LWT - Food Science and Technology 201 (2024) 116246

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Rapid and non-destructive quantification of meat content in the legs of live red king crab (*Camtschaticus paralithodes*) by near-infrared spectroscopy

Jens Petter Wold [a,*], Marion O'Farrell [b], Jon Tschudi [b], Grete Lorentzen [a]

[a] Nofima AS - Norwegian Institute of Food, Fisheries and Aquaculture Research, PB 210, NO-1431, Aas, Norway
[b] SINTEF Digital, Smart Sensor Systems, Forskningsveien 1, 0373, Oslo, Norway

ARTICLE INFO

Keywords:
Near-infrared spectroscopy
Red king crab
Prediction of meat content
Quality control
Sustainable red king crab fishery
Live holding with feeding

ABSTRACT

Red king crab (RKC) is one of the most widely distributed and well-known of all king crabs. The edible meat of the RKC is in the legs and claws and is considered a delicacy. Occasionally, the content of meat is low, and this is regarded as the single most negative quality attribute. In this study, we elucidated how rapid and non-destructive near-infrared spectroscopy (NIRS) on live RKCs can be used to predict the meat content in the cooked legs. A NIRS prototype (wavelength range 760–1080 nm) was used to obtain spectra from the interior tissue of the crab legs. 99 live crabs (380 legs) were measured over a 10-month period. Calibration models were developed with a prediction error for meat content of 6.4 %-points, which is accurate enough to classify the crabs into low (<80 %) and high meat content. The amount of cooked meat is proportional to the amount protein in the muscle. A limitation with the method is that it may overestimate the meat content if the legs have lost free water due to e.g. injuries in the exoskeleton. The technology described can facilitate a more sustainable RKC fishery and improve the subsequent trade and processing.

1. Introduction

Red King Crab (RKC) (*Camtschaticus paralithodes*) is one of the most widely distributed and well-known of all king crabs, ranging from British Columbia in the north through the Bering Sea, around the Kamchatka Peninsula and in the Barents Sea. The edible meat of RKC is in the legs and claws. Occasionally, the content of meat is low, especially after molting, which occurs in late spring. However, access to food, population density, and location of capture, are also factors that influence the meat content. A low meat content, i.e. < 80%, is regarded as the single most negative quality attribute when trading RKC (Voldnes, Kvalvik, & Nøstvold, 2020). Hence, it would helpful to have information of the meat content in live RKCs. In this study, an objective, and non-destructive method to quantify the meat content, while the RKCs are alive, have been developed. In detail, we elucidated how near-infrared spectroscopy (NIRS) can be employed for this purpose.

Quantification of the meat content in live RKCs is needed along the entire value chain. Firstly, the fisherman gets paid according to the crabs' meat content, thus, being able to assess the meat content on the vessel is relevant. Next, when delivering the RKCs to land-based facilities, the buyers manually assess the meat content by squeezing the legs. This is a highly subjective and inaccurate method, time- and labour consuming, and a potentially contentious variable in negotiations between the employees and fishermen. Squeezing the legs may also lead to poor crab welfare, loss of value in terms of damage to or loss of legs, and spots of blue and black discoloration on the meat due to melanosis, which reduces market value (Lorentzen et al., 2018). Feeding of RKCs in captivity, both juveniles and adults, show positive prospects to improve the meat content and overall welfare (Lorentzen et al., 2023; Lorentzen et al., 2024). Being able to assess the meat content periodically during live holding, facilitates optimised feeding routines and generally improved conditions, and can help pinpoint slaughter-readiness.

The legs of live crabs contain fibrous muscle tissue, which provides the crab with the ability to move and manipulate its environment. Additionally, like other parts of the crab's body, the legs also contain haemolymph, which circulates throughout the crab's body cavity and serves various physiological functions (Medler & Mykles, 2015). The main component of haemolymph is water. Although the amount of meat varies according to the season, the moisture, protein, and fat content in *cooked* meat do not seem to change significantly (Lian et al., 2021). The actual meat content in the legs - the quantitative reference measurement – can only be assessed after slaughter and cooking of the legs. The cross

* Corresponding author.
E-mail addresses: jens.petter.wold@nofima.no (J.P. Wold), marion.ofarrell@sintef.no (M. O'Farrell), jon.tschudi@sintef.no (J. Tschudi), grete.lorentzen@nofima.no (G. Lorentzen).

https://doi.org/10.1016/j.lwt.2024.116246
Received 13 March 2024; Received in revised form 9 May 2024; Accepted 22 May 2024
Available online 23 May 2024

sections of the legs are then evaluated, and the meat content is defined as the percentage of cooked muscle relative to the total inner cross-section. Today, this procedure is done manually but can alternatively be performed by using digital image analysis (Lorentzen, Lian, & Siikavuopio, 2019).

NIRS is a widely used technique for rapid and non-destructive determination of food quality (Porep, Kammerer, & Carle, 2015). The method is an excellent tool for determination of fat, water, and protein by measuring molecular vibrations involving hydrogen bonds (e.g. C–H, O–H, and N–H) (Osborne & Fearn, 1986). Today, NIRS is an advanced technology that allows in-line grading and sorting of complex foods such as meat, fish, cereals, and fruits. The meat content in the legs of cooked king crabs most likely relies on the amount of protein and water in the raw legs. NIRS is known to be well-suited for measuring the ratio of protein and water in muscle (Isaksson, Miller, & Næs, 1992; Wold, Måge, Løvland, Sanden, & Ofstad, 2019), however, measuring these properties non-destructively, through the exoskeleton of live crabs, is challenging. Commercial NIRS is most often measured in reflection mode, meaning that the surface of the sample is illuminated by NIR light, and the light reflected from the same spot on the surface is detected for quantitative analysis. This means that mainly the surface is characterized, and not the interior of the sample. Successful quantification of the meat content in a crab leg would require *sub-surface measurements*. This can be achieved by measuring the spectra in interactance mode, where the detected light signal is measured a short distance away from the region that is illuminated. Applications that rely on the detection of deeply penetrating NIR light are already established in the food processing industry for in-line quality grading of fat in salmon fillets (Segtnan, Høy, Lundby, Narum, & Wold, 2009), fat in meat trimmings (Wold, O'Farrell, Høy, & Tschudi, 2011) and protein in chicken fillets (Wold, Veiseth-Kent, Høst, & Løvland, 2017). Another relevant example is the determination of the edible meat content in the carapace of live brown crabs, measured through the dark exoskeleton (Wold, Kermit, & Woll, 2010). In this case, the meat content can be estimated based on the amount of fat, protein and water, which is information that is contained in the NIR spectrum. Recently it was also shown that NIRS can capture signals from the muscle in live mussels (Ghidini et al., 2024).

The objective of this study was to evaluate how NIR interactance spectroscopy, measured on live crabs, can be used to predict the meat content in the subsequently cooked legs. The possibility of achieving this will depend on two factors: 1) the ability to properly probe the interior of the crab legs with NIRS, and 2) a sufficient correlation between the measured properties in live crabs and the amount of muscle in cooked crabs. A prototype NIR system designed for measurements in depth, which was previously developed for in-line determination of core temperature in sausages (Wold et al., 2020) and dry matter in potatoes (Wold, O'Farrell, Andersen, & Tschudi, 2021), was used to establish calibrations for meat content in cooked crab legs, based on measurements on live crabs. Experiments have been designed to extract the physiochemical information, which is the basis for estimating the meat content. Finally, we have evaluated the reliability of the spectroscopic method on crabs fished directly out of water and crabs after a period storage in air.

2. Materials and methods

2.1. Crabs and experiments

All measurements on live crabs were done at the crab processing company Capefish AS in Honningsvåg, Norway. At this site, hundreds of live crabs were kept in water tanks (600 ltr) with running seawater before further processing and shipping. All crabs had been captured during commercial fishery. In experiments with NIRS it was important to include samples with a large span in meat content to develop and validate a robust, quantitative NIRS calibration model. Crabs were sampled to obtain this variation, with the help from an experienced grader who picked a range of crabs with low, medium, and high meat content. The NIRS measurements were done in the vicinity of the processing line immediately after the sampling. The room temperature varied between 4 and 10 °C, depending on the outdoor temperature. The temperature of the crabs in the seawater was about 6 °C. Three different experimental trials were performed.

2.1.1. *Trial 1* (April 2019)

Fifty live crabs were measured with NIRS. The measured crabs were picked from the water and kept live in a vessel without water before the measurements. The midpoint of the legs on the right side was measured for each crab (Fig. 1). The measurements were done on the back side of the legs, where the exoskeleton is light beige/pink/yellowish and the surface is mostly flat, with a slight curvature. This is a more optimal measurement location, since the upper side of the legs are dark brown and absorbs more of the NIR radiation than the back side. Moreover, the front surface contains exoskeletal spikes that would likely interfere with the NIRS measurements. After the legs were measured, they were then cooked in a commercial boiler obtaining a core temperature of approximately 92 °C. The legs were then cooled in refrigerated sea water to approx. 2 °C. After a quick draining of the legs, they were cut across the midpoint (Fig. 1), where the NIR measurement had previously been performed, and the meat content was estimated visually.

2.1.2. *Trial 2* (October 2019)

The shipping of live RKCs involves packing them in styrofoam boxes, which enables survival for up to 48 h. However, a prerequisite for survival is to maintain the welfare of the crab by packing them at humid and low-temperature conditions. Trial 2 was conducted to investigate if the storage of crabs in such conditions affected the NIRS measurements compared to measurements on crabs removed directly out of the water. A total of 28 crabs were measured, on all six legs (midpoint of leg, Fig. 1), using the NIRS prototype at four different stages: 1) Live crabs taken directly from water, 2) live crabs after 4 h of dry storage, 3) live crabs after 24 h of dry storage, 4) drained legs after slaughter. Draining the legs involved putting them in a vertical position, with the shoulder joint facing downward, enabling drainage of the water and haemolymph. The measured legs were then cooked, cooled, cut, and inspected as described for Trial 1.

2.1.3. *Trial 3* (January 2020)

The aim of this experiment was to better understand the physiochemical properties that enable quantification of the meat content in cooked crabs based NIRS on live crabs. A total of 21 live crabs were measured on all six legs (Fig. 1). They were measured immediately after removal from the water. The crabs were then slaughtered. The three left

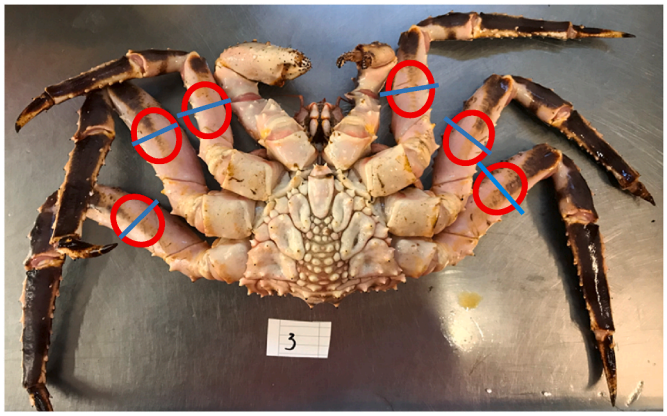

Fig. 1. Red ellipses indicate areas of NIR measurements on live crabs. Measurements were done on the back side of the legs. Blue lines indicate cross sections for evaluation of meat content after cooking.

legs of each crab were cooked as described above, frozen and shipped to our lab overnight. Upon arrival, they were thawed, and meat content in all legs was evaluated visually. Also, the water content in each leg muscle was determined.

The three right legs were frozen in their raw state, to maintain the free water/haemolymph and the muscles. They were then shipped overnight to the lab. Drip loss (the amount of haemolymph) from the legs was measured during thawing. Brix measurements can be used as an indicator of dissolved protein and were performed on the haemolymph. Water content was determined in the raw muscles after draining of the legs. The muscles were also weighted, and length and diameter of the exoskeleton legs (the *merus* part where the measurements were performed) were measured.

2.2. NIR spectroscopic measurements

The NIR prototype measured in interactance mode. A halogen light source of 50 W was used to illuminate the leg in two rectangular regions of approximately 2 mm*20 mm. The distance between the two illuminated regions was 24 mm. Between the two illumination regions, there was a 4 × 4 mm field-of-view that was imaged at the spectrometer. The light travelled from the illuminated regions through the leg and was then detected as it exited the crab through the field of view (Fig. 2). As can be seen in Fig. 2 a large part of the *merus* volume was illuminated. The photo shows visible (VIS) light, and it is assumed that the near infrared (NIR) light, which is at slightly longer wavelengths and more absorbed, probed to at least the inner centre of the leg. Exposure time per measurement was 2 s, and the collected spectra consisted of twenty evenly spaced wavelengths in the region 761–1081 nm. Working distance between instrument and crab leg was 20 cm, so there was no physical contact between them. The NIR instrument was placed on a stand to measure downwards. The live crabs were held up-side down under the instrument during measurement. Total measurement time per crab was typically 20 s when all six legs were measured. However, this could sometimes take longer if the crab was very active and difficult to handle. If the crab moved too much during a measurement, the measurement was repeated.

2.3. Visual assessment of meat content

The meat content was assessed visually after cooking and cooling. A sharp knife with small barbs was used to cut at the mid-point of the legs without squeezing and destroying the exoskeleton and to obtain clean cuts of the muscle. Three people were trained to visually estimate the proportion of muscle in the two cross-sections pr leg. The three judgements were averaged and rounded to nearest 5%. Specifically, the meat content was expressed as the portion of cooked muscle in the two cross-sections, and it was calculated as:

Meat content (%) = [Inner area occupied by meat/Total inner area of exoskeleton leg] × 100.

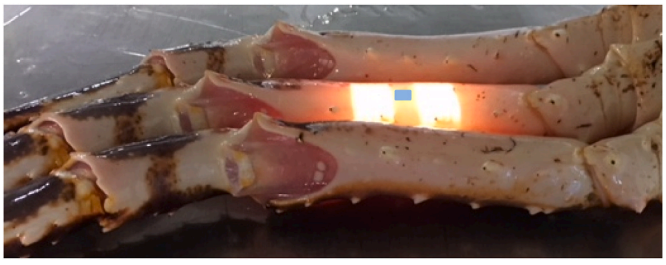

Fig. 2. Illustration of illumination pattern and the field of view of the spectrometer (blue rectangle). Measurements were performed on the backside of the leg, in this case on the middle leg on the right side of the crab. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2.4. Data analysis

2.4.1. Spectral pre-processing

The NIR spectra were linearized using the logarithm of the inverse of the interactance spectrum (T), log10 (1/T). To reduce the effects of light scattering, the absorption spectra were normalized by standard normal variate (SNV) (Barnes, Dhanoa, & Lister, 1989): For each spectrum the mean value was subtracted, and the spectrum was then divided by the standard deviation of the spectrum. Since the spectra were of rather low resolution, we did not apply derivation for pre-processing.

2.4.2. Calibration for meat content

NIRS calibrations for meat content was made by partial least squares regression (PLSR) (Martens and Næs, 1989). The calibration models were based on one spectrum from each leg. Performance of the models was evaluated by the root mean squared error (RMSE) and the squared correlation (R^2) between measured and estimated meat content, where the estimated values were obtained either by full cross-validation in the calibration stage (RMSECV) or by using the calibration on an independent test set (RMSEP). Cross validation was used when establishing calibrations in Trial 1 and 2. Validation of calibration with test sets was done in trials 2 and 3.

The software The Unscrambler ver. 9.8 (CAMO Software AS, Oslo, Norway) was used for regression modelling.

2.5. Physiochemical measurements

2.5.1 Water content in the muscle was determined gravimetrically. Each individual muscle was cut into smaller pieces with a knife. Two sub-samples (20 g) of each muscle were immediately placed in aluminium pans and dried for 48 h at 105 °C in a forced fan oven. Water percentages were calculated based on the weight before and after drying.

2.5.2 Drip loss from each leg was collected during thawing. The frozen leg was placed vertically in a cup and the drip loss was expressed in per cent as the weight of the collected liquid in the cup (g) of the total weight of the frozen leg.

2.5.3 The Brix value of the liquid lost during thawing was determined by a refractometer (PAL-BX|ACID1, Atago Co., Ltd., Tokyo, Japan) and expressed as °Brix.

2.5.4 Physical dimensions of the legs (the merus) were measured with a caliper, length and diameter, and used to calculate the approximate leg volume.

3. Results and discussion

3.1. Spectral properties

Fig. 3a shows SNV normalized spectra from three crab legs with different amount of meat content. The water absorption peak at around 980 nm originates from the second overtone of the OH stretching bond and dominates the spectra. There is also a small water peak (shoulder) at about 840 nm, which is a combination of the third overtone OH stretching and OH bending bands and one at about 760 nm.

The spectra reveals an apparent shift in the 980 nm peak towards shorter wavelengths with reduced amount of meat. This spectral change can most likely be attributed to two phenomena: 1) Protein has absorption peaks at about 910 and 1020 nm (Osborne & Fearn, 1986), and subtle increases in these regions can be seen for crabs with much meat. 2) It is well known that a shift in the water peak at 980 nm occurs depending on the strength of the bond between hydrogen in the water and other molecules, such as protein (Libnau, Kvalheim, Christy, & Toft, 1994). More loosely bound water creates a shift towards shorter wavelengths, and in a crab leg with little muscle there will be more loosely bound and free water, creating such a shift. Stronger hydrogen bonding between water and proteins causes a spectral shift toward longer

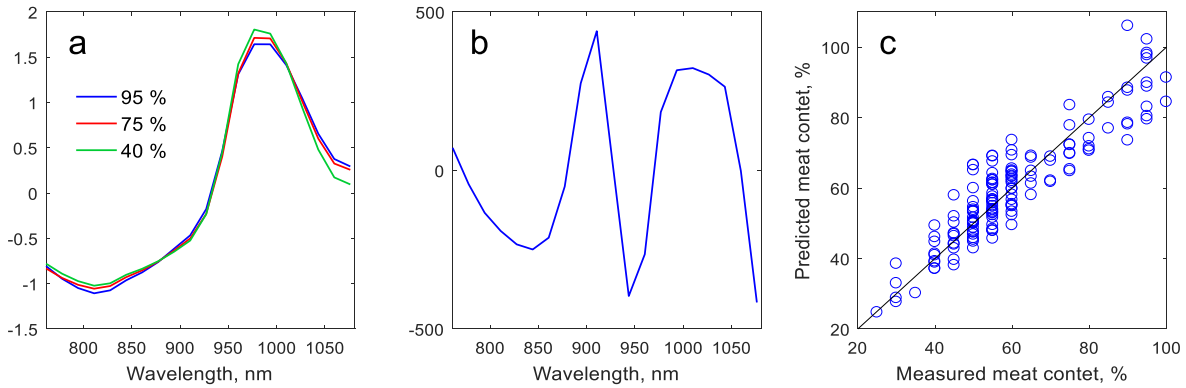

Fig. 3. a) Typical NIR spectra from king crab legs. Meat content indicated at legends. b) Regression coefficient for meat content calibration, c) Predicted versus measured meat content in crab legs from Trial 1.

wavelengths as well as a peak broadening (Chung et al., 2008). This effect is seen in legs where there is more muscle, since the water is more tightly bound to the protein. A crab leg does not contain air, which means that if it contains little muscle tissue, the remaining space contains free water. In a leg full of muscle, most of the comprising water is bound to the muscle protein. The same spectral features, which are observed in this system, are used for in-line detection of the muscle syndrome Woody Breast in chicken fillets since this syndrome is characterised by lower protein content and more loosely bound water (Wold et al., 2017). These features have also been proposed as a marker to detect human breast cancer since cancerous tissue contains a larger share of less bound water compared to normal breast tissue (Chung, Yu, Su, Cerussi, & Tromberg, 2012).

In addition to 1) the amount of protein and water, and 2) the degree of hydrogen bonding between protein and water, the spectra will to some extent be affected also by other properties. The size and geometry of the leg might affect the signals. Light scattering will most likely increase with increasing amount of muscle due to a denser cellular structure. The fat content in the muscle is very low, about 0.6%, and does not seem to affect the spectra significantly. The colour, thickness and transparency of the exoskeleton can also vary quite a bit, and since the light must travel through it two times (in and out), we must assume that these factors introduce some variation in the spectra that must be accounted for in a quantitative calibration. The measured spectra are rather featureless, since we are measuring a more subtle shift in the peak rather than a clearly increasing or decreasing absorption peak, so it is difficult to pinpoint the effects of the mentioned properties.

3.2. Meat content calibration based on NIRS

Fig. 3c shows predicted versus measured meat content in the legs of the 50 crabs from Trial 1 based on cross validation (leave one crab out). Six PLS factors were required to build a calibration and a prediction error (RMSECV) of 6.5% was obtained. This accuracy is sufficient to classify the meat content into high, medium, and low. The regression vector (Fig. 3b) mostly resembles the shape of a protein spectrum with peaks at about 910 and 1020 nm, which indicates that the amount of protein in the raw legs determines the meat content in the cooked legs, which means the biochemistry confirmed our assumptions.

3.3. Effect of storage in air

In Trial 2, 28 crabs were measured under different storage conditions to study possible effects on the calibration robustness and accuracy. Table 1 summarises the calibration results for crabs at different time intervals after removal from water. Calibrations for crabs taken directly from water and for those stored in air for 4 h were quite similar, with approximately the same number of components as the model from Trial 1 and prediction errors in the same range. The regression coefficients were also very similar (not shown). Using data from crabs stored in air for 24 h and drained legs after slaughter, the calibrations were less accurate, and the shape of the regression coefficients changed significantly (not shown). The change can be illustrated more clearly by using the calibration based on crabs taken directly from the water to predict the meat content in the stored crabs (shaded part of Table 1). Predictions for crabs stored for 4 h were good with a low prediction error. The error increased for crabs stored for 24 h and became very high for drained legs.

Table 1
Calibration and test results for meat content in cooked crabs based on NIRS measurements on live crabs (except for the drained legs).

Calibrations	Validation	# PLS components	R^2	RMSE
Trial 1 model (n = 150)	Cross-val	6	0.84	6.5
Trial 2 models (n = 167)				
1. Directly from water	Cross-val	7	0.83	6.7
2. Storage in air 4 h	Cross-val	6	0.85	6.4
3. Storage in air 24 h	Cross-val	5	0.70	9.1
4. Drained legs	Cross-val	5	0.71	8.8
Predictions using model 1, Trial 2				
Storage in air 4 h	Test	7	0.86	6.1
Storage in air 24 h	Test	7	0.70	8.8
Drained legs	Test	7	0.09	22.3
Predictions using model 1, Trial 2				
Trial 3 (n = 63)	Test	7	0.90	6.2

n – number of legs.

Since the prediction errors were quite remarkable, the prediction versus measured plots for the three conditions are shown in Fig. 4. Predictions on crabs stored for 4 h were very good, while after 24 h some of the meat estimates were far too high. For drained legs, most of the meat content estimates were around 100%, which shows that the prediction errors were systematically overestimated and not based on random noise. The reason for this is that the crab legs contain two main fractions: muscle and free water/haemolymph. When the legs are drained of free water, the NIR signal is mainly from the muscle, and the calibration will estimate around 100% muscle. Remember that the calibration is based on the condition that the crab leg is always full, with muscle and haemolymph, and a drained leg deviates from that condition. This is not a problem when the crabs are measured directly from the water, since the part of the leg that is not filled with muscle will be filled with water. During storage, some crabs can lose the free water due to holes and damage to the exoskeleton. This was the case for two of the crabs after 24 h of storage, and in these cases, the meat content was overestimated. It is important to be aware of this limitation of the method when measurements are to be made on crabs after storage in air. It seems to be possible to make calibrations for meat content in drained crab legs (Table 1), but then it is necessary to know or detect that the

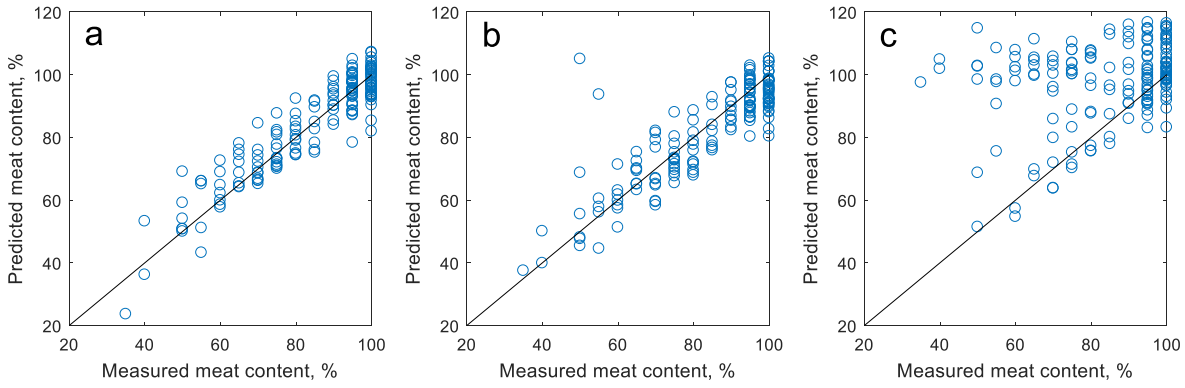

Fig. 4. Predicted meat content in a) crabs stored 4 h in air, b) crabs stored 24 h in air, and c) drained legs.

legs are in fact drained.

3.4. Connection between NIRS estimated meat content and physiochemical measures

To support the hypotheses based on the results above, we studied the relation between predicted meat content in cooked crabs with physiochemical properties in raw crabs (Trial 3). A NIRS calibration made in October (Trial 2) was used to predict the meat content in the legs of the 21 crabs in Trial 3. The predictions were validated on the left legs, which were cooked and inspected for meat content. The prediction results were quite accurate and in line with the results obtained in Trial 1 and 2 (Table 1, Fig. 5). The result verified that the calibration worked well on a set of new crabs. The calibration was used to predicted meat content also in the right legs for comparison with the physicochemical properties. The correlations between predicted meat content and the different physicochemical properties are summarised in Fig. 6. The distribution of each parameter is shown in the diagonal, and each range at the border. The amount of free water (measured as driploss) correlated closely (negatively) with the predicted meat values. The same was the case for the water content in the muscle, which varied in the range 75–88%. The protein content varied consequently in the approximate range of 12–25%. It is clear that the amount of muscle in the cooked crab relied

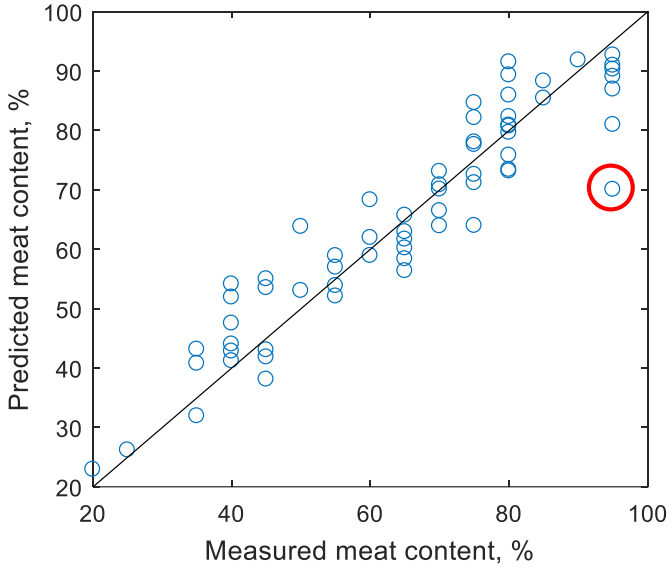

Fig. 5. Predicted meat content in left legs of January crabs based on calibration from October. Red circle marks sample with porous meat. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

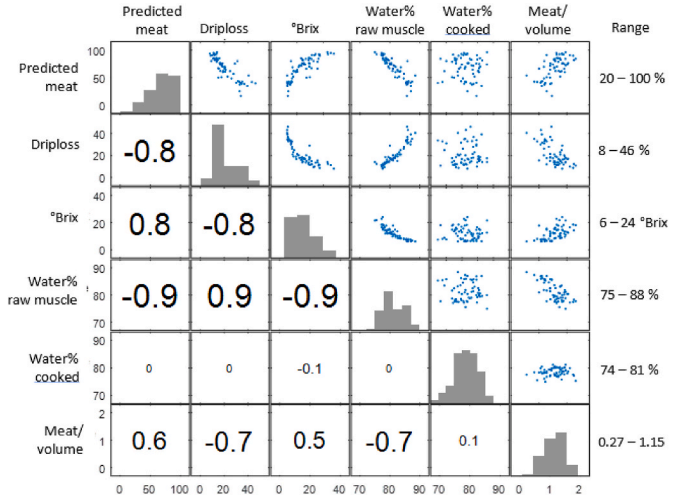

Fig. 6. Correlations between predicted meat content and physiochemical properties in right legs from January crabs. Water in cooked muscle was measured on samples from the left legs.

on both the amount of free water and the amount of water in the raw muscle. This corresponds to the meat content varying with the total protein content in the biomass, which explains why NIRS works for this application. It also explains why the regression coefficient in Fig. 3b resembles the absorption spectrum for protein. Water and protein content in the cooked muscle (in the left side legs) varied, but not systematically with water content in the raw muscle. The parameter meat/volume is an indication of the amount of muscle relative to the volume (size) of the leg. This measure correlated positively with predicted meat content, but not very strongly. The amount of protein in the muscle was more important. This is valuable information to measure, as larger size is not indicative of more meat.

Brix measurements of haemolymph has been previously studies as a potential measure of the physical state of crustaceans (Berry, Simon, Foote, Jerry, & Wade, 2019). A higher concentration of protein in the haemolymph could be expected with more protein in the legs, and Brix measurement could therefore potentially be an alternative rapid method for meat content determination. Fig. 6 shows that the Brix value decreases with increasing free water and bound water in the legs. The relations are slightly non-linear. The Brix value correlates positively with predicted meat content (0.8). From these data it seems that Brix could be used to measure meat content, however, the main disadvantage is that the leg must be pierced to drain it, which also influences the crab's welfare negatively. Another uncertainty is that the composition of haemolymph can change also with other physiological conditions in the crabs, such as during the molting cycle (Berry et al., 2019).

NIR interaction spectroscopy seems to be a sound non-destructive method for rapid assessment of meat content in king crabs. Non-contact, subsurface measurements, through the exoskeleton, work well, just as it was reported for brown crabs (Wold et al., 2010). The varying size of the legs does not seem to influence the results, but more thorough studies can be done to investigate potential effects of size, colour and translucency of the exoskeleton.

Table 1 shows that results from crabs caught and measured in April, October and January were similar in terms of accuracy of the calibration models. The calibration from October also performed well on the crabs in January. Spectral data from April 2019 was unfortunately not comparable with data from October 2019 and January 2020 due to an upgrade in the optical instrumentation.

A limitation to the method reported here can be that it relies on the legs not having lost free water due to injuries in the exoskeleton. Loss of free water/haemolymph will introduce overestimation of the meat content. It is important that this is handled and controlled by the operators and might not be a big problem in practice. During our trials, only a few legs lost the free water during normal storage, and the water loss took quite some time, as observed in Trial 2 (Fig. 4).

One source of error in the calibration is the manual referencing of meat content. Accurate judgement of the percentage of meat in the cross section of the legs requires training and can be subjective. An alternative would be to quantify the meat content with digital camera and image analysis, as was done by Lorentzen et al. (2019). However, this imaging method does also have sources of error since it is not always easy to obtain representative images of the cross sections. Furthermore, the meat content can vary along the leg, so that a single cross section of the leg may not be representative of the whole muscle. The NIR system, in comparison, used in this work collects signals from a leg section of at least 24 mm in length. Considering the uncertainties presented here, we regard the manual method used as sufficient and, since it is quite fast, efficient. It is also the standard method used by operators in the industry.

In Fig. 5, one sample is marked with a red circle. This sample/leg was from a crab where all three legs were manually judged to contain 95% meat. The NIRS calibration estimated the three legs to be 94%, 70% and 93%, where the middle leg was the one marked in Fig. 5. Fig. 7 shows the cross sections of two of the legs. The leg that was underestimated had a porous muscle with large holes. These holes would contain free water and the NIRS estimated meat content would be lower than manually assessed. This can be seen on the spectra from the two samples (Fig. 7), where the water peak at 980 nm was narrower and slightly shifted towards shorter wavelengths for the sample with porous meat. It illustrates potential uncertainties by using a physical quality feature (and not a chemical) as a reference for NIR spectroscopy. The porous meat was in a leg that had an old damage, so it was apparently not of normal quality.

The protein content in cooked muscle is typically about 18.5% (Lian et al., 2021). However, in the case of reduced access to nutrition, the crab utilizes its body reserves to compensate. For example, for RKCs kept at 10 °C, with no access to feed for 3 months, the protein content in the cooked leg meat decreased from 18.0 to 14.8 %, the moisture levels increased from 78.5 to 82.9%, and the meat content decreased from approximately 85 %–40 %. (Lian et al., 2022; Lorentzen et al., 2019). It is assumed that utilization of the body reserves, entails changes in the microstructure of the crab muscle which promotes loss of liquid loosely bound or physically entrapped during the cooking process (Lorentzen et al., 2019). Also, during the molting, the crab absorbs water, assuming to interfere the water content of the muscle (Stevens & Jewett, 2014). These factors might lead to uncertainties in the meat content estimates produced by NIRS. However, in our study, the water content in cooked muscle varied from 74 to 81% water in Trial 3 (Fig. 6), indicating that the method does handle such variations.

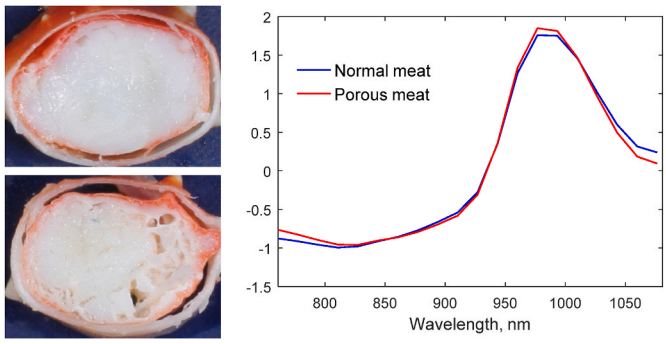

Fig. 7. Cross sections of two legs from the same crab. Both estimated manually to have a meat content of 95%. The meat content in leg at the lower image was estimated lower by NIR (marked sample in Fig. 5). To the right: NIR spectra from the two samples.

4. Conclusion

The technology described here can represent a significant improvement towards a more sustainable RKC fishery, feeding of juvenile and adult RKCs, and in trading. NIRS is rapid (1–2 s per measurement), non-destructive and objective. Applying NIRS does not require experienced employees, and it will reduce the time and potential contention during "meat content negotiations" with the fishermen. The ability to check that crabs have sufficient meat content before live transport will reduce the risk of mortality due to poor welfare and thereby prevent food loss, ensure high quality, and reduce the risk of complaints from the market. During processing, the back side of each crab is routinely visually inspected as part of the weighing and sorting process, and this can easily be combined with a meat content measurement. When employing live holding systems, it is also important to monitor the meat content over time, and frequent NIRS measurements enable the industry to know when the RKC is ready for trading. Also, in the processing of RKCs, knowledge of the meat content is important as it enables the industry to adjust the cooking regimen (time and temperature) to minimise the yield loss.

CRediT authorship contribution statement

Jens Petter Wold: Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Marion O'Farrell:** Writing – review & editing, Software, Methodology. **Jon Tschudi:** Writing – review & editing, Software, Methodology. **Grete Lorentzen:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

We would like to thank Katinka Dankel, Karen Wahlstrøm Sanden, Tina Thesslund, and Anette Hustad for excellent technical assistance and Capefish AS for access to crabs and processing facilities. Ragni Ofstad is thanked for fruitful discussions.

This work was funded by the Research Council of Norway through

the projects Optimal and modern processing of snow and king crab clusters (RCN no. 269218), KingCrab-NIR: Development of instrument to measure meat content in King Crab (RCN no. 300466), SFI Digital Food Quality (RCN no. 309259), and the Nofima funded project "SIS Helt Konge" (NOFIMA no. 13618).

References

Barnes, R. J., Dhanoa, M. J., & Lister, S. J. (1989). Standard normal variate transformation and de-trending of near-infrared diffuse reflectance spectra. *Applied Spectroscopy, 43*, 772–777.

Berry, S. E., Simon, C. J., Foote, A. R., Jerry, D. R., & Wade, N. M. (2019). Evaluation of baseline haemolymph biochemistry, volume and total body energetics to determine an accurate condition index in the black tiger shrimp, Penaeus monodon. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 228*, 1–9. https://doi.org/10.1016/j.cbpb.2018.10.003

Chung, S. H., Cerussi, A. E., Klifa, C., Baek, H. M., Birgul, O., Gulsen, G., et al. (2008). In vivo water state measurements in breast cancer using broadband diffuse optical spectroscopy. *Physics in Medicine and Biology, 53*, 6713–6727.

Chung, S. H., Yu, H., Su, M. Y., Cerussi, A. E., & Tromberg, B. J. (2012). Molecular imaging of water binding state and diffusion in breast cancer using diffuse optical spectroscopy and diffusion weighted MRI. *Journal of Biomedical Optics, 17*, Article 071304. https://doi.org/10.1117/1.JBO.17.7.071304

Ghidini, S., Varrà, M. O., Bersellini, D., Conter, M., Fabrile, M. P., Ianieri, A., et al. (2024). Real-time and non-destructive control of the freshness and viability of live mussels through portable near-infrared spectroscopy. *Food Control, 160*, Article 110353. https://doi.org/10.1016/j.foodcont.2024.110353

Isaksson, T., Miller, C. E., & Næs, T. (1992). Nondestructive NIR and NIT determination of protein, fat, and water in plastic-wrapped, homogenized meat. *Applied Spectroscopy, 46*, 1685–1694.

Lian, F., Siikavuopio, S. I., Harrison, S. M., Vang, B., Brunton, N. P., Esaiassen, M., et al. (2022). Fatty acid composition of cooked leg meat and raw hepatopancreas of red king crab (*Paralithodes camtschaticus*) during three-month live holding without feeding at 5 and 10 °C. *Journal of Food Composition and Analysis, 105*, Article 104206. https://doi.org/10.1016/j.jfca.2021.104206

Lian, F., Siikavuopio, S. I., Hustad, A., Thesslund, T., Lindberg, S. K., & Lorentzen, G. (2021). Comparative quality evaluation of processed clusters obtained from red king crab (Paralithodes camtschaticus) typical of spring and autumn harvests in the Barents Sea. *Food Control, 123*, Article 107826.

Libnau, F. O., Kvalheim, O. M., Christy, A. A., & Toft, J. (1994). Spectra of water in the nearinfrared and midinfrared region. *Vibrational Spectroscopy, 7*, 243–254.

Lorentzen, G., Lian, F., Hustad, A., Thesslund, T., Esaiassen, M., & Siikavuopio, S. I. (2023). Evaluation of morphological and quality parameters in adult male red king crabs (*Paralithodes camtschaticus*) raised to commercial weight from juveniles. *Aquaculture Research*, 1–11. https://doi.org/10.1155/2023/6884272, 2023.

Lorentzen, G., Lian, F., & Siikavuopio, S. I. (2019). Quality parameters of processed clusters of red king crab (Paralithodes camtschaticus) - effects of live holding at 5 and 10 °C up to 92 days without feeding. *Food Control, 95*, 142–149. https://doi.org/10.1016/j.foodcont.2018.07.041

Lorentzen, G., Samuelsen, T.-A., Lian, F., Hustad, A., Thesslund, T., Rotabakk, B. T., et al. (2024). Morphology, processing attributes, fatty acid and amino acid composition in cooked leg meat and raw hepatopancreas from juvenile male red king crab (*Paralithodes camtschaticus*) after 12 months feeding. In *prep*.

Lorentzen, G., Voldnes, G., Whitaker, R. D., Kvalvik, I., Vang, B., Gjerp Solstad, R., et al. (2018). Current status of the red king crab (*Paralithodes camtchaticus*) and snow crab (*Chionoecetes opilio*) industries in Norway. *Reviews in Fisheries Science and Aquaculture, 26*, 42–54. https://doi.org/10.1080/23308249.2017.1335284

Martens, H., & Næs, T. (1989). *Multivariate calibration*. Wiley.

Medler, S., & Mykles, D. L. (2015). Muscle structure, fiber types, and physiology. In E. S. Chang, & M. Thiel (Eds.), *The natural history of the Crustacea* (Vol. 4). Oxford University Press USA. Physiology.

Osborne, B. G., & Fearn, T. (1986). *Near infrared spectroscopy food analysis*. Longman Group UK Limited.

Porep, J. U., Kammerer, D. R., & Carle, R. (2015). On-line application of near infrared (NIR) spectroscopy in food production. *Trends in Food Science & Technology, 46*(Part A), 211–230. https://doi.org/10.1016/j.tifs.2015.10.002

Segtnan, V. H., Høy, M., Lundby, F., Narum, B., & Wold, J. P. (2009). Fat distribution analysis in salmon fillets using non-contact near infrared interactance imaging: A sampling and calibration strategy. *Journal of Near Infrared Spectroscopy, 17*, 247–253.

Stevens, B. G., & Jewett, S. C. (2014). Growth, molting, and feeding og king crabs. In B. G. Stevens (Ed.), *King crabs of the world, biology and fisheries management* (pp. 315–354). Boca Raton, FL, US: CRC Press. Taylor & Francis group.

Voldnes, G., Kvalvik, I., & Nøstvold, N. (2020). Taking care of a highly valuable resource throughout the value chain - lack of market orientation in red king crab export? *Marine Policy, 117*, Article 103965. https://doi.org/10.1016/j.marpol.2020.103965

Wold, J. P., Kermit, M., & Woll, A. (2010). Rapid nondestructive determination of edible meat content in crabs (Cancer Pagurus) by Near-Infrared Imaging spectroscopy. *Applied Spectroscopy, 64*, 691–699.

Wold, J. P., Måge, I., Løvland, A., Sanden, K. W., & Ofstad, R. (2019). Near-infrared spectroscopy detects woody breast syndrome in chicken fillets by the markers protein content and degree of water binding. *Poultry Science, 98*, 480–490. https://doi.org/10.3382/ps/pey351

Wold, J. P., O'Farrell, M., Tschudi, J., Eskildsen, C. E., Andersen, P. V., & Ottestad, S. (2020). In-line and non-destructive monitoring of core temperature in sausages during industrial heat treatment by NIR interaction spectroscopy. *Journal of Food Engineering, 277*, Article 109921. https://doi.org/10.1016/j.jfoodeng.2020.109921

Wold, J. P., O'Farrell, M., Andersen, P. V., & Tschudi, J. (2021). Optimization of instrument design for in-line monitoring of dry matter content in single potatoes by NIR interaction spectroscopy. *Foods, 10*, 828. https://doi.org/10.3390/foods10040828

Wold, J. P., O'Farrell, M., Høy, M., & Tschudi, J. (2011). On-line determination and control of fat content in batches of beef trimmings by NIR imaging spectroscopy. *Meat Science, 89*, 317–324.

Wold, J. P., Veiseth-Kent, E., Høst, V., & Løvland, A. (2017). Rapid on-line detection and grading of wooden breast myopathy in chicken fillets by near-infrared spectroscopy. *PLoS One, 12*, Article e0173384.