Analytical Methods

AMC TECHNICAL BRIEFS

View Article Online
View Journal | View Issue

Seafood nitrogen factors

Analytical Methods Committee AMCTB No 62

Cite this: *Anal. Methods*, 2014, **6**, 4490

Received 29th April 2014

DOI: 10.1039/c4ay90042a

www.rsc.org/methods

The determination of nitrogen as a quantitative marker for seafood fat-free protein, allowing the calculation of seafood content of seafood products, is well established, and is the official chemical enforcement method. It is also is widely used by food producers to check the specification and added water of their seafood raw materials. A "nitrogen factor" is the average nitrogen content of seafood tissues, on a fat free basis unless the fat content is low as in white fish. Most seafood is prepared by using wet processes as part of good hygienic practice (GHP). Therefore, in establishing nitrogen factors for seafood, keeping water uptake to a minimum and using good manufacturing practice (GMP) has to be taken into account.

Introduction

Labelling rules[1] maintain the option of declaring the species of fish or using the generic description "fish". Where ingredients are highlighted in the name of the food, the amount of that ingredient must be declared as a percentage of the final product (QUID – quantitative ingredient declaration). Products that look like a fillet or piece of seafood, but which have more than 5% added water, have to declare added water in the name of the product, in addition to the seafood content. Although the proportion of an ingredient is calculated on a recipe basis, enforcement authorities usually check the declaration by analysis of the finished product. The analysis determines the nitrogen content (mainly on a fat free basis) and converts this to seafood content by using a previously determined nitrogen factor. Added water can be calculated by the difference. This approach to measuring meat content is almost 100 years old.[2] Manufacturers of seafood products usually check the specification of their raw materials, and nitrogen factors are used to determine if they contain excess added water. The use of nitrogen factors to calculate the fish content of coated fish products has also been accepted at an international level as detailed in Codex Alimentarius.[3] A considerable amount of research already has been undertaken to determine robust nitrogen factors for the common meat and poultry species, and some seafood species. Determining and using seafood nitrogen factors is more challenging than dealing with meat nitrogen factors. There are many more species of seafood. Most is still caught wild, and there is greater natural variation in the life-cycle and geographic origin than with meat animals.

amc technical briefs
www.rsc.org/amc

AMC Technical Briefs are produced by the Analytical Methods Committee, the Technical Subcommittee of the Analytical Division of the Royal Society of Chemistry.

Wet processing of seafood is a requirement of good hygienic practice. Changes that alter the chemical composition of the seafood, that is the degree of water absorption and loss of soluble nitrogen, can take place at virtually all stages of processing. Storing seafood on ice can reduce the nitrogen content of fish, but the principal processing variables that have an effect on nitrogen or water contents are washing and freezing. The further along the processing chain the more vulnerable seafood is to changes in soluble nitrogen and water content.

Nitrogen factors for seafood ingredients

Because of the importance of good manufacturing practice in the determination of nitrogen factors for seafood ingredients, a Code of Practice[4] on the Declaration of Fish Content in Fish Products was agreed between UK food industry organisations and enforcement authorities. The Code gives a table, based on industry data, of interim nitrogen factors for commonly commercialised fish, which represents fish ingredients produced under GMP. It is important to include the nitrogen factor of the unprocessed fish or seafood if possible to use as a baseline comparison with the ingredient, to verify that the fish ingredient nitrogen factor has been produced by GMP/GHP. Since the publication of the Code of Practice there have been several studies to determine agreed nitrogen factors. The use of a seafood ingredient nitrogen factor especially for frozen fish blocks (fillet or mince), which are the main raw material for coated fish products, reduces natural variability to some extent because the factor is based on a composite sample.

Measurement of nitrogen content is done according to an internationally accepted method.[5] Most laboratories in the UK use the rapid Dumas method for nitrogen determination. This measures the non-protein nitrogen as well as the protein nitrogen, and hence gives higher results than the Kjeldahl method.[6] The difference is small, and Dumas is higher by a factor of 1.014, which is more important for seafood because of its higher non-protein nitrogen content than meat. Therefore it is usual to quote the nitrogen results for both methods of analysis as Kjeldahl is still widely used in laboratories worldwide.

Scampi (*Nephrops norvegicus*)

Scampi is a valuable and popular shellfish, in the UK it is usually consumed as a coated cooked product. It was the subject of a study[7] by the AMC, which collected samples over a 16 month period from two different fishing grounds (North Sea and Irish Sea). The overall *N* factor of untreated (straight from the sea) scampi was 2.90, and this was diminished by a total of 0.46%N by GMP processing of storage on ice, peeling, cleaning and draining (that is, the *N* factor for scampi after GMP preparation of 2.45).

Atlantic cod (*Gadus morhua*)

Processed frozen blocks of cod fillet and cod mince are the main ingredients for the industrial production of cod products. The AMC study[8] compared UK-produced cod blocks with imported ones from cod caught in the Barents Sea and Norwegian waters of the North Sea. In UK-produced blocks, the *N* factor for both fillet and mince blocks was 2.78, and for the unprocessed fillet ingredient was 2.88. In imported blocks, which covered European producers and Chinese producers, the overall *N* factor for fillet blocks was 2.74, and for mince blocks was 2.67. As virtually all cod blocks are imported into the UK, the overall recommended *N* factor for cod fillet block was 2.75, and for cod mince block was 2.67.

Atlantic salmon (*Salmo salar*)

LGC undertook this study[9] as part of its Government Chemist function. Both fillets and recovered frame mince from farmed Scottish and Norwegian salmon were analysed. As the average fat content of fillets and mince is around 12%, a fat-free *N* factor was determined. The nitrogen content was determined by the rapid Dumas method. A general *N* factor for fillets of 3.80 (Kjeldahl 3.75) was recommended, and an *N* factor of 2.85 (Kjeldahl 2.81) for salmon frame mince.

Tilapia (*Oreochromis nilotica*)

A study[10] on tilapia was undertaken by Thailand as part of the work by the Codex Committee on Fish and Fishery Products (CCFFP). Tilapia is a tropical fish used for the manufacture of fish sticks (fingers). In the first study, tilapia were obtained from three different provinces in Thailand, and the nitrogen content measured at each stage of processing – filleting, icing and washing, and frozen fish blocks production. The results of this study showed that the nitrogen content varied more with aquaculture and feeding regimes than with processing, and gave an average factor of 3.00. A parallel study by Malaysia, from a more mixed aquaculture system of earthen ponds and cage systems, gave an average factor of 2.62. A ring trial was undertaken to ensure that the Thai and Malaysian labs were giving satisfactory results.

A second larger study of tilapia with fish from both Malaysia and Thailand was undertaken. Tilapia were collected from 80 locations, which included all 10 provinces, and 3 wholesale markets, to cover a wider range of aquaculture practices and feeds. Fillets were prepared and analysed, and the results combined with those of the first study. There was more effect from location on the nitrogen content of the fish because of feed difference. The average nitrogen value from pond culture which predominates in Thailand was 2.92 g/100 g. Malaysia also collected fish farmed by both cage and pond, and fillets were prepared and analysed. The average nitrogen content was found to be 2.76 g/100 g. As pond culture dominates the market, after statistical analysis of all the results, a *N*-factor for tilapia of 2.88 was recommended.

South Atlantic hake (*Merluccius capensis* and *Merluccius paradoxus*)

South Africa[11] presented data of a large trial to determine the *N*-factor of South Atlantic hake to the CCFFP. Samples (360) were taken of headed and gutted fish, unprocessed fillets, fillet and mince blocks from two fishing grounds and 3 seasons during 2011 and 2012. The samples were composited down to 90 samples, from which were selected 84 composite samples because of some errors. The results were analysed and the average nitrogen content of whole fish or unprocessed fillets was 2.60 g/100 g, fillet blocks was 2.46 g/100 g, and for mince blocks 2.33 g/100 g. A recommended *N*-factor of fillet blocks of 2.45 was agreed by the CCFFP.

Alaska pollack (*Theragra chalcogramma* (Pallas))

Seventy five samples[12] each of whole Alaska pollack, fillet and mince blocks were obtained from both the Russian sector and the US sector of the Bering Sea, over different periods in both the Spring (A) season and Autumn (B) season. The average nitrogen content of the fillet blocks was 2.68 g/100 g (Kjeldahl 2.64), which again was about 5% less than the average nitrogen content of the fillets prepared from the whole fish (2.81 g/100 g (Kjeldahl 2.77)). The mince block average nitrogen content was 2.51 g/100 g (Kjeldahl 2.47), which was around 11% less than the whole fish fillet.

Table 1 Summary of published nitrogen factors for GMP fish ingredients

Fish species	Kjeldahl nitrogen%
Cod fillet block	2.75
Minced cod block	2.67
Scampi (*Nephrops*)	2.45
Atlantic salmon (fat-free) fillet	3.75
Atlantic salmon mince (fat-free)	2.81
Tilapia fillet	2.88
S. Atlantic hake fillet block	2.45
S. Atlantic hake mince block	2.33
Pangasius fillet	2.66
Alaska pollack fillet block	2.64
Alaska pollack mince block	2.47

Table 2 Interim nitrogen factors[4] awaiting confirmation

Species	Nitrogen%
Coley/saithe	2.69
European hake	2.64
Haddock	2.72
Ling	2.78
Whiting	2.68
White fish mean	2.65

Pangasius (*Pangasius hypophthalmus*)

Pangasius (Basa, Panga or river cobbler) is a catfish reared in the Vietnamese Mekong Delta. Seventy five[13] each of whole fish and commercially prepared frozen fillets made to GMP were procured from two main aquaculture sites on the Mekong Delta in Vietnam, over two periods in 2012. The average nitrogen content for the fillets was 2.70 g/100 g (Kjeldahl 2.66), which is 5% lower than the average nitrogen content of fillets removed from whole fish (2.85 g/100 g (Kjeldahl 2.80)).

Determining seafood content by chemical analysis

The use of nitrogen factors to determine the seafood content of coated fish or seafood products is given below. A summary of published nitrogen factors for seafood is given in Table 1. Table 2 gives the remaining interim nitrogen factors from the UK Code of Practice.[4]

The percentage fish content, corrected for the non-fish nitrogen contributed by the carbohydrate coating, is calculated as follows:

$$\%\ \text{Fish} = \frac{(\%\ \text{total nitrogen} - \%\ \text{non-fish nitrogen})}{\text{appropriate } N \text{ factor}} \times 100$$

The non-fish nitrogen is calculated as follows:

$$\%\ \text{non-fish nitrogen} = \%\ \text{carbohydrate} \times 0.02$$

where the carbohydrate is calculated by difference:

$$\%\ \text{carbohydrate} = 100 - (\%\ \text{water} + \%\ \text{fat} + \%\ \text{protein} + \%\ \text{ash})$$

References

1 Regulation (EU) No. 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, OJ L304, 22.11.2011, pp. 18–63.
2 G. Stubbs and A. More, *Analyst*, 1919, **44**, 125.
3 Codex Standard for Quick Frozen Fish Sticks (Fish Fingers), Fish Portions and Fish Fillets – Breaded or in Batter. CODEX STAN 166 – 1989, Rev 2011.
4 Code of Practice on the Declaration of Fish Content of Fish Products, UKAFFP, BFFF, BHA, BRC, Sea Fish Industry Authority, LACOTS, Association of Public Analysts, http://www.seafish.org/media/Publications/Fish_Content_CoP.pdf.
5 British Standard, BS 4401:1980 ISO 937:1978, "Methods of Test for Meat and meat products–Part 2: Determination of nitrogen content (reference method)".
6 M. Thompson, L. Owen, K. Wilkinson, R. Wood and A. Damant, A Comparison of the Kjeldahl and Dumas Methods for the Determination of Protein in Foods, using Data from a Proficiency Testing Scheme, *Analyst*, 2002, **127**, 1666–1668.
7 AMC, Nitrogen Factor for *Nephrops norvegicus* (scampi), *Analyst*, 2000, **125**, 347–352.
8 AMC, Nitrogen Factors for Cod Ingredient in Fish Products, *J. Assoc. Public Analysts*, 2007, **35**, 1–16.
9 P. Colwell, L. R. Stephen Ellison, M. J. Walker, S. Elahi, D. Thorburn Burns, K. Gray and D. Craston, Nitrogen factors for Atlantic Salmon, Salmo salar, Farmed in Scotland and in Norway and for the Derived Ingredient, "Salmon Frame Mince" in Fish Products, *J. Assoc. Public Analysts*, 2011, **39**, 44–78.
10 CX/FFP 11/31/12 Proposed Draft Amendment to the Standard for Quick Frozen Fish Sticks (Nitrogen Factors). Thirty-first Session CCFFP, Tromsø, Norway 11–15 April 2011, http://ftp://ftp.fao.org/codex/Meetings/CCFFP/ccffp31/fp31_12e.pdf.
11 CX/FFP 12/32/9 Proposed Draft Amendment to the Standard for Quick Frozen Fish Sticks (Nitrogen Factor for South Atlantic Hake), Thirty-Second Session of the CCFFP, Bali, Indonesia, 1–5 October 2012, http://ftp://ftp.fao.org/codex/meetings/ccffp/ccffp32/fp32_09e.pdf.
12 AMC, A Nitrogen Factor for Alaska Pollack Ingredient in Fish Products, *Anal. Methods*, 2014, **6**, 1279–1283.
13 AMC, A Nitrogen Factor for Commercial Pangasius (*Pangasius hypopthalmus*) Fillets, *Anal. Methods*, 2014, **6**, 1284–1287.

Mark Woolfe

This Technical Brief was drafted for the Nitrogen Factors Sub-Committee (Chair M. Woolfe) and approved by the Analytical Methods Committee on 25/04/14.

CPD Certification I certify that I have studied this document as a contribution to Continuing Professional Development.

Name……………………………………………………………
Signature…………………………….Date…………

Name of supervisor…………………………………..
Signature…………………………….Date…………

