified them on the entire data set.

Due to the small data set used while developing the de-

tectors, we consider their performance to be a lower bound on the melanin spot detection performance that is possible to achieve using computer vision. We therefore consider it important to continue refinement and development of this computer vision application.

It is important to observe that a melanin spot detector does not need near-perfect detection rates and false-alarm rates, in order to be useful in the salmon processing plants. At high detection rates, where almost many melanin spots are detected and all large spots are detected, even a false-alarm rate of 25% will be useful. At such a false-alarm rate, the number of fillets that need to be inspected by humans is reduced to one fourth of all fillets.

Judging from the results in this paper, we believe that computer vision has the potential to enable fully automated melanin spot detection in Atlantic salmon processing plants.

4 Conclusion

An important inspection task in Atlantic salmon processing plants is the detection of melanin spots in fillets. Ths is done manually today and is labor intensive and thus costly. It is therefore desirable to automate this inspection. Towards this end, we have presented two computer vision based melanin spot detectors. The performance of these detectors indicate that a computer vision system has the potential to automate the detection of melanin spots in Atlantic salmon fillets.

5 Acknowledgements

The project was funded by the Research Council of Norway (NFR project No. 145634/140-'Efficient and economic sustainable fish processing industry').

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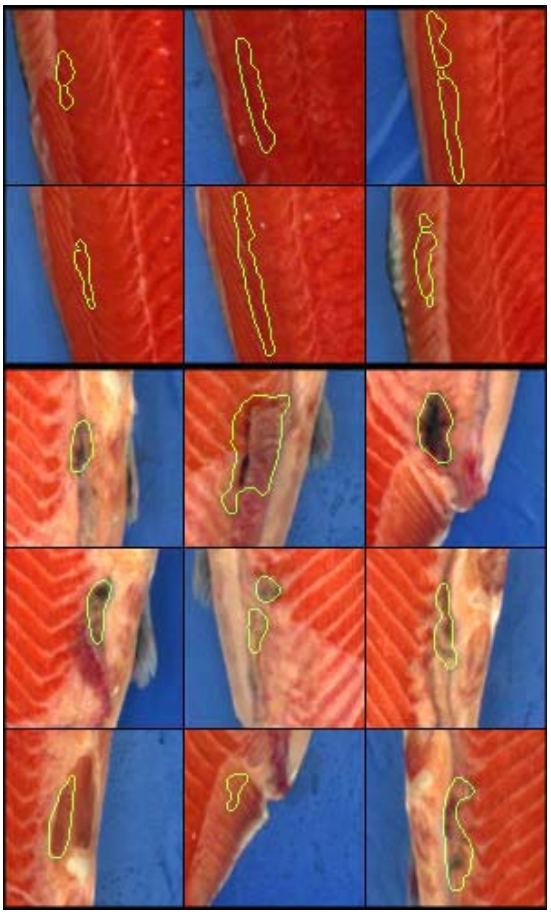

Figure 7. Falsely detected melanin spots using the LDA features, at a detection rate of 93%. The top two rows illustrate false detections due to uneven illumination, and the bottom three rows illustrate false detections due to blood, viscera and fin remnants on the belly flap.