Journal of the Association of Public Analysts (Online) 2011 **39** 44-78
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Nitrogen Factors for Atlantic Salmon, *Salmo salar*, farmed in Scotland and in Norway and for the derived ingredient, “Salmon Frame Mince”, in Fish Products.

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Summary

Pre-packed named fish products are required to be labelled with a declaration of the amount of named fish present as a percentage of the final weight of the product, (quantitative ingredient declaration, QUID). The basis of independent analytical assessment of QUID claims for fish is comparison of the fish-specific nitrogen content of a particular food sample with the known species nitrogen content, the “nitrogen factor”. The only extant species nitrogen content datum for Atlantic salmon, Salmo salar, is that reported in 1973 and it has been claimed that this nitrogen factor is not representative of modern farmed Atlantic salmon. In addition, there is no published nitrogen factor for the commercial ingredient derived by machine recovery in minced form of the residual salmon flesh remaining on the salmon “frames" after removal of the fillets. Pursuant to his statutory duties, the Government Chemist instigated at the request of both trade and enforcement interests, a review of the appropriate nitrogen factors for farmed Atlantic salmon and salmon frame mince. We present data confirming that the 1973 nitrogen factor is not appropriate for farmed Atlantic salmon and suggest that the estimation of salmon content should be based on nitrogen factors expressed on a fat-free basis. Dumas nitrogen factors on a fat-free basis for various cuts of Norwegian and Scottish farmed salmon are given (Table 7) and a general Dumas factor, on a fat-free basis of 3.80 (3.75 Kjeldahl) is suggested. For salmon frame mince a general factor, on a fat-free basis, of 2.85 (2.81 Kjeldahl) is suggested.

Introduction

UK and European food law underpins the concept of informed consumer choice in the purchase of food[1,2,3]. The European Food Labelling Directive[4] includes the requirement for a Quantitative Ingredients Declaration (QUID) which means that most pre-packed fish products are required to be labelled with a declaration of the amount of fish present as the percentage of the final weight of the product. If a specific named fish is in the name of the food, then the percentage of the named fish in the product has to be declared. The basis of independent analytical assessment of QUID claims for fish (and meat) is comparison of the fish-specific-nitrogen content of a particular food with the known species nitrogen content. For readers unfamiliar with the calculation an outline is shown in Appendix 1; standard works give a fuller treatment[5,6]. Typically, the food nitrogen is determined using a “standard method” whose performance characteristics are well documented. However, difficulties can arise in the absence of an appropriately validated database against which interpretation of the sample data can be made. When interpretations have to be able to withstand challenges in a court of law, it is essential that the reference databases have proven validity. The difficulties of enforcement officers who are charged with verifying product label declarations have recently been discussed by Hargin[7].

The Kjeldahl process was introduced in 1883[8] for the determination of nitrogen in food and since then has been used as a marker to measure the protein content of food samples. The method is well characterised[9,10,11] and accepted for legislative purposes[12]. Since the introduction of automated tube combustion elemental analysers[13] the Dumas method is often used as an alternative to the Kjeldahl process. However, the two methods are not quite equivalent when used for the estimation of protein in a food sample. On average, by examination of results of proficiency test data on cereals, milk powder, canned meat and canned fish or paste, the Dumas method was found to provide results that are relatively higher than that of the Kjeldahl method by about 1.4% of the mean Kjeldahl nitrogen, i.e. by a factor of 1.014[14]. The generally accepted explanation is that non-protein forms of nitrogen are converted into elemental nitrogen in the Dumas method.

Validated databases for nitrogen factors for meat, poultry or fish are, by definition, those published in peer reviewed journals. A recent major review of the literature has confirmed that the only extant data is that produced in association with the Association of Public Analysts (formerly the Society of Public Analysts) or by the Analytical Methods Committee (AMC) of the Analytical Division of the Royal Society of Chemistry (formerly the Society for Analytical Chemistry)[15]. The review identified extensive databases for meat and poultry however the species variation of nitrogen factors for fish has been less studied, due to difficulties in obtaining sufficient fish on which to base a recommendation[16]. Data from the literature and from unpublished sources for a wide range of fish collected by the Fish Products Sub Committee of AMC were provided to assist analysts who were required to examine fish products[16] but given with the clear statement that the absolute reliability of the data could not be attested. It should be noted that the current factor for Atlantic salmon, 3.60, is that given in the non-attestable data set in 1973. Validated Kjeldahl nitrogen factor data is available for cod (*Gadus morhua*)[17], cod ingredient[18], coal fish (saithe; coley; *Pollachius virens*)[19], seven British commercial white fish[20] and for scampi (*Nephrops norvegicus*)[21] but no work later than 1973 on Atlantic salmon has been published.

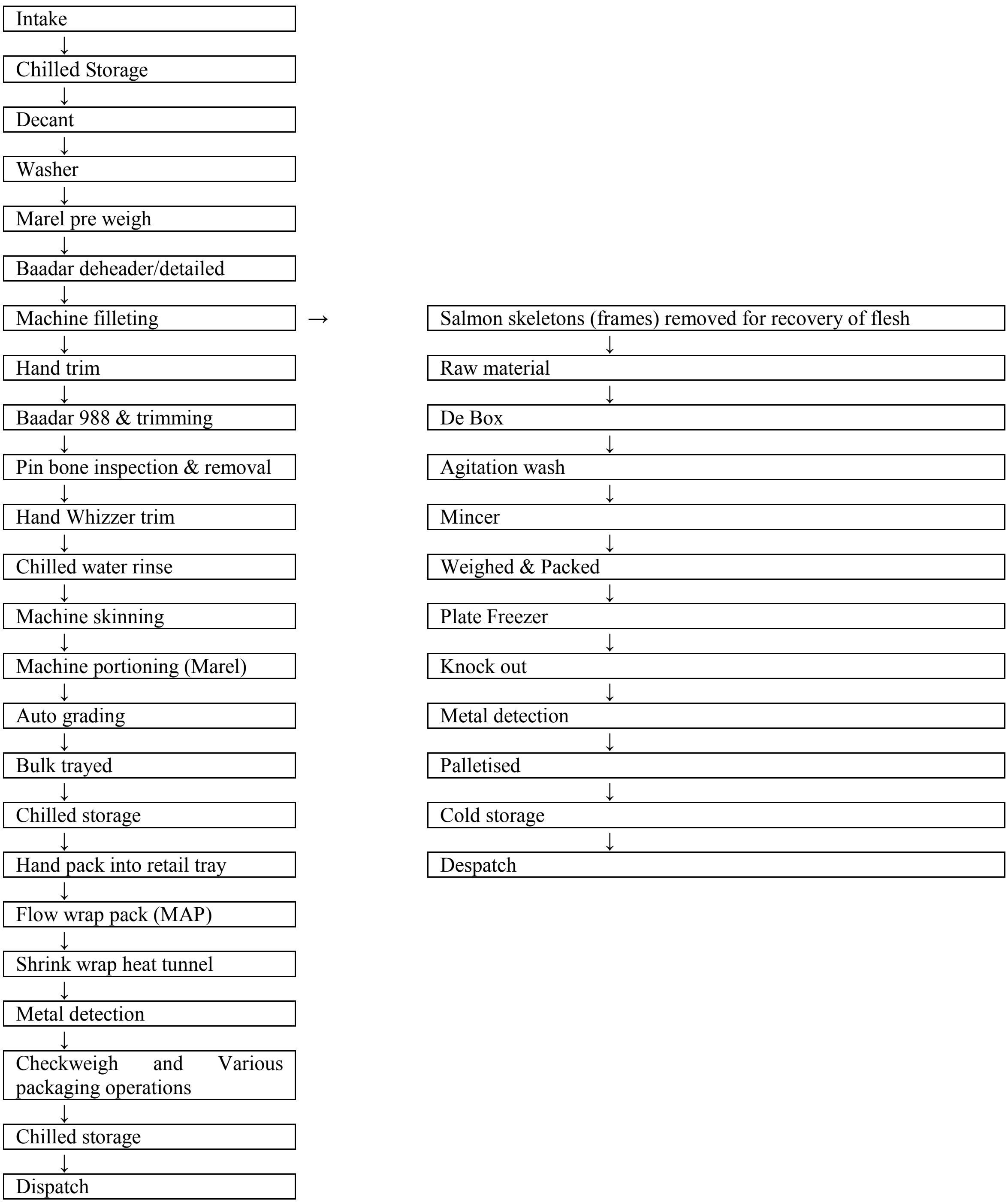
In 1998 a Code of Practice[22] was drawn up in the United Kingdom, in agreement with enforcement, analytical and industrial representatives that outlined good manufacturing practices with respect to water uptake and soluble nitrogen losses during fish processing. Interim nitrogen factors were agreed for the main commercial species of fish, but excluding that for Atlantic salmon, either farmed or wild.

The Atlantic salmon (*Salmo salar*), the only native salmon species in the Atlantic Ocean, exhibits a temporal variation in its proximate composition, a function of its complex life cycle. The life cycle and processing of Salmo salar is described in detail in Appendix 2. The overwhelming majority of Atlantic salmon consumed in the UK is farmed, mainly in Scotland and Norway. Processing of the harvested salmon is now largely mechanical; Figure 1 summarises the process. There is an appreciable amount of salmon flesh remaining on the "frames" after mechanical filleting. This flesh can also be recovered by machine after washing of the frames. We propose to use the term "salmon frame mince" for such product.

In the UK the Government Chemist is required by statute to act as the national focus of independent technical appeal in specified areas where there is an actual or potential dispute between food businesses and a regulator. The specified areas are broadly drafted but in practice tend to focus on the results of chemical analysis or their interpretation in the agrifood sector[23].

It was represented to the Government Chemist by both trade and enforcement sources that a robust modern dataset representative of modern farmed Atlantic salmon was required as the 1973 nitrogen factor of 3.60 (based on wild salmon) was suspected of leading to apparently artificially low calculated fish contents. In addition there was no published basis for the use of a nitrogen factor of 3.60 for salmon frame mince. Hence the present study, to provide a validated database of variations of nitrogen factors for farmed Atlantic salmon and salmon frame mince from a range of locations and sampled at different times of the year. For convenience and efficiency the nitrogen determinations were made using an automated Dumas system from which the equivalent Kjeldahl nitrogen factors can, if required, be calculated.

Figure 1 – Schematic Diagram for the Factory Process for Fillets and Salmon Frame Mince



Experimental

General Sampling Procedure

The study benefited from cooperation from two large companies in the food industry, a Local Authority Trading Standards Department, and their Public Analyst, with experience of fish composition and labelling issues, and an independent consultant with experience of AMC and *Codex Alimentarius* approaches. The independent consultant provided assurance that the practices observed were industry norms.

Field work commenced in 2006 with a full scale inspection of a large plant processing harvested farmed Atlantic salmon. Sampling of farmed Atlantic salmon harvested in Scotland and in Norway and transported to the processor on ice was carried out (then and subsequently) by a senior Trading Standards Officer who ensured a forensic chain of custody of the samples taken. The sampling plan took into account relevant variables such as season (although time of year sampling took place was the only viable proxy for season), size of fish, country of origin and product (whole fish[*], fillets, mince); sampling followed a full factorial design including four seasons, three fish sizes, two countries of origin (for whole fish and fillet - country of origin was assumed mixed for frame mince), and the three product types, with equal numbers of replicate samples per sub-group.

During factory visits samples of factory produced fillets were taken skin-on after pin bone removal and before cutting into portions. Further separate neck portions, middle portions and tail portions of skin-on fillet were also taken, together with plate frozen 7.5 kg blocks of salmon frame mince. Figure 2 shows the general sampling plan used on each sampling occasion; there were four such occasions, in December 2006 and in March, June and September 2007. It is not uncommon for fish product manufacturers to treat fish with a polyphosphate solution, though no polyphosphate use was observed on any of the sampling occasions in this study. An additional sample of fillet was taken on the first sampling occasion, homogenised and frozen for use as a quality control material throughout the entire course of the work.

The samples were frozen at the factory for at least 24 hours before dispatching to LGC by courier. Samples were stored frozen at LGC until ready for preparation. Whole fish, after thawing, were filleted by hand to replicate an industry norm “trim level D” (see Figure 3 – reproduced courtesy of Young’s Bluecrest Seafood Ltd).

- Skin was removed from fillets (removed whilst partially frozen and trimmed by knife)
- Skinless fillets were homogenised
- Salmon frame mince blocks were cut whilst frozen (but after softening for 1 hour) into six aliquots
- Salmon flesh was “spooned” from the salmon frames and homogenised
- Wash water (sampled in December 2006) from the bath where the salmon frames were washed prior to processing [automated removal of salmon frame mince] was analysed for nitrogen both before and after passing through 0.45 micron filters

Replicate aliquots of the materials prepared were placed in plastic tubes and stored frozen until ready for analysis.

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[*] Salmon, gutted and with heads removed were obtained during factory visits for removal of fillets manually in

Figure 2 General Sampling Plan

Norwegian			**Scottish**		
3 Individual SMALL (2-3 kg) WHOLE FISH			3 Individual SMALL (2-3 kg) WHOLE FISH		
3 Individual MEDIUM (3-4 kg) WHOLE FISH			3 Individual MEDIUM (3-4 kg) WHOLE FISH		
3 Individual LARGE (4-5 kg) WHOLE FISH			3 Individual LARGE (4-5 kg) WHOLE FISH		
3 Individual FILLETS from SMALL fish			3 Individual FILLETS from SMALL fish		
3 Individual FILLETS from MEDIUM fish			3 Individual FILLETS from MEDIUM fish		
3 Individual FILLETS from LARGE fish			3 Individual FILLETS from LARGE fish		
1kg NECK Fillet Cuts from SMALL fish	1kg NECK Fillet Cuts from MEDIUM fish	1kg NECK Fillet Cuts from LARGE fish	1kg NECK Fillet Cuts from SMALL fish	1kg NECK Fillet Cuts from MEDIUM fish	1kg NECK Fillet Cuts from LARGE fish
1kg MIDDLE Fillet Cuts from SMALL fish	1kg MIDDLE Fillet Cuts from MEDIUM fish	1kg MIDDLE Fillet Cuts from LARGE fish	1kg MIDDLE Fillet Cuts from SMALL fish	1kg MIDDLE Fillet Cuts from MEDIUM fish	1kg MIDDLE Fillet Cuts from LARGE fish
1kg TAIL Fillet Cuts from SMALL fish	1kg TAIL Fillet Cuts from MEDIUM fish	1kg TAIL Fillet Cuts from LARGE fish	1kg TAIL Fillet Cuts from SMALL fish	1kg TAIL Fillet Cuts from MEDIUM fish	1kg TAIL Fillet Cuts from LARGE fish
9 x 1 kg Frame Mince Samples – fresh from machine - Regardless of the country of origin					
3 x 7.5 kg Frozen Frame Mince Block Samples (each from 3 different production batches) - Regardless of the country of origin					

Figure 3 Trimming of Fillet

Backbone off, Ribcage removed, Collar bone off, All fins removed, Tail tip squared, Belly fat removed, Pin bones removed

Analytical Methods

All analytical methods employed were third party accredited to ISO/IEC 17025 by UKAS[24] and based on BSI methods.

Moisture – Samples were oven dried at 103°C ± 2°C for 2 hours and then to constant weight[25].

Ash, (Mineral Matter) – Samples were ashed at 550°C ± 50°C for 8 hours and then reweighed[26].

Nitrogen – Nitrogen was determined by Dumas combustion using a LECO 2000 CNS analyser. In-house method FFF/B1-0010. The nitrogen was converted to protein using the factor 6.25.

Fat – Fat was determined using a standard acid hydrolysis and extraction method[27].

Analytical Quality Assurance – The certified reference material (CRM) LGM 7000, pork and beef (no fish based CRM was available) was analysed in duplicate in each analytical run (ash 122 results, fat 114 results, moisture 80 results, nitrogen 79 results). All replicates (study, CRM and drift samples) were assessed for repeatability against preset criteria and found to be acceptable except where noted in the statistical commentary below. The drift sample results for nitrogen and fat were reasonably normally distributed and showed no between-day variation that cast doubt on the validity of the study.

Allocation of Test Items to Analytical Runs

Nitrogen content determination used a randomised block design for each separate sampling occasion; every combination of Product type, Country and Size appeared the same number of times in each run and the run order was randomised for each run. Two test samples per frozen block of frame mince were assigned randomly to each run. This results in a total of 18 test samples run in duplicate in each of 3 runs. The individual whole fish or fillet test portions were randomly assigned to runs from each group of 3 samples per process/size/country combination. Each run additionally contained two QC samples, run in duplicate, and one CRM observation, also in random order.

For fat, available run length was up to 18 observations (including duplication and QC). Fat determinations were accordingly allocated among 9 runs of 6 samples each. Allocation to runs used stratified random sampling from each nitrogen run with Product type as strata, so that two samples each of Whole, Fillet and Mince material appeared in duplicate per run. One QC (in duplicate) and one CRM were included in each run, and run order, including QC materials, randomised separately for each run.

Moisture determination equipment allowed for approximately 20 observations (including duplication). Moisture determination was accordingly run as a randomised block design with 18 observations per run and 6 runs (two moisture runs for each set of nitrogen samples). Run order was randomised separately for each run and two QC samples and 1 CRM were included in each run.

For ash, the available equipment allowed up to 12 determinations per run, including duplication and QC observations. Duplicate observations for ash determination were therefore randomly assigned to runs from within the three nitrogen sets, with all duplicates randomly distributed across runs, giving a total of four ash runs per nitrogen run. One duplicated QC sample and one CRM observation were included per run to provide precision and bias checks, and the run order randomised for each run.

Data Handling and Preliminary Inspection

For each sampling occasion, laboratory data (including sample identifiers, full description, run identifiers and associated analytical results) were collated and appropriate fields added to each set to identify the sampling season (“Season”), Product type, Size, Country of origin and Production batch (from the description) with automated error checking to confirm absence of anomalous/non-matching descriptions. Field names and level identifiers used for each factor were as shown in Table 1.

Data were expressed to four decimal places and saved in text format for import to the statistical software used. Prior to statistical assessment, data were inspected for anomalies using scatter plots and box plots. Nitrogen data (figure 4) showed one marked outlier; (1.75% against typical data of about 2.2-3.5% for the remainder of the observations). Inspection showed this to be one observation from a pair with unacceptable dispersion. The point was marked as an outlier and removed in subsequent treatment. Some observations for fat and moisture (Figures 5 and 6 respectively) appeared to be modest outliers within a particular group (for example, for a particular product type) but since none appeared disproportionate for the data set as a whole and no other technical reason was noted, the data were retained except where otherwise noted below. Ash showed comparatively frequent low outliers (Figure 7). The majority of these, however, were confirmed by their associated duplicate observation, indicating material variation rather than analytical procedure variation as causes. The outlying data for ash were accordingly retained.

For fat-free nitrogen assessment, fat-free nitrogen was calculated from the mean fat and nitrogen content for each test sample after outlier treatment as indicated above. Analytical run information was not retained for fat-free nitrogen assessment as sub-samples for nitrogen crossed runs (that is, replicates were allocated to different runs) and there was no simple relationship between fat and nitrogen runs for different sub-samples.

Table 1 Statistical Field Names and Level Identifiers

Feature	Field Name	Values
Product type	Cut	Fillet, Whole or Mince
Size	Size	Small, Medium or Large for fillet or whole fish; not applicable for Mince
Country of Origin	Country	Scottish or Norwegian for fillet or whole fish, not applicable for mince
Production Batch	ProdBatch	Batch 1-3 for mince, (considered nested within Season).

Software

All statistical analysis was performed in R version 2.10.1[28]. Mixed effects modelling used the lme4 package, version 0.999375-35[29].

Results

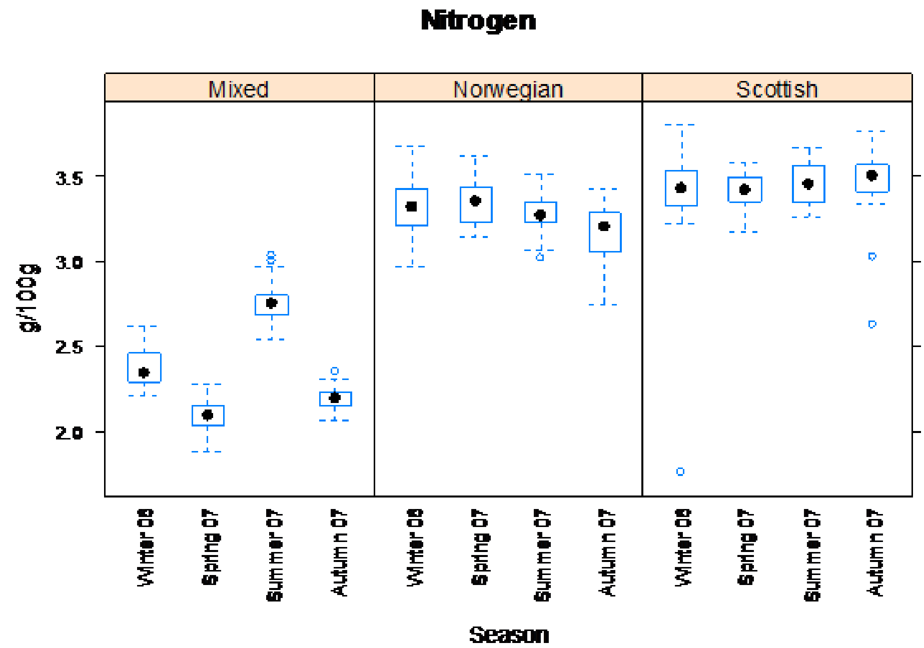
Overview

Collated results of analyses for nitrogen, fat, moisture and ash are given in full in Appendix 3 and are presented graphically in Figures 4 to 8. Complete raw data, in machine-readable format, are also available as a supplementary data file.

In reviewing the results, it is important to note that "season" identifies the season in which sampling took place, but only one sampling event occurred in each season. It follows that while this report refers to season as a grouping factor, it is not possible from this study data alone to distinguish true seasonal variation in composition from other variation in content between different sampling times.

The pairs plot (Figure 8) shows appreciable structure in the data set as a whole. Whole fish and fillet generally form overlapping contiguous groups. There is strong visible evidence of inverse correlation between fat and moisture for the whole fish and fillet, and a suggestion of inverse correlation between fat and ash and between fat and nitrogen. This is not unexpected as the proximates are expected to sum to approximately 100% for typical fish flesh. For the salmon frame mince the pairs plots show clear subsets attributable to variation by season. These variations are also clearly visible in the box plots (Figure 4 to Figure 7).

Figure 4 – Nitrogen by Country and Season



- Note: In Figures 4-7 "Mixed" denotes salmon frame mince as no country of origin information was available.

Figure 5 – Fat by Country and Season

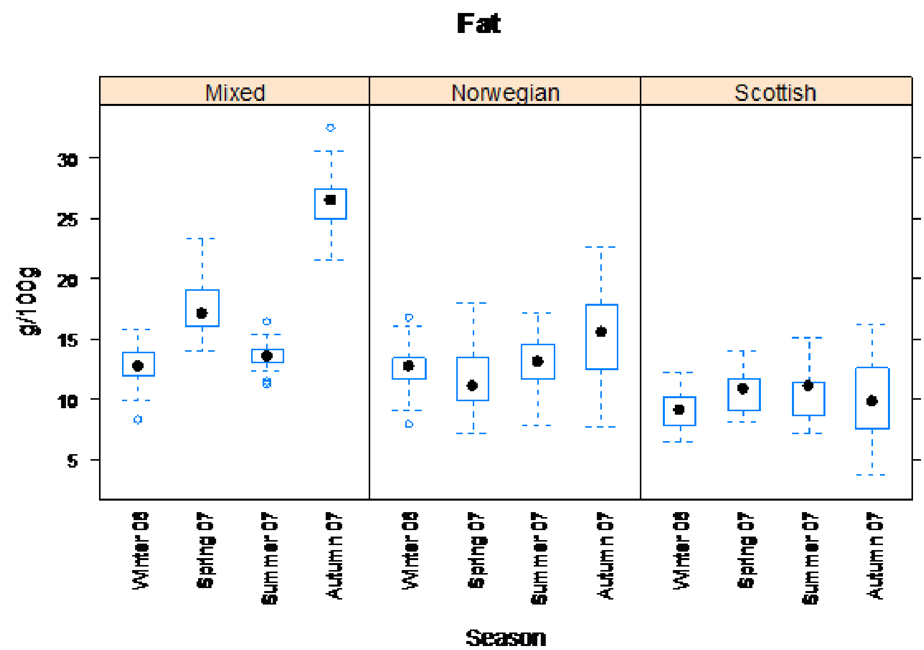


Figure 6 – Moisture by Country and Season

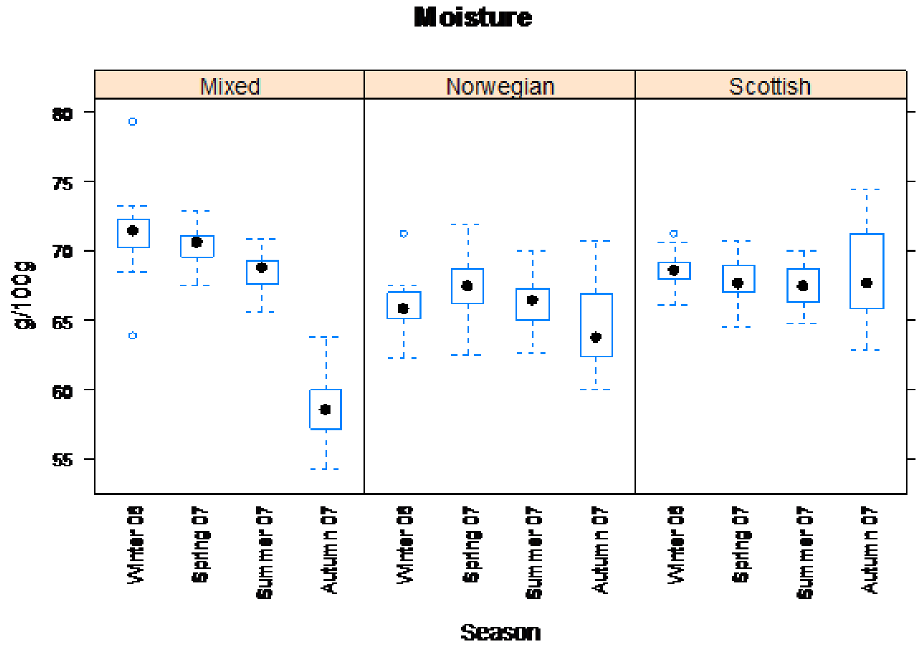


Figure 7 – Ash by Country and Season

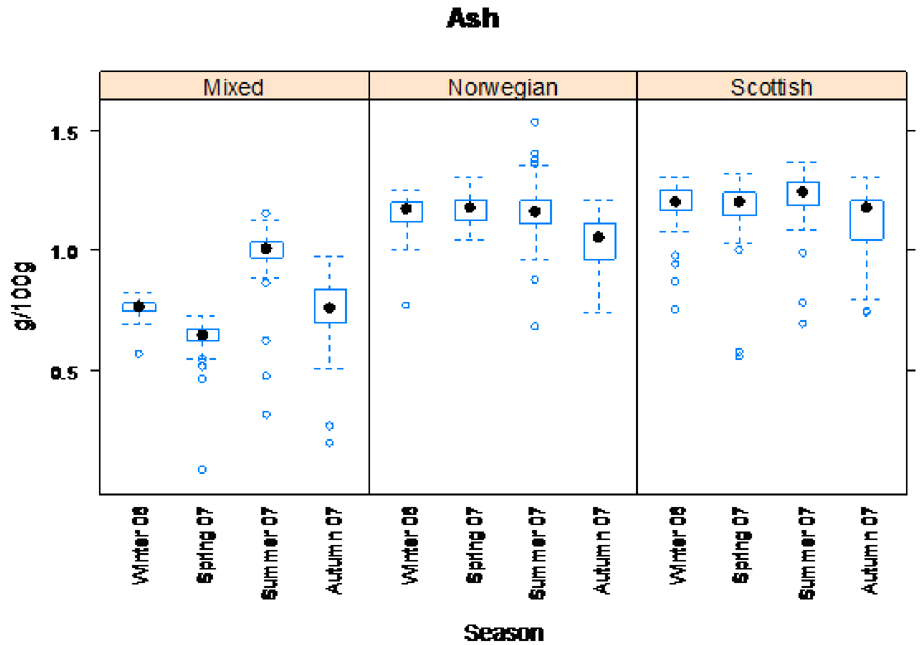
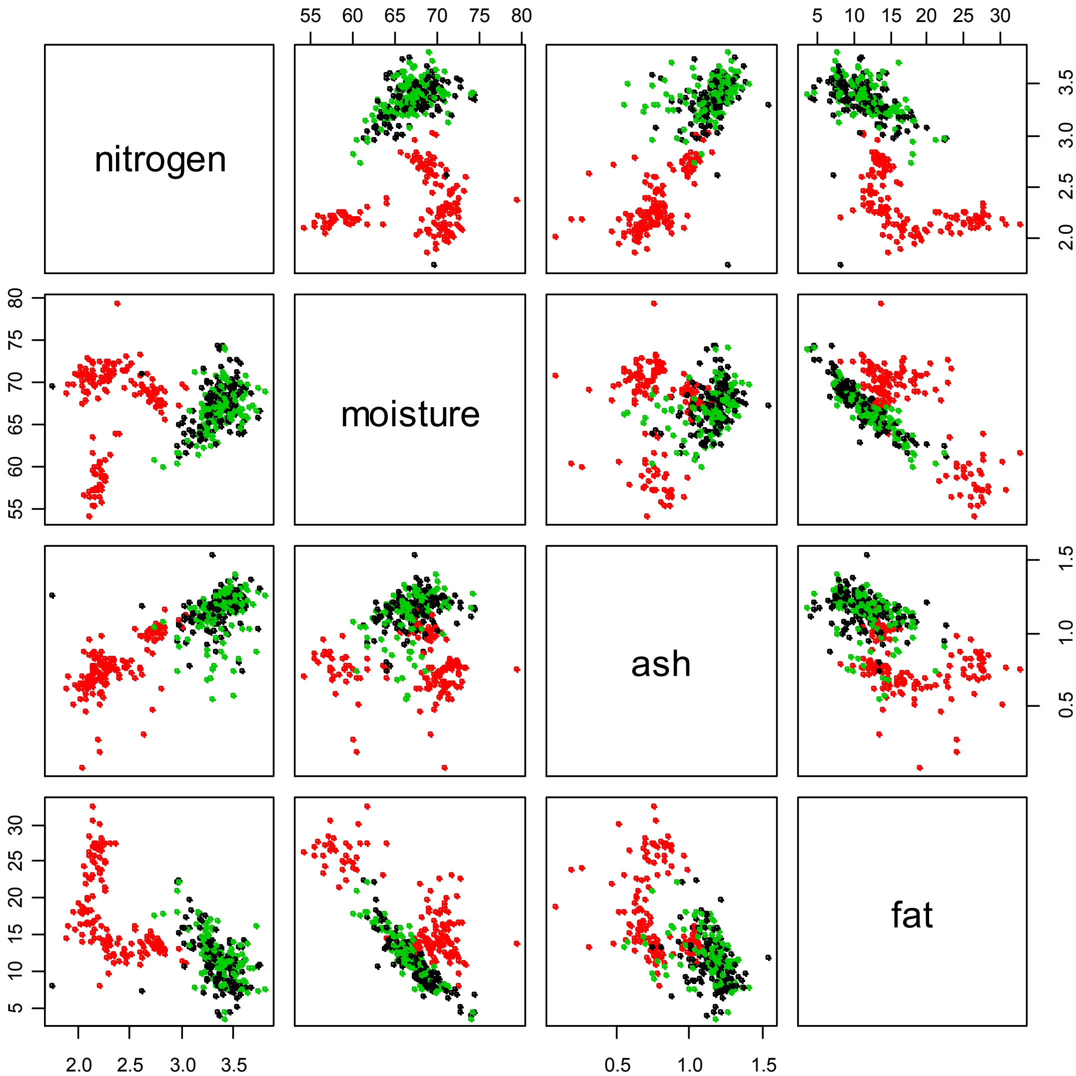


Figure 8 – Proximates Pairs Plots – All Data



The figure shows pairwise scatter plots of each proximate against another. Colour-coding is by cut: Red: Salmon frame mince; Black: Fillet; Green: Whole fish. The axis units are percentages read horizontally and vertically against the named parameter.

Factors Affecting Proximate Content

Whole Fish and Fillet

The sample acquisition followed a balanced factorial design with factors for season, product type ("Cut"), country of origin and size of fish. Laboratory analysis following sampling used randomised block designs as far as possible. Initial assessment of significance therefore applied a linear model, testing all possible factors and interactions, including run effects, against the residual variance. The results are summarised as ANOVA tables in Appendix 4. The most important features of these preliminary tests are:

- Season is very strongly significant, compared to residual variation, for essentially all proximates and product types.
- Analytical run ("Batch") (shown nested within season as season:batch in the tables) is very strongly significant, compared to residual variation, for all proximates and product types other than moisture in whole fish and fillet.
- Country of origin shows very strongly significant effects throughout, other than for salmon frame mince for which the country of origin was not recorded.

Season is a potentially important effect on fish composition, but in the present design season is unavoidably confounded with sampling event, normally introducing a random effect. Further, in an enforcement context, the analyst will normally be unable to determine the season of origin of the fish used in a fish product, making the season an essentially random effect at the point of analysis of a product. Analytical run effects also constitute a random effect and while the randomised block designs used are expected to remove the adverse effects of batch variation on internal comparisons, the analytical run effect contributes to standard errors in estimated mean values.

We have therefore calculated mean proximate content for fillet and "whole" fish by country, size of fish and product type ("Cut") using a mixed effects model with season and analytical run taken as random effects, with analytical run nested in season. In addition, noting that all two-way interactions involving season were significant for at least one proximate, all two-way interactions involving season were also taken as random. This leads to mean proximate content across sampling time, with conservative standard errors incorporating random seasonal variations and interactions. The model additionally provides variance estimates for seasonal and analytical run variation to give an indication of the size of each effect. The resulting mean values and associated standard errors are summarised in Table 2.

Table 2 Mean Values and Associated Standard Errors

Country	Size	Cut	Nitrogen	Moisture	Fat	Ash
Norwegian	Large	Fillet	3.236	65.12	14.15	1.126
Norwegian	Large	Whole	3.196	63.87	15.50	1.098
Norwegian	Medium	Fillet	3.280	66.55	12.48	1.163
Norwegian	Medium	Whole	3.298	65.95	13.31	**1.108**
Norwegian	Small	Fillet	3.300	66.79	11.88	1.120
Norwegian	Small	Whole	3.340	68.08	10.58	1.184
Scottish	Large	Fillet	3.433	66.57	11.56	1.153
Scottish	Large	Whole	3.477	67.12	11.24	1.149
Scottish	Medium	Fillet	3.437	69.18	9.02	1.193
Scottish	Medium	Whole	3.448	67.38	10.93	1.137
Scottish	Small	Fillet	3.399	69.34	8.60	1.172

Country	Size	Cut	Nitrogen	Moisture	Fat	Ash
Scottish	Small	Whole	3.469	68.78	9.36	1.197
Standard error (Notes 1,2)			0.044	0.74	0.92	0.035

- Note 1: Standard errors are rounded to two significant figures. The standard errors for different groups sometimes differed in the third place owing to outlier removal (one) or differences in allocation to analytical runs; with one exception, the standard errors were identical on rounding to two figures. The single ash figure in bold had a rounded standard error of 0.036 instead of 0.035.
- Note 2: Although the standard errors suggest critical differences of approximately 0.1%, 1.5%, 2% and 0.07% for nitrogen, moisture, fat and ash respectively, correlation (due to run and seasonal effects) within the data set causes the standard error for differences between groups to be appreciably smaller. These critical differences should therefore be taken as a conservative guide; any values that differ by more than these indicative differences are significant to at least the 95% level, but smaller differences may also be statistically significant.

Considering the comparatively small differences within Table 2 and the specific interest in nitrogen, the statistical model for nitrogen was re-examined with a view to simplification. It proved possible to drop product type ("Cut") (whole versus fillet) and associated season/cut interaction, leading to a reduced model including only the country and size of fish. It was not possible to drop size; a likelihood ratio test indicated that size and its associated interaction with country were, taken together, very strongly significant (p=0.0057). Means by country and size are accordingly given in Table 3, with standard errors calculated from a mixed effects model with two-way Seasonal interactions taken as random. From the data in the table, it is evident that the significance of the size effect largely arises from the clear differences in nitrogen content for different sizes of Norwegian salmon; there is essentially no size effect in the Scottish salmon.

Alternative models with no random effects, with only a seasonal main effect taken as random, or with selected seasonal interactions taken as random all returned smaller standard errors than the model used. For nitrogen, the least conservative (no random effects) returned standard errors of approximately 0.03%N per group compared to approximately 0.044%N for the model used.

Table 3 Nitrogen Content by Country and Size (% by mass, whole sample basis)

Country	Size	Estimate	Standard Error (note 1)
Norwegian	Large	3.216	0.037
Norwegian	Medium	3.289	0.037
Norwegian	Small	3.320	0.037
Scottish	Large	3.455	0.037
Scottish	Medium	3.443	0.038
Scottish	Small	3.434	0.037

- Note 1: Correlation within the data set causes the standard error for differences between groups to be significantly smaller than the standard error associated with the group mean.

Salmon Frame Mince

For salmon frame mince, different sampling points included three different production batches each, but no additional information on fish size, cut or country of origin was available. There was, however, a strong seasonal effect, and production batch effects appear very strongly significant for all proximates other than ash. In this instance, there is reason to believe that seasonal effects may be dominated by manufacturing practice (especially for moisture content), whereas production batch effects are essentially random. We have therefore calculated seasonal averages, with standard errors based on treating production batch and analytical run as (crossed) random effects. The results are summarised, Table 4.

Table 4 Proximates - Frame Mince (% by mass, whole sample basis)

Season	Nitrogen	Moisture	Fat	Ash
Winter 06	2.371	71.24	12.85	0.760
Spring 07	2.087	70.36	17.75	0.618
Summer 07	2.749	68.45	13.60	0.956
Autumn 07	2.189	58.52	26.32	0.744
Standard Error (Note 1)	0.041	0.58	0.72	0.028

- Note 1: Standard errors are rounded to two significant figures.

The salmon frame mince shows very different proximate content to the whole fish and fillet, and in particular shows reduced nitrogen content. The effect of washing during processing was accordingly investigated. Salmon meat spooned from one set of frames taken prior to washing at the first sampling visit yielded the results in Table 5.

Table 5 Proximates - Salmon Flesh Spooned from Unwashed Frames (mean ± SD, n = 6)

Nitrogen %	Moisture %	Fat %	Ash %
3.13 ± 0.18	68.92 ± 0.81	11.36 ± 0.25	0.97 ± 0.02

A sample of the water in the wash bath through which the salmon frames passed before recovery of the salmon mince was also taken in the first sampling visit. Analysis for nitrogen (n = 6) on the sample as taken and the liquid after passing through a 0.45 micron filter yielded the results in Table 6.

Table 6 Salmon Frame Wash Water

	Nitrogen %	SD
Wash water unfiltered	1.52	0.08
Wash water filtered	1.60	0.02

Calculation of Fat-free Nitrogen

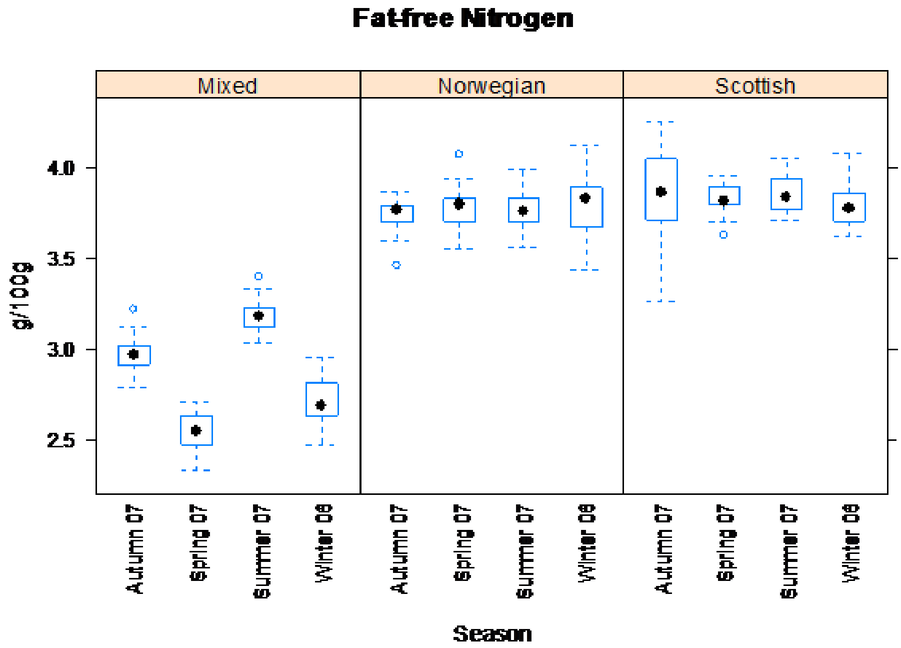
Nitrogen (N_{ff}) calculated on a fat-free basis was calculated from observed nitrogen N_{obs} and fat F_{obs} using

$$N_{ff} = \frac{100 \times N_{obs}}{100 - F_{obs}} \quad [1]$$

where all values are expressed as percentages. Equation 1 was applied to mean nitrogen and fat content for each sample, leading to one fat-free nitrogen value per sample.

Figure 9 summarises the resulting fat-free nitrogen data, broken down by country and season; as before, "Mixed" origin denotes frame mince which is derived from a mixture of Scottish and Norwegian sources.

Figure 9 – Fat-Free Nitrogen by Season and Origin



- Note: "Mixed" denotes salmon frame mince as no country of origin information was available.

Discussion

The temporal variation of whole body proximate composition of Atlantic salmon, a feature of the animals' anadromous life cycle has been the subject of several studies. Karalazos[30] confirmed that in general fat deposition increases as the fish grow larger. In a more detailed study Shearer *et al*[31] analysed homogenised whole fish, (as opposed to the edible portion derived from the whole fish in this work) and described the changes in proximate composition through the entire life cycle. Relatively large changes were noted at the following specific points: first feeding, smoltification, entry into sea water and during sexual maturation, events that are associated with periods of rapid tissue building, metamorphosis, high energy demand and transfer of somatic material to the gonads. As farmed Atlantic salmon are harvested as smolts Shearer's data on the on-growing phase at sea after smoltification are particularly relevant and show that nitrogen content was unaffected by seawater entry and showed relatively little variation during the maturation phase. Lipid content dropped to below 5% at seawater entry, increased as the fish gained weight to almost 20% at maturation, decreased thereafter to about 10% and fluctuated about that level over the next 8 months, the fish gaining weight throughout the entire period. It should be borne in mind that these data include visceral and offal lipids. Shearer found that moisture content, following an inverse trend to lipids, increased at saltwater entry, decreased thereafter to maturation and increasing again. Ash mirrored the lipid trend. Thus the widest variation in proximate composition during the period under study in this work is expected to occur in the lipid content, a fact also reported by industry.

Recommended Fat-Free Nitrogen Factor, N_{ff}, for "Whole" Fish and Fillet

Inspection of Figure 9 shows some differences compared to the features seen for total nitrogen in Figure 4. In particular, the figure suggests that mean fat-free nitrogen content differs less between countries of origin, and that the effect of season is less marked, especially for Norwegian salmon. The fat-free nitrogen data show fewer outliers, in part due to averaging of duplicates and in part because the fat-free calculation has removed some of the more extreme effects of fat variation, shown by Shearer *et al* to be the largest proximate variable in growing smolts. We therefore suggest that the estimation by analysis of the salmon content of compound food products should be based on the calculation of a "defatted salmon content" using a fat-free nitrogen factor, N_{ff}. An appropriate fraction of the determined fat added to the "defatted salmon content" thus obtained would yield "total salmon content". Bearing in mind that the smoking business dominates how fish are grown and that very fatty fish do not smoke well the upper ranges of the data for the fat content of farmed Atlantic salmon may well reflect a typical upper limit on the proportion of fat that should be added to a fat-free salmon content derived from a fat-free nitrogen factor. The influence of fish diet remains unresolved. Karalazos found evidence both for and against dietary changes (vegetable oil replacement for fish oils) on proximate composition of the fish whole body. As a guide, we estimate that grounds for further investigation of the composition of the fat would be raised if the amount of fat found exceeded some 15% of the "defatted salmon content".

It remains to decide upon a suitable N_{ff}. Simple analysis of variance broadly supports the impression that the country effect is relatively weaker for fat-free nitrogen (see Appendix 4), at least to the extent that the *p*-values indicate the relative strength of effects. Product type ("Cut") is significant at the 95% level and size and country are very strongly significant with *p*-values close to 0.002. Seasonal variation is less prominent, though visible in an apparent three-way season:size:country interaction. Note that the country where the farmed fish is cultivated is not required to be declared for composite fish products. Several alternative reduced models were investigated, including mixed effects models treating seasonal effects as random as before. The simplest model, a fixed effects model with main effects only for product type ("Cut") size and country proved sufficient and returned standard errors for group means essentially identical to the corresponding mixed model with allowance for random seasonal effects. The group means and standard errors are summarised in Table 7.

Table 7 – Fat-Free Nitrogen (Whole Fish and Fillet) by Country, Product Type ("Cut") and Size

Country	**Size**	**Cut**	**Nitrogen (% fat-free)** (Note 1)	**Std. Error** (Note 1)
Norwegian	Large	Fillet	3.778	0.039
Norwegian	Large	Whole	3.789	0.039
Norwegian	Medium	Fillet	3.742	0.039
Norwegian	Medium	Whole	3.808	0.039
Norwegian	Small	Fillet	3.751	0.039
Norwegian	Small	Whole	3.738	0.039
Scottish	Large	Fillet	3.883	0.039
Scottish	Large	Whole	3.924	0.039
Scottish	Medium	Fillet	3.776	0.039
Scottish	Medium	Whole	3.861	0.039
Scottish	Small	Fillet	3.716	0.039
Scottish	Small	Whole	3.821	0.039

- Note 1: Means and standard errors were calculated from a simple linear model with no random effects. Standard errors are associated with the mean value; correlation within the data set may lead to smaller standard errors associated with differences between group means.

Recalling that whole fish were filleted in the laboratory, by inspection of the means of proximates (Table 2) four out of 6 results have less water in the "whole" (i.e. laboratory filleted) than processed fillets and small Norwegian "whole" is higher in moisture because of one replicate. However the inverse relationship with fat also holds in these instances (when moisture is lower in the "whole" fat is higher). If not a random effect this suggests an intrinsic compositional difference rather than more water uptake in the factory processed fillet. The range of group means in Table 7 (approximately 0.2% m/m) is modest compared to the repeatability standard deviation, estimated as approximately $\sqrt{0.0142}$= 0.12% m/m from the analysis of variance table in Appendix 4. This suggests that if country and product type information is available the data in Table 7 may be used to select a suitable N_{ff} for most practical purposes and if not a single fat-free nitrogen factor of 3.80 g/100 g (Dumas) would suffice for fillets of Atlantic salmon. Nitrogen determined by the Dumas method is in general higher than that determined by the Kjeldahl method by 1.4% of the mean Kjeldahl result, i.e. by a factor of 1.014. Thus a single fat-free nitrogen factor of 3.75 g/100 g (Kjeldahl) would suffice for fillets of Atlantic salmon and the data in Table 7 should also be similarly corrected if used to assess a nitrogen content determined by Kjeldahl.

Recommended Fat-Free Nitrogen Factor, N_{ff}, for Salmon Frame Mince

For salmon frame mince, seasonal effects are clearly evident in Figure 9. Simple two-way ANOVA (Appendix 4) indicates that Season is the only significant effect ($p<0.001$). Table 8 therefore summarises the seasonal means, together with the mean defatted nitrogen content for salmon frame mince. We do not consider the seasonal variation to be a function of the salmon life cycle, rather we suspect it results from changes in the raw material fed into the salmon frame mince production process, although that may in itself be due to anatomical variations in fat deposition in the smolts.

Table 8 – Fat-Free Nitrogen (Frame Mince) (g/100g)

Season	Estimate (g/100g)	Std. Error
Autumn 07	2.972	0.025
Spring 07	2.539	0.025
Summer 07	3.182	0.025
Winter 06	2.721	0.025
Mean	2.853	0.141 (Note 1)

- Note 1: Calculated from the dispersion of group means. The standard deviation of group means is 0.282 g/100g

Production batch effects were strongly significant for nitrogen as a fraction of total mass, but were not significant for fat-free nitrogen content. The mean fat-free nitrogen content for salmon frame mince was 2.85 g/100 g, with a seasonal standard deviation of 0.28 g/100g. This is much lower than the N_{ff} found for fillets. However it is well known that the types of proteins in fish flesh differ from those of meat from land animals. The amounts of sarcoplasmic proteins and non-protein nitrogen, NPN, (creatin, creatinine, nucleotides, amino acids and other bases), all water soluble, are higher in fish and NPN differs between farmed and wild fish of the same species. Fish muscle fibres lack the connective tissue sheath possessed by meat muscle fibres. These factors coupled with the wet processing of product used to generate salmon frame mince render loss of soluble nitrogen and uptake of water more likely[7]. This was confirmed by the analytical results presented in Tables 5 and 6 above. We consider (data not shown) that the major reason for the significantly lower nitrogen concentration in salmon frame mince recovered by machine following water washing is from loss of soluble nitrogen rather than uptake of water.

The joint trade and regulatory Code of Practice on the Declaration of Fish Content in Fish Products [22] recognises two principles: (1) that fish treated in accordance with good manufacturing practice (GMP) is regarded as fish as an ingredient for fish products, and (2) fish content is determined in the post production supply chain by nitrogen content based on fish processed by GMP followed if necessary by in-factory inspection[7,22]. Thus, recognising that water washing of salmon frames for production of minced fish, adopted by the processor in this study, was authoritatively advocated by Torry Research Station[32] as long ago as 1979, it is reasonable to regard salmon frame mince as a legitimate ingredient as produced but with a lower nitrogen content (factor) than either whole or filleted fish. Thus the authors suggest that a nitrogen factor N_{ff} of 2.85 (Dumas) is appropriate for salmon frame mince with an equivalent Kjeldahl N_{ff} of 2.81. That this will result in little disadvantage to a manufacturer if the pattern of proximate composition found in this study is typical as is shown in Table 9 below.

It is not possible from the results of this study to indicate a cut-off value for allowable salmon fat in a compound product in which salmon frame mince is incorporated because the ratio of fat to defatted salmon content (DSC) varies considerably with "season" as shown in Table 10 below.

Table 9 Apparent Total Salmon Frame Mince Content (based on data in Table 4)

	Nitrogen % N	**Fat %**	**DSC** (based on Nff = 2.85) (Note 1)	**DSC + Fat %** (i.e. salmon frame mince content)
Winter 06	2.371	12.85	83.2	96.0
Spring 07	2.087	17.75	73.2	91.0
Summer 07	2.749	13.60	96.5	110.1
Autumn 07	2.189	26.32	76.8	103.1
			mean	100.1

- Note 1: DSC here denotes Defatted Salmon Frame Mince content, [N x 100)/N_{ff}]

Table 10 Ratio of Fat to Defatted Salmon Content for Salmon Frame Mince Content (based on data in Table 9)

	Fat %	**DSC** (based on N_{ff} = 2.85)	**Fat/DSC %**
Winter 06	12.85	83.2	15.4
Spring 07	17.75	73.2	24.2
Summer 07	13.60	96.5	14.1
Autumn 07	26.32	76.8	34.2

Tables 2, 3, 4 and 5 demonstrate that there are marked differences in proximate composition between salmon frame mince on the one hand and whole, fillet or spooned-off salmon flesh on the other. Therefore we further suggest that principle (1) of the Code of Practice[22] (see above) is not applicable in this instance. Moreover Directive 2000/13/EC implemented in Great Britain by the Food Labelling Regulations 1996 requires the name of a food to be accompanied by an indication of any treatment it has received if a purchaser could be mislead by omission of such an indication. An ingredient used in a compound product must be named in the same manner as it would be if sold as a food in its own right. Therefore we recommend that the ingredient of commerce derived by automated recovery in minced form of salmon flesh from the water-washed frames of salmon after the removal of their fillets must be distinguished from salmon proper and identified in ingredients lists as "salmon frame mince". Following this practice will also help to avoid disputes on salmon content as a more appropriate N_{ff} can be chosen.

QUID Appraisal

In assessing compliance against a QUID declaration, if an apparent deficiency is revealed by end-product official analysis, we suggest an in-factory investigation, with the benefit of recipe information, trade data and official sampling and analysis of ingredients. At the same time a series of observations should be obtained which if necessary may be used either to inform advice to the manufacturer or in a subsequent prosecution. For example, three separate formal samples taken at appropriate intervals of time, say, a month apart, or of three separate production batches, should be analysed to build up an official dataset.

Conclusions

The results presented herein demonstrate that the nitrogen factor of 3.60 currently in use based on a 1973 study, is not appropriate for farmed Atlantic salmon. The species nitrogen concentration of farmed Atlantic salmon is lower than that reported in 1973 for fish in the wild. Our data on fat-free nitrogen content is consistent with the known relative stability of nitrogen and variability of the fat in smolts during the on-growing phase of their development at sea prior to harvest. Therefore Nitrogen factors on a fat free basis, N_{ff}, are recommended to be used for the estimation of the amount of salmon (*Salmo salar*) in compound products. Where the country of origin (Scotland or Norway) and product type are known Table 7 herein may be consulted for a specific Dumas N_{ff} failing which a general N_{ff} for Dumas N determinations of 3.80 (3.75 Kjeldahl) is suggested for salmon (*Salmo salar*) flesh processed as described in Appendix 2 . For salmon frame mince a general Dumas factor, N_{ff} for *Salmo salar* of 2.85 (2.81 Kjeldahl) is suggested. It is further suggested that salmon frame mince must be separately identified in the list of ingredients of compound products in which it is incorporated. Anomalous analytical findings against QUID declarations of salmon content must be followed up by in-factory investigation. Further work on the quantitative estimation of salmon and non-salmon lipids in compound products is required.

Acknowledgements

Funding for this case study was wholly provided by the UK National Measurement Office Government Chemist Programme. The authors thank the Government Chemist, Derek Craston, for permission to publish the work and the Food Chemistry Team at LGC, particularly Malcolm Burn and Kam Lee, for technical assistance. We also thank Darren Andrews and Jenny Douglas, Young's Bluecrest Seafood Ltd, David Wroe, Princes Foods, Cliff Morrison, Jane Tomlinson, Principal Trading Standards Officer, North East Lincolnshire Trading Standards Dept and Chris Hunt, Public Analyst, West Yorkshire Analytical Services. We thank peer reviewers for helpful comments that have improved the clarity of the paper.

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Appendix 1 – Calculation of Salmon Content

The calculation is based on the determination of the nitrogen content of a sample and its comparison, corrected for non-flesh nitrogen, with the species specific nitrogen concentration, the "Nitrogen Factor" and is based on the approach originally demonstrated by Stubbs and More working in the Laboratory of the Government Chemist[33]. An example of a simple "Stubbs and More" calculation of the apparent salmon content of a compound product is shown below. Nitrogen factors for red meat species and poultry (but not white fish) are expressed on a fat-free basis, N_{ff}, following the example of Stubbs and More as this approach simplifies subsequent calculations. We suggest this approach is taken for salmon because the amounts of fat present are variable and much higher than for white fish.

The calculation is straightforward if the only source of non-salmon N is that from the rusk, potato or flour binder – say in a fish cake. It is assumed that all data is as determined by standard laboratory procedures on a representative sample that has been homogenised. In routine analysis nitrogen, moisture, fat and mineral matter are determined.

The following parameters are used:

Parameter	Symbol
"Carbohydrate" fraction, %	CHO
Total nitrogen determined on the sample, %	N_t
Protein (N_t x 6.25), %	P
Fat, %	F
Moisture, %	M
Ash, %	A
Nitrogen determined on sample and corrected for non-fish nitrogen from the "carbohydrate" fraction, %	N_{corr}
Nitrogen factor on a fat free basis	N_{ff}
Defatted salmon content, %	DSC
Apparent total salmon content, %	ATSC
Multiply	*

Note "Carbohydrate" fraction is not pure carbohydrate and some practitioners prefer the term "rusk"

The "Carbohydrate" fraction is calculated by difference:

$$CHO = 100 - (P + F + M + A)$$

The nitrogen content found by analysis is corrected for the nitrogen in the "Carbohydrate" fraction:

$$N_{corr} = N_t - ([2*CHO]/100)$$

This assumes that the nitrogen content of the "carbohydrate" fraction is 2%, if it is known by analysis to be otherwise then the known nitrogen content should be used.

The next step is the calculation of "defatted salmon content", a hypothetical intermediate figure:

$$DSC = [N_{corr}*100]/N_{ff}$$

Apparent Total Salmon Content, ATSC is then calculated by:

$$ATSC = DSC + F$$

The word "apparent" is used if other sources of N are present (e.g. soya protein, milk powder etc.). In practice soya can be corrected for if it is determined in a separate procedure e.g. ELISA but as each extra source of potential N is examined the cost of the analysis goes up.

Similarly, extra lipid (e.g. olive oil) must be discounted which is not straightforward and so we emphasise that a suspect analytical result must be followed up by in-factory inspection where the recipe and formulation data are available – it is more efficient to do this than to analyse for all the possible extra sources of N or lipid.

Further Considerations

For completeness it should be noted that for red meat and poultry hydroxyproline is also determined to estimate the amount of connective tissue and there are legislative limits for the amount of fat and connective tissue that are allowed to be included in the ingredient declarations of beef, pork, chicken etc. These limits must be taken into account in the determination and calculation of meat content. There are currently no such legislative limits in the UK for the fat content of salmon.

Finally it should be added that there are limitations in the use of nitrogen factors. They are average values, and when deciding whether declarations of meat or fish content are accurate, it is important to bear in mind the possible variability of natural values and the analytical variability of their determination. Pre-packed fish products may still use the generic ingredient description "fish" or give the type of fish used, and in either case the percentage of these ingredients present in the product. It is not possible to analyse accurately products containing mixed species of meat or of fish using nitrogen factors, nor products containing mixtures of meat and offal. Readers are referred to standard works for a fuller treatment, see below.

Standard Reference Works

B. McClean, "Meat and meat products: the calculation of meat content, added water and connective tissue content from analytical data", Campden & Chorleywood Food Research Association, Chipping Campden. 2007.

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Appendix 2 – Atlantic Salmon - Life Cycle, Farming and Processing

Salmon are native to the world's two biggest oceans and the rivers draining into them. The Atlantic Ocean has only one species, the Atlantic salmon (*Salmo salar*), while in the Pacific Ocean there are several species: pink salmon (*Oncorhynchus gorbuscha*), chum (*O. keta*), sockeye (*O. nerka*), coho (*O. ksutch*), chinook (*O. tschawytscha*) and amago (*O. rhodurus*)[34] . The life cycle of the Atlantic salmon is complex[35]; spawning occurs in freshwater in late autumn, eggs hatch the following early spring producing alevin which mature into fry that reach some 5-8 cm in their first year. Salmon over a year old are known as parr, measure only a fraction of adult size and can remain in the parr stage, in the wild (freshwater) for up to 4 years, (usually 2)[36] . At a length of between 10 and 24 cm a springtime transformation of parr into smolt occurs. Smolt can survive in seawater to become adult. Wild salmon that return to fresh water (the river where they were born) after one year at sea are called grilse and spent/spawned salmon are termed kelt. Farmed Atlantic salmon are reared in freshwater facilities to the fingerling or smolt stage and then they are transferred to seawater where the fish are grown in sea cages. The on-growing phase can be up to two years until the fish reach the desired market size, which may vary from 2 to 6 kg (see Karalazos[30]). In the wild adult males can reach up to 150 cm in length and 36 kg in weight, while females may attain lengths and weights of about 120 cm and 20 kg.

The overwhelming majority of salmon consumed in the United Kingdom is farmed, mainly in Scotland but Norway is a not unsubstantial source. By way of illustration, globally in 2004 the major producing countries in order of tonnage were Norway, Chile, Scotland, Canada, Faeroe Islands, Ireland, Australia and USA[30]. The total output of the Scottish sector was estimated in 2009 to be around £412 million for farmed Atlantic salmon, £14.34 million for rainbow trout and £7.7 million for shellfish. Brown trout, sea trout, halibut and Arctic charr are also farmed in Scotland. There has been a steady year-on-year increase in salmon production. In 2009, for example, 144,247 tonnes of Atlantic salmon was produced at 254 active marine sites by 31 companies in Scotland, compared with 28,553 tonnes in 1989[37,38].

The majority of farmed salmon goes for smoking, the remainder is sold fresh as whole fish, fillets, cuts of fillets and as the by-product salmon frame mince, which is sold to non-retail businesses for inclusion in their own products (e.g. pates and spreadable products) and meals in the food chain. The fresh salmon processing plant from which fish were taken for this study takes salmon from 12 suppliers across Scotland and Norway. Different sizes of fish are required for different styles of product and are sourced appropriately from the stock held by farms. The fish feed used in the farms from which the salmon were derived for this study was said not to include genetically modified material, colour (additives) nor avian protein and to contain more soya now than previously to take pressure off marine protein sources. Karalazos discusses fishmeal composition in more detail. For Scottish salmon, the size range 1-2 kg is not processed as all are used as entire fish. The overall shelf life of Atlantic salmon is 13-14 days from slaughter hence processing time must be minimised[39].

In a visit to a major salmon processor in 2006 some of the authors (MJW, SE, PC) observed that head-on gutted fish were received at the processing plant packed in ice or ice/brine slurry. The weight was recorded and the head and tail were removed by machine. Filleting was also largely automatic, resulting in less handling and better visual quality. The back section and belly trim were removed, the latter, with high fat/oil content were exported to countries where such items are esteemed. Water washing up to this stage was minimal. Pin bones were removed from the fillets by water and vacuum, and this waterlogged by-product was used for fishmeal.

Salmon Frame Mince

There is an appreciable amount of salmon flesh still attached to the skeleton ("salmon frame") after machine fillet removal. This flesh can be recovered either manually ("spooning") or, more quickly, by machine. Salmon frames are washed to remove adhering debris and improve the microbiological keeping quality, drained and further water removed with the assistance of air blowers. They are then fed from a hopper to pass between a moving rubber belt and the outside of a revolving perforated drum of stainless steel. The flesh is forced through the perforations into the drum from where it is expelled as a coarse mince by a screw. Skin and bone are retained on the outside of the drum and removed continuously by a scraper blade. The drum perforations are most commonly 5 mm in diameter (3 mm observed) but drums with smaller or larger holes are available, which produce mince of different texture. Yield can be increased by increasing the tension on the belt, at the expense of some increase in the degree of fragmentation of the flesh, and in the amounts of bone, pieces of skin and other debris [32]. The minced product is packed into boxes and plate frozen over a period of some 2 hours. During this time some fluid is expelled from the block. There is a variety of nomenclature options for this product such as “recovered salmon mince”, “salmon frame mince” or “salmon frame mince ingredient”. We use the term “salmon frame mince” throughout this paper.

Figure 1 shows in schematic form the main steps in processing salmon after it has been harvested.

Appendix 3 – Collated Results

- Note: The collated results do not include analytical run information.

Description	Winter December 2006				Spring April 2007				Summer August 2007				Autumn November 2007			
	Ash %	Moisture %	Fat %	N %	Ash %	Moisture %	Fat %	N %	Ash %	Moisture %	Fat %	N %	Ash %	Moisture %	Fat %	N %
7.5 Kg Frozen Frame Mince Block - Production Batch 1	0.72	70.03	14.64	2.21	0.71	69.82	16.21	1.91	0.87	68.27	14.74	2.71	0.19	60.35	23.93	2.20
7.5 Kg Frozen Frame Mince Block - Production Batch 1	0.56	70.13	14.36	2.24	0.64	69.59	14.94	2.03	1.01	68.87	14.24	2.66	0.26	59.96	24.07	2.20
7.5 Kg Frozen Frame Mince Block - Production Batch 1	0.69	68.44	15.79	2.54	0.63	70.92	20.02	2.08	0.95	68.74	13.60	2.75	0.87	56.69	24.49	2.06
7.5 Kg Frozen Frame Mince Block - Production Batch 1	0.70	63.85	14.61	2.39	0.71	70.08	22.83	2.12	1.00	69.10	13.10	2.77	0.96	56.40	25.79	2.11
7.5 Kg Frozen Frame Mince Block - Production Batch 1	0.76	70.76	14.69	2.29	0.46	67.82	21.93	2.08	0.99	66.45	13.52	2.80	0.76	57.35	27.31	2.17
7.5 Kg Frozen Frame Mince Block - Production Batch 1	0.76	70.68	14.69	2.30	0.63	72.33	20.33	2.15	1.01	67.03	14.17	2.76	0.82	57.33	27.12	2.19
7.5 Kg Frozen Frame Mince Block - Production Batch 1	0.72	70.12	12.81	2.27	0.66	69.00	18.59	2.05	1.09	67.24	13.12	2.96	0.76	57.32	30.61	2.14
7.5 Kg Frozen Frame Mince Block - Production Batch 1	0.74	70.23	13.71	2.29	0.67	70.89	18.47	2.12	1.16	67.37	12.89	2.83	0.51	60.65	30.14	2.20
7.5 Kg Frozen Frame Mince Block - Production Batch 1	0.77	68.88	13.85	2.28	0.70	70.14	17.86	2.04	1.02	67.81	14.99	2.70	0.78	59.19	25.81	2.16
7.5 Kg Frozen Frame Mince Block - Production Batch 1	0.78	71.48	13.77	2.24	0.62	68.98	18.18	2.09	0.47	69.57	13.85	2.72	0.71	59.46	25.05	2.14
7.5 Kg Frozen Frame Mince Block - Production Batch 1	0.72	70.29	13.84	2.51	0.62	70.76	19.28	1.99	1.05	67.27	13.91	2.80	0.84	59.03	27.39	2.18
7.5 Kg Frozen Frame Mince Block - Production Batch 1	0.76	70.57	13.66	2.31	0.55	71.16	18.22	2.02	1.06	67.91	14.01	2.79	0.88	57.25	26.58	2.22
7.5 Kg Frozen Frame Mince Block - Production Batch 2	0.75	70.75	12.67	2.60	0.64	70.42	18.28	2.09	0.98	68.65	13.99	2.61	0.85	56.45	23.33	2.20
7.5 Kg Frozen Frame Mince Block - Production Batch 2	0.77	73.27	11.06	2.60	0.08	70.89	18.99	2.03	0.98	69.76	13.83	2.61	0.70	60.84	27.48	2.26
7.5 Kg Frozen Frame Mince Block - Production Batch 2	0.77	71.93	12.21	2.48	0.62	68.63	14.54	1.88	1.07	67.80	13.28	2.76	0.75	61.67	32.52	2.14
7.5 Kg Frozen Frame Mince Block - Production Batch 2	0.80	71.48	11.91	2.46	0.67	72.85	16.70	2.09	0.98	69.44	13.50	2.80	0.78	63.57	23.09	2.14
7.5 Kg Frozen Frame Mince Block - Production Batch 2	0.79	71.29	12.23	2.31	0.55	71.66	23.31	2.06	1.02	68.59	14.15	2.68	0.98	58.66	24.03	2.23
7.5 Kg Frozen Frame Mince Block - Production Batch 2	0.82	72.32	12.31	2.34	0.63	72.73	22.68	2.11	1.00	70.31	13.51	2.71	0.76	58.89	24.94	2.26
7.5 Kg Frozen Frame Mince Block - Production Batch 2	0.71	69.61	11.94	2.62	0.64	71.12	15.72	1.97	0.94	69.23	13.06	2.73	0.83	55.41	25.85	2.15
7.5 Kg Frozen Frame Mince Block - Production Batch 2	0.77	73.07	11.33	2.47	0.58	71.13	16.45	2.07	0.31	69.16	13.37	2.64	0.74	57.29	27.62	2.16

Description	Winter December 2006				Spring April 2007				Summer August 2007				Autumn November 2007			
	Ash %	Moisture %	Fat %	N %	Ash %	Moisture %	Fat %	N %	Ash %	Moisture %	Fat %	N %	Ash %	Moisture %	Fat %	N %
7.5 Kg Frozen Frame Mince Block - Production Batch 2	0.82	71.42	11.29	2.34	0.65	71.80	16.53	2.02	0.97	67.76	14.17	2.69	0.70	54.15	26.38	2.10
7.5 Kg Frozen Frame Mince Block - Production Batch 2	0.79	72.11	12.41	2.35	0.67	70.62	16.41	2.10	0.96	70.94	14.82	2.66	0.69	57.50	26.79	2.17
7.5 Kg Frozen Frame Mince Block - Production Batch 2	0.81	71.40	12.34	2.44	0.60	70.54	16.24	2.23	1.00	68.80	13.50	2.72	0.87	56.50	27.36	2.16
7.5 Kg Frozen Frame Mince Block - Production Batch 2	0.81	72.32	12.33	2.51	0.66	70.72	15.86	2.27	0.98	69.22	12.54	2.65	0.58	57.78	26.44	2.20
7.5 Kg Frozen Frame Mince Block - Production Batch 3	0.78	72.25	14.21	2.29	0.69	68.79	19.77	2.09	1.05	69.68	11.37	3.00	0.70	57.62	28.23	2.22
7.5 Kg Frozen Frame Mince Block - Production Batch 3	0.80	71.96	13.09	2.26	0.73	68.07	17.35	2.19	1.13	69.40	11.22	3.03	0.78	59.50	27.10	2.22
7.5 Kg Frozen Frame Mince Block - Production Batch 3	0.77	71.54	12.66	2.45	0.58	70.38	15.29	2.15	0.62	67.89	12.81	2.80	0.83	58.78	25.22	2.26
7.5 Kg Frozen Frame Mince Block - Production Batch 3	0.72	72.41	11.35	2.39	0.65	70.78	14.04	2.12	1.04	69.00	13.24	2.83	0.80	55.71	27.65	2.23
7.5 Kg Frozen Frame Mince Block - Production Batch 3	0.77	71.26	14.34	2.31	0.64	69.17	21.10	2.26	1.00	65.55	12.43	2.83	0.82	61.51	27.49	2.31
7.5 Kg Frozen Frame Mince Block - Production Batch 3	0.75	71.54	13.74	2.30	0.52	69.68	18.27	1.96	0.89	69.12	12.46	2.67	0.75	63.84	27.48	2.35
7.5 Kg Frozen Frame Mince Block - Production Batch 3	0.77	69.32	13.04	2.45	0.68	69.48	16.84	2.16	1.03	67.26	12.69	2.78	0.86	55.33	26.95	2.13
7.5 Kg Frozen Frame Mince Block - Production Batch 3	0.75	79.32	13.75	2.38	0.66	71.47	16.70	2.15	1.01	69.22	13.27	2.77	0.85	57.05	28.37	2.11
7.5 Kg Frozen Frame Mince Block - Production Batch 3	0.76	72.47	11.94	2.28	0.67	69.69	15.77	2.15	1.03	66.98	14.01	2.76	0.69	60.35	25.70	2.20
7.5 Kg Frozen Frame Mince Block - Production Batch 3	0.76	70.99	12.05	2.35	0.73	72.47	16.64	2.13	1.04	67.18	14.60	2.77	0.75	59.98	24.95	2.15
7.5 Kg Frozen Frame Mince Block - Production Batch 3	0.79	72.54	8.20	2.21	0.62	67.53	13.99	2.04	1.03	68.00	15.44	2.70	0.72	58.30	21.52	2.26
7.5 Kg Frozen Frame Mince Block - Production Batch 3	0.78	72.44	9.84	2.28	0.66	70.79	14.58	2.16	1.03	69.70	16.34	2.54	0.67	59.01	22.59	2.23
Individual Large (4-6 kg) Whole Norwegian fish - Replicate 1	1.13	65.93	13.04	3.35	1.20	66.16	13.88	3.28	1.10	66.35	12.32	3.35	0.74	60.41	21.14	2.95
Individual Large (4-6 kg) Whole Norwegian fish - Replicate 1	1.21	66.22	13.42	3.37	1.13	65.91	14.04	3.25	0.88	66.82	12.14	3.37	0.91	61.71	22.32	2.98
Individual Large (4-6 kg) Whole Norwegian fish - Replicate 2	1.10	62.19	16.78	3.20	1.12	66.91	12.03	3.32	1.15	62.57	17.24	3.27	1.04	60.83	17.75	2.74
Individual Large (4-6 kg) Whole Norwegian fish - Replicate 2	1.14	63.22	16.05	3.30	1.18	66.62	11.10	3.42	1.17	62.88	16.96	3.35	0.97	61.69	18.10	2.95
Individual Large (4-6 kg) Whole Norwegian fish - Replicate 3	1.18	65.06	13.06	3.21	1.09	62.50	16.53	3.14	1.20	63.99	15.62	3.23	1.08	59.94	17.93	2.82
Individual Large (4-6 kg) Whole Norwegian fish - Replicate 3	1.18	65.82	12.97	3.34	1.14	63.79	17.98	3.15	1.16	64.00	15.74	3.22	1.04	61.46	18.14	3.15

Description	Winter December 2006				Spring April 2007				Summer August 2007				Autumn November 2007			
	Ash %	Moisture %	Fat %	N %	Ash %	Moisture %	Fat %	N %	Ash %	Moisture %	Fat %	N %	Ash %	Moisture %	Fat %	N %
Individual Large (4-6 kg) Whole Scottish fish - Replicate 1	1.19	67.65	10.09	3.48	1.21	66.80	10.93	3.58	1.19	70.02	7.33	3.57	1.22	65.74	13.80	3.63
Individual Large (4-6 kg) Whole Scottish fish - Replicate 1	1.21	68.50	9.96	3.51	1.30	67.47	10.62	3.47	1.29	69.82	7.15	3.63	1.26	64.65	13.50	3.57
Individual Large (4-6 kg) Whole Scottish fish - Replicate 2	1.20	69.12	9.36	3.31	1.20	67.61	12.75	3.37	1.33	66.04	11.67	3.62	1.15	65.20	12.34	3.59
Individual Large (4-6 kg) Whole Scottish fish - Replicate 2	0.75	69.19	9.16	3.29	1.18	66.90	12.34	3.28	1.31	66.28	11.14	3.56	1.06	65.59	12.55	3.49
Individual Large (4-6 kg) Whole Scottish fish - Replicate 3	1.14	68.62	10.21	3.40	1.21	65.87	9.74	3.19	1.15	65.56	15.04	3.26	1.03	67.05	10.49	3.65
Individual Large (4-6 kg) Whole Scottish fish - Replicate 3	1.16	68.82	9.86	3.54	1.21	66.00	12.46	3.48	0.69	65.73	13.85	3.28	0.87	66.67	16.14	3.71
Individual Large Fillet from large Norwegian fish - Replicate 1	1.12	64.21	13.63	3.11	1.12	63.26	17.74	3.23	1.54	67.34	11.82	3.30	1.05	61.15	22.58	2.98
Individual Large Fillet from large Norwegian fish - Replicate 1	1.11	64.36	14.53	3.20	1.05	63.63	17.24	3.17	1.19	67.89	12.29	3.28	0.95	62.41	22.23	2.96
Individual Large Fillet from large Norwegian fish - Replicate 2	1.12	66.28	12.83	3.33	1.23	67.77	10.43	3.45	1.01	63.71	13.22	3.11	1.12	63.07	17.98	3.18
Individual Large Fillet from large Norwegian fish - Replicate 2	1.18	66.24	12.68	3.31	1.12	68.66	10.40	3.28	1.12	65.92	13.02	3.25	1.08	62.72	18.35	3.09
Individual Large Fillet from large Norwegian fish - Replicate 3	1.14	64.23	14.39	3.46	1.07	67.45	11.00	3.33	1.12	67.09	11.58	3.22	1.10	62.93	10.77	3.02
Individual Large Fillet from large Norwegian fish - Replicate 3	1.16	64.23	14.35	3.60	1.16	67.02	11.63	3.39	1.16	68.11	12.36	3.24	1.12	63.20	16.90	3.18
Individual Large Fillet from large Scottish fish - Replicate 1	0.94	66.49	12.06	3.33	1.04	67.37	11.32	3.42	1.21	64.78	11.02	3.55	1.17	62.78	15.65	3.33
Individual Large Fillet from large Scottish fish - Replicate 1	1.09	67.17	10.79	3.24	1.15	67.68	11.26	3.38	1.24	65.43	11.39	3.60	1.18	63.85	16.07	3.03
Individual Large Fillet from large Scottish fish - Replicate 2	1.17	68.39	10.88	3.26	1.19	67.94	11.09	3.35	1.21	65.33	11.46	3.47	1.31	66.64	10.94	3.76
Individual Large Fillet from large Scottish fish - Replicate 2	1.19	67.99	10.52	3.44	1.22	68.44	10.66	3.41	1.22	66.77	10.96	3.32	1.04	65.72	10.78	3.74
Individual Large Fillet from large Scottish fish - Replicate 3	1.17	66.09	11.88	3.29	1.28	68.39	10.08	3.49	1.30	67.97	10.04	3.50	0.80	63.91	13.44	3.56
Individual Large Fillet from large Scottish fish - Replicate 3	1.20	66.79	12.23	3.26	1.21	68.09	10.33	3.50	1.26	69.57	10.09	3.58	0.75	63.97	13.33	3.59
Individual Medium (3-4 kg) Whole Norwegian fish - Replicate 3	1.18	66.40	12.13	3.53	1.24	68.52	10.56	3.34	1.07	64.14	15.99	3.17	0.88	63.66	15.94	3.24
Individual Medium (3-4 kg) Whole Norwegian fish - Replicate 3	1.17	66.77	11.99	3.49	1.18	68.64	10.54	3.36	1.08	64.08	16.66	3.24	0.96	63.18	15.68	3.05
Individual Medium (3-4 kg) Whole Norwegian fish - Replicate 5	1.20	65.80	11.43	3.43	1.19	66.33	12.39	3.19	1.17	65.49	15.95	3.30	1.11	66.84	15.13	3.26
Individual Medium (3-4 kg) Whole Norwegian fish - Replicate 5	1.20	65.14	11.46	3.43	1.17	67.42	13.69	3.20	0.97	65.25	13.60	3.32	1.06	65.72	14.76	3.19

Description	Winter December 2006				Spring April 2007				Summer August 2007				Autumn November 2007			
	Ash %	Moisture %	Fat %	N %	Ash %	Moisture %	Fat %	N %	Ash %	Moisture %	Fat %	N %	Ash %	Moisture %	Fat %	N %
Individual Medium (3-4 kg) Whole Norwegian fish - Replicate 6	1.22	64.60	12.95	3.33	1.25	70.86	8.00	3.33	1.20	64.74	14.72	3.24	0.85	64.52	15.35	3.24
Individual Medium (3-4 kg) Whole Norwegian fish - Replicate 6	1.26	65.39	13.11	3.35	1.18	70.47	8.45	3.41	0.68	65.25	14.70	3.24	1.07	63.64	15.65	3.29
Individual Medium (3-4 kg) Whole Scottish fish - Replicate 1	1.28	69.11	7.67	3.40	1.23	67.84	11.72	3.35	1.31	66.22	11.36	3.46	1.20	66.03	11.12	3.58
Individual Medium (3-4 kg) Whole Scottish fish - Replicate 1	1.25	69.74	7.71	3.44	1.17	67.10	10.82	3.39	1.28	67.34	11.35	3.45	0.82	66.64	10.64	3.54
Individual Medium (3-4 kg) Whole Scottish fish - Replicate 2	1.21	68.22	9.22	3.32	1.03	67.29	11.05	3.38	1.26	66.60	10.67	3.51	1.15	66.26	13.07	3.48
Individual Medium (3-4 kg) Whole Scottish fish - Replicate 2	1.17	68.43	10.12	3.22	1.26	68.15	10.94	3.40	1.26	66.61	10.78	3.51	1.21	65.88	12.71	3.46
Individual Medium (3-4 kg) Whole Scottish fish - Replicate 3	1.21	68.48	8.90	3.60	0.57	66.42	14.07	3.50	1.20	66.36	11.91	3.33	1.03	68.14	7.72	3.58
Individual Medium (3-4 kg) Whole Scottish fish - Replicate 3	1.19	68.91	8.47	3.62	0.55	66.29	13.48	3.30	1.17	67.06	11.52	3.40	1.23	67.99	9.62	3.51
Individual Medium Fillet from medium Scottish fish - Replicate 1	1.26	69.59	8.18	1.75	1.20	64.49	13.71	3.17	1.28	69.51	8.09	3.48	1.18	74.28	6.92	3.40
Individual Medium Fillet from medium Scottish fish - Replicate 1	1.25	70.34	7.87	3.48	1.18	64.71	13.43	3.38	1.19	69.30	7.83	3.61	1.07	72.31	6.96	3.56
Individual Medium Fillet from medium Scottish fish - Replicate 2	1.27	68.00	8.83	3.35	1.20	67.56	11.63	3.27	1.37	68.71	7.94	3.67	1.11	73.97	4.11	3.36
Individual Medium Fillet from medium Scottish fish - Replicate 2	0.98	68.58	9.26	3.37	1.00	66.59	11.58	3.43	1.32	68.85	8.06	3.57	1.17	74.41	4.51	3.34
Individual Medium Fillet from medium Scottish fish - Replicate 3	1.18	66.98	11.34	3.44	1.26	68.97	8.73	3.51	1.24	65.44	12.08	3.45	1.18	72.64	5.28	3.53
Individual Medium Fillet from medium Scottish fish - Replicate 3	1.18	67.78	11.30	3.37	1.23	69.19	8.63	3.49	1.28	65.35	12.32	3.36	1.21	72.71	4.52	3.47
Individual Medium Fillets from medium Norwegian fish - Replicate 1	1.00	67.30	10.85	3.27	1.09	69.36	9.83	3.19	1.20	65.70	13.32	3.30	1.18	64.06	14.30	3.29
Individual Medium Fillets from medium Norwegian fish - Replicate 1	1.25	67.51	9.05	3.31	1.21	68.91	10.03	3.48	1.11	66.48	12.79	3.26	1.18	65.09	14.20	3.26
Individual Medium Fillets from medium Norwegian fish - Replicate 2	1.24	65.43	13.05	3.38	1.29	68.50	9.17	3.32	1.21	66.76	10.70	3.46	1.11	62.41	16.11	3.08
Individual Medium Fillets from medium Norwegian fish - Replicate 2	1.18	65.50	12.33	3.38	1.20	69.91	9.82	3.37	1.38	67.53	9.79	3.51	1.05	63.86	16.02	3.18
Individual Medium Fillets from medium Norwegian fish - Replicate 3	1.16	67.07	12.37	3.26	1.21	71.69	7.21	3.20	1.13	66.12	13.19	3.26	0.93	61.53	17.37	3.04
Individual Medium Fillets from medium Norwegian fish - Replicate 3	1.10	66.96	12.06	3.30	1.27	71.95	7.55	3.38	1.21	64.75	14.47	3.18	1.21	62.74	19.84	3.08
Individual Small (2-3 kg) Whole Norwegian fish - Replicate 1	1.21	67.17	10.47	3.68	1.19	66.78	13.48	3.23	1.20	65.89	11.84	3.28	0.99	70.68	7.67	3.43
Individual Small (2-3 kg) Whole Norwegian fish - Replicate 1	1.20	67.19	10.56	3.47	1.16	66.63	13.18	3.22	1.16	66.88	11.99	3.22	1.19	70.56	7.98	3.39

Description	Winter December 2006				Spring April 2007				Summer August 2007				Autumn November 2007			
	Ash %	Moisture %	Fat %	N %	Ash %	Moisture %	Fat %	N %	Ash %	Moisture %	Fat %	N %	Ash %	Moisture %	Fat %	N %
Individual Small (2-3 kg) Whole Norwegian fish – Replicate 2	1.20	65.30	11.71	3.29	1.31	69.90	7.77	3.45	1.36	69.99	7.88	3.47	1.10	67.02	12.79	3.31
Individual Small (2-3 kg) Whole Norwegian fish – Replicate 2	1.19	66.20	11.58	3.27	1.31	70.81	7.66	3.61	1.41	69.83	7.75	3.51	1.17	67.05	14.32	3.21
Individual Small (2-3 kg) Whole Norwegian fish – Replicate 3	1.17	71.26	7.86	3.13	1.13	68.12	11.61	3.36	1.18	66.57	13.50	3.07	1.18	66.98	10.60	3.35
Individual Small (2-3 kg) Whole Norwegian fish – Replicate 3	1.23	71.24	7.86	3.21	1.18	67.86	11.11	3.38	1.03	66.86	13.20	3.25	0.94	67.07	10.91	3.39
Individual Small (2-3 kg) Whole Scottish fish - Replicate 1	1.27	68.52	8.48	3.56	1.31	67.64	11.23	3.42	1.26	67.47	11.54	3.32	1.18	71.31	8.01	3.40
Individual Small (2-3 kg) Whole Scottish fish - Replicate 1	1.31	68.86	8.65	3.57	1.32	67.35	11.39	3.48	1.26	67.41	11.42	3.36	1.22	70.78	8.24	3.42
Individual Small (2-3 kg) Whole Scottish fish - Replicate 2	1.26	69.01	7.71	3.80	1.29	67.25	10.73	3.53	1.13	66.45	11.29	3.37	0.74	68.60	9.89	3.49
Individual Small (2-3 kg) Whole Scottish fish - Replicate 2	1.28	69.32	7.48	3.73	1.14	67.11	10.73	3.52	0.78	67.21	11.34	3.30	0.84	68.01	9.55	3.41
Individual Small (2-3 kg) Whole Scottish fish - Replicate 3	1.28	67.26	7.70	3.42	1.20	69.03	8.76	3.51	1.30	67.65	11.11	3.44	1.18	73.97	3.63	3.40
Individual Small (2-3 kg) Whole Scottish fish - Replicate 3	1.30	67.82	8.18	3.51	1.28	68.98	8.26	3.51	1.29	69.57	10.65	3.37	1.27	74.09	4.54	3.41
Individual Small Fillets from small Norwegian fish - Replicate 1	1.03	65.15	15.91	3.01	1.22	66.29	11.64	3.51	1.12	65.85	13.96	3.01	1.01	66.12	13.60	3.27
Individual Small Fillets from small Norwegian fish - Replicate 1	1.07	65.00	15.31	2.97	1.25	65.75	11.52	3.45	1.14	66.60	14.16	3.10	0.92	66.08	14.02	3.24
Individual Small Fillets from small Norwegian fish - Replicate 2	0.77	64.29	13.41	3.08	1.19	64.79	13.50	3.56	1.36	67.77	8.89	3.45	1.02	69.89	8.75	3.24
Individual Small Fillets from small Norwegian fish - Replicate 2	1.08	65.45	13.28	3.10	1.20	65.71	13.94	3.46	1.33	67.85	9.19	3.37	1.20	70.00	9.74	3.28
Individual Small Fillets from small Norwegian fish - Replicate 3	1.17	67.18	11.76	3.42	1.15	67.33	11.06	3.46	1.26	67.34	9.23	3.36	1.07	66.90	12.15	3.32
Individual Small Fillets from small Norwegian fish - Replicate 3	1.14	67.56	11.83	3.46	1.11	67.38	10.89	3.42	1.23	69.14	9.64	3.37	1.01	67.55	11.60	3.31
Individual Small Fillets from small Scottish fish - Replicate 1	1.22	69.72	7.18	3.50	1.03	69.62	8.84	3.42	1.25	69.33	7.39	3.46	1.15	67.17	10.93	3.51
Individual Small Fillets from small Scottish fish - Replicate 1	1.22	69.77	7.52	3.54	1.23	69.90	9.00	3.49	1.23	69.68	7.94	3.51	1.20	67.29	9.58	3.49
Individual Small Fillets from small Scottish fish - Replicate 2	1.22	71.19	6.43	3.53	1.23	70.65	8.15	3.35	1.08	68.33	8.66	3.34	1.18	69.30	8.57	3.51
Individual Small Fillets from small Scottish fish - Replicate 2	1.25	70.61	7.83	3.54	1.26	70.59	8.25	3.44	0.99	68.58	8.52	3.44	1.30	68.54	8.09	3.54
Individual Small Fillets from small Scottish fish - Replicate 3	0.87	68.25	6.73	3.33	1.15	70.27	9.03	3.24	1.22	67.51	11.05	3.35	1.19	71.08	7.26	2.62
Individual Small Fillets from small Scottish fish - Replicate 3	1.08	68.26	9.95	3.40	1.06	69.51	9.07	3.35	1.13	68.21	11.18	3.30	1.25	70.76	7.74	3.42

Appendix 4 – Analysis of Variance Results

The following listings show ANOVA output for linear models, as output from the statistical analysis package used; "prox.pct" denotes % proximate by mass. Significance indicators and the interpretation used in this study follow the conventions below:

Calculated p-value	Interpretation	Indicator
p from 0 to 0.001	Very Strongly Significant	***
p from 0.001 to 0.01	Strongly Significant	**
p from 0.01 to 0.05	Significant	*
p from 0.05 to 0.1	Marginal	.
p>0.1	Not significant	None

Fillet and Whole Fish

Nitrogen

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Season	3	0.1610	0.0537	3.8325	0.010474	*
Cut	1	0.0420	0.0420	2.9967	0.084770	.
Size	2	0.0876	0.0438	3.1265	0.045731	*
Country	1	2.0478	2.0478	146.2544	< 2.2e-16	***
Season:Cut	3	0.1316	0.0439	3.1330	0.026353	*
Season:Size	6	0.1831	0.0305	2.1791	0.045863	*
Cut:Size	2	0.0371	0.0185	1.3236	0.268180	
Season:Country	3	0.5724	0.1908	13.6276	3.236e-08	***
Cut:Country	1	0.0227	0.0227	1.6229	0.203963	
Size:Country	2	0.1991	0.0996	7.1100	0.001008	**
Season:Batch	8	0.5219	0.0652	4.6590	2.679e-05	***
Season:Cut:Size	6	0.0715	0.0119	0.8512	0.531710	
Season:Cut:Country	3	0.0601	0.0200	1.4319	0.234206	
Season:Size:Country	6	0.8130	0.1355	9.6769	1.673e-09	***
Cut:Size:Country	2	0.0242	0.0121	0.8655	0.422199	
Season:Cut:Size:Country	6	0.1177	0.0196	1.4013	0.214962	
Residuals	231	3.2344	0.0140			

Moisture

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Season	3	53.49	17.83	9.2797	8.363e-06	***
Cut	1	11.15	11.15	5.8042	0.0168109	*
Size	2	324.43	162.21	84.4287	< 2.2e-16	***
Country	1	287.87	287.87	149.8276	< 2.2e-16	***
Season:Cut	3	12.43	4.14	2.1567	0.0940584	.
Season:Size	6	165.33	27.56	14.3418	8.722e-14	***
Cut:Size	2	29.27	14.64	7.6180	0.0006325	***
Season:Country	3	130.13	43.38	22.5769	8.805e-13	***
Cut:Country	1	3.12	3.12	1.6247	0.2037880	
Size:Country	2	6.28	3.14	1.6339	0.1975331	
Season:Batch	20	40.70	2.04	1.0592	0.3947606	
Season:Cut:Size	6	27.43	4.57	2.3793	0.0301245	*
Season:Cut:Country	3	8.70	2.90	1.5086	0.2131932	
Season:Size:Country	6	116.47	19.41	10.1030	7.322e-10	***
Cut:Size:Country	2	45.53	22.76	11.8476	1.298e-05	***
Season:Cut:Size:Country	6	123.51	20.58	10.7137	1.918e-10	***
Residuals	220	422.69	1.92			

Fat

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Season	3	111.39	37.13	13.1945	6.404e-08	***
Cut	1	20.79	20.79	7.3876	0.0071214	**
Size	2	481.30	240.65	85.5143	< 2.2e-16	***
Country	1	656.69	656.69	233.3556	< 2.2e-16	***
Season:Cut	3	52.76	17.59	6.2494	0.0004412	***
Season:Size	6	192.90	32.15	11.4246	4.937e-11	***
Cut:Size	2	39.48	19.74	7.0138	0.0011278	**
Season:Country	3	199.88	66.63	23.6752	3.184e-13	***
Cut:Country	1	2.98	2.98	1.0605	0.3043037	
Size:Country	2	13.39	6.69	2.3783	0.0952205	.
Season:Batch	32	245.21	7.66	2.7229	1.095e-05	***
Season:Cut:Size	6	4.58	0.76	0.2712	0.9499246	
Season:Cut:Country	3	7.44	2.48	0.8816	0.4514701	
Season:Size:Country	6	122.78	20.46	7.2717	4.594e-07	***

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Cut:Size:Country	2	45.00	22.50	7.9948	0.0004517	***
Season:Cut:Size:Country	6	92.17	15.36	5.4587	2.887e-05	***
Residuals	208	585.34	2.81			

Ash

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Season	3	0.51124	0.17041	13.9515	2.786e-08	***
Cut	1	0.01376	0.01376	1.1264	0.289850	
Size	2	0.07185	0.03593	2.9413	0.055131	.
Country	1	0.06565	0.06565	5.3746	0.021464	*
Season:Cut	3	0.16850	0.05617	4.5983	0.003911	**
Season:Size	6	0.07443	0.01240	1.0156	0.416334	
Cut:Size	2	0.13739	0.06869	5.6239	0.004218	**
Season:Country	3	0.07442	0.02481	2.0308	0.110886	
Cut:Country	1	0.00014	0.00014	0.0111	0.916203	
Size:Country	2	0.00096	0.00048	0.0394	0.961362	
Season:Batch	44	1.32208	0.03005	2.4599	1.337e-05	***
Season:Cut:Size	6	0.13952	0.02325	1.9037	0.081980	.
Season:Cut:Country	3	0.01227	0.00409	0.3349	0.800124	
Season:Size:Country	6	0.22551	0.03758	3.0770	0.006693	**
Cut:Size:Country	2	0.01149	0.00574	0.4702	0.625593	
Season:Cut:Size:Country	6	0.07738	0.01290	1.0559	0.390613	
Residuals	196	2.39407	0.01221			

Frame Mince

Nitrogen

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Season	3	9.1827	3.0609	556.7057	< 2.2e-16	***
Season:ProdBatch	8	0.2692	0.0336	6.1197	1.228e-06	***
Season:Batch	8	0.2636	0.0330	5.9936	1.691e-06	***
Residuals	124	0.6818	0.0055			

Moisture

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Season	3	3714.7	1238.2	524.1834	< 2.2e-16	***
Season:ProdBatch	8	71.0	8.9	3.7588	0.0006198	***
Season:Batch	20	110.7	5.5	2.3428	0.0026432	**
Residuals	112	264.6	2.4			

Fat

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Season	3	4121.4	1373.8	782.0320	< 2.2e-16	***
Season:ProdBatch	8	65.7	8.2	4.6733	7.024e-05	***
Season:Batch	32	269.8	8.4	4.8001	8.570e-10	***
Residuals	100	175.7	1.8			

Ash

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Season	3	2.18210	0.72737	62.1002	< 2.2e-16	***
Season:ProdBatch	8	0.11837	0.01480	1.2633	0.2730466	
Season:Batch	44	1.17954	0.02681	2.2888	0.0004947	***
Residuals	88	1.03072	0.01171			

Fat-free Nitrogen

Whole Fish and Fillet

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Season	3	0.00878	0.002926	0.2053	0.892475	
Cut	1	0.08593	0.085935	6.0315	0.015851	*
Size	2	0.18254	0.091271	6.4060	0.002446	**
Country	1	0.14122	0.141219	9.9116	0.002189	**
Season:Cut	3	0.01421	0.004737	0.3325	0.801891	
Season:Size	6	0.21756	0.036261	2.5450	0.024896	*
Cut:Size	2	0.01507	0.007535	0.5289	0.590983	
Season:Country	3	0.08751	0.029171	2.0474	0.112366	

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Cut:Country	1	0.02798	0.027981	1.9639	0.164324	
Size:Country	2	0.06174	0.030872	2.1668	0.120114	
Season:Cut:Size	6	0.05919	0.009866	0.6924	0.656239	
Season:Cut:Country	3	0.08338	0.027792	1.9506	0.126616	
Season:Size:Country	6	0.48059	0.080099	5.6218	4.913e-05	***
Cut:Size:Country	2	0.01751	0.008756	0.6145	0.543015	
Season:Cut:Size:Country	6	0.13709	0.022848	1.6036	0.154432	
Residuals	96	1.36779	0.014248			

Frame Mince

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Season	3	4.2827	1.42758	128.6334	<2e-16	***
Season:ProdBatch	8	0.1177	0.01471	1.3253	0.2487	
Residuals	60	0.6659	0.01110			