Health promoting, safe seafood of high eating quality in a consumer driven fork-to-farm concept

EU Integrated Project no 506359

Evaluation of the profile of lipids as a tool to discriminate wild from farmed fish

Iciar Martinez

SEAFOODplus Publication Series
Report 6.3.8 - 2006
ISBN 978-87-7075-001-1

Health promoting, safe seafood of high eating quality in a consumer driven fork-to-farm concept

EU Integrated Project no 506359

A report from RTD area 6

'Seafood traceability to ensure consumer confidence'

Project 6.3 VALID

Evaluation of the profile of lipids as a tool to discriminate wild from farmed fish

Deliverable D8

Iciar Martinez

SINTEF Fisheries and Aquaculture Ltd
7465 Trondheim
Norway

SEAFOODplus Publication Series
Report 6.3.8 - 2006
ISBN 978-87-7075-001-1

Deliverable 8

Evaluation of the profile of lipids as a tool to discriminate wild from farmed salmon

Open Information Only

by

Iciar Martinez
SINTEF Fisheries and Aquaculture Ltd
June 2006

Deliverable 8

Evaluation of the profile of lipids as a tool to discriminate wild from farmed salmon

Iciar Martinez
SINTEF Fisheries and Aquaculture Ltd.

Summary

This is a report on the evaluation of the profile of lipids from muscle of salmon as a tool to discriminate wild from farmed Atlantic salmon based on published data and on our original on-going research work using NMR-based profiling of lipids. Accordingly, the report is divided in two parts: one part based on published data that may be open and a **second part based on our ongoing research that is confidential and has not been included in this short version.**

The fatty acid profiles of oils extracted from Atlantic salmon muscle always reflected the profile of the diet and even after a washing out period the n3/n6 ratio was not fully recuperated. Farmed from wild Atlantic salmon were easily differentiated by the ratio n3/n6 calculated as follows: [(C20:5n3+C22:5n3+C22:6n3)/C18:2n6], in those works were the composition of farmed and wild fish were provided. However, when combining the results of all the publications used for this review it lost is value. This may be due to differences in the analytical procedures used by different research groups, to the great differences in the composition of the farmed Atlantic salmon and also to the much larger number of farmed than wild Atlantic salmon examined here.

Index

##########

Abbreviations

CLA	Conjugate linoleic acid
DHA	Docosahexaenoic acid: C226n-3
DPA	Docosapentaenoic acid: C22:5n3
EPA	Eicosapentaenoic acid,: C205n-3
FA	Fatty acid
FAME	Fatty acid methyl ester
GC	Gas chromatography
MUFA	Monounsaturated fatty acid
NMR	Nuclear magnetic resonance
PCBs	Polychlorinated biphenyls
PUFA	Polyunsaturated fatty acid
SFA	Saturated fatty acid
n3/n6	(C20:5n3+C22:5n3+C22:6n3)/C18:2n6

1. PART 1. OPEN. PUBLISHED WORKS

1.1 Fatty acid composition of farmed and wild fish: Published works

1.1.1 Introduction

It is well known the fatty acid profile of the skeletal muscle in Atlantic salmon reflect the fatty acid composition of the feed [Hardy et al., 1987; Thomassen & Røsjø, 1989; Jobling et al., 2002; Bell et al., 2002, 2003a,b; Jobling 2004]. In the past, the source of dietary lipids for farmed salmon has been oil from marine fish, usually from pelagic species such as herring, menhaden, capelin or anchoveta. More recently, however, the overexploitation of pelagic fish and the high price of their oils have led to the search for alternative sources and the introduction of vegetable oils, which are more abundant and cheaper, in newer formulations of fish feeds.

There are several analytical methods suitable to discriminate farmed form wild *Salmo salar* [Martinez, 2006; Sægrov et al., 1997]. The present reports focuses only on the evaluation of the profile of lipids from muscle of salmon as a tool to discriminate wild from farmed Atlantic salmon. To do so, we have compared the fatty acid profile of lipids extracted from the white skeletal muscle of wild salmon with that of farmed. The information has been obtained from published works and from our on going work.

1.1.2 Fatty acid composition of vegetable and fish oils

Figure 1 shows the composition of some vegetable oils such as corn, cottonseed, linseed, olive, palm, rapeseed, soybean and sunflower, used as partial substitutes for fish oils in Atlantic salmon diets [Data from Gunstone et al., 1994, Henderson et al., 1996; Dosanjh et al., 1998; Grisdale-Helland et al., 2002; Torstensen, et al., 2004b]. A common feature to these vegetable oils is the very low or undetectable amount of the long chain omega-3 PUFAS C20:5n3 (EPA) and C22:6n3 (DHA) characteristic from fish oils [Figure 2] and believed to exert a preventive role in cardiovascular diseases, inflammation and cancer [Drevon et al., 1995; Vognild et al., 1998] and the fact that often a single fatty acid may account for about 50% of the total fatty acid content in these oils: about 40% of the total fatty acids in palm oil is C16:0; in rapeseed oil C18:1n9 accounts for over 50% and in olive oil for almost 80% of the total; in soybean, corn and cottonseed oils C18:2n6 makes up over 50%, as it does C18:3n3 in linseed oil.

As shown in Figures 2 and 3, the composition of fatty acids in fish oils is more complex, i.e., there are more fatty acids present in detectable amounts and it is seldom that only one of them makes over 25% total. As in vegetable oils, which fatty acid is more abundant is species dependant. C16:0 and C18:1n9 are relatively abundant in all fish oils, but C22:1 (several isomers) is relatively more abundant in coho salmon, capelin, herring and sandeel and C22:6n3 is more abundant (over 10%) in Atlantic and coho salmon, Peruvian PUFA, sandeel and sardines than in capelin, herring or menhaden. All these species are also a good source of C20:5n3, where it makes up about 10% of the total.

1.1.3 Fatty acid composition of farmed and wild fish: Published works

Aursand et al. [2000] published the fatty acid composition of Norwegian wild Atlantic salmon and of specimens farmed in Norway and Scotland as well as the composition of feeds used at these two locations and Figure 4 shows the results of principal component analysis performed on the data reported by these authors. The first principal component (PC1) explained 67% of the total variability of the model and separated almost all wild from farmed fish. The main contributor to PC1 was C22:1 a fatty acid abundant in both diets and also in capelin, herring and sandeel [Figure 4]. Inclusion of the ratio n3/n6 [(C20:5n3+C22:5n3+C22:6n3)/C18:2n6] as a variable in the principal component analysis classified all the wild as farmed samples correctly except for two of the samples: one wild clustered together with the farmed fish and one farmed appeared with the wild ones [Figure 5]. Consultation to the authors of the manuscript indicated that the samples might have been mislabelled in the original work. In the model including n3/n6 as a variable, PC1 explained 71% of the variability where n3/n6 and C22:1 were the two variables with the highest loading on PC1 and the highest relevance for the classification: high n3/n6 ratios and low amounts of C22:1 being characteristic for wild salmon and the opposite for farmed salmon. The two diets analyzed in this work were relatively rich in C22:1, poorer than the salmon in the content of C22:6n3, and contained between 2 and 3 times more C18:2n6

than the wild fish, which would account for the low n3/n6 ratio. The authors reported C18:2n6 to be significantly different in the wild and farmed groups [Aursand et al, 2000] however the main influence this fatty acid exerted on the model seemed to be the mentioned lowering of the n3/n6 ratio.

Nichols et al., [2002] examined the effect of the amount of oil content (38% and 24% of oil content in the feeds) of three different feeds on the composition of Atlantic salmon farmed in Australia. In all cases, the fish had proportionally higher levels of C18:1n9 and C22:6n3 than the feed, while the feed had higher levels of C20:5n3 [see Figure 6]. The authors reported that there was no evidence of increased oil levels in the flesh of fish fed diets with higher oil levels.

The relationship between composition of the feeds and flesh from Norwegian farmed salmon from the works of Eienen et al. [1998]; Bjerkeng et al. [1999] and Refsgaard et al. [1998] is shown in Figure 7. Feeds D-06 and D-56 were rich in C20:5n3 and feed D-07 in C22:1. The flesh of the fish, on the other hand, had higher levels of C18:1n9 and C22:6n3 than the diet and in the case of diet D-07 there did not seem to be a direct relationship between the amount of C22:1 in the diet (which was relatively high) and the flesh (where it was lower).

Berge et al. [2004] examined the effect of the addition of different amounts of CLA to the feed of fry salmon [Figure 8]. The composition of the fish at the beginning of the test trials was clearly different from those sampled 12 weeks later, the main differences being their higher content in C22:6n3 (almost twice) and lower of C20:1n9 and C22:1 (almost half). At the end of the experiment, fish fed the higher 1% and 2% doses of CLA deposited more n-3 fatty acids than those fed none or low (0.5%) CLA and C22:6n3 was the fatty acid that increased most, although it never reached the higher values of the fry at the beginning of the experiment. Whether this difference is to be expected due to normal physiological changes of the fish as it grows (since fish fed standard control diet also had lower C22:6n3 values after 12 weeks) was not addressed by the authors, however, as they do indicate, this experiment should be repeated with fish of commercial size.

In the search for alternative sources of fish oils, Olsen et al. [2004] examined the suitability of feeding *Calanus* oil to Norwegian farmed salmon in seawater and compared their fatty acid composition to that of salmon fed a diet containing fish oil instead. Although the diet containing *Calanus* oil had higher contents in C14:0 and C18:4n3, and lower in C22:1, C18:1n9 and C20:1n9, the composition of the two groups of fish was more similar than what the differences in the diet might have indicated [Figure 9] and both diets produced salmon that were a good source of the desirable C20:5n3 and C22:6n3.

Menoyo et al. [2003] examined dietary fatty acid strategies of metabolic relevance (*sic*) in 1.8 kg Atlantic salmon by using feeds varying in the levels of n-3 and saturated fatty acids. They used a 2x2 factorial design, where diets contained: low levels of n-3 and either low of saturated fatty acids (D-15) or high levels of saturated fatty acids (D-16) and high levels of n-3 and either low of saturated fatty acids (D-17) or high levels of saturated fatty acids (D-18). The fatty acid composition of the diet was reflected in the fillet [Figure 10] except for the saturated fatty acids where high amounts of C16:0 did not lead to similarly high contents in the fillet. Again, the fish muscle contained proportionally higher amounts of C18:1n9 and C22:6n3 than in the diet and relatively lower amounts of C22:1 and C20:5n3.

The effect of feeding for a year alternative lipid sources to Atlantic salmon was examined by Rosenlund et al. [2001]. The scores plot [Figure 11] shows the composition of the muscle is a clear reflection of the diet as the fish samples form clusters with their respective feeds. One clearly segreagated cluster was made up of the salmon and diet containing only capelin oil (D-23 and F-23) and characterized by the high contents in C22:1. Another segregated cluster was formed by the diets and fish fed soybean oil (D-24 and F-24), characterized in this case by the high amounts of C18:2n6. Fish fed linseed (D-20and F-20) and palm (D-22 and F-22) oils had somehow higher levels of C16:0, C18:1n9 but, mostly, C18:3n3. Fish fed rapeseed (D-19 and F-19) and poultry (D-21 and F-21) oils were mostly characterized by higher levels of C18:1n9. As in the previous works, the fillets of all the experimental diets had proportionally higher levels of C18:1n9 and C22:6n3 and lower of C22:1 and C20:5n3 than their respective diets.

Torstensen et al., [2004, 2004b] showed the effect of replacing dietary fish (capelin) oil with increasing amounts of rapseed oil and olive oil in a 42 weeks feeding trial in seawater in Norway using post-smolt fish [Figures 12 and 13]. PC1 explains 80% of the variability of the models and the variables with the highest loadings are C18:1n9, C18:2n6 and C18:3n3. After a washout period of

1788 days degree (°C) when the salmon diets used contained only fish oil, only the fish that had been fed the highest amount of rapeseed oil (75% and 100% of the oil source) retained higher levels of C18:1n9 and C18:2n6. All fish at the end of this period had similar levels of the PUFAs C20:5n3 and C22:6n3 and high levels of C20:1n9 [Figure 14]. In all cases, between 13 and 18% of the variability seemed to be explained by PC2 on which C20:1n9 and C22:1 and C16:0 had the highest positive loadings.

The same authors examined in a later study the effect of a 75% and 100% substitution of fish oil by vegetable oil in a 2 years' trial in Norway and Scotland [Torstensen et al., 2005]. The vegetable oil diet was made up of a mixture of rapeseed oil, palm oil and linseed oil designed to produce balanced levels of saturated, monounsaturated and polyunsaturated fatty acids. The control diet contained capelin oil and all fish received 100% fish oil during the last 5-6 months prior to slaughter. The main differences in the fatty acid composition of the flesh after harvesting were that salmon fed the vegetable oils contained higher amounts of C18:1n9, C18:2n6 and C18:3n3 and lower amounts of 22:1; 20:1n9, C22:6n3 and C20:5n3, the differences being proportional to the degree of substitution [Figure 15].

Bell et al. [2003a] examined the changes in the fatty acid composition of Atlantic salmon flesh by substituting fish oil with different amounts of rapeseed oil: from 10% to 100% substitution followed by a 100% fish oil diet. Figure 16 shows the results of only the 100% substitution. As in the previous works, the main changes were the high increase in the amount of C18:1n9, C18:2n6 and C18:3n3 in the rapeseed oil fed fish and higher amounts of C16:0, C20:1n9, C22:1, C20:5n3, C22:5n-3, C22:6n3 in the salmons fed the fish oil diet, the differences being proportional to the degree of substitution. However, in this work, the reduction in the levels of C20:5n3 and C22:6n3, that was progressive as the amount of rapeseed oil was increased, did not reach dramatic levels up to a 50% substitution. A 100% substitution on the other hand reduced by half the flesh content in these fatty a ids. Switching to a commercial diet containing only marine fish oil showed that the time necessary to restore the levels of C18:2n6, C20:5n3 and C22:6n3 to values similar to those of Atlantic salmon fed fish oil were different for each fatty acid. Thus, the levels of C20:5n3 was restored fastest, after 4 weeks on the wash diet; while restoration of C22:6n3 required 12 weeks and even after this period, the levels of C18:2n6 were still higher than in the salmon fed the fish oil diet.

Grisdale-Helland et al. [2002] examined the effect of feeding diets containing 100% capelin oil, 100% crude rapeseed oil and a 50-50 mixture of the two to Norwegian Atlantic salmon for about 1050 days degree (°C). In this case, the most dramatic effect was the high increase in the amount of C18:2n6, characteristic for soybean oil and, to a lesser degree, C18:1n9 [Figure 17].

The effect of feeding dietary blends of menhaden and canole oils to Atlantic salmon was examined by Dosanjh et al. [1998]. Again, the muscle lipid composition mirrored that of the feeds and at the end of the treatment salmon fed blended oils had higher levels of C18:1n9 and C18:2n6 and lower levels of C16:0, C20:5n3, C22:5n-3 and C22:6n3 than fish fed 100% menhaden oil, and the degree of difference was proportional to the canole oil content in the feeds [Figure 18]. Similarly, inclusion of rapeseed and crude palm oil in feed formulations induced an increase in the amounts of C18:1n9 C18:2n6 and a decrease of C20:5n3 C22:5n-3 C22:6n3 in the salmon flesh.

The fatty acid composition of Norwegian Atlantic salmon fed for 12 weeks diets containing blends of fish, rapeseed and palm oils was reported by Ng et al. [2004]. The flesh composition mirrored that of the feed and two principal components explained 98% of the total variability of the model. PC1, explained 84% with the fatty acid characteristic for salmon C22:6n3 showing negative loadings and the characteristic vegetable fatty acids C18:1n9; C18:2n6 and C16:0 having positive loadings on the same factor. PC2 was mainly attributed to the amount of C16:0 [Figure 19].

Figures 1 to 19

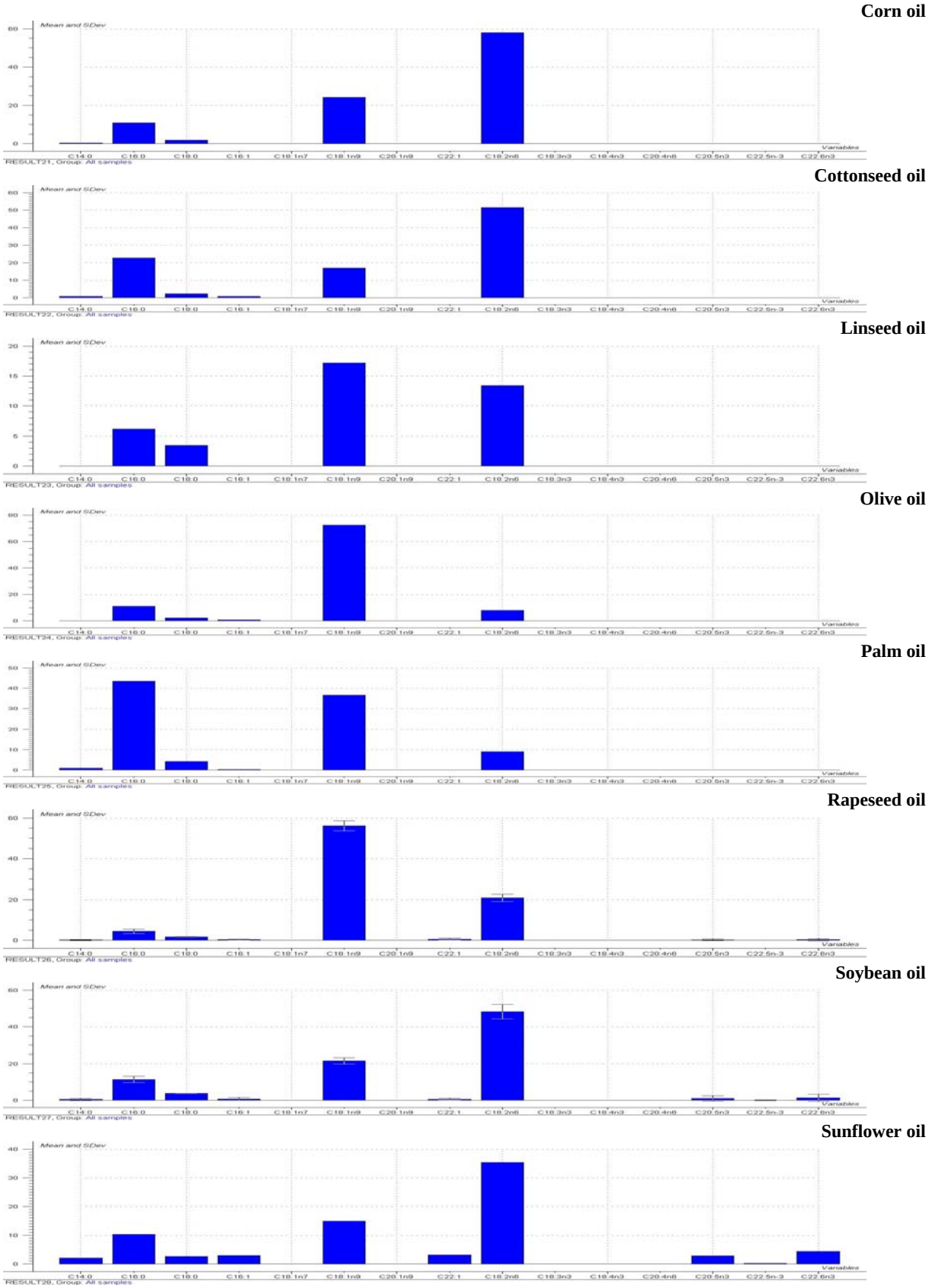


Figure 1. From top to bottom, fatty acid composition of the indicated vegetable oils [Data from Gunstone et al., 1994; Henderson et al., 1996; Dosanjh et al., 1998; Grisdale-Helland et al., 2002; Torstensen, et al., 2004b].

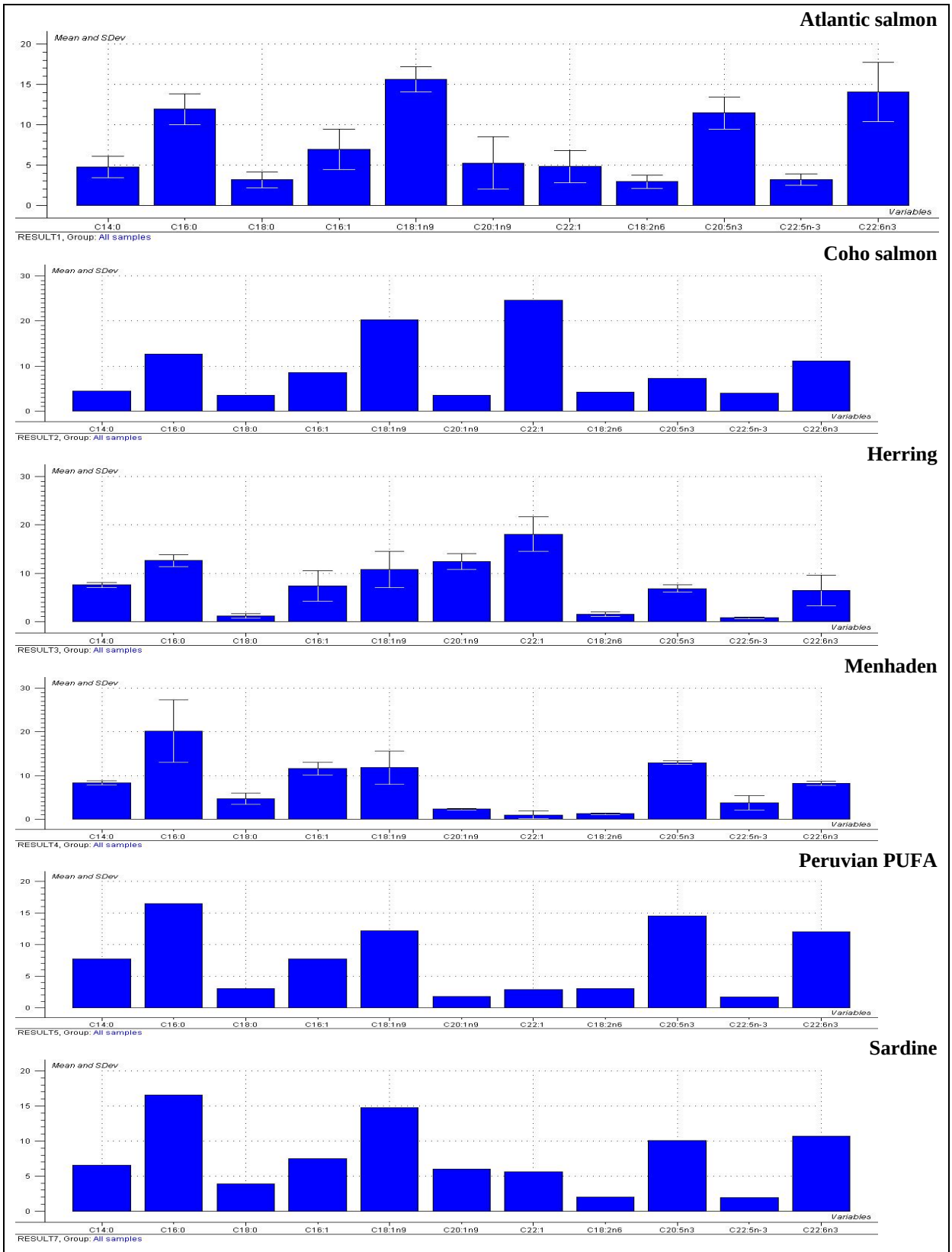


Figure 2. Fatty acid composition of the indicated fish oils [Data from Gunstone et al., 1994, Alessandri et al., 1993; Dosanjh et al., 1998; Bjerkeng et al., 1999b; Aursand et al., 2000; Grisdale-Helland et al., 2002; Torstensen et al., 2004b].

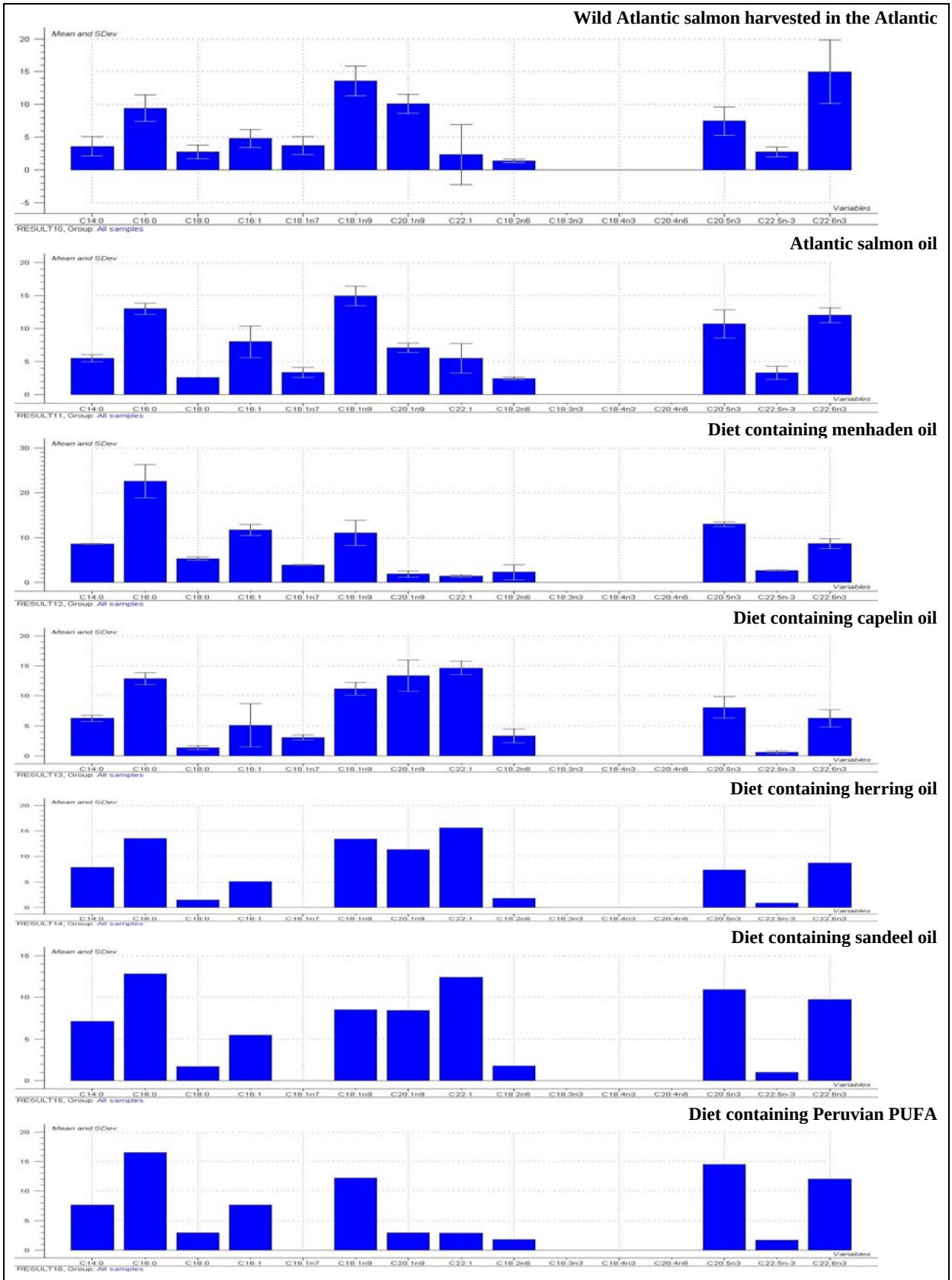


Figure 3. Fatty acid composition of the indicated oils [Data from Alessandri et al., 1993; Henderson et al., 1996; Dosanjh et al., 1998; Bjerkeng et al., 1999b; Aursand et al., 2000; Rosenlund et al., 2001; Grisdale-Helland et al., 2002; Bell et al., 2003a,b; Olsen et al., 2004; Torstensen et al., 2004,2004b,2005].

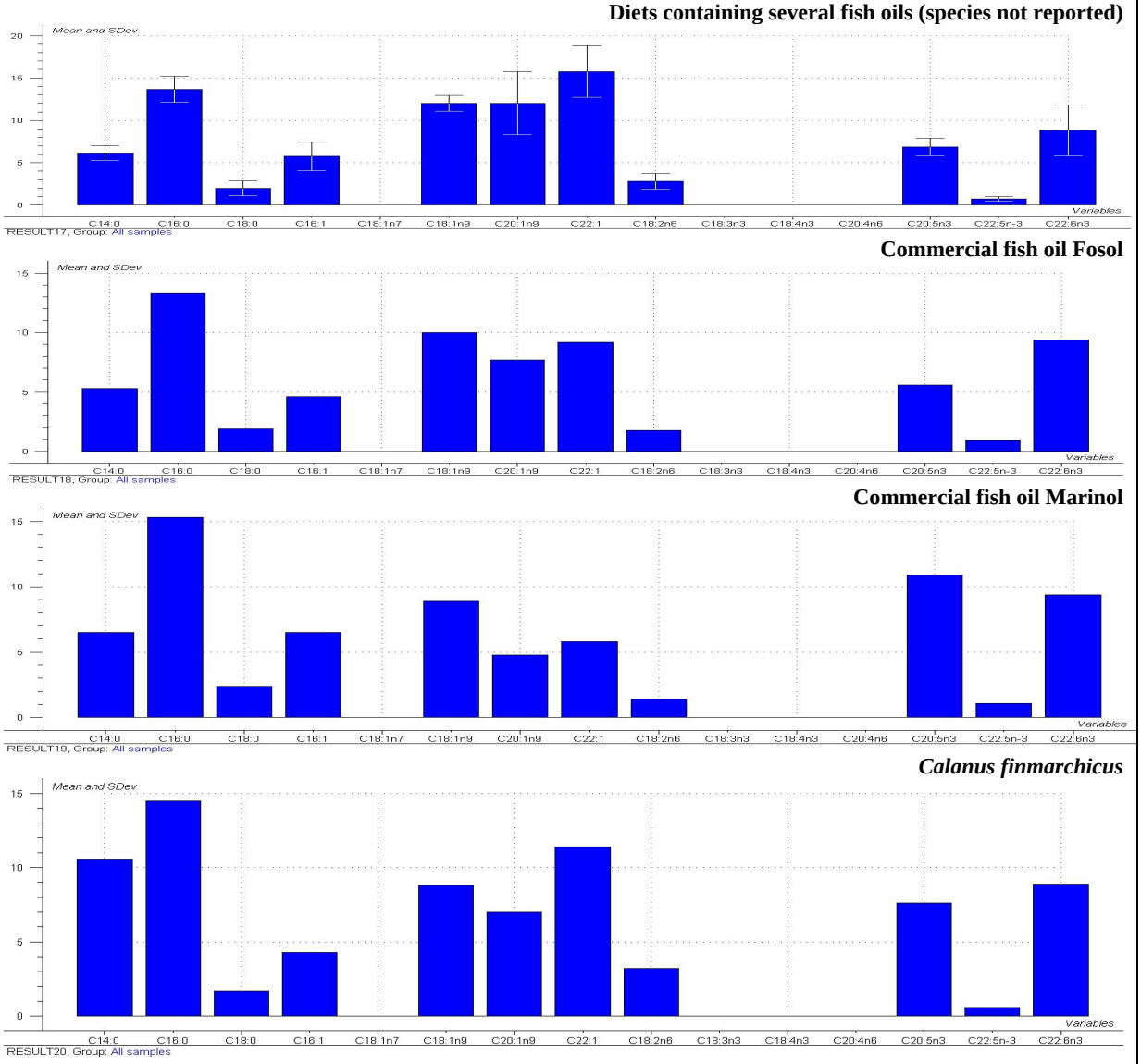


Figure 3. Cont. Fatty acid composition of diets containing different fish oils, as indicated in the figure [Data from Alessandri et al., 1993; Henderson et al., 1996; Dosanjh et al., 1998; Bjerkeng et al., 1999b; Aursand et al., 2000; Rosenlund et al., 2001; Grisdale-Helland et al., 2002; Bell et al., 2003; Olsen et al., 2004; Torstensen et al., 2004; Torstensen et al., 2004b; Torstensen et al., 2005].

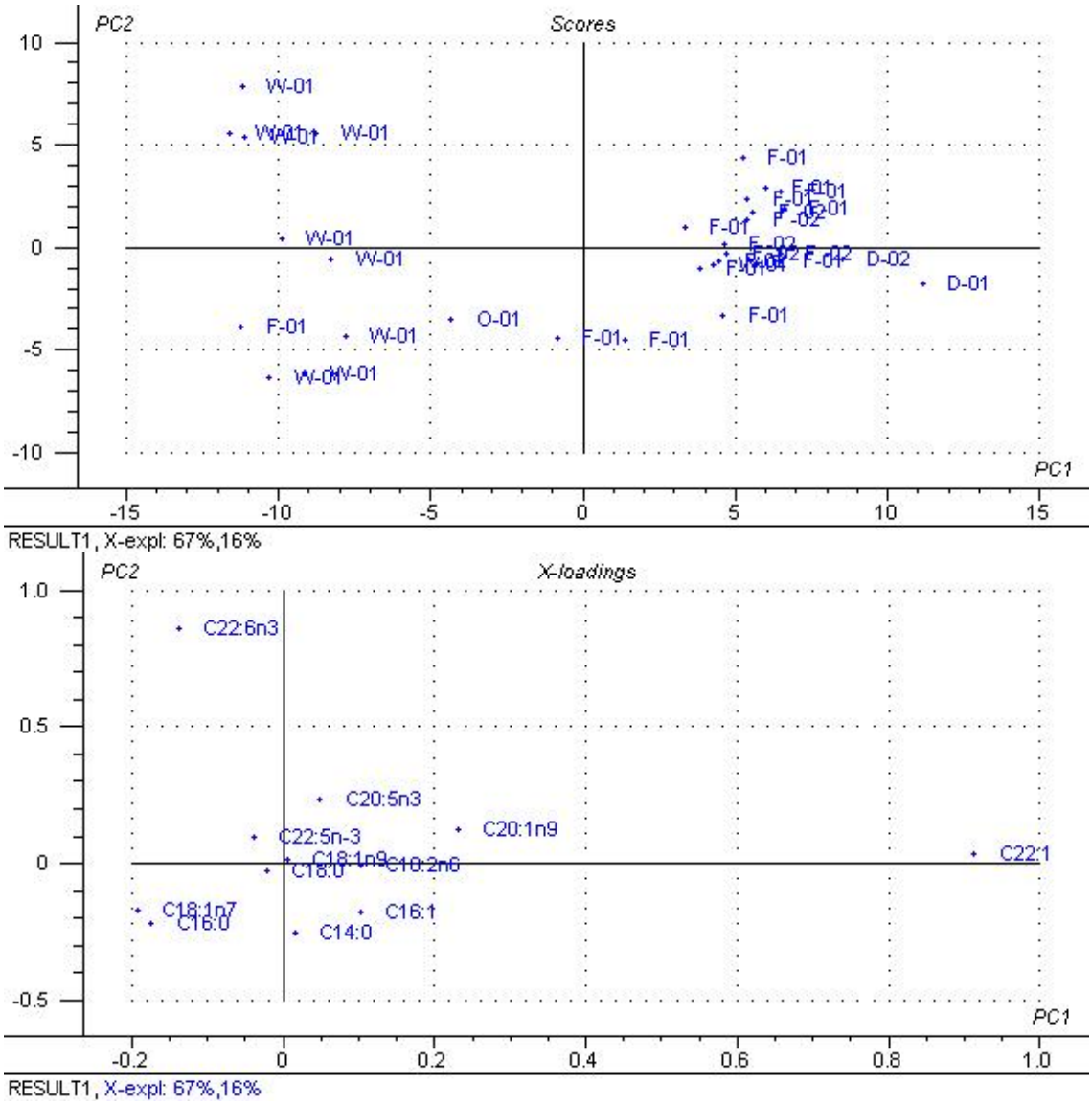


Figure 4.-Principal component analysis on the fatty acid composition of Norwegian wild Atlantic salmon (W-01) and of specimens farmed in Norway (F-01) and Scotland (F-02) as well as the composition of feeds used at these two locations (D-01 and D-02 respectively) and of Atlantic salmon oil (O-01). The model was fully cross validated and each variable was given a weight of 1. Scores plot is shown on top and loadings plot on the bottom. The first at second principal components explained 67% and 16% of the total variability respectively. Data from Aursand et al. [2000].

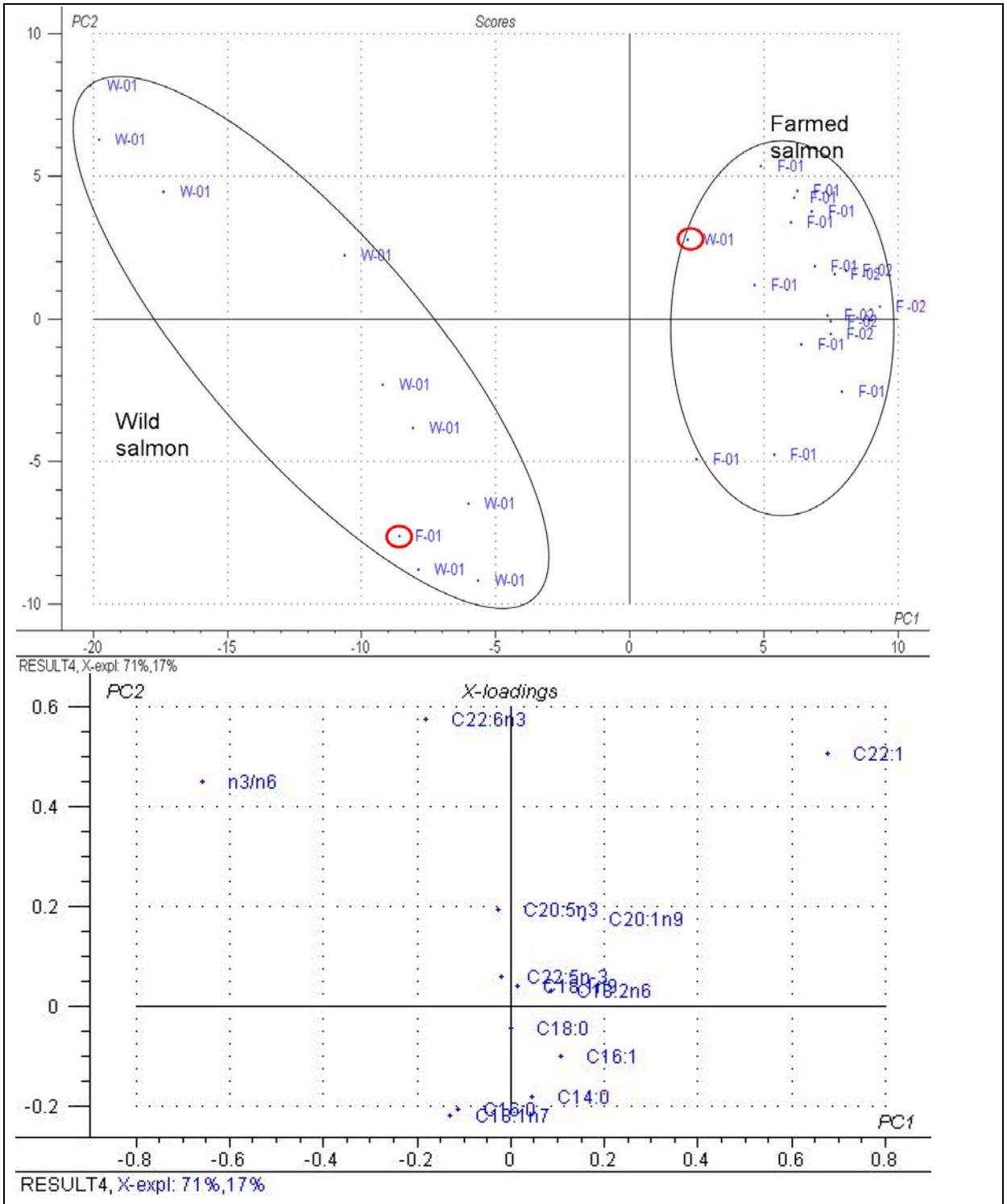


Figure 5.-Principal component analysis on the fatty acid composition of Norwegian wild Atlantic salmon (W-01) and of specimens farmed in Norway (F-01) and Scotland (F-02). In addition to the fatty acids, the ratio n3/n6 was also included as a variable. The model was fully cross validate and each variable was given a weight of 1. Scores plot is shown on top and loadings plot on the bottom. The first at second principal components explained 71% and 17% of the total variability respectively. The small red circles enclose 2 samples that apparently clustered wrongly: one claimed-wild sample clustered together with the farmed fish and one claimed-farmed clustered with the wild ones. Data from Aursand et al. [2000].

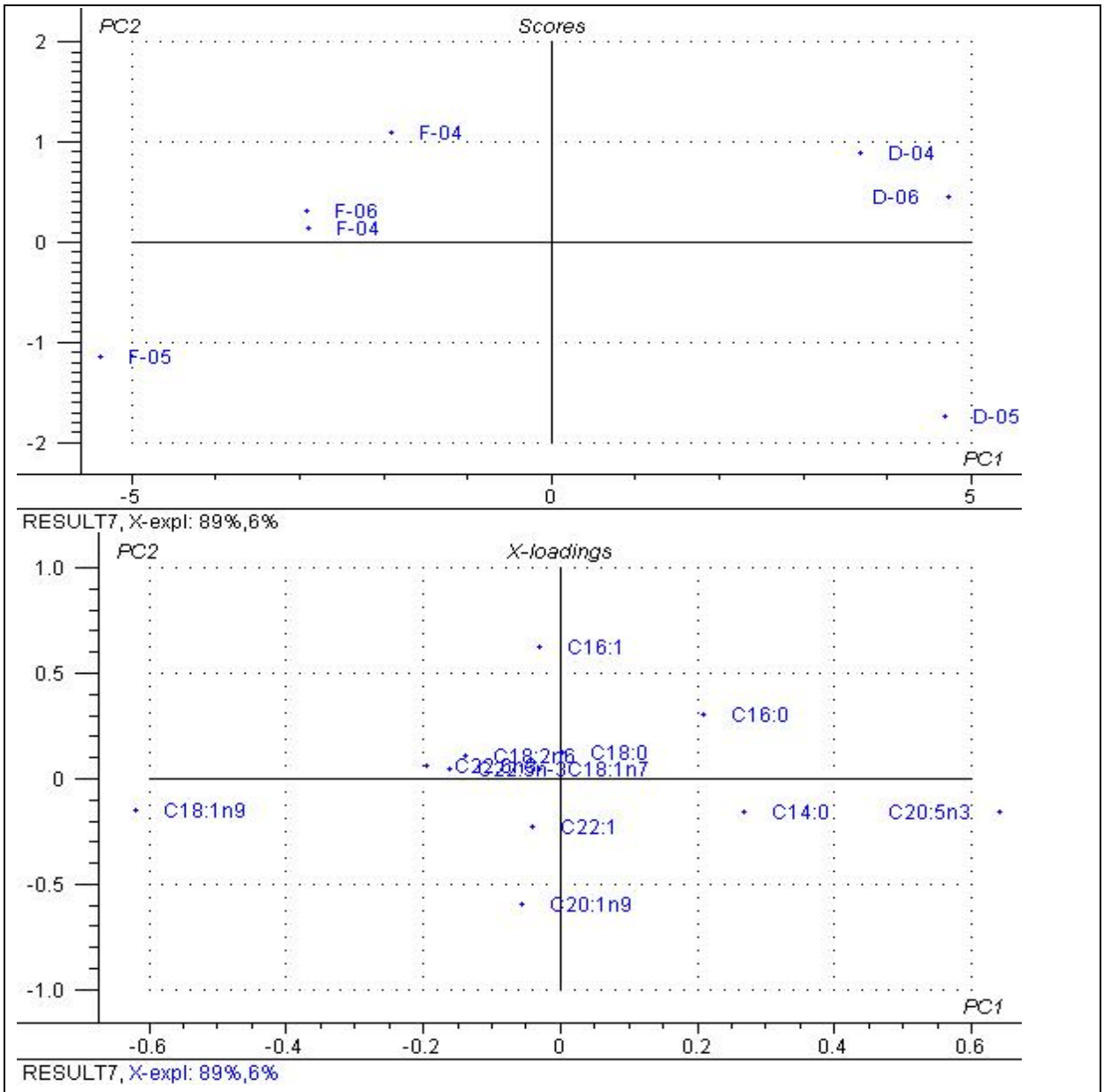


Figure 6.-Principal component analysis on the fatty acid composition of Australian farmed Atlantic salmon (F-04 to F-06) and of feeds used (D-04 to D-06) during 5 months. Feed F-04 had a 24% oil and feeds D-05 and D-06 a 38%. The model was fully cross validated and each variable was given a weight of 1. Scores plot is shown on top and loadings plot on the bottom. PC1 and PC2 explained 89% and 6% respectively of the total variability in the model. Data from Nichols et al., [2002].

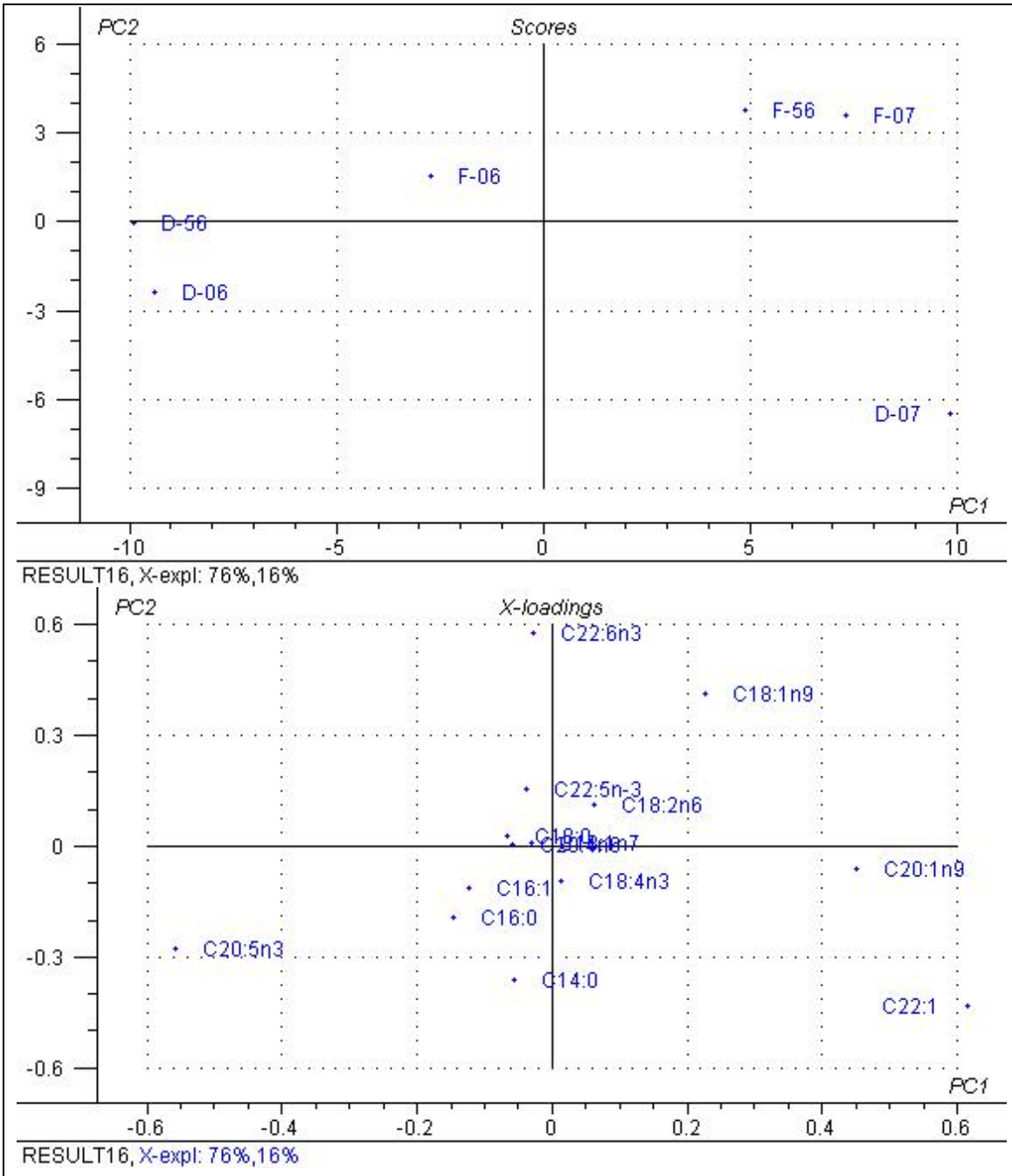


Figure 7.-Principal component analysis on the fatty acid composition of Norwegian farmed Atlantic salmon (F-06, F-07 and F-56) and of feeds used (D-06, D-07 and D-56) during about 12 weeks. The model was fully cross validated and each variable was given a weight of 1. Scores plot is shown on top and loadings plot on the bottom. PC1 and PC2 explained 76% and 16% respectively of the total variability in the model. Data from Einen et al., [1998]; Bjerkeng et al., [1999] and Refsgaard et al. [1998]

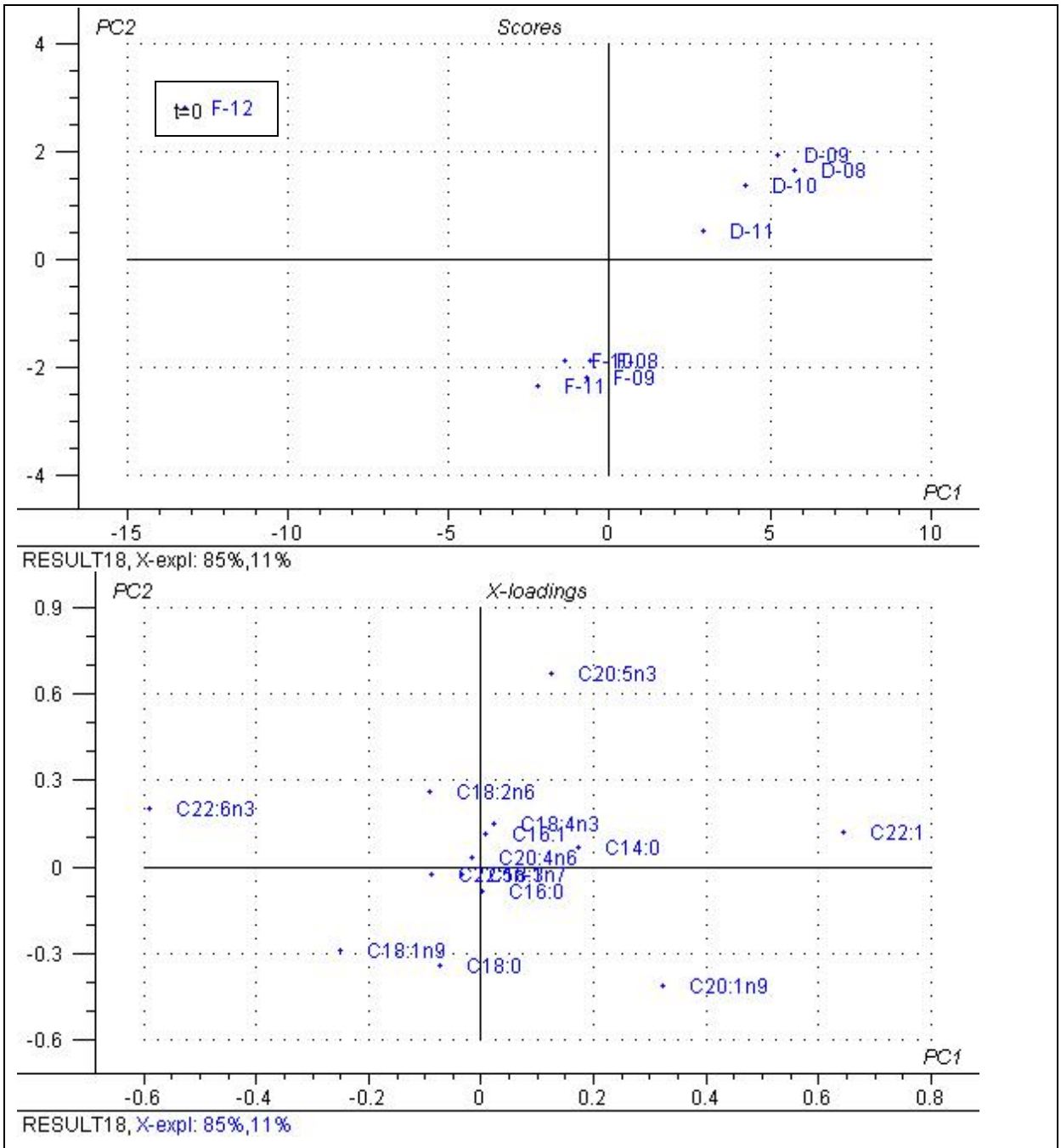


Figure 8.-Principal component analysis on the fatty acid composition of Norwegian farmed Atlantic salmon (F-08 to F-12)) and of feeds used (D-08, D-09, D-10 and D-11 contained 0%, 0.5%, 1 and 2% CLA respectively) for 12 weeks. Fish F-12 was sampled at the beginning of the feeding trials (t=0). The model was fully cross validated and each variable was given a weight of 1. Scores plot is shown on top and loadings plot on the bottom. PC1 and PC2 explained 85% and 11% respectively of the total variability in the model. Data from Berge et al. [2004]

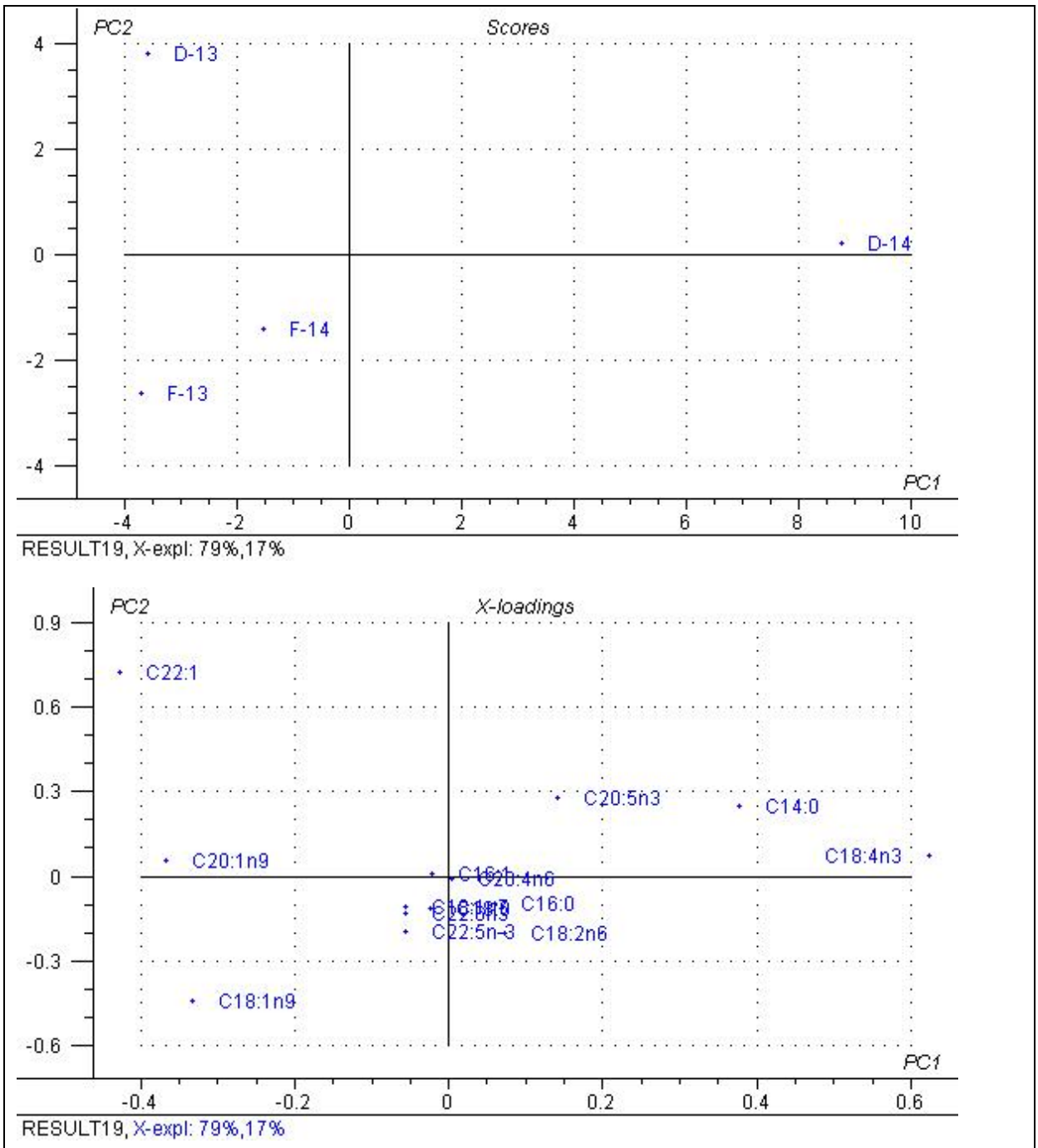


Figure 9.-Principal component analysis on the fatty acid composition of Norwegian farmed Atlantic salmon (F-13 and F-14) feed respectively a diet containing fish oil (D-13) or *Calanus* oil (D-14) during 20 weeks. The model was fully cross validated and each variable was given a weight of 1. Scores plot is shown on top and loadings plot on the bottom. PC1 and PC2 explained 79% and 17% respectively of the total variability in the model. Data from Olsen et al. [2004]

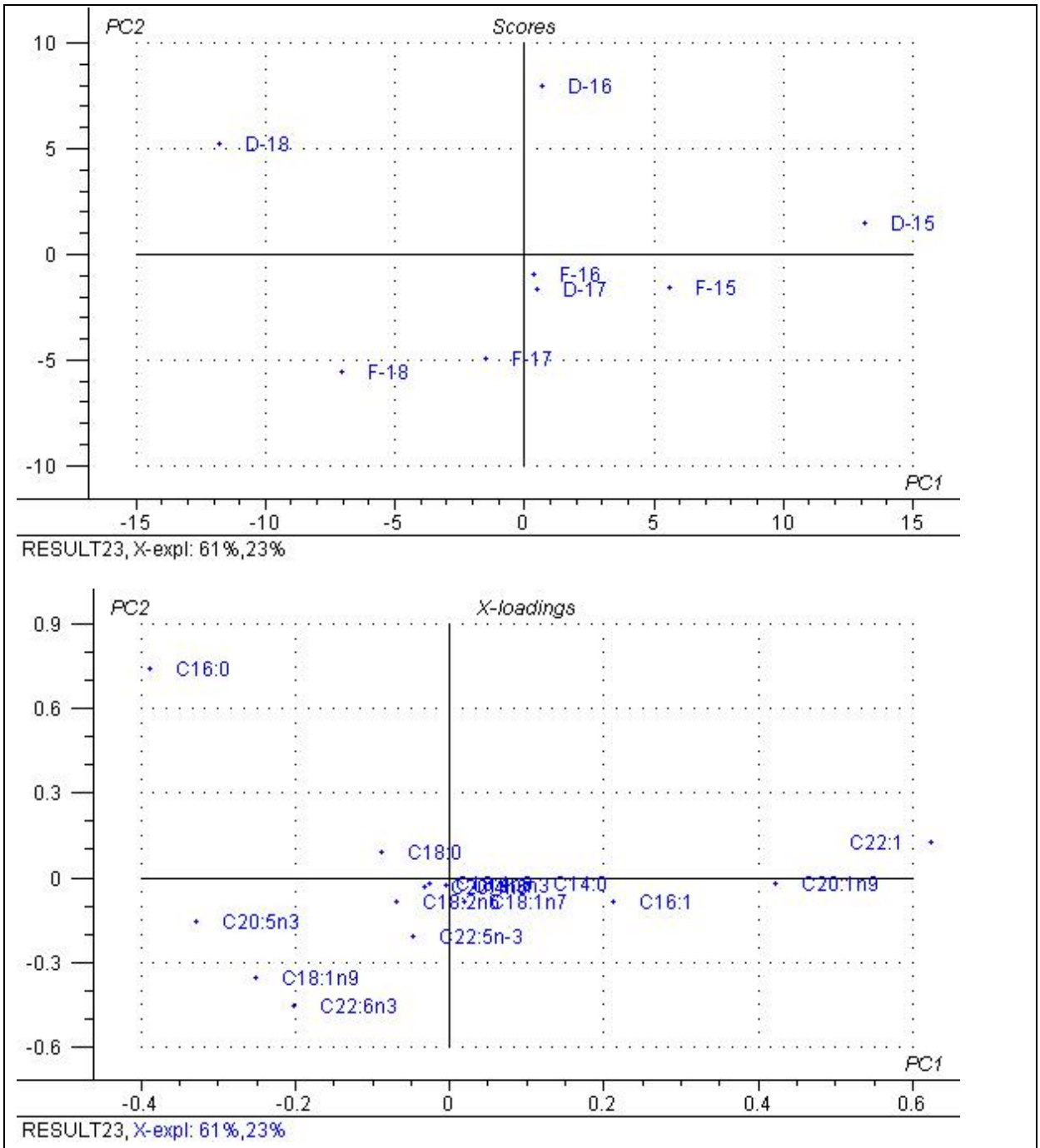


Figure 10.-Principal component analysis on the fatty acid composition of Norwegian farmed Atlantic salmon (F-15 to F-18) fed with diets containing low levels of n-3 and either low of saturated fatty acids (D-15) or high levels of saturated fatty acids (D-16) and high levels of n-3 and either low of saturated fatty acids (D-17) or high levels of saturated fatty acids (D-18) respectively, during a period of 24 weeks. The model was fully cross validated and each variable was given a weight of 1. Scores plot is shown on top and loadings plot on the bottom. PC1 and PC2 explained 61% and 23% respectively of the total variability in the model. Data from Menoyo et al. [2003].

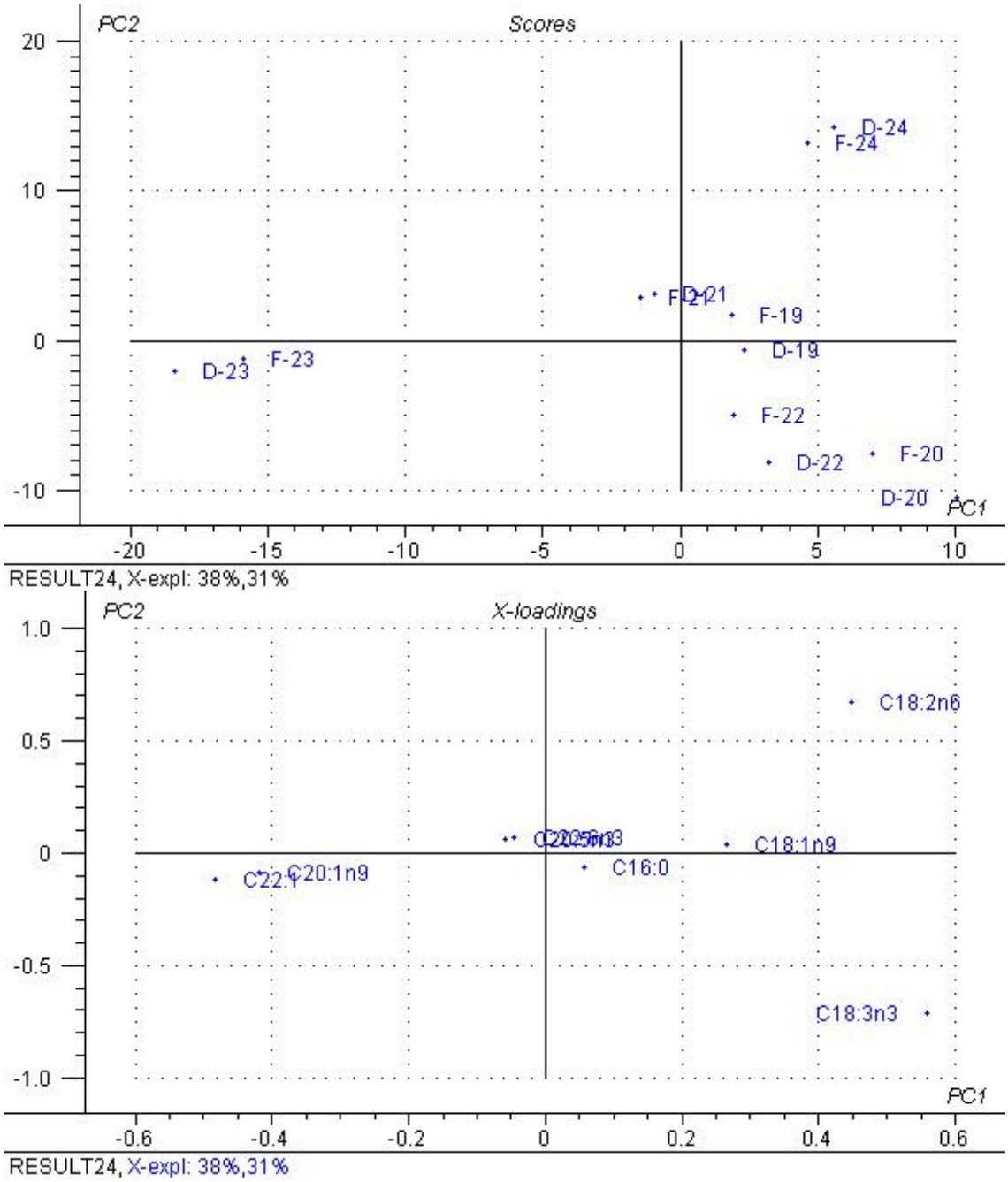


Figure 11.-Principal component analysis on the fatty acid composition of Norwegian farmed Atlantic salmon (F-19 to F-24) fed for 12 months with diets containing, respectively, a 40:50% combination of fish oils (capelin and anchovy) and rapeseed oil (D-19), linseed oil (D-20), poultry oil (D-21), palm oil (D-22), with capelin oil only (D-23) and with soybean oil (D-24). The model was fully cross validated and each variable was given a weight of 1. Scores plot is shown on top and loadings plot on the bottom. PC1 and PC2 explained 38% and 31% respectively of the total variability in the model. Data from Rosenlund et al. [2001]. Cont.

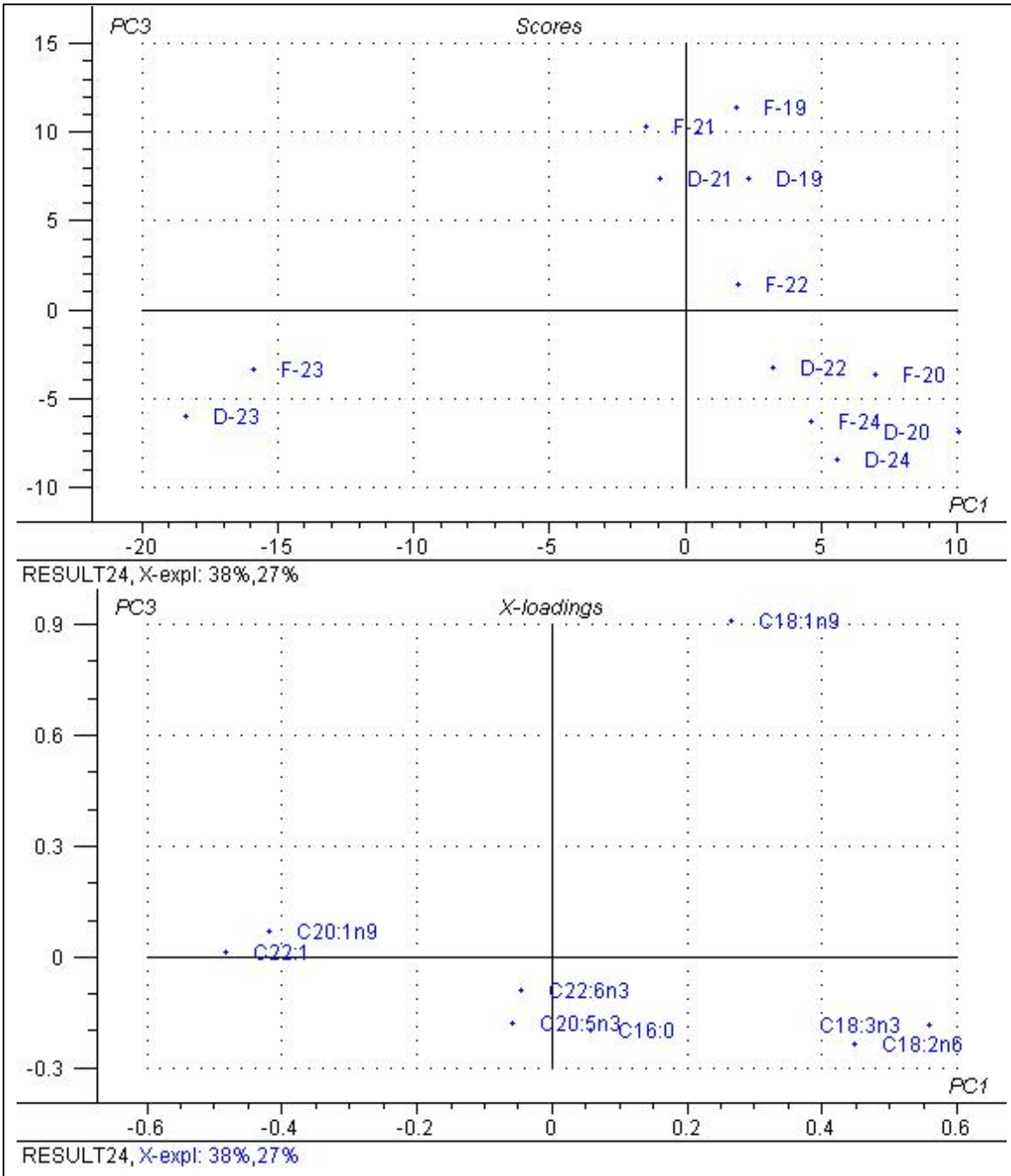


Figure 11 - Cont. Same as Figure 11 but showing PC1 and PC3. Principal component analysis on the fatty acid composition of Norwegian farmed Atlantic salmon (F-19 to F-24) fed for 12 months with diets containing, respectively, a 40:50% combination of fish oils (capelin and anchovy) and rapeseed oil (D-19), linseed oil (D-20), poultry oil (D-21), palm oil (D-22), with capelin oil only (D-23) and with soybean oil (D-24). The model was fully cross validated and each variable was given a weight of 1. Scores plot is shown on top and loadings plot on the bottom. PC1 and PC3 explained 38% and 27% respectively of the total variability in the model. Data from Rosenlund et al. [2001]. Cont.

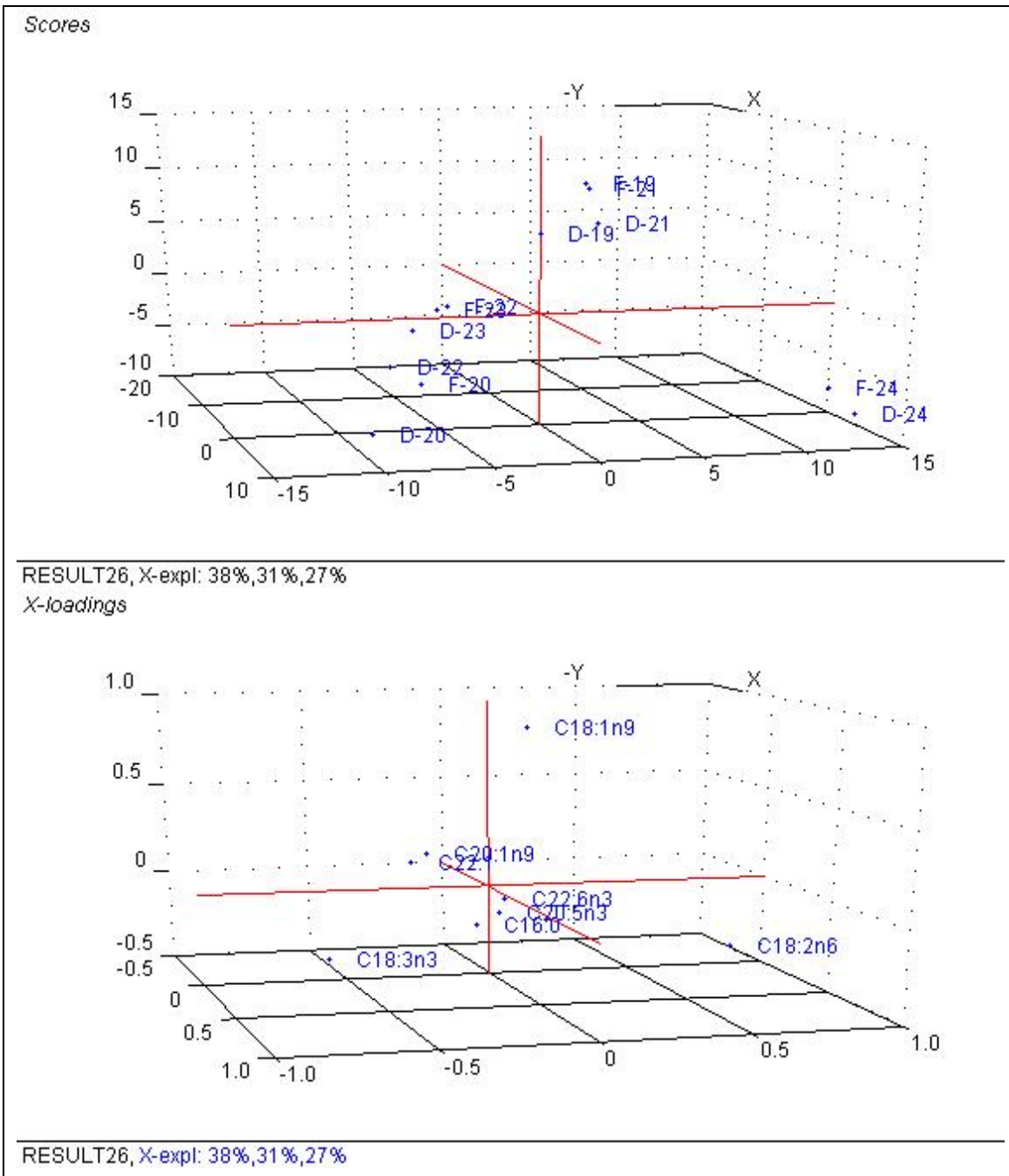


Figure 11 - Cont. Same as Figure 11 but showing PC1,2 and 3. Principal component analysis on the fatty acid composition of Norwegian farmed Atlantic salmon (F-19 to F-24) fed for 12 months with diets containing, a 40-50% combination of fish oils (capelin and anchovy) and rapeseed oil (D-19), linseed oil (D-20), poultry oil (D-21), palm oil (D-22), with capelin oil only (D-23) and with soybean oil (D-24). The model was fully cross validated and each variable was given a weight of 1. Scores plot is shown on top and loadings plot on the bottom. PC1 and PC3 explained 38% and 27% respectively of the total variability in the model. Data from Rosenlund et al. [2001].

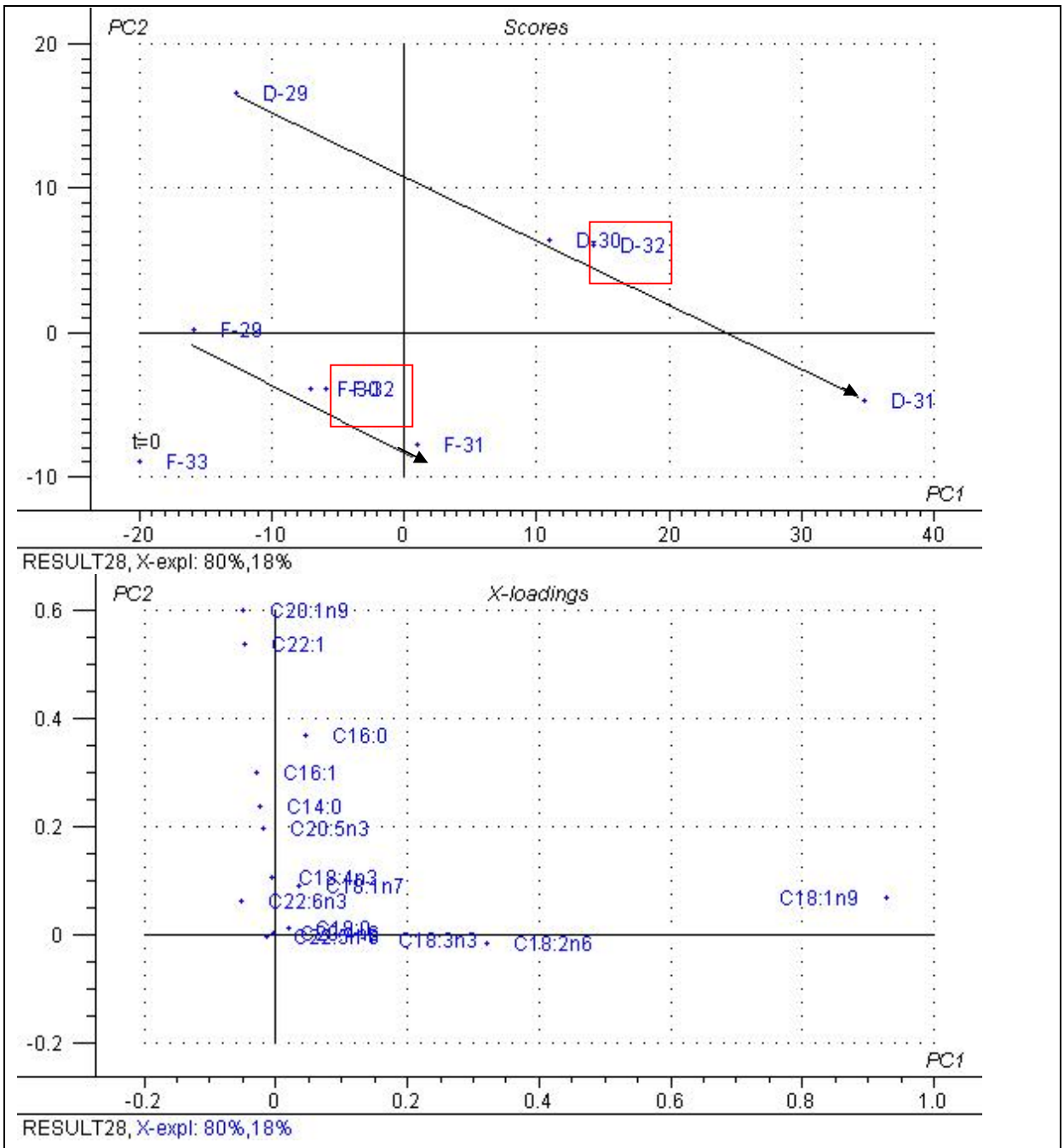


Figure 12. Principal component analysis on the fatty acid composition of Norwegian farmed Atlantic salmon (at the beginning of the experiment (F-33, t=0) and after being fed (F-29 to F-32) for 42 weeks with diets containing, respectively, only fish oil (D-29); a mixture of 50-50% of fish oils and rapeseed oil (D-30); only rapeseed oil (D-31) and a mixture of 50-50% of fish oils and olive oil (D-32, in the red square). The model was fully cross validated and each variable was given a weight of 1. Scores plot is shown on top and loadings plot on the bottom. PC1 and PC2 explained 80% and 14% respectively of the total variability in the model. The arrows in the scores plot indicate the trends in both the diets and flesh according to the amount of vegetable oil mixed in. Data from Torstensen et al. [2004].

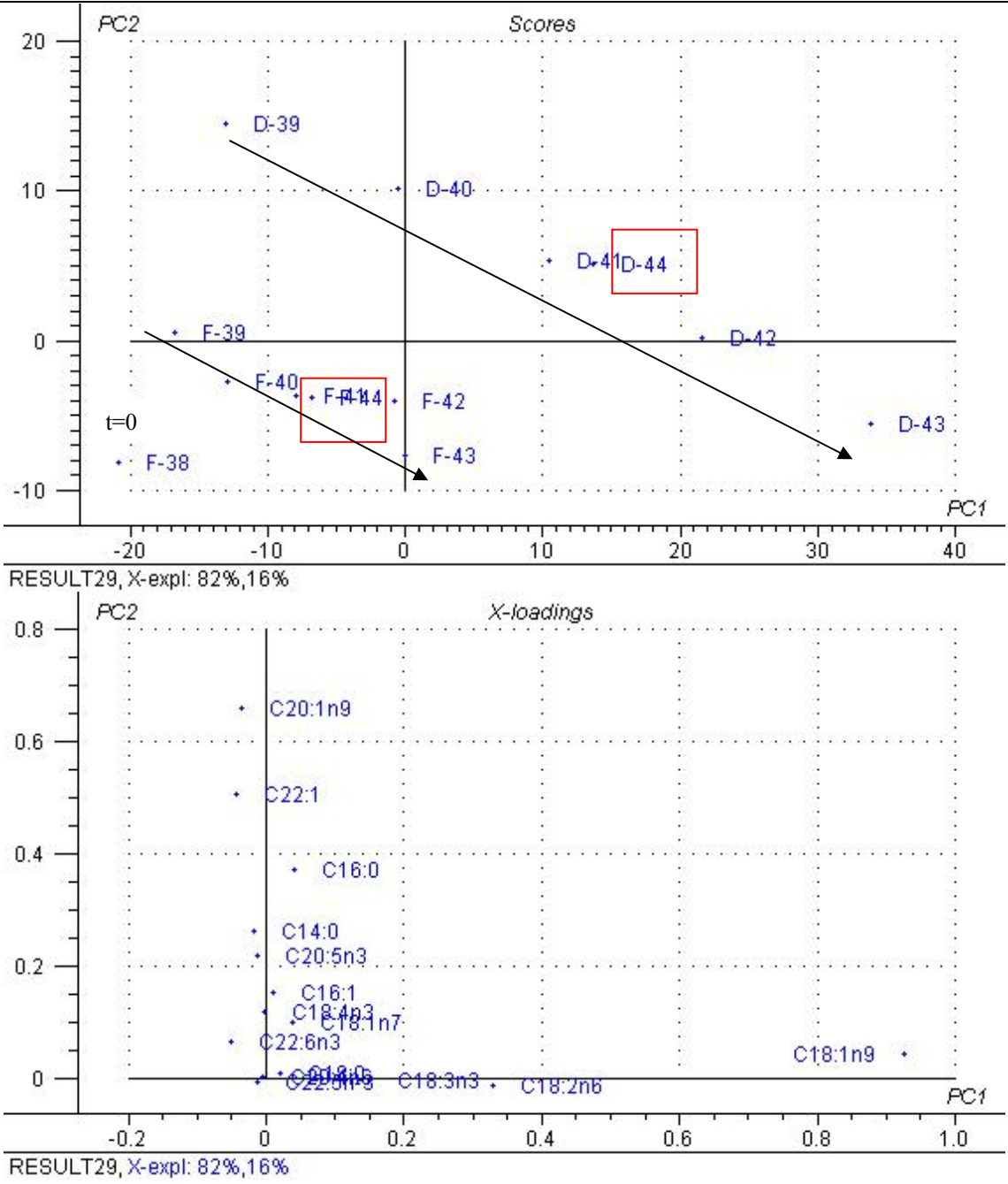


Figure 13. Principal component analysis on the fatty acid composition of Norwegian farmed Atlantic salmon (at the beginning of the experiment (F-38, t=0) and after being fed (F-39 to F-44) for 42 weeks with diets containing, respectively, only fish (capelin) oil (D-39); a mixture of 75:25% of fish oils and rapeseed oil (D-40); 50:50% of fish oils and rapeseed oil (D-41); 25:75% of fish oils and rapeseed oil (D-42); 100% rapeseed oil (D-43); and 50%-50% of fish oils and olive oil (D-44 in the red square). The model was fully cross validated and each variable was given a weight of 1. Scores plot is shown on top and loadings plot on the bottom. PC1 and PC2 explained 83% and 16% respectively of the total variability in the model. The arrows in the scores plot indicate the trends in both the diets and flesh according to the amount of vegetable oil mixed in. Data from Torstensen et al. [2004b].

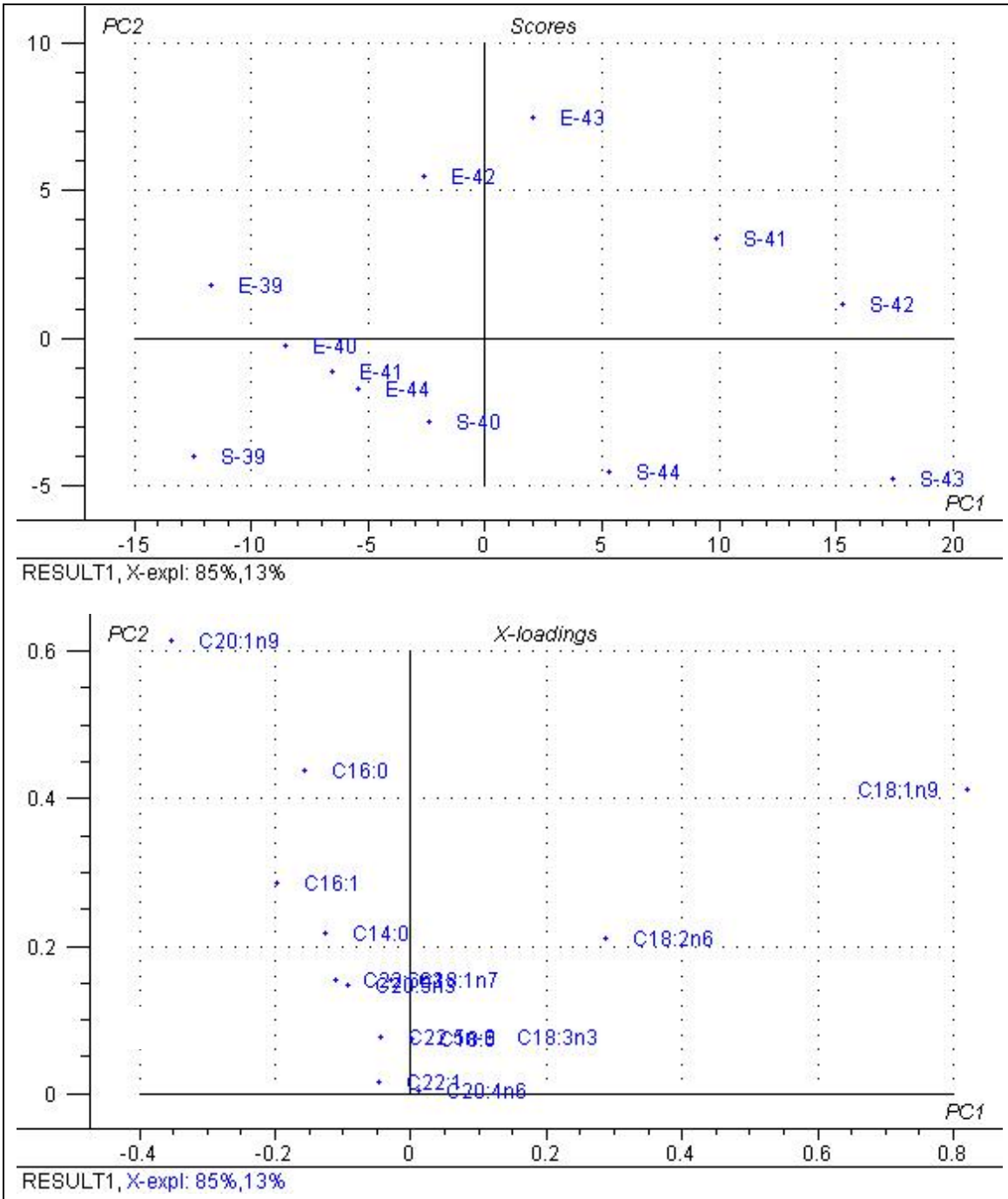


Figure 14. Principal component analysis on the fatty acid composition of Norwegian farmed Atlantic salmon fed for 42 weeks with diets containing, respectively, only fish (capelin) oil (S-39); a mixture of 75:25% of fish oils and rapeseed oil (S-40); 50:50% of fish oils and rapeseed oil (S-41); 25:75% of fish oils and rapeseed oil (S-42); 100% rapeseed oil (S-43); and 50:50% of fish oils and olive oil (S-44 in the red square) and the same fish at the end of a washout period of 1788 days degree (^{o}C) with only fish oil as source of dietary lipid (Samples E-39 to E-44 respectively). The model was fully cross validated and each variable was given a weight of 1. Scores plot is shown on top and loadings plot on the bottom. PC1 and PC2 explained 85% and 13% respectively of the total variability in the model. The lines in the scores plot indicate the trends (from upper left to lower right) in both the diets and flesh according to the amount of vegetable oil mixed in. Data from Torstensen et al. [2004b].

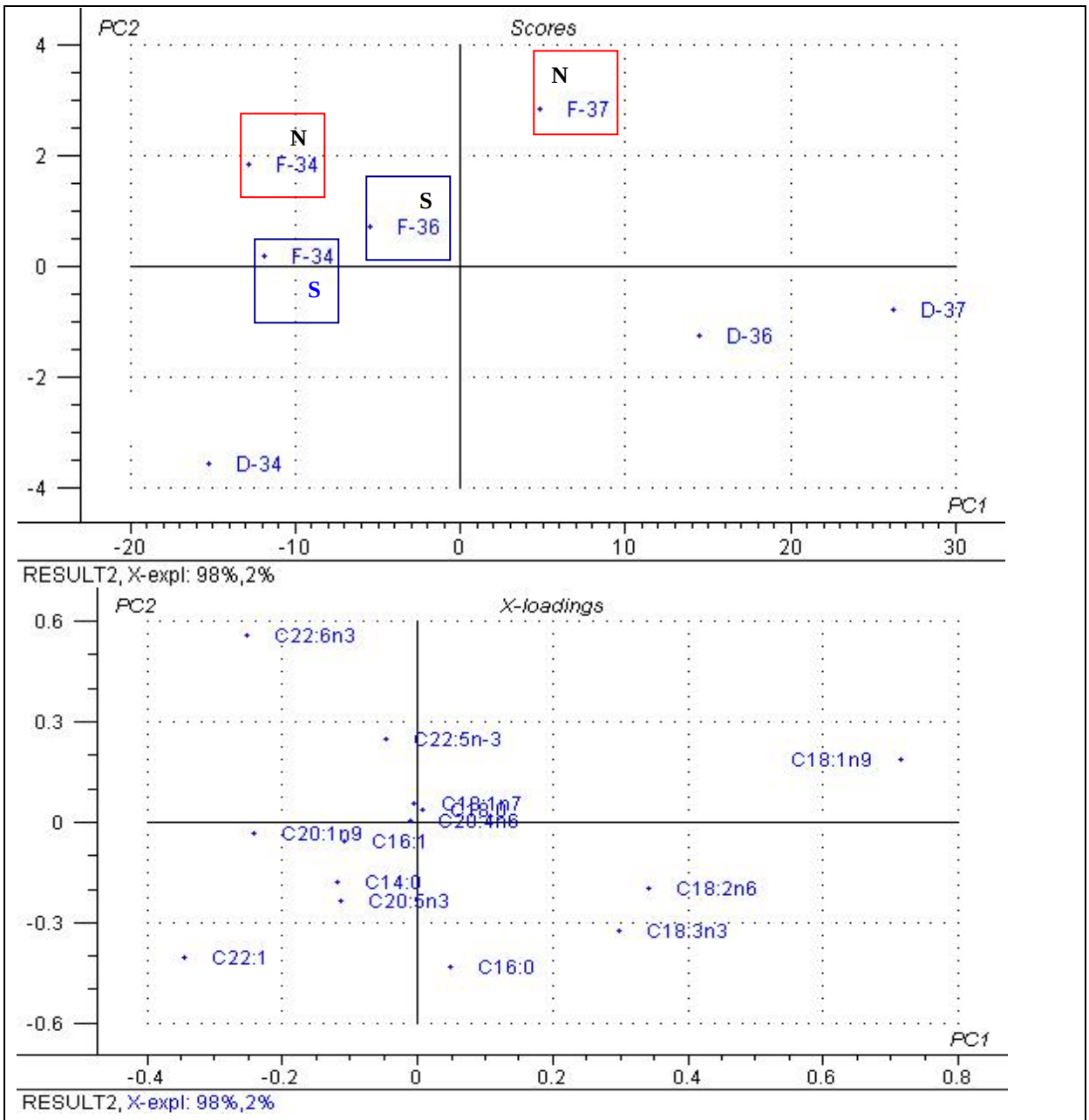


Figure 15. Principal component analysis on the fatty acid composition of Norwegian farmed Atlantic salmon fed for a 2 year production cycle in Seawater in Scotland and Norway (F-34, F-36 and F-37) with diets containing, respectively, 100% fish oil (D-34); 75% of a blend of vegetable oils (D-36) and 100% of the vegetable oil blend (D-37). The 75% replacement was tested in Scotland (S), the 100% in Norway (N) and the control diet (D-34) at both locations. The model was fully cross validated and each variable was given a weight of 1. Scores plot is shown on top and loadings plot on the bottom. PC1 and PC2 explained 98% and 2% respectively of the total variability in the model. Data from Torstensen et al. [2005].

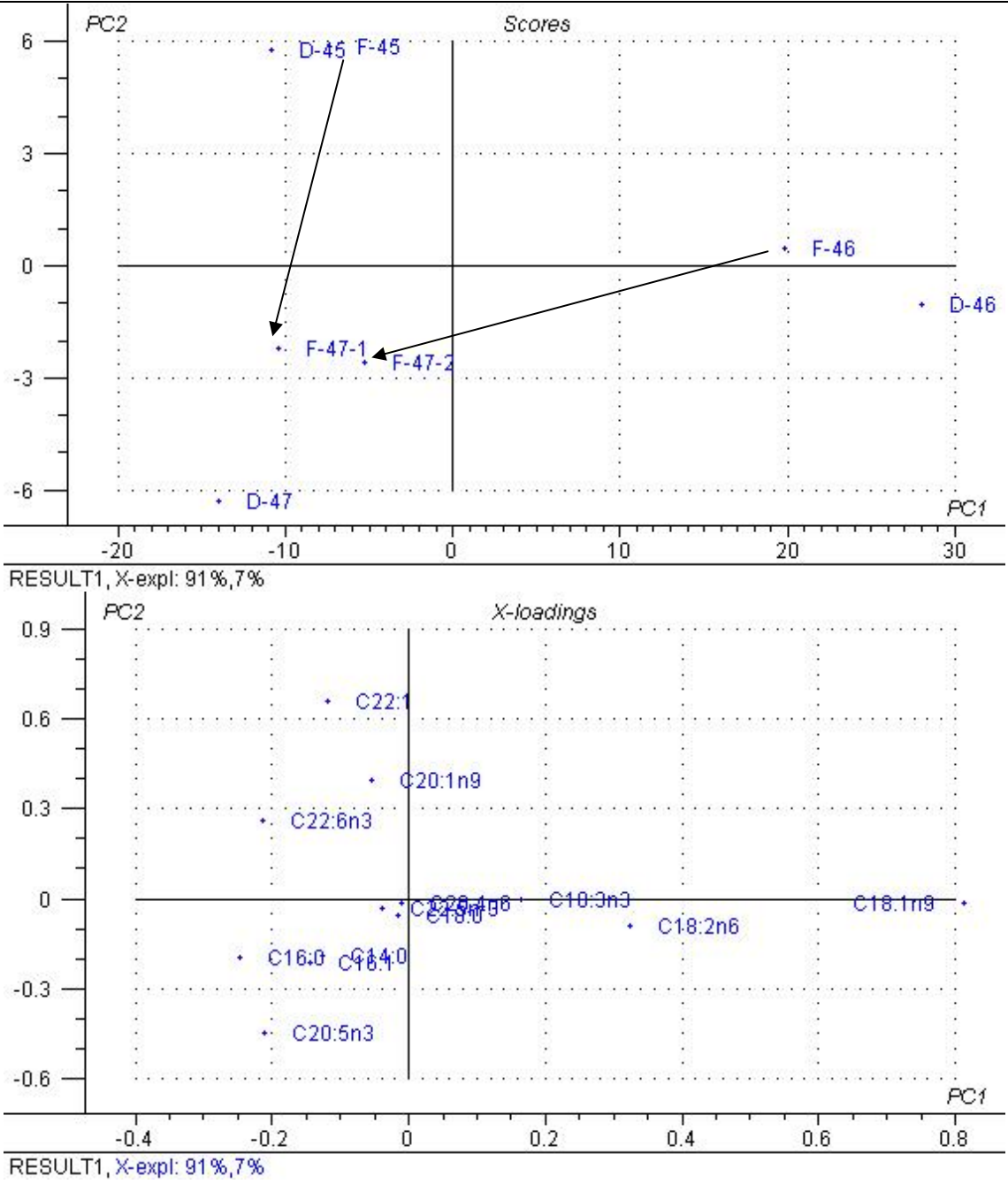
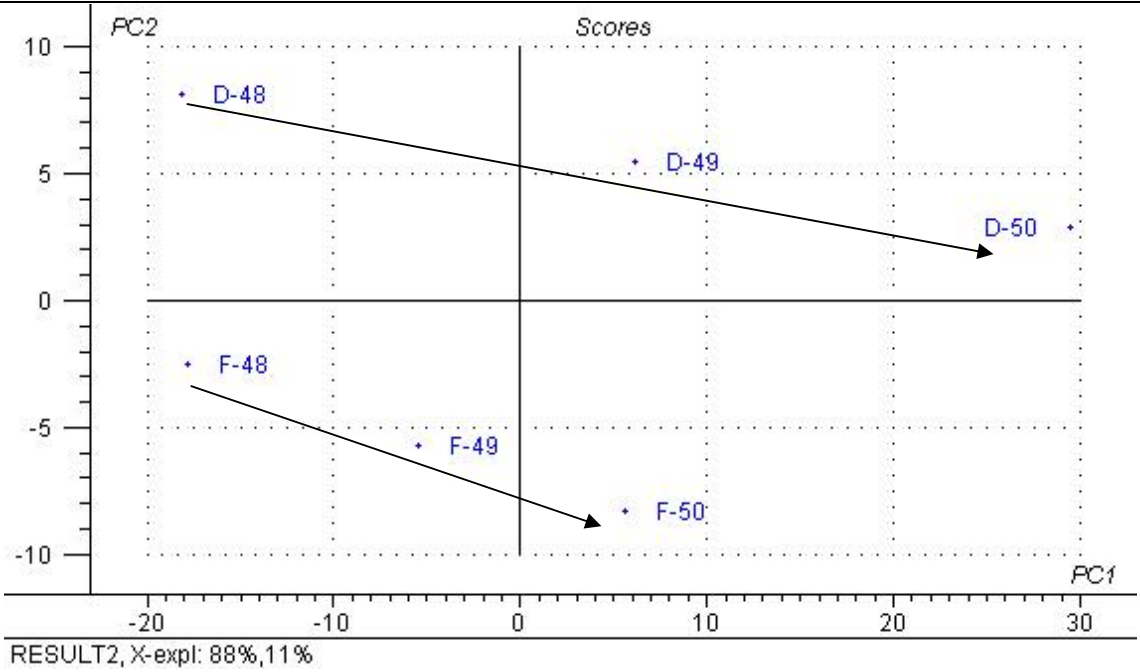


Figure 16. Principal component analysis on the fatty acid composition of Scottish farmed Atlantic salmon fed for 8 months a diet of 100% fish oil (D-45 for the diet and F-45 for the fish) or 100% rapeseed oil (D-46 and F-46). Prior to slaughter all fish were fed a commercial salmon grower diet containing only fish oil (D-47): F-47-1 had been fed D-45 (fish oil) and F-47-2 had been fed D-46 (rapeseed oil). The model was fully cross validated and each variable was given a weight of 1. Scores plot is shown on top and loadings plot on the bottom. PC1 and PC2 explained 91% and 7% respectively of the total variability in the model. Data from Bell et al. [2003].



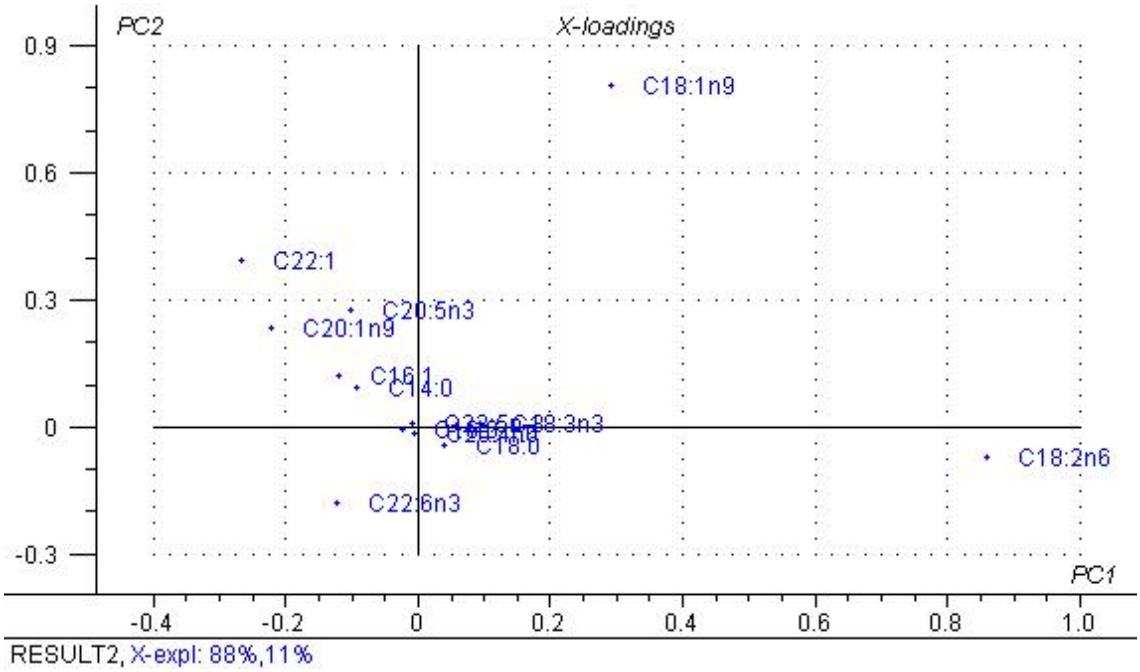


Figure 17. Principal component analysis on the fatty acid composition of Norwegian farmed Atlantic salmon fed for about 1050 days degree (°C) a diet of 100% capelin oil (D-48 for the diet and F-48 for the salmon), 100% crude soybean oil (D-50 and F-50) and a 50:50% mixture of the two (D-49 and F-49). The model was fully cross validated and each variable was given a weight of 1. Scores plot is shown on top and loadings plot on the bottom. PC1 and PC2 explained 88% and 11% respectively of the total variability in the model. The arrows indicate the effect of increasing the amount of soybean oil on the position of the samples. Data from Grisdale-Helland et al. [2002]

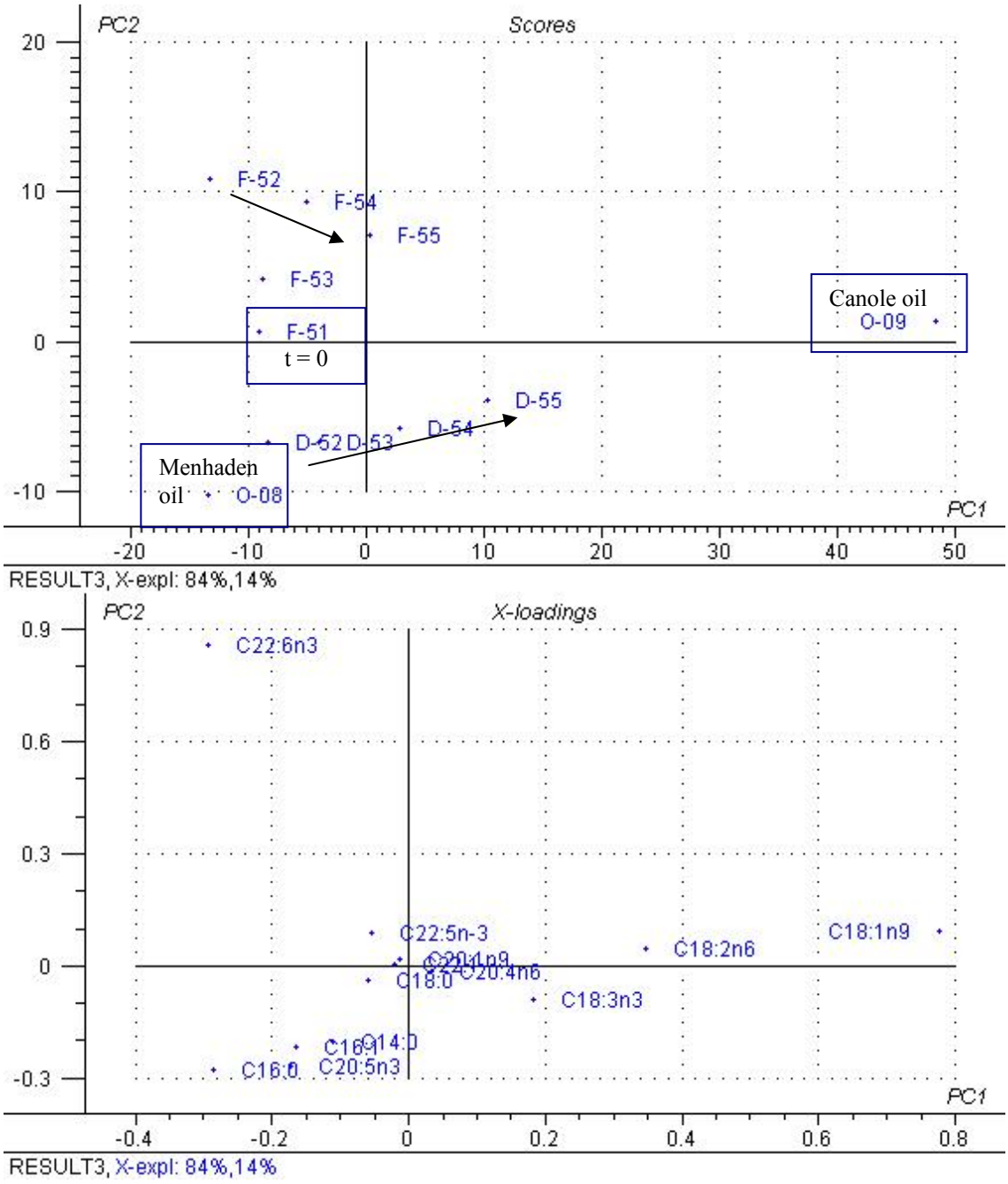


Figure 18. Principal component analysis on the fatty acid composition of Canadian farmed Atlantic salmon fed for 16 weeks a diet containing 100% menhaden oil (D 52 for the diet and F-52 for the fillet) and of mixtures of menhaden and canole oils in the ratio 83:17%, (D-53, F-53); 66:34% (D-54, F-54) and 49:51% (D-55, F-55). O-08 and O-09 are the menhaden and canole oil respectively. F-51 is the salmon at the beginning of the experiment. The model was fully cross validated and each variable was given a weight of 1. Scores plot is shown on top and loadings plot on the bottom. PC1 and PC2 explained 84% and 14% respectively of the total variability in the model. The arrows indicate the effect of increasing the amount of canole oil on the position of the samples. Data from Dosanjh et al. [1998].

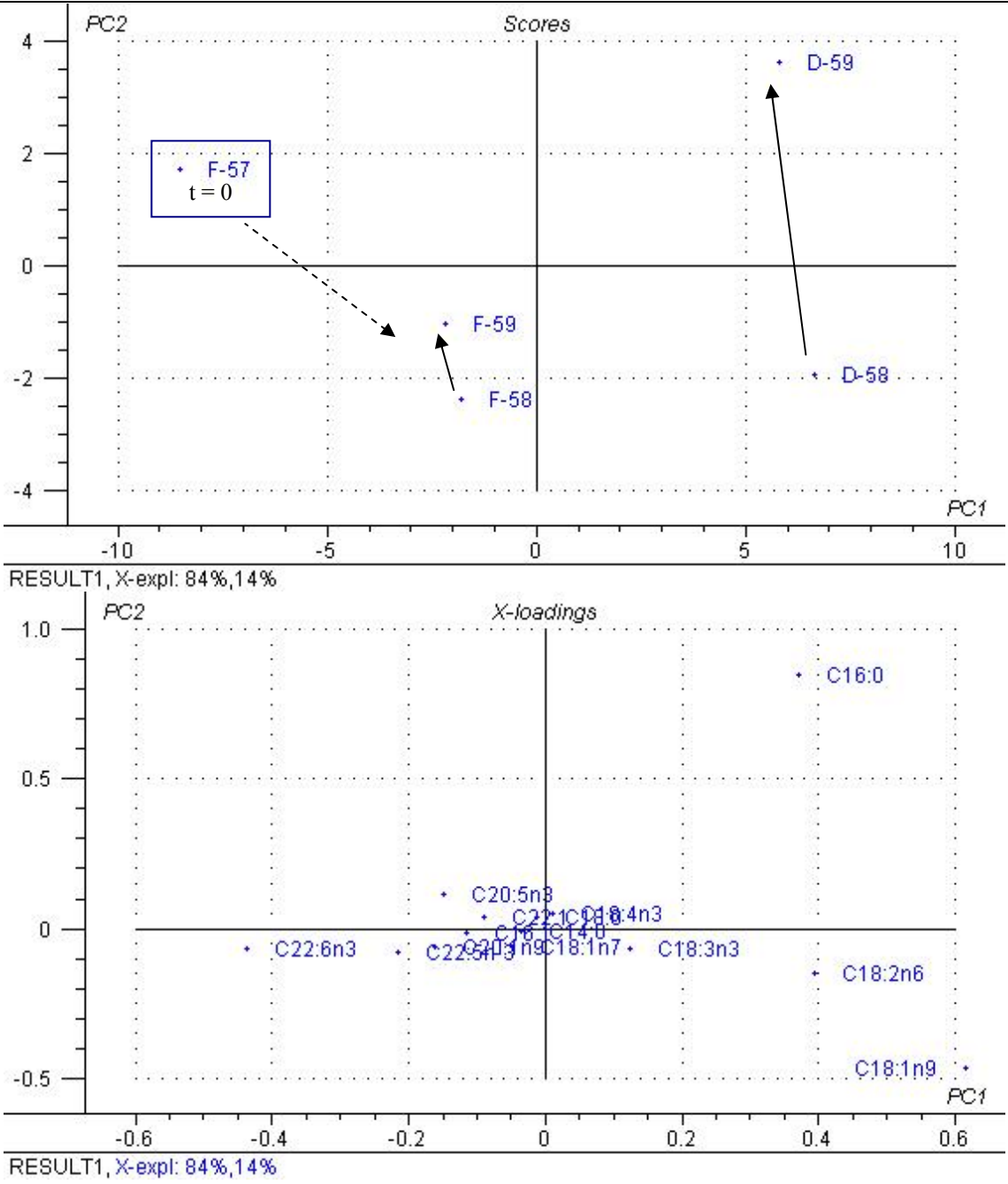


Figure 19. Principal component analysis on the fatty acid composition of Norwegian farmed Atlantic salmon fed for 12 weeks diets containing blends of fish oil:rapeseed oil:crude palm oil in the proportions 50:40:10 (D-58 for the diet and F-58 for the flesh) and 50:25:25 (D-59 and F-59). F-57 is the salmon at the beginning of the experiment. The model was fully cross validated and each variable was given a weight of 1. Scores plot is shown on top and loadings plot on the bottom. PC1 and PC2 explained 84% and 14% respectively of the total variability in the model. The arrow with broken line indicates the effect of increasing the amount of vegetable oil in the diets, the other arrows indicate the effect of increasing the amount of crude palm oil on the position of the samples. Data from Ng et al. [2004].

1.2 Fatty acid positional distribution for authentication

Fatty acid positional distribution for authentication

The fatty acid positional distribution in triglycerols [Pfeffer et al., 1977; Ng, 1985, Hidalgo and Zamora, 2003] has been used to identify contaminants in oils: for example 5% hazelnut oil in virgin olive oils has been detected by ^{13}C NMR by Zamora et al. [2001] and Siddiqui et al. [2003] using ^{13}C NMR were also able to distinguish natural fish oils from oils subjected to industrial refining and addition of synthetic ethyl esters of C22:6n3 and C20:5n3 to encapsulated oil supplements.

Differences in the positional distribution of n3 fatty acids on the glycerol backbone of triacylglycerol from depot fat of farmed Atlantic salmon, cod liver and seal oils by ^{13}C NMR was successfully used for species identification by Aursand et al. [1995]: 73-74% of the C22:6n3 was found to be preferentially esterified at the β position of the triacylglycerols in cod liver oil and farmed Altantic salmon; about 38% of the C22:5n3 was located in the β position in lipids of cod liver oil and farmed Altantic salmon (which means that this fatty acid is nearly randomly distributed in triacylglycerols compared to C22:6n3). DPA (C22:5n3) was preferentially esterified (69%) at the β position of the triacylglycerols in muscle lipids of farmed Altantic salmon but the amount of this fatty acid in cod liver oil was too low to analyze its positional distribution. These findings corroborate previous works by Brockerhoff et al. [1968] and Litchfield [1969] demonstrating the general tendency of C20:5n3, C22:6n3 and C22:5n3 to be preferentially esterified at the β position of fish and invertebrate triacylglycerols. Ando et al. [1992] showed that the positional distribution of C22:6n3 and C22:5n3 is related to the amount of C22:1 and C20:1 fatty acids in the triacylglycerols: in fish lipids with high contents of C20:1 and C22:1 nearly 70-80% of the C22:6n3 was in the β position of the glycerol moiety. The distribution in marine mammals was quite different: in harp seal oil nearly 100, 97 and 95% of C22:5n3, C22:6n3 and C20:5n3 respectively were esterified to the α (1 and 3) positions of the glycerol moiety, in accordance with positional data for triacylglycerols of harp sea blubber [Brockerhoff et al., 1968, Litchfield, 1969].

Diet-induced changes in the positional distribution of fatty acids have been shown in bovine adipose tissues [Smith et al., 1998]: long term feeding of cattle was sufficient to produce significant alterations in fatty acid composition in bovine adipose tissue consisting in changes both in the distribution and in the composition of the triacylglycerol species, which, in turn, accounted for marked differences in melting points among treatment groups.

In the rotifer *Brachionus plicatilis* and in *Artemia franciscana* on the other hand, the positional distribution of fatty acids in the dietary fish oils triacylglicerols was not held: once the fatty acids were released from the dietary fish oils they were incorporated into *Artemia* and *B. plicatilis* in a consistent manner independent of their origin: while C22:6n3 was preferentially esterified in the position sn-2 in the fish oils, it ended up in the sn-3 in both *Artemia* and *B. plicatilis* [Ando et al., 2004a,b].

We have been unable to find published works on how the fatty acid composition of feeds affects the positional distribution of fatty acids in farmed Atlantic salmon, or works comparing the fatty acid positional distribution in wild and farmed Atlantic salmons.

Thus, while the fatty acid positional distribution seems to be a good candidate to identify species in oils, its potential use to identify the feed source and therefore discriminate farmed from wild Atlantic salmon remains to be investigated.

2. CONCLUSIONS

Conclusions

The fatty acid profiles of oils extracted from Atlantic salmon muscle always reflected the profile of the diet and even after a washing out period the n3/n6 ratio were not fully recuperated. Farmed and wild Atlantic salmon were clearly differentiated by the ratio n3/n6 in some works, but when we combined the results of all the publications used for this review it lost is value. This may be due to differences in the analytical procedures used by the different research groups and also in the much higher number of farmed than wild Atlantic salmon used.

3. ACKNOWLEDGMENTS

This report was performed within the Integrated Research Project SEAFOODplus, contract No FOOD-CT-2004-506359. The partial financing of the work by the European Union is gratefully acknowledged, as well as the financial support of the Norwegian Research Council.

4. REFERENCES

References

Alessandri, J.M., Joannie, J.L. and Durand, G.A. (1993) Polyunsaturated fatty acids as differentiation markers of rat jejunal epithelial cells: a modeling approach. J. Nutr. Biochem., 4: 97-104.

Ando, T., Nishimura, K., Aoyanagi, N. and Takagi, T. (1992) Stereospecific analysis of fish oil triacyl-sn-glycerols. JAOCS, 69: 417-424 .

Ando, Y., Kobayashi, S., Sugimoto, T. and Takamaru, N. (2004a) Positional distribution of n-3 highly unsaturated fatty acids in triacylglycerols (TAG) of rotifers (Brachionus plicatilis) enriched with fish and seal oils TAG. Aquaculture, 229: 275-288.

Ando, Y., Samoto, H. and Murayama, Y. (2004b) Positional distribution of DHA and EPA in triacyl-sn-glycerols (TAG) of *Artemia franciscana* nauplii enriched with fish oils ethyl esters and TAG. Aquaculture, 233: 321-335.

Aursand, M., Mabon, F. and Martin, G.J. (2000) Characterization of farmed and wild salmon (*Salmo salar*) by a combined use of compositional and isotopic analyses. JAOCS., 77: 659-666.

Aursand, M., Jørgensen L. and Grasdalen, H. (1995) Positional distribution of ω-3 fatty acids in marine lipid triacylglycerols by high resolution ^{13}C nuclear magnetic resonance spectroscopy. JAOCS, 72: 293-297.

Barker, M. and Rayens, W. (2003) Partial Least Squares for Discrimination. J. Chemometrics,17: 166-173.

Bell, J.G., Henderson, R.J., Tocher, D.R., McGhee, F., Dick, J.R., Porter, A., Smullen, R. and Sargent, J.R. (2002) Substituting fish oil with crude palm oil in the diet of Atlantic salmon (*Salmo salar*) affects tissue fatty acid compositions and hepatic fatty acid metabolism. J. Nutr., 132: 222–230.

Bell, J.G., McGhee, F., Campbell, P.J. and Sargent, J.R. (2003a) Rapeseed oil as an alternative to marine fish oil in diets of post-smolt Atlantic salmon (*Salmo salar*): changes in flesh fatty acid composition and effectiveness of subsequent fish oil ‘‘wash out’’. Aquaculture 218: 515–528.

Bell, J.G., Tocher, D.R., Henderson, R.J., Dick, J.R. and Crampton. V.O. (2003b) Altered fatty acid compositions in Atlantic salmon (*Salmo salar*) fed diets containing linseed and rapeseed oils can be partially restored by a subsequent fish oil finishing diet. J. Nutr., 133: 2793–2801.

Bell., J.G., MsEnvoy, J., Tocher, D.R., McGhee, F., Campbell, P.J. and Sargent, J.R. (2001a) Replacement of fish oil with rapeseed oil in diets of Atlantic salmon (*Salmo salar*). J. Agric. Food Chem., 46: 119-127.

Berge, G.M., Ruyter, B. and Asgard, T. (2004) Conjugated linoleic acid in diets for juvenile Atlantic salmon *(Salmo salar*); effects on fish performance, proximate composition, fatty acid and mineral content. Aquaculture, 237: 365-380.

Bishop, C. M. and G. D. James (1993). Analysis of multiphase flows using dual-energy gamma densitometry and neural networks. Nuclear Instruments and Methods in Physics Research A327: 580--593.

Bishop, C.M., Svensen, M. and Williams, C.K.I. (1996). GTM: The generative topographic mapping. Technical Report NCRG/96/015, Neural Computing Research Group, Aston University, Birmingham, UK.

Bishop, C. M., M. Svensen, and C. K. I. Williams (1996b). GTM: The generative topographic mapping. Technical Report NCRG/96/015, Neural Computing Research Group, Aston University, Birmingham, UK.

Bjerkeng, B., Storebakken, T. and Wathne, E. (1999) Cholesterol and short-chain fatty acids in diets for Atlantic salmon *Salmo salar* (L.): effects on growth, organ indices, macronutrient digestibility, and fatty acid composition. Aquaculture Nutr., 5: 181-191.

Bjerkeng, B., Hatlen, B. and Wathne, E. (1999b) Deposition of astaxanthin in fillets of Atlantic salmon (*Salmo salar*) fed diets with herring, capelin, sandeel, or Peruvian high PUFA oils. Aquaculture, 180: 307-319.

Brockerhoff, H., Hoyle, R.J., Hwang, P.C. and Litchfield, C. (1968) .Lipids, 3: 24. Reference from Aursand et al. (1995).

Dosanjh, B.S., Higgs, D.A., McKenzie, D.J., Randall, D.J., Eales, J.G., Rowshandeli, N., Rowshandeli, M. and Deacon, G. (1998). Influence of dietary blends of menhaden oil and canola oil on growth, muscle lipid composition, and thyroidal status of Atlantic salmon (*Salmo salar*) in sea water. Fish Physiol. Biochem., 19: 123-134.

Dosanjh, B.S., Higgs, D.A., Plotnikoff, M.D., Markert, J.R., and Buckley, J.T. (1988) Preliminary evaluation of canola oil, pork lard and marine lipid singly and in combination as supplemental dietary lipid sources for juvenile fall Chinook salmon (*Oncorhynchus tshawytscha*). Aquaculture, 68: 325-343.

Drevon, C.A., Nenseter, M.S., Brude, I.R., Finstad, H.S., Kolset, S.O. and Rustan A.C. (1995) Omega-3 fattya cids-Nutritional aspects. Can. J. Cardiol., 11: 47G-54G.

Einen, O., Waagan, B. and Thomassen, M.S. (1998) Starvation prior to slaughter in Atlantic salmon (*Salmo salar*) - I. Effects on weight loss, body shape, slaughter- and fillet-yield, proximate and fatty acid composition Aquaculture, 166: 85-104.

Geladi, P. and Kowalski, B. (1986) Partial least squares regression: a tutorial. Anal. Chim Acta, 185: 1-17.

Grisdale-Helland, B., Ruyter, B., Rosenlund, G., Obach, A., Helland, S.J., Sandberg, M.G., Standa,l H.and Rosjo, C. (2002) Influence of high contents of dietary soybean oil on growth, feed utilization, tissue fatty acid composition, heart histology and standard oxygen consumption of Atlantic salmon (*Salmo salar*) raised at two temperatures. Aquacultue, 207: 311-329.

Hardy, R.W., Scott, T.M. & Harrell, L.W. (1987) Replacement of herring oil with menhaden oil, soybean oil, or tallow in the diets of Atlantic salmon raised in marine net-pens. Aquaculture, 65: 267-277.

Helland, I.S. (1992) On the structure of partial least squares regression. Commun. Statist. Soc. B, 54: 637-645.

Henderson, R.J., Bell, J.G. & Park, M.T. (1996) Polyunsaturated fatty acid composition of the salmon *(Salmo salar* L.) pineal organ: modification by diet and effect on prostaglandin production. Biochimica et Biophysica Acta (BBA), 1299: 289-298.

Hidalgo, F.J. and Zamora, R. (2003) Edible oil analysis by high-resolution nuclear magnetic resonance spectroscopy: recnt advances and future perspective. Trends Food Sci. Technol., 14: 499-506.

Hoskuldsson, A. (1988) PLS regression methods, J Chemometrics, 2: 211-228.

Jobling, M. (2004) Are modifications in tissue fatty acid profiles following a change in diet the result of dilution? Test of a simple dilution model. Aquaculture, 232: 551-562.

Jobling, M., Larsen, A.V., Andreassen, B., Sigholt, T. and Olsen, R.L. (2002) Influence of a dietary shift on temporal changes in fat deposition and fatty acid composition of Atlantic salmon post-smolt during the early phase of seawater rearing. Aquaculture Res., 33: 875-889.

Jolliffe, I.T., (1986) Principal Component Analysis. Springer-Verlag, New York.

Kemsley, E.K., (1996) Discriminant analysis of high dimensional data: a comparison of principal components analysis and partial least squares data reduction methods, Chem Intell. Lab. Syst., 33: 47-61.

Kohonen, T. (1982) Self-organized formation of topologically correct feature maps. Biological Cybernetics, 43: 59-69.

Kohonen, T. (1997)Self-Organizing Maps. Second Ed. Berlin: Springer Verlag, 1-448.

Lie, Ø., Waagbø, R., and Sandnes, K. (1988) Growth and chemical composition of adult Atlantic salmon (*Salmo salar*) fed dry silage based diets. Aquaculture, 69: 343-353.

Litchfield, C. (1969), Lipids, 3: 417. Reference from Aursand et al. (1995).

Livingstone, D.J. and Manallack, D.T. (1993) Statistics using neural networks: chance effects, J. Med. Chem. 36: 1295–1297.

Martinez, I. (2006) Revision of analytical methodologies to verify the production method of fish. In: Seafood from fish to dish, Quality, safety and processing of wild and farmed fish. Luten, J.B.; Jacobsen, C.; Bekaert, K.; Sæbø, A.. and Oehlenschlager J. (Eds.). ISBN 9086860052 Wageningen Academic Publishers. The Netherlands, in press.

Masters, T. (1993) Practical Neural Network Recipes in C++; Academic Press Inc.: Boston, MA,

Masters, T. (1995) Advanced Algorithms for Neural Networks; John Wiley: New York.

Menoyo, D., Lopez-Bote, C.J., Bautista, J.M. and Obach, A. (2003) Growth, digestibility and fatty acid utilization in large Atlantic salmon (*Salmo salar*) fed varying levels of n-3 and saturated fatty acids. Aquaculture, 225: 295-307.

Ng, S. (1985) Analysis of positional distribution of fatty acids in palm oil by ^{13}C NMR spectroscopy. Lipids, 20: 778-182.

Ng, W.K., Sigholt, T. and Bell, J.G. (2004) The influence of environmental temperature on the apparent nutrient and fatty acid digestibility in Atlantic salmon (*Salmo salar* L.) fed finishing diets containing different blends of fish oil, rapeseed oil and palm oil. Aquaculture Res., 35: 1228-1237.

Ng, W.K., Lim, P.K. and Boey, P.L. (2003) Dietary lipid and palm oil source affects growth, fatty acid composition and α-tocopherol concentration in African catfish, *Clarias gariepinus*. Aquaculture, 215: 229-243.

Ng, W.K., Lim, P.K. and Sidek, H. (2001) The influence of a dietary lipid source on growth, muscle fatty acid composition and erythrocyte osmotic fragility of hybrid tilapia. Fish Physiol. Biochem., 25: 301-310.

de Noord, O.E. (1994) The influence of data preprocessing on the robustness and parsimony of multivariate calibration models, Chemom. Intell. Lab. Syst., 23: 65–70.

Nichols, P.D., Mooney, B.D. and Elliot, N.G. (2002) Nutritional Value of Australian Seafood II. CSIRO Marine Research and FRDC, Hobart, Tasmania, Australia. 198 pp. ISBN 1 876 996 07 2.

Olsen, R.E., Henderson, R.J., Sountama, J., Hemre, G., Ringo, E., Melle, W. and Tocher, D.R. (2004) Atlantic salmon, *Salmo salar*, utilizes wax ester-rich oil from *Calanus finmarchicus* effectively. Aquaculture, 240: 433-449.

Pfeffer, P.E., Sampugna, J., Schwartz, D.P. and Schoolery, J.N. (1977) Analytical ^{13}C NMR: detection, quantitation and positional analysis of butyrate in butter oil. Lipids, 12: 869-871.

Refsgaard, H.H.F., Brockhoff, P.B. and Jensen, B. (1998) Biological variation of lipid constituents and distribution of tocopherols and astaxanthin in farmed Atlantic salmon (*Salmo salar*). J. Agric. Food Chem., 46: 808-812.

Rosenlund, G., Obach, A., Sandberg, M.G., Standal, H. and Tveit, K. (2001). Effect of alternative lipid sources on long-term growth performance and quality of Atlantic salmon (*Salmo salar* L.). Aquaculture Res., 32 (Suppl. 1): 323-328.

Seaholtz, M.B. and Kowalski, B. (1992) The parsimony principle applied to multivariate calibration, Anal. Chim. Acta, 277:165–177.

Siddiqui, N., Sim, J., Silwood, C.J.L., Toms, H., Iles, R.A. and Grootveld, M. (2003) Multicomponent analysis of encapsulated marine oil supplements using high-resolution ^{1}H and ^{13}C techniques. J. Lipid Res., 44: 2406-2427.

Smith, S.B., Yang, A., Larsen, T.W. and Tume, R.K. (1998) Positional analysis of triacylglycerols from bovine adipose tissue lipids varying in degree of unsaturation. Lipids, 33: 197-207.

Specht, DF. (1990) IEEE Transactions on Neural Network, 1: 111-121.

Specht, D.F. (1990b) Probabilistc Neural Networks (original contribution), Neural Networks, vol. 3, no. 1, 1990, pp. 109–118.

Specht, D. (1991) A generalized regression neural network. IEEE transactions on neural networks, 2: 568-576.

Sægrov H, Hindar K. Kålås S, and Lura H. (1997) Escaped farmed Atlantic salmon replace the original salmon stock in the River Vosso, western Norway. ICES J Mar Sci 54: 1166-1172.

Thomassen, M. and Røsjø, C. (1989) Different fats in feed for salmon: Influence on sensory parameters, growth rate and fatty acids in muscle and heart. Aquaculture, 79: 129-135.

Todeschini, R., D.Ballabio, D., Consonni, V., Mauri, A. and Pavan, M. (2006) CAIMAN (Classification and Influence Matrix Analysis): a new approach to the classification based on leverage-scaled functions. Chemom.Intell.Lab.Syst., in press.

Torstensen, B.E., Bell, J.G., Rosenlund, G., Henderson, R.J., Graff, I.E., Tocher, D.R., Lie, Ø., Sargent, J.R. (2005) Tailoring of Atlantic salmon (*Salmo salar* L.) flesh lipid composition and sensory quality by replacing fish oil with a vegetable oil blend. J. Agric Food Chem., 53: 10166-10178.

Torstensen, B.E., Froyland, L. and Lie, Ø. (2004) Replacing dietary fish oil with increasing levels of rapeseed oil and olive oil - effects on Atlantic salmon (*Salmo salar* L.) tissue and lipoprotein lipid composition and lipogenic enzyme activities. Aquaculture Nutr., 10: 175-192 .

Torstensen, B.E., Froyland, L., Ørnsrud, R. and Lie Ø (2004b) Tailoring of a cardioprotective muscle fatty acid composition of Atlantic salmon (*Salmo salar*) fed vegetable oils. Food Chem., 87: 567-580.

Torstensen, B.E., Lie, Ø. and Froyland, L. (2000) Lipid metabolism and tissue composition in Atlantic salmon (*Salmo salar* L.) – effects of capelin oil, palm oil and oleic acid-enriched sunflower oil as dietary lipid sources. Lipids, 35: 653-664.

Vandeginste, B.G.M., Massart, D.L., Buydens, L.M.C., Jong, S.D.E., Lewi, P.J. and Smeyers-Verbeke, J. (1998) Principal component analysis. In: Handbook of Chemometrics and Qualimetrics: Part B, Elsevier, Amsterdam, The Netherlands, pp. 88–149 (Chapter 31.1).

Vognild, E., Elvevoll, E.O., Olsen, R.L., Barstad, H., Aursand, M. And Østerud, B. (1998) Effects of dietary marine oils and olive oil on composition, platelet membrane fluidity, platelet responses and serum lipids in healthy humans. Lipids, 33: 427-436.

Zamora, R., Alba, V. and Hidalgo, F.J. (2001) Use of high-resolution ^{13}C nuclear magnetic resonance spectroscopy for the screening of virgin olive oils. JAOCS, 78: 89-94.

Wold, S., Esbensen, K. and Geladi, P. (1987) Principal Component Analysis. Chem. Intell. Lab. Syst., 2: 37-52.