

Simultaneous determination of two major snow crab aeroallergens in processing plants by use of isotopic dilution tandem mass spectrometry

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Abstract Snow crab is a major fishery in the North Atlantic region. During crab processing the proteins are aerosolized and some are responsible for development of occupational asthma. Tropomyosin and arginine kinase have recently been reported as major snow crab allergens. A liquid chromatographic tandem mass spectrometric method has been developed for simultaneous analysis of these two proteins in air samples collected from processing plants. These proteins were initially isolated then characterized by use of mass spectrometry to determine their primary structure and signature peptides. The signature peptides were chemically synthesized in light and heavy forms and used as standards for developing the multiple-reaction monitoring transitions to monitor allergen levels. A validation study was performed; precision and accuracy were 1.8–8% and 91–104%, respectively. Replicate air samples were collected on air filters from two crab-processing plants in Newfoundland and Labrador (NL) and four located in Quebec. In NL, measured levels of both tropomyosin and arginine kinase were between 1 and 20 ng m⁻³. In Quebec plants, however,

levels were found to be much higher at 2–2400 ng m⁻³. Significant differences were also observed among the plants and individual processing workstations. For the first time arginine kinase has been detected in its aerosolized form in processing plants. In general, levels of the allergens were highest in the butchering and cooking areas; plant design can, however, have a significant effect on levels of the allergens.

Keywords Aeroallergens · Bioanalytical method · Tandem mass spectrometry · Proteins · Peptides · High throughput screening · Occupational health · Asthma

Introduction

Seafood ingestion can cause severe acute hypersensitivity reactions and seafood is recognized as one of the most common food allergies. Workers involved in crab, prawn, shrimp, and fish processing are commonly exposed to airborne allergens that increase the risk of developing occupational asthma [1]. In general, occupational exposure to seafood causes a variety of health problems; one problem reported is a significant prevalence of occupational asthma [2–4]. Dermatitis and asthma are the major hazards caused by both irritant and immunological reaction to occupational exposure in seafood production.

The prevalence of snow crab occupational asthma was reported as early as 1980 by *Cartier et al.*; it was 15.6% among workers [5]. However, the prevalence of protein contact dermatitis ranges from 3 to 11% and from 7 to 36% for asthma and allergy, respectively, in workers exposed to arthropoda [6–8]. Occupational asthma has been

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reported among Polish fishermen working in processing plants on factory ships [9]. In addition, other reports have shown sensitization to red soft corals among spiny lobster fishermen and occupational asthma in workers employed in fish-freezing and fish-smoking factories [10, 11].

In 2001, a multidisciplinary study commenced on Canada's east coast to evaluate the prevalence of occupational asthma and allergies in workers from four snow crab-processing plants in Newfoundland and Labrador (NL) [5]. This study was challenging partly because no regulation had been established on the subject. Samples were analyzed using both total protein assay (Bradford method) and immunological-based quantification approaches. But these techniques are non-specific and not sufficiently sensitive for mapping exposure levels among workstations [12]. These techniques are valid for quantifying single allergen or total allergen when the antibody or sensitized patients' IgE, respectively, are used. The multistep immuno-reactivity complicates the assay with poor accuracy and reproducibility. These methods also have limitations owing to the requirements for radioactive or colorimetric detectors.

Recently, mass spectrometry has been of crucial importance in protein identification after evaluation of allergenicity using direct immunoblotting. For snow crab, several allergens were identified by mass spectrometry [13–15]. These have been reviewed in a recent textbook [16].

In this study, a novel multi-allergenic approach was developed to quantify the aerosolized forms of both snow crab tropomyosin (TM) and arginine kinase (AK) in air samples. The approach was developed using isotopic dilution tandem mass spectrometry (MS–MS). A linear calibration curve with wide dynamic range was developed for these allergens. The method sensitivity, accuracy, reproducibility, and linearity were studied to evaluate the validity of the method for multi-allergen analysis. Real air samples were collected from two snow crab plants in NL and four other plants from Quebec. The aerosolized allergens were eluted from the air filters, treated by tryptic digestion, and the signature peptides were analyzed by LC–MS–MS.

As will be demonstrated in this paper, use of the LC–MS–MS to monitor both TM and AK at the same time is a useful means of determining workers' exposure to these aeroallergens. Environmental monitoring must be performed in the workplace during these processing tasks to map the area containing allergens and to identify sectors and assignments where improvements are needed. Workplace mapping is a mandatory step to evaluate adequately the risk of exposure of the workers. With such field assessment, improvements for personal protection, handling procedures, and workplace design can be proposed. This paper reports the development of methods for analysis of occupational exposure and the manner it could be applied in the field by the industrial hygienist to monitor workplaces.

Experimental

Chemicals and materials

All chemicals were purchased and used without further purification. Ammonium sulfate, acetonitrile, hydrochloric acid, and methanol were supplied from ACP (Montreal, Canada). Trypsin proteomics grade, Tris(hydroxymethyl) aminomethane (Tris), dithiothreitol (DTT), ethylenediaminetetraacetic acid (EDTA), formic acid (FA), ammonium bicarbonate, *O*-methylisourea hemisulfate, ammonium formate, iodoacetamide (IAA), and α -cyano-4-hydroxycinnamic acid (HCCA) matrix were purchased from Sigma–Aldrich (St Louis, MO, USA). The Bradford assay kit was from BioRad (Hercules, CA, USA). Dialysis bags were purchased from Fischer Scientific (Roncho Dominguez, CA, USA). For desalting, ZipTip C₁₈ filters were purchased from Millipore (Bedford, MA, USA), RapiGest SF surfactant from Waters Corporation (Milford, MA, USA), and 33-mm polytetrafluoroethylene (PTFE) filters for air sampling were bought from SKC (Eighty Four, PA, USA). The AK signature peptide (AKSP), LVSAVNEIEK (purity >98.33%; molar mass=1101.27 Da) and its deuterated isotopic homolog (d₃-AKSP) using d₃-L-alanine (purity >96.80%; molar mass=1104.27 Da), and the TM signature peptides (TMSP), SQLVENELDHAQEQL-SAATHK (purity >95.3%; molar mass=2348.53 Da) and its deuterated isotopic homolog (d₉-TMSP) using d₃-L-alanine (purity >97.1%; molar mass=2357.53 Da) were purchased from GeneMed Synthesis (San Francisco, CA, USA). Snow crab TM and AK were extracted and purified from fresh frozen meat samples as described elsewhere [12–14]. These extracts were used to determine the signature peptide for each allergen and were also used as positive controls during routine analyses.

Signature peptide solutions

A solution mixture of TMSP and AKSP was prepared in distilled water (dH₂O). This working solution was used to prepare the calibration curve and quality-control samples. The internal standard solution, d₉-TMSP and d₃-AKSP, was also prepared in dH₂O and added to the samples at 500 nmol L⁻¹ final concentration. A linear calibration curve ranging from 1–1000 nmol L⁻¹ and quality control samples at three concentrations, 20, 250, and 850 nmol L⁻¹ were prepared using 0.05% RapiGest SF in 0.1 mol L⁻¹ ammonium bicarbonate solution.

Sample preparation

Air sample collection

Air samples were collected from several snow crab-processing plants; two plants in NL (30 samples in total) and four in

Quebec (75 samples in total) during the 2010 and 2011 fishing seasons, respectively. The air samplers, Leland Legacy Sample Pump (SKC, Rochester, MN, USA), were deployed among five to seven different locations inside each plant, i.e., butchering, cleaning, cooking, packing, triaging, and storage areas in addition to field blank samples collected outside the plants. The samplers were programmed to collect particulates for an 8-h working shift at a flow rate ranging from 4–5 Lmin⁻¹. In NL, this collection was repeated over three consecutive days for each plant, to give a good statistical daily result, whereas collection in Quebec was done over 1 day, either singly or up to six times, depending on the workstation. Personal breathing zone (PBZ) air samples and ambient air samples were also collected on PTFE filters in the Quebec plants at flow rates ranging from 2–3 Lmin⁻¹. The flow rate of each sampler was calibrated before and after collection by use of a Defender 510 air sampling pump calibrator (Air-Met Scientific, Victoria, Australia). The filters from the sites were shipped in dry ice to the lab and stored at -80 °C until protein extraction.

Protein extraction and tryptic digestion

The proteins were eluted from the filter surface by overnight shaking at 4 °C in 500 µL 0.05% RapiGest SF in 0.1 mol L⁻¹ ammonium bicarbonate, pH 7.8. The internal standards were added to each sample at 500 nmol L⁻¹ final concentration. The eluted protein, calibration, and quality control samples were freeze dried, then reconstituted with 100 µL of 50 mmol L⁻¹ ammonium bicarbonate. The disulfide bonds were reduced and methylated using DTT and IAA, respectively, and the samples were then tryptic digested by adding 1 µg trypsin to each sample in 50 mmol L⁻¹ ammonium bicarbonate solution form. The sample mixture was incubated at 37 °C overnight (~18 h). The digestion was quenched by adding an equivalent volume of 1% formic acid (pH ~2) to degrade the acid-labile surfactant (RapiGest SF) and heated to 37 °C for 30 min [16]. Samples were then vortex-mixed and centrifuged for 15 min at 23,500 R.C.F. The supernatant was transferred, freeze-dried, and stored at -80 °C for LC-MS-MS analysis.

Multiple-reaction monitoring tandem mass spectrometry

The detergent-free samples were reconstituted with LC mobile phase and introduced to the tandem mass spectrometer. The multiple reaction monitoring transitions for the standards and internal standards were developed using the signature peptide solutions. The samples were introduced to a Waters Alliance 2795 HPLC system coupled to a Micro-mass Quattro Ultima (Waters, Milford, MA, USA) tandem mass spectrometer (triple-quad) operated in electrospray positive-ionization mode for analysis. The tryptic peptides

were separated on a reversed-phase chromatography column (Kinetex C₁₈, 2.1 mm×100 mm, 2.6-µm particle size; Phenomenex) at 20 °C. Peptide separation was performed by use of a mobile phase gradient at constant flow rate (100 µL min⁻¹). The aqueous mobile phase (A) consisted of HPLC-grade water containing 0.1% formic acid; the organic phase (B) was ACN containing 0.1% formic acid. The gradient was from 5% to 90% B in 3 min, held for 4 min, then back to 5% in 3 min (11 min run time). At normal flow rate, 20 µL sample was injected, after pre and post-injection needle washing with 200 µL mobile phase B then 600 µL mobile phase A. The eluted peptides were aerosolized with a constant flow of nitrogen gas to form light droplets, which then desolvated in the ESI source with a gas flow of 400 Lh⁻¹ at a temperature of 250 °C. The ions were accelerated through the capillary and orifice cone at 3.02 kV and 40 V, respectively. The precursor ions were selectively isolated and fragmented via low-energy collision-induced dissociation (CID) in the middle quadrupole using argon gas with a collision energy of 13 eV.

Results and discussion

Developing a quantitative tandem mass spectrometric method for simultaneous determination of two key snow crab aeroallergens in processing plants was the objective of this study. The isotopic dilution tandem mass spectrometric approach used requires assigning signature peptides for the allergens, which are used to develop this method.

Tropomyosin and arginine kinase have been identified as major snow crab airborne allergens [15–18]. These two proteins were extracted and purified by use of precipitation techniques. The individual allergen extracts were exposed to tryptic digestion for determination of their representative signature peptides. It is imperative to note that the ideal signature peptide should be unique for the target protein, have a short amino acid sequence, and be free from any chemically reactive residues. The best peptides for representing snow crab TM and AK are ⁵⁰SQLVENELDHAQEQLSAATHK⁷⁰ and ²⁴⁶LVSANVEIEK²⁵⁵, respectively, as they meet the above criteria [13, 14].

Method development

The signature peptides of both aeroallergens were chemically synthesized in both light and heavy forms for use as external and internal standards, respectively. Working solutions of these peptides were used to develop the MRM transitions using the triple-quad MS. The product ion spectra of the highest-intensity precursor ions were collected for the peptides as shown in Fig. 1. The selected

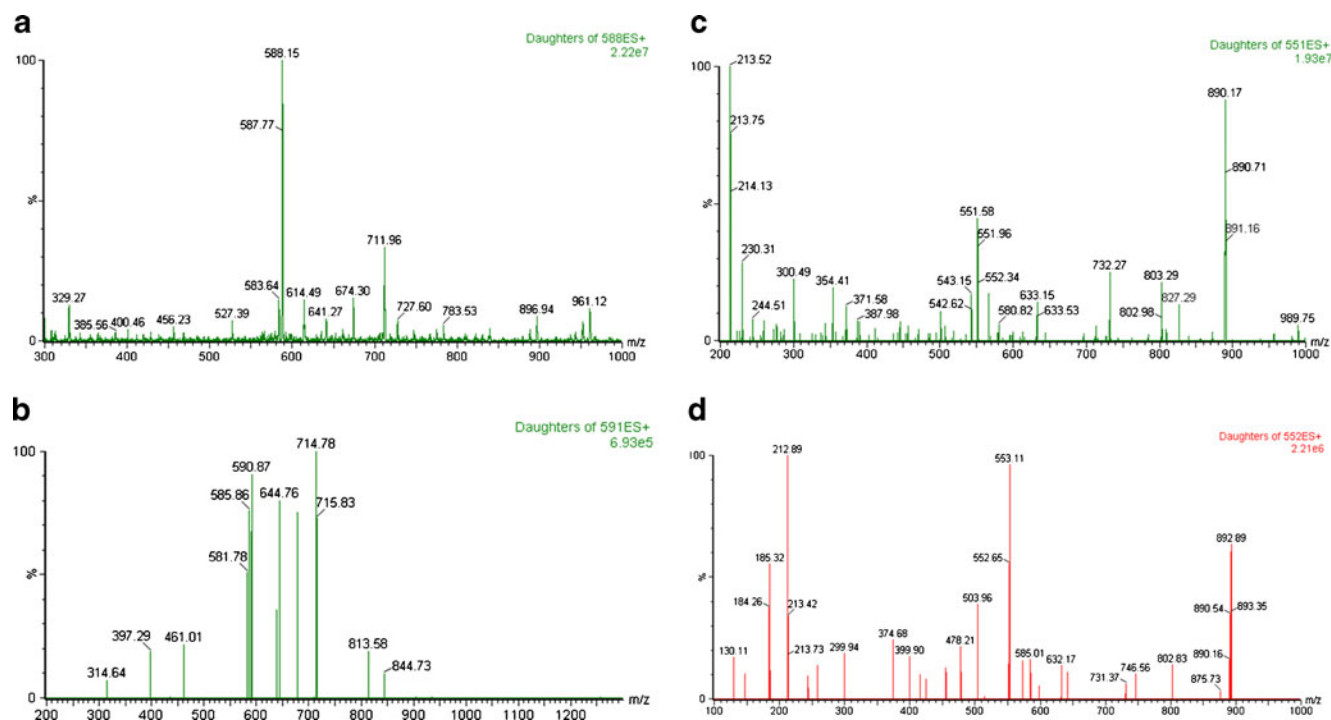


Fig. 1 Product-ion spectra of: (a) TMSP $[M+4H]^{4+}$, m/z 588; (b) d₉-TMSP $[M+4H]^{4+}$, m/z 590; (c) AKSP $[M+2H]^{2+}$, m/z 551; (d) d₃-AKSP $[M+2H]^{2+}$, m/z 552

precursor ions were in different charge states—quadruple and double for TMSP and AKSP, respectively. With attention to the charge state, that mainly depends on the proton affinity and length of the peptide itself. The $[M+2H]^{2+}$ precursor ions of the unlabeled and labelled forms of the AK signature peptide were 551.52 and 552.64 m/z , respectively. The $[M+4H]^{4+}$ precursor ions of the unlabeled and labelled forms of the TM signature peptide were 588.15 and 590.25 m/z , respectively.

The peptide mixture was chromatographed on a reversed-phase column using a mobile phase gradient; the run time was 11 min. Representative MRM chromatograms for the TMSP and AKSP allergens and their labelled forms are shown in Fig. 2. Linear calibration curves were developed for the mixture of allergens over a wide dynamic range (1–1000 nmol L⁻¹).

In-solution digestion was performed on snow crab crude extracts to study the reliability of choosing these signature peptides to surrogate their proteins. This method was also used to standardize the purified SCTM and SCAK for further use in studying the extraction recovery and to be used as positive controls in routine analysis.

Method validation

This bioanalytical method was validated for both allergens in accordance with International Conference of Harmonization (ICH) guidelines.

Linearity

Under the method conditions, linear relationships over a wide dynamic range were verified for the signature peptides. For both allergen signature peptides, analysis of variance regression r^2 values were >0.99 for seven calibration points. The correlation coefficient, y -intercept, and slope of the regression line over four different days are summarized in Table 1, and a representative calibration curves are shown in Fig. 3.

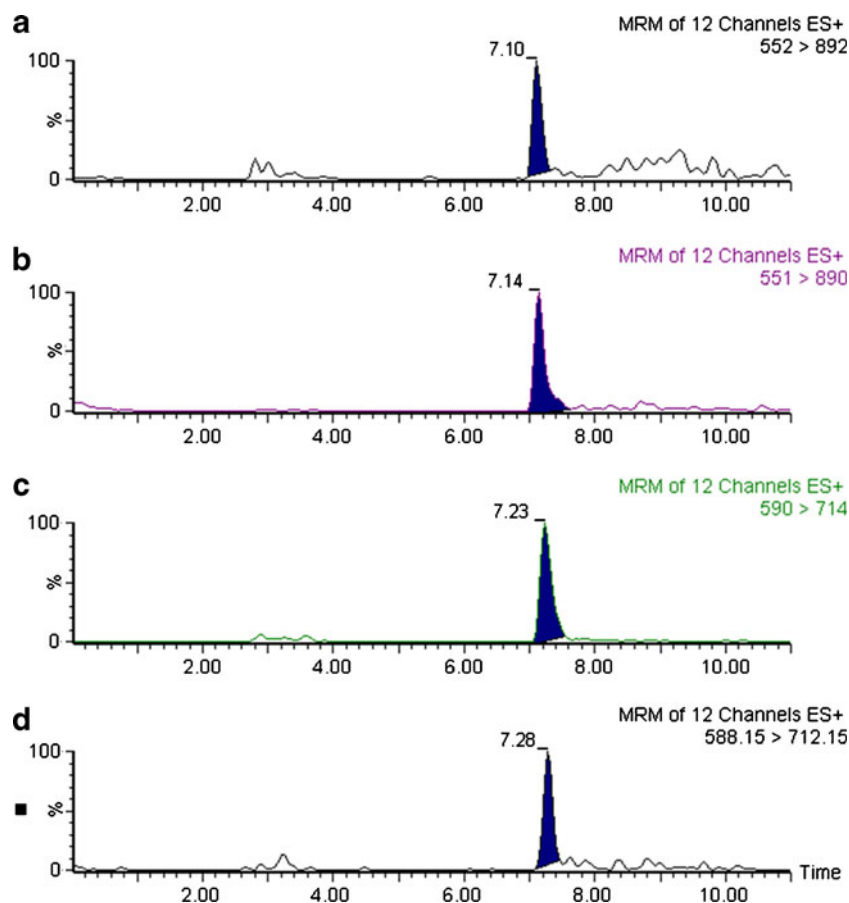
Sensitivity

In determination of the sensitivity of the method, the lower limit of quantification (LOQ), which is the lowest calibration point (1 nmol L⁻¹), showed consistent precision and accuracy of ≤20% of the nominal value determined at a 10:1 signal-to-noise ratio. The lower limit of detection (LOD), was calculated to be 0.2 nmol L⁻¹ and 0.15 nmol L⁻¹ for TM and AK, respectively; the values were calculated on the basis of the standard deviation of the response (σ) and the slope of the calibration curves (m), where $LOD=3.3\sigma/m$ [19].

Accuracy and precision

The accuracy and precision of the method were evaluated by analysis of six replicates of three different levels of quality-control samples, (low, medium, and high) during the sample

Fig. 2 Representative MRM chromatograms obtained from: (a) d₃-AKSP, (b) AKSP, (c) d₉-TMSP, (d) TMSP



analysis. Sample concentrations were back-calculated and the deviation of the mean from the nominal values served as the measure of method accuracy and precision, as summarized in Table 2.

Recovery

A solution of each allergen was standardized as described elsewhere [18]. Briefly, a mixture of standard allergens (50 nmol L⁻¹) was used to study the recovery of the protein by elution from PTFE filters. This solution was sprayed using a detached mass spectrometry ESI setup. Spiked filters were then processed, in parallel with equivalent reference solution, as described in the “Experimental” section. The recoveries (average from three runs) of the allergens

Table 1 Summary of results from validation of the linearity of calibration curves for the snow crab allergens

Allergen	<i>R</i>	<i>y</i> (intercept)	<i>M</i> (slope)	Range
TM	0.99941	0.14708	0.002567	1–1000 nmol L ⁻¹
AK	0.99834	0.04076	0.00214	

These results are averages from different four curves on four different days

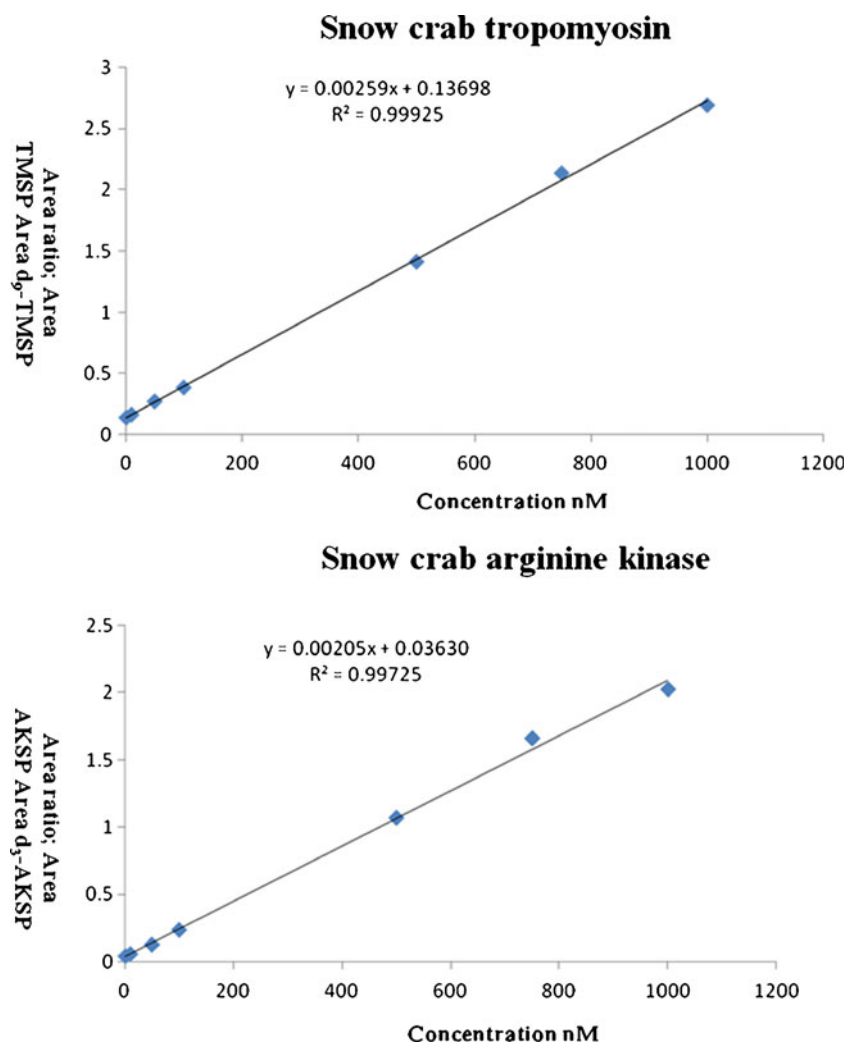
from PTFE filters were 95.7% and 93.2% for TM and AK, respectively.

Sample collection and analysis

Sampling from Newfoundland and Labrador plants

Air samples were collected in summer 2010 from two different snow crab-processing plants in NL located on the Avalon Peninsula. Snow crab processing begins with the butchering or cracking step, in which the leg section of the body is broken off and the ends of the legs are cleaned and trimmed with a rotary brush. After cleaning of the whole leg sections with brushes the leg portions are cooked in an open, steaming kettle. The cooked snow crab legs are then cooled and packed in boxes according to size. The processed crab is stored in freezers for shipping to market [20]. Sampling was performed in five different locations inside these plants—the butchering, cleaning, cooking, packing, and storage areas. This sampling process was repeated on three consecutive days (8-h shift/day). The collected proteins were eluted and subjected to tryptic digestion. Optimum conditions were found to ensure complete digestion, to maintain the 1:1 stoichiometric relationship between the signature peptides and their proteins. The air samples were analyzed by LC–

Fig. 3 Representative calibration curves of signature peptides for snow crab tropomyosin (*top*) and arginine kinase (*bottom*)



MS–MS with a set of calibration curve and quality-control samples in the same batch. Experimental variables were controlled by maintaining method reproducibility by analyzing negative controls with a set of field, extraction, and laboratory blank samples in the same manner as actual air samples. Finally, purified protein TM and AK extract samples were used as positive controls.

The levels of TM and AK aeroallergens detected are summarized in Table 3. The allergens were detected in all locations. The method shows a significant improvement in sensitivity compared with previous work in which the allergens were only detected in the butchering area and near the cooking areas [21]. The immunoassay method is more sensitive than total protein-based methods, for example the

Table 2 Summary of the precision and accuracy of the method

Allergen	Statistical data	QC, low (20 nmol L ⁻¹)	QC, medium (250 nmol L ⁻¹)	QC, high (850 nmol L ⁻¹)
Tropomyosin	Mean ^a	19.06	245.34	888.45
	SD	1.81	25.96	54.64
	Precision CV%	4.72	1.86	4.52
	Accuracy%	95.28	98.14	104.52
Arginine kinas	Mean ^a	18.23	248.13	870.52
	SD	4.55	26.10	35.63
	Precision CV%	8.84	0.75	2.41
	Accuracy%	91.16	99.25	102.41

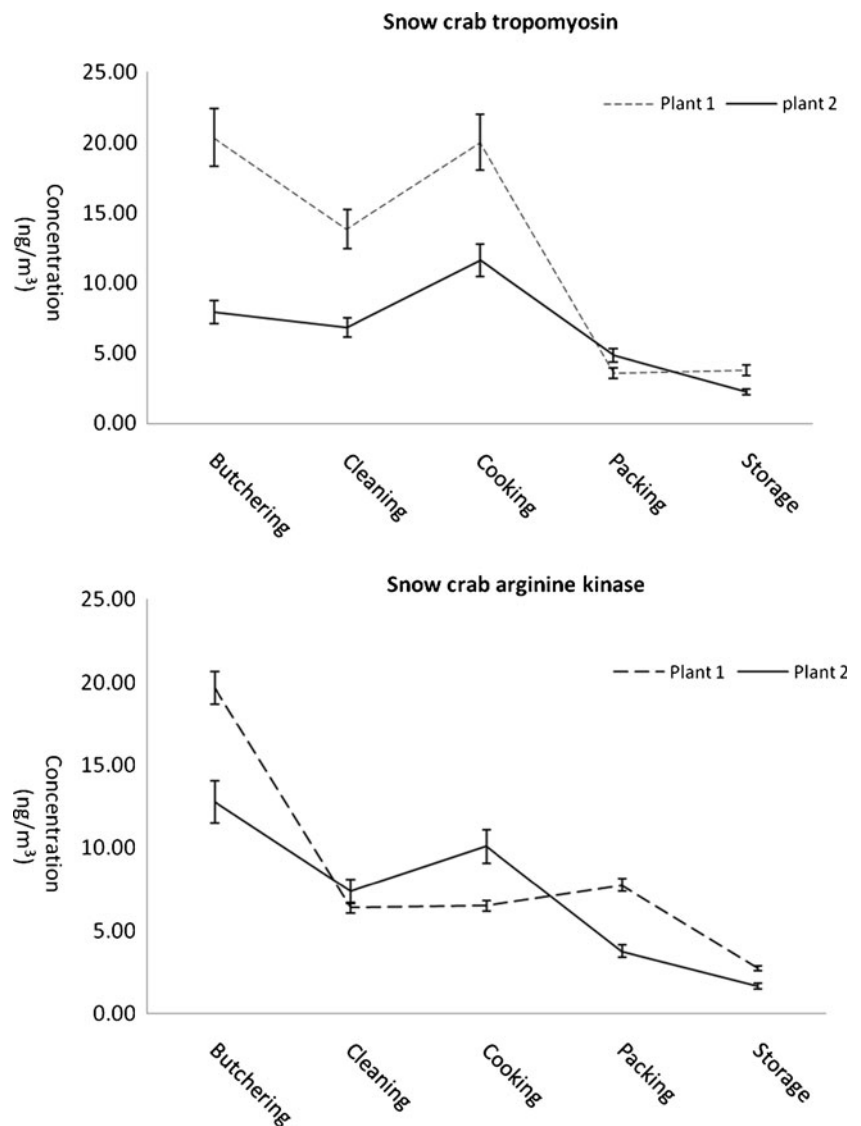
^aThese results are averages from six samples ($n=6$) on four different days

Table 3 Results from analysis of air samples collected from two different NL plants with different designs

	Processing area	TM (ng m ⁻³) ^a	AK (ng m ⁻³) ^a
Plant 1	Butchering	20.34±0.64	19.68±0.13
	Cleaning	13.83±1.07	6.38±1.34
	Cooking	20.01±0.44	6.50±0.79
	Packing	3.59±0.05	7.75±0.04
	Storage	3.78±0.12	2.75±0.09
Plant 2	Butchering	7.94±0.53	12.79±0.24
	Cleaning	6.85±0.53	7.37±0.58
	Cooking	11.60±0.59	10.09±0.52
	Packing	4.85±0.05	3.76±0.07
	Storage	2.26±0.11	1.64±0.02

^a Each result is the average ± SD for three samples ($n=3$) collected on three consecutive days

Fig. 4 Profiles of allergen levels in the two NL crab plants: TM exposure profile (*top*) and the AK exposure profile (*bottom*)



concept design, poorer ventilation, and lack of physical and preventive separators between the different workplaces. In plant 2, where the packing and storage areas were designed as separate working areas, the allergen level was lower, particularly for the AK protein. However, these plants still need to improve their air ventilation to reduce exposure to these aeroallergens.

Sampling from Quebec plants

After the successful sampling activity in NL, it was decided to scale up sampling and analysis by measuring TM and AK simultaneously in the snow crab-processing plants in Quebec. In Quebec, snow crab processing is mainly conducted in the La Cote-Nord, La Gaspésie/Iles-de-la-Madeleine, and Le Bas-St-Laurent regions. This processing activity involves approximately 2,000 staff working for between 8 and 10 weeks per year at 15 different workstations. It has been found that more than 15% of these workers develop asthma when they are exposed to aerosolized snow crab meat and cooking water. In this work eight plants in Quebec were investigated; the most relevant findings are reported and discussed below.

Each plant investigated in this study was designed differently. The same workstations were not present in all the plants because of their specific layout. In addition to the layout, the amount of snow crab processed could be different because of factors affecting the fisheries, for example the

weather, the quota, and other uncontrolled factors. Effort has been made to present the results from the plants with the largest similarities in terms of amount of crab processed, sampling period, and overall tasks accomplished. PBZ samples were taken in all processing workstations, and ambient samples were taken at two workstations.

The samples taken at the most relevant workstations in the four most important snow crab plants are presented in Fig. 5. The two known allergenic proteins, TM and AK, were detected at all the workstations. However, important differences are observed among the mean levels of TM and AK detected in each plant. Mean levels of TM were higher than the mean levels of AK in plants 1 and 2 whereas mean levels of both allergens were almost equal in plant 4. Levels of AK were significantly higher than those of TM in plant 3. Levels of allergenic proteins at all the workstations were generally less in plant 2 than in the other plants, and mean levels of allergenic proteins were highest plant 3. This could be explained by the design of the plants and work habits. Plant 2 is smaller than the other plants and the ventilation system is different. Also, it is common practice to work slowly in this plant, in contrast with the other plants. The other plants are larger and the workers have more room to work, which accelerates the rhythm of the work. These factors could explain why the presence of allergen proteins in the air is different in each plant. At this point the extent of allergenic protein in the workplace is known, so emphasis

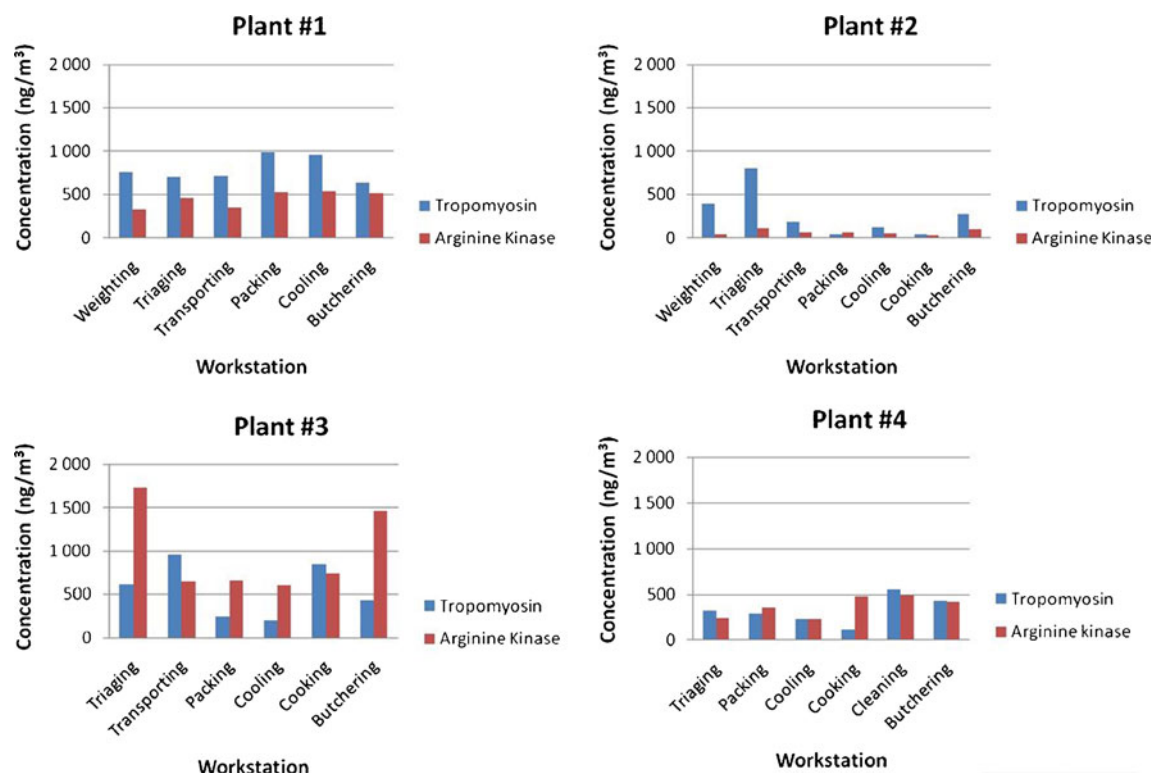


Fig. 5 TM and AK PBZ levels in snow crab plants in Quebec

Table 4 Results from air sampling performed in Québec showing the range min–max for each relevant station of each plant

Plant	Station	Allergen	Mean (ng m ⁻³)	Range min–max (ng m ⁻³)
Plant #1	Weighing	TM	760	536–984
		AK	331	210–452
	Triaging	TM	700	375–941
		AK	462	245–599
	Transporting	TM	709	596–860
		AK	353	205–484
	Butchering	TM	638	551–774
		AK	512	484–541
Plant #2	Triaging	TM	802	189–1712
		AK	103	22–209
	Transporting	TM	191	114–267
		AK	60	n.a. ^a
	Packing	TM	44	12–76
		AK	68	32–103
	Cooling	TM	122	n.a. ^a
		AK	48	16–80
	Cooking	TM	43	29–56
		AK	26	n.a. ^a
Plant #3	Triaging	TM	621	290–1336
		AK	1727	613–2205
	Transporting	TM	962	220–1632
		AK	652	96–1355
	Packing	TM	246	78–334
		AK	665	172–1456
	Butchering	TM	427	285–643
		AK	1466	954–2312
Plant #4	Triaging	TM	326	75–609
		AK	244	75–396
	Cleaning	TM	553	395–711
		AK	493	467–519

^a Range min–max not available. The mean is based on one sample only

must now be on collection of statistically relevant data to propose adequate modification to current workplace habits or design.

In combination with the differences between the plants, more information can be obtained from the range of data obtained. This is shown for each workstation. Table 4 shows the minimum and the maximum values obtained at each workstation of each plant when enough data were available. In contrast with the sampling performed in NL, activity in Quebec was studied during a single day on which almost the entire workforce was sampled by use of PBZ cassettes. Significant differences are observed among workers at the same workstation. This could be explained by different work habits, by the agility of some workers, and by the size of the workers. The proximity of the workers to each other is also a source of variation, because cross contamination could occur between workers.

In parallel with the PBZ sampling, measurement of TM and AK was also possible by ambient air sampling. Because limited resources were available, ambient air sampling focused on the cooking and packing workstations in all the plants. As shown in Fig. 6, significant levels were detected for both allergens during cooking and packing. Levels of both aeroallergens vary from one plant to another. Nevertheless, the amounts detected show once again that improvements to reduce the amounts of allergenic proteins in the air of the plants investigated could be introduced to minimize, as much as possible, exposure of the workers to allergen proteins.

Arginine kinase as airborne allergen

Arginine kinase one of the phosphogen kinase enzymes. These enzymes catalyze phosphorylation transfer from guanidine phosphagen (in AK this is arginine) to adenosine diphosphate (ADP) giving adenosine triphosphate (ATP) [22, 23]. AK is of significant importance in cell energy use, and is known to be a ubiquitous protein throughout the whole body (tissue, hemolymph, etc.) occurring at concentrations higher than those of ADP and ATP. To the best of our knowledge, this is the first time arginine kinase has

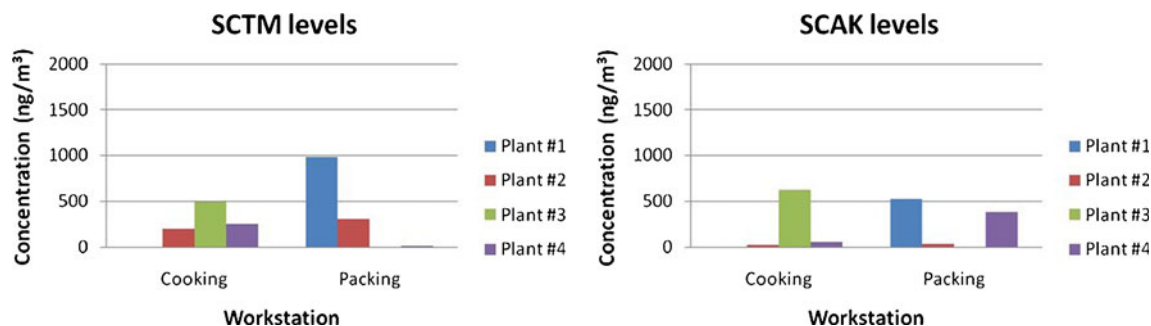


Fig. 6 TM and AK ambient levels near cooking and the packing

been reported as a snow crab aeroallergen. The function and chemistry of AK can explain its high levels in the butchering area; levels were similar to those of TM except in plant 2 of NL and plant 3 of Quebec, where AK was significantly higher than TM. Arginine kinase levels in hemolymph are usually quantified using an enzyme activity-based method, which is very indirect and only recognizes the active forms of AK. However, the inactive form has the specific epitope that causes an allergic reaction once inhaled or ingested [24].

The arginine kinase of the black tiger prawn has been reported to be a heat labile protein (inactive) compared with TM (unpublished work in the laboratory of Dr Lopata, James Cook University, Australia). This gives the MS method an advantage over immune activity-based methods, because it can detect trace amounts of AK even in its inactive form by detecting its signature peptide. Indeed, this supports the idea that AK levels in other processing areas, for example packing and storage areas, are also detected as contaminants from other areas.

Conclusion

This study shows the suitability of LC–MS–MS for quantification of a mixture of allergenic proteins in their airborne state by use of isotopic dilution. This strategy starts by extracting potential allergens from snow crab tissue. These allergens were screened by immunological techniques and characterized (amino acid sequencing) by use of MS. The signature peptides were selected and used for developing MRM transitions in LC–MS–MS. The allergens collected from snow crab-processing plants were analyzed using this approach after optimizing the elution, digestion, and sample-preparation procedures. An extensive study was performed to validate linearity, sensitivity, selectivity, and accuracy. Real air samples collected from crab plants show the strength of the MS method for quantifying airborne multi-allergenic proteins. Interestingly, the allergen levels measured are in agreement with those from a previous study that used immunology-based techniques, although our method is more specific and sensitive. For the first time arginine kinase has been detected in its aerosolized form in snow crab-processing plants; it is generally known to be the second major allergen after tropomyosin. This novel MS-based approach is very effective for industrial hygienists wishing to perform a field assessment. It has been clearly shown in this paper that the aeroallergens TM and AK are present in workplace environments at significant levels, and that employees in snow crab plants are exposed seasonally in both provinces.

This monitoring method can now be used in common industrial hygiene practice to identify the hazards of each task and propose adequate preventive solutions. This work showed that snow crab allergenic airborne proteins can be detected in

the air of crab-processing plants at all workstations. This knowledge is important in study of the occupational health and safety (OHS) of crab asthma. The challenge now is to adjust the statistical design to take into account individual tasks, sampling capacity, plants design, ventilation, work habits, amount of raw material, work shifts, and personal protective equipment, to name only a few. The methods used to collect samples and the number of samples per workstation are also important considerations.

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