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A simulated e-commerce cold chain for fresh cod (*Gadus morhua* L.) products: Applicability of selected TTIs and effects of pre-treatment and packaging

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ABSTRACT

The objective of this work was to study 1) the applicability of two selected time and temperature integrators (TTIs) and 2) the quality effects of different pre-treatments and packaging of fresh cod in a simulated e-commerce cold chain of 16 days. TTIs enables consumers to evaluate the product quality based on its time and temperature history. The first 6 days, the fish was either stored as gutted or gutted and filleted, representing "from business to business" (B2B), while the remaining days "from business to consumer" (B2C) value chain, the fish was packaged and stored at 4 °C in modified atmosphere (MA) or in air. One TTI recorded the temperature, and the other TTI was based on an enzymatic reaction. In B2B, the product core temperature was app. −0.5 °C. In the B2C, shelf life of 7–10 and 5–7 days for MA- and air-packaged products were obtained, respectively. Time for filleting, 1 or 6 days *post-mortem* for the MA products, did not influence the shelf life in the B2C. The TTI recording the temperature showed a good coherence with the reference temperature, while the reaction based TTI was inconsistent with the actual shelf life. To ensure consistency, the TTI could have been activated earlier. From a consumer's point of view, the information generated by the TTIs is beneficial to avoid the purchase of products of poor sensory quality.

1. Introduction

Traditional food systems are experiencing changes due to the expansion of e-commerce. This poses a transformation in how we buy, sell, and distribute food (Gee, Heard, Webber & Miller, 2020). In recent years, successful development in fresh seafood e-commerce has been observed in China (Zhong & Zhao, 2020). Seafood is an important segment of this fresh food market in China, where 46% of respondents in a study stated that they often or very often buy seafood online (Norwegian Seafood Council, 2021). Corresponding figures for European countries are a bit lower with 19%, 18%, 14%, and 10% for Great Britain, Spain, Germany, and France, respectively. In Norway, only 2% reported buying seafood often or very often online. The reason for the differences between the countries has not been explored, but there are indications of skepticism related to poor fish quality bought online (Norwegian Seafood Council, 2021).

E-commerce can be defined as all types of commercial transactions and business activities taking place over electronic online systems (Voldnes et al., 2020). The commercial transactions include both business to business (B2B), as well as business to consumer (B2C), making the options and alternatives for e-commerce widespread. Overall, there are many organizations involved in a seafood value chain, for instance, foreign exporters, domestic importers, wholesalers, resellers, and clearance companies (Wang, Somogyi, & Charlebois, 2019).

The perishable nature of seafood and the complexity of its value chain with a range of processing steps make e-commerce challenging compared to other food products. Today, the assortment of products available in the traditional market ranges from fresh, frozen to processed products like canned, and salt-cured seafood. Currently, both fresh and frozen seafood products are offered through many of the existing e-commerce channels. The time and temperature exposure in the cold chain is coherent with the risk potential, shelf life, and quality of the fish product. To communicate the time and temperature history, an assortment of time and temperature integrators (TTIs) or indicators have been developed. TTIs has been developed for more than 20 years and has shown a great potential to continuously monitor temperature conditions

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along the food chain (Kreyenschmidt, Christiansen, Hübner, Raab & Petersen, 2010). A TTI is commonly attached to the package, and it provides the time and temperature history from a given point in the cold chain, and such a TTI is often related to time and temperature-sensitive reactions between an enzyme and a substrate leading to color changes (Pandian, Chaturvedi, & Chakraborty, 2020). A control strategy for controlling hazard of *Clostridium botulinum* using TTI on seafood packages is provided by FDA (2021). Alternatively, temperature recordings generating temperature profile e.g., on a mobile app is also available. Such devices are small, inexpensive, and can be critical in building trust among consumers.

In the traditional seafood trade, literature and guidelines describing quality and food safety aspects due to processing, packaging, and distribution are available (Codex Alimentarius, 2012; European Food Safety Authority, 2015; Tsironi & Taoukis, 2018; Yu et al., 2020). Among these, only a few considered challenges related to e-commerce. E-commerce challenges the established systems for processing, packaging, and distribution as the products are traded online. Such trading requires a low temperature, especially in the B2C interval, as seafood is highly perishable.

To the best of our knowledge, papers describing the effect of e-commerce on fresh seafood have not been published. Hence, the aim of the present study has been to 1) evaluate the applicability of selected TTIs and 2) study the quality effects of different pre-treatments and packaging of fresh cod. Both packaging in air and modified atmosphere (MA) will be applied. The time and temperature conditions applied will reflect a simulated e-commerce cold chain of fresh cod from northern Norway to countries in southern Europe. The chain includes ice storage of gutted cod for up to 6 days, followed by different process and product categories, i.e., fillets and cutlets, stored at 4 °C up to 16 days after the catch in the air or MA.

2. Materials and methods

2.1. Fresh cod

Cod (*Gadus morhua* L.) were caught by demersal seine, longline on the 13th of October 2020 by a commercial fishing vessel off the coast of Vannøy, northern Norway. A selection of 13 fish was headed and gutted, added ice upon landing, and transported to Nofima the same day. Upon arrival at Nofima, the iced cod were stored at 1 °C overnight. The following day, the cod were distributed into four groups with app. 15 kg in each.

2.2. Simulated e-commerce cold chain

The experimental setup for the simulated e-commerce cold chain in terms of product groups and days after processing, i.e., sampling days, is illustrated in Fig. 1. The cod in group “F-1-MAP” were filleted on day one, *post-mortem*, while the remaining groups “F-6-MAP”, “C-6-MAP”, and “F-6-Air”, were filleted (F), or cut into cutlets (C) on day 6, *post-mortem*, and stored in modified atmosphere packages (MAP) or ambient air (Air). Hereafter, sampling days refer to days *post-mortem*.

For all product groups, the sample size varied from 250 to 350 g, mimicking commercial package units. On sampling day 6, all product groups were packaged, either in MA or with air. For the MA groups (F-1-MAP, F-6-MAP, and C-6-MAP), the products were individually packaged (T200/Multivac AS, Germany) in CPET (C2187-1F, 680 ml, Færch Plast AS, Denmark). The atmosphere was evacuated (99% vacuum) and subsequently flushed with a gas mixture of 60% CO_2 and 40% N_2 before heat sealing with a top web (Cryovac OSF33ZA, Sealed Air Norge AS, Norway). The gas to volume (g/v) ratio was 1.5 ± 0.7.

For the air-packaged products (F-6-Air), cod fillets were put in polystyrene trays (Silver-Plastics® GMBH & Co, Troisdorf, Germany) and wrapped with a PVC film (Global Plastics International, Fécamp, France). After the package, all products were stored at 4 °C.

2.3. Time and temperature integrators (TTIs) and reference logger

Time and temperature in the B2B and B2C intervals were logged applying the TTI’s Keep-it® and Tag Sensor. The principle with Keep-it® is a time and temperature-dependent reaction between an immobilized and a mobile reactant (keep-it.com), and the version used in this trial was calibrated to fresh fish as a product, with a shelf life of 14 days at 4 °C. The Tag Sensor records temperature data, and the tag is configured, started, and red using smartphone (tag-sensors.com). Both Keep-it® and Tag Sensor measured the ambient temperature as they were attached to the inner side of the polystyrene lid and on the outer side of

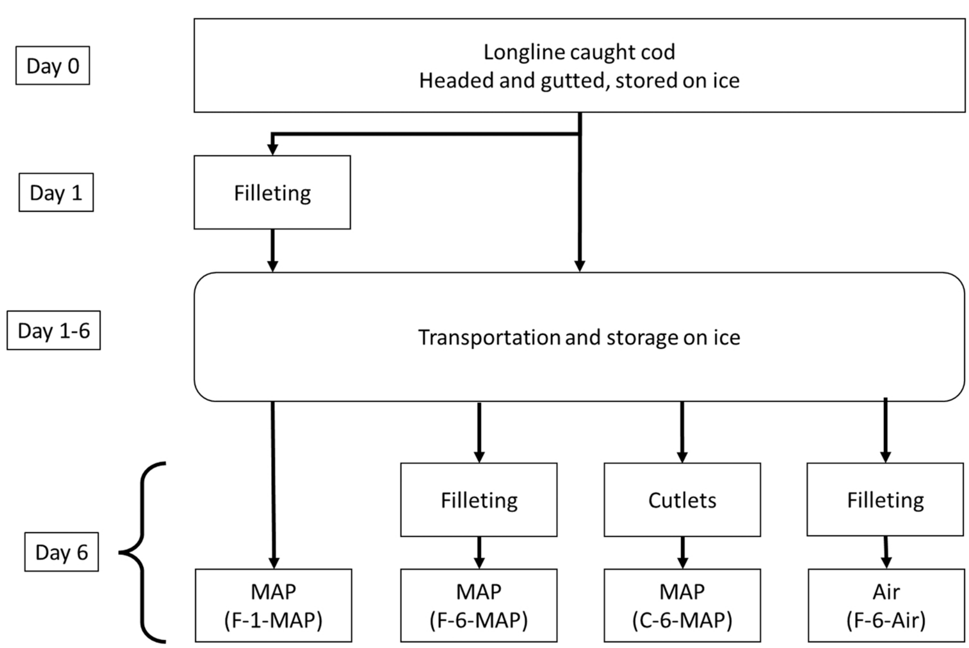

Fig. 1. Experimental setup for the simulated e-commerce cold chain for fresh cod, filleted (F), made cutlets (C), and packed in air (Air) or in modified atmosphere (MAP).

the MA package, respectively.

As a reference, the ambient and core temperature of the products were logged using electronic temperature loggers (TrackSense Pro®, Ellab AS, Denmark).

2.4. Quality analyses

2.4.1. TVB-N and TMA

Samples of approximately 100 g were cut from the fillets and cutlets during the storage. The samples were with skin. The fish were packaged in zip-lock plastic bags and transferred to −80 °C immediately. Before analyses, the samples were thawed, de-skinned, and minced. Analyses for TVB-N were performed according to Mai et al. (2011). The TVB-N includes ammonia, monoethyl amine, dimethylamine, and TMA. Besides, the TVB-N is part of the non-protein nitrogen fraction of the fish muscle as well (Debevere & Boskou, 1996). The TMA analyses were carried out according to Conway and Byrne (1933).

2.4.2. Odor

The odor of the MA products was evaluated by a trained expert panel with at least three assessors on days 6, 13, and 16. On each sampling day, the evaluation was performed with four parallel samples in each product group. A scoring system obtained from the filet index method was applied (Esaiassen, Dahl, Eilertsen, Gundersen & Sivertsvik, 2008; Lorentzen, Ageeva, Heide & Esaiassen, 2020). The demerit scores were 0: sea fresh, 1: neutral, 2: fishy, 3: ammonia/sour. Scores up to 2 were defined to be sensory acceptable and thereby within the shelf life. Prior to the evaluation, the samples were stored at room temperature (20 ± 2 °C) for 20 min.

2.4.3. Microbial analyses

Microbial analyses were performed on days 6, 13, and 16 for the MA products and on days 6, 11, 13, and 16, for the air-packaged products. Sterile saline water 0.85% NaCl and 0.1% peptone (Bacto Peptone, Merck KGaA, Darmstadt, Germany) was added to the fish sample (5–10 g) to obtain a 1:10 dilution. The samples were with skin. The samples were then blended in a Lab Blender 400 Stomacher (Seward Medical Ltd., London, UK) for 2 min followed by 10-fold serial dilutions as required. The total aerobic psychrotrophic count (APC) were quantified in accordance with the NMKL method No. 184 (NMKL, 2006) applying Long and Hammer (L&H) agar, incubated at 15 °C for 5–7 days, and iron agar plates (TVC) (Iron Agar Lyngby, CM 964, Oxoid, Basingstoke, UK), incubated at 25 °C for 24–48 h. The last media was also applied to determine the level of sulfide producing bacteria (SPB), i.e., *Shewanella putrefaciens*, observed as black colonies. The detection limit for L&H and iron agar were log 2 CFU/g. The results were reported as the decimal logarithm of colony-forming units per gram of sample ($\log_{10}$ CFU/g).

2.4.4. MA products; measurement of gas, drip loss, and color

Immediately after the MA packaging on day 6, the initial gas composition (%) was 60 and 40 for CO_2 and N_2, respectively. On days 13 and 16, triplicates from each product group were analyzed for residual gas using an oxygen and carbon dioxide analyzer (Checkmate 9900 analyzer, PBI-Dansensor, Ringsted, Denmark) according to a method described by the group of Rotabakk (2006).

The drip loss was calculated as the weight of exudates in the trays after storage relative to the initial product weight (%). The drip loss was calculated from triplicates.

The surface color (L*, a*, and b*) was measured as described by the group of Chan et al. (2021). Chroma (C), representing the color saturation and Hue, the color angel between a* and b*, where H* = 0° for reddish. C and Hue were calculated by Eqs. (1) and (2), respectively.

$$C = \sqrt{(a*^2 + b*^2)} \tag{1}$$

$$\text{Hue} = \tan^{-1}(b*/a*) \tag{2}$$

2.5. Statistical analyses

All data were analyzed using multivariate analyses, applying the software Unscrambler version 10.3 (CAMO Process AS, Oslo, Norway). Prior to the analyses, the variables were weighted with the inverse of all objects' standard deviation to compensate for the different scales of the variables. Principal component analysis (PCA) (Martens & Næs, 1989) was used to identify similarities and differences amongst samples based on sensory evaluation of odor, microbiological parameters, TVB-N, TMA, color parameters, and drip loss. Multivariate calibration models based on Partial Least Square Regression (PLS) were developed to study the effects of storage on both duration and condition, time of filleting, and temperature. Full cross-validation leave-one-out was employed as the validation technique. Model performance was assessed using the values of RMSEC and RMSEP with corresponding coefficients of determination, R^2_c, and R^2_p.

One-way ANOVA was carried out to explore the significant differences between groups at each sampling day. The statistical interpretation of data was carried out using SYSTAT 13 for Windows version 13.1 (Systat Software Inc., San Jose, CA, US). The results are given as mean ± standard deviation.

3. Results and discussion

3.1. Storage conditions and TTIs

The product core and ambient temperature in the simulated e-commerce cold chain are presented in Fig. 2. According to the product core temperature, the aimed temperature of 0 and 4 °C in the B2B and B2C intervals, respectively, were achieved. In fact, in the B2B interval, the product core was app. −0.5 °C. The Tag sensor measured the ambient temperature in the cold chain from day 1–16. In the B2B interval, this temperature was slightly lower than the product core temperature, while for the B2C interval, the Tag sensor temperatures varied in the range from 3 to 4 °C. Although not following the exact same pattern, the temperature profile generated by this TTI is considered to be adequate for its purpose.

Immediately after the catch, storage at 0 °C is crucial to ensure a safe and sensory acceptable seafood product as long as possible. Today, transport of only gutted or gutted and headed fish in the B2B interval is commonly performed by putting the cod in a box with ice. For fresh cod fillets, the ice represents about 33% of the total box weight when

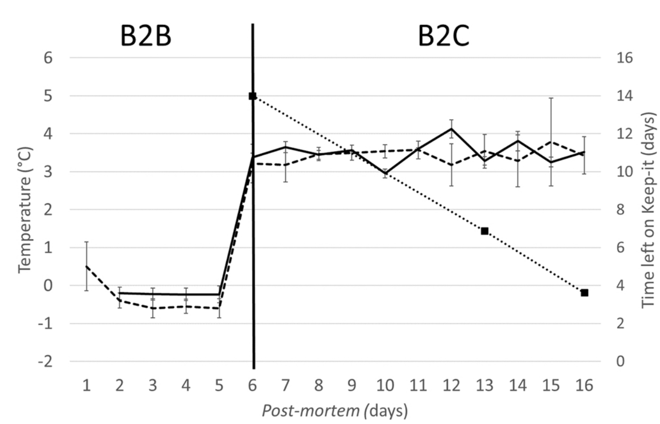

Fig. 2. Core temperature (—) of cod and ambient temperature recorded by reference logger and Tag sensor (– – – – –), respectively, from day 1 to day 16, *post-mortem*. Remaining shelf life is showed by Keep-It (■).Day 1–6 simulates business to business (B2B), while day 6–16 simulates from business to consumer (B2C).

transported from northern Norway to the European continent (Lorentzen et al., 2020). Upon arrival, a product temperature as low as −1 °C has been reported (Lorentzen et al., 2020). However, this is assumed to be related to ice in excess at the arrival point. Afterwards, the fish is commonly cut into fillets or cutlets and then packaged.

On day 6, the TTI "Keep-it®" was activated and attached to the surface of the MA package for the B2C interval (Fig. 2). On day 13, these fish products were still sensory acceptable, while the TTI indicated a remaining shelf life of 6.8 days. On day 16, the products were considered not sensory acceptable, although the TTI indicated a remaining shelf life of 3.6 days. This inconsistency might be related to the time for activating the TTI. To ensure consistency with the actual shelf life of the MA product, 1) the TTI could have been activated earlier, like 3–4 days *post-mortem*, 2) a narrower scale from 7 to 0 days for the remaining shelf life could have been applied, or 3) an enzymatic reaction with a faster progression could have been used.

TTIs based on enzymatic reactions, must be activation at a certain point in the cold chain. The activation is commonly done immediately after the packaging. In case the product has been exposed to elevated temperatures before this point, the shelf life will be less than indicated by the TTI. Hence, a thorough knowledge about the time and temperature history before the packaging is essential to ensure consistency between the information given by the TTI and the actual shelf life is a prerequisite.

3.2. Quality changes of the products along the cold chain

The quality changes of the product groups from day 6–16 are presented in Table 1. The time for samplings were selected according to the expected shift from acceptable to non-acceptable odor. Hence a differentiation of the sampling days following the package categories was made.

As expected, TVB-N and TMA were continuously produced during storage. No significant differences in the TVB-N levels on day 6 were observed. For the air-packaged cod, the levels corresponded well with results obtained previously (Lorentzen et al., 2020). The main reason for

Table 1
Developments of TVB-N, TMA, odor, total volatile count (TVC), sulfide producing bacteria (SPB), aerobic psychrotrophic count (APC), CO_2, O_2, drip loss, and color (L*, C, Hue) during storage at 4 °C. The product categories include MA packaged cod; F-1-MAP, F-6-MAP, and C-6-MAP, in addition to air packed cod; F-6-Air.

Variable (unit)	Product category	*Post-mortem* (days)			
		6 (before packaging)	11	13	16
TVB-N (mg N/100 g)	F-1-MAP	10.75 ± 1.50	na	58.25 ± 6.70[ab]	80.50 ± 12.97
	F-6-MAP	10.25 ± 2.06	na	61.00 ± 12.75[ab]	84.25 ± 5.50
	C-6-MAP	12.50 ± 1.91	na	71.50 ± 10.15[a]	86.25 ± 15.33
	F-6-Air	10.50 ± 0.58	20.25 ± 9.60	39.50 ± 11.90[b]	79.75 ± 16.03
TMA(mg N/100 g)	F-1-MAP	4.33 ± 2.08[ab]	na	47.25 ± 6.90[ab]	70.25 ± 12.89
	F-6-MAP	2.67 ± 1.53[ab]	na	51.50 ± 12.77[ab]	74.25 ± 7.37
	C-6-MAP	4.75 ± 1.50[a]	na	61.50 ± 8.50[a]	73.75 ± 11.90
	F-6-Air	1.50 ± 0.58[b]	12.25 ± 8.85	31.00 ± 11.89[b]	64.00 ± 17.98
Odor	F-1-MAP	0.4 ± 0.3	na	1.9 ± 0.1[ab]	2.7 ± 0.20
	F-6-MAP	0.4 ± 0.2	na	1.7 ± 0.2[b]	2.7 ± 0.20
	C-6-MAP	0.5 ± 0.3	na	2.0 ± 0.0[a]	2.8 ± 0.16
	F-6-Air	na	na	na	na
APC (log cfu/g)	F-1-MAP	4.50 ± 0.33[a]	na	7.41 ± 0.23	8.13 ± 0.11
	F-6-MAP	4.80 ± 0.23[a]	na	7.23 ± 0.26	7.85 ± 0.27
	C-6-MAP	5.09 ± 0.28[a]	na	7.05 ± 0.64	7.83 ± 0.31
	F-6-Air	3.45 ± 0.22[b]	6.20 ± 0.22	7.16 ± 0.09	7.69 ± 0.22
TVC (log cfu/g)	F-1-MAP	4.39 ± 1.08[ab]	na	5.70 ± 0.34[ab]	6.14 ± 0.41[c]
	F-6-MAP	4.66 ± 0.33[a]	na	5.10 ± 0.68[a]	5.28 ± 0.40[a]
	C-6-MAP	4.92 ± 0.17[a]	na	5.29 ± 0.98[a]	5.15 ± 0.52[a]
	F-6-Air	3.45 ± 0.22[b]	5.67 ± 0.35	6.77 ± 0.46[b]	7.58 ± 0.22[b]
SPB (log cfu/g)	F-1-MAP	2.99 ± 0.69[a]	na	5.44 ± 0.42	6.03 ± 0.44[b]
	F-6-MAP	3.73 ± 0.40[a]	na	4.90 ± 0.84	4.74 ± 0.57[a]
	C-6-MAP	3.34 ± 0.33[a]	na	5.18 ± 1.04	4.77 ± 0.85[a]
	F-6-Air	2.00 ± 0.35[b]	4.72 ± 0.39	6.13 ± 0.59	6.82 ± 0.28[b]
CO_2 (%)	F-1-MAP	59.5 ± 0.1*	na	26.93 ± 2.67[a]	27.48 ± 1.24[ab]
	F-6-MAP		na	34.88 ± 4.08[b]	33.23 ± 4.77[b]
	C-6-MAP		na	26.38 ± 3.84[a]	24.28 ± 4.73[a]
	F-6-Air		na	na	na
O_2 (%)	F-1-MAP	0.01 ± 0.0*	na	0.01 ± 0.00	0.02 ± 0.02
	F-6-MAP		na	0.01 ± 0.01	0.00 ± 0.00
	C-6-MAP		na	0.07 ± 0.10	0.00 ± 0.00
	F-6-Air		na	na	na
Drip loss (%)	F-1-MAP	–	na	3.37 ± 1.96[ac]	5.91 ± 3.21[ab]
	F-6-MAP	–	na	8.25 ± 2.09[b]	6.45 ± 2.91[a]
	C-6-MAP	–	na	5.10 ± 0.72[a]	7.30 ± 1.01[a]
	F-6-Air	–	1.65 ± 0.64	1.62 ± 0.35[c]	1.84 ± 0.18[b]
L*	F-1-MAP	78.01 ± 4.00	na	77.61 ± 3.85	79.65 ± 1.44[b]
	F-6-MAP	77.00 ± 0.29	na	75.24 ± 3.06	79.48 ± 1.73[b]
	C-6-MAP	na	na	73.33 ± 3.21	74.19 ± 1.51[a]
	F-6-Air	na	na	na	na
C	F-1-MAP	8.72 ± 0.21	na	9.37 ± 0.88	9.68 ± 0.39[a]
	F-6-MAP	9.66 ± 1.34	na	10.27 ± 0.58	10.38 ± 0.99[a]
	C-6-MAP	na	na	12.13 ± 4.06	10.94 ± 1.61[a]
	F-6-Air	na	na	na	na
Hue	F-1-MAP	1.35 ± 0.14	na	1.32 ± 0.14	1.43 ± 0.09[b]
	F-6-MAP	1.30 ± 0.07	na	1.32 ± 0.13	1.40 ± 0.05[ab]
	C-6-MAP	na	na	1.19 ± 0.10	1.28 ± 0.05[a]
	F-6-Air	na	na	na	na

na: not analyzed. *Representative for all MAP categories. Lowercase letters show significant differences between different groups on the same day, *post-mortem*.

this is assumed to be due to the low storage temperature in the B2B interval (Fig. 2). Interestingly, the time for processing, i.e., filleting on day one or 6, did not lead to significant differences in the TVB-N nor TMA levels. On day 13, a significant production of TVB-N and TMA was observed for the MA-packaged products, while lower levels were observed for their air-packaged counterparts. Elevated TVB-N and TMA levels for the MA products align well with results previously reported (Sivertsvik, 2007). In detail, farmed cod added a gas mix with 67% and 17% for CO_2 and N_2, respectively, resulted in TVB-N and TMA levels of 44 and 24.4 mg N/100 g, respectively, after 14 days of storage at 4 °C. Overall, the TVB-N and TMA contribute to a negative effect on the organoleptic quality of the fish products. The increase in TVB-N is mainly ascribed to the TMA formation rather than the production of other volatile bases (Debevere et al., 1996). For air-packaged seafood, TVB-N and TMA levels above 35 mg N/100 g and 15 mg N/100 g are considered spoiled (European Commission, 1995). In our study, the TVB-N threshold was passed between days 11 and 13 for the air-packaged cod. Corresponding thresholds for TVB-N and TMA for MA products are, to our knowledge, not reported.

On day 6, no off-odors were noticed, and this aligns nicely with the low levels of TVB-N and TMA. Only on day 13, the cutlets (C-6-MAP) obtained a significantly distinct off-odor compared to the fillets (F-6-MAP). Odor with scores up to 2 is considered sensory acceptable (Lorentzen et al., 2020). The odor score was close to this limit on day 13, while on day 16, this limit was crossed for all MA product categories. Hence, the shelf life for these products was between 13 and 16 days.

On day 6, the total aerobic psychrotrophic count (APC) was significantly lower for the fish to be packaged in the air compared to the MA-packaged fish. Compared to the corresponding counts for TVC from the same day, the levels were within the same log unit, showing no additional APC than those already being detected applying the TVC agar. However, on day 13, the APC level was about 2 log units above the TVC level. The group of Dalgaard, Mejlholm, and Huss (1997) showed that the psychrotrophic organism *Photobacterium phosphoreum* was the dominant part of the spoilage flora in cod when packaged in 60% CO_2 and 40% N_2. After an initial storage period at 0 °C followed by 5 °C, the *P. phosphoreum* and TVC were app. log 7.5 and 7.2 CFU/g at the time of sensory rejection, respectively. They also discovered that the MA-packaged cod fillets did not spoil until the numbers of *P. phosphoreum* had reached and remained at their maximum level for a while. This fits well with the APC levels (Table 1), showing that the maximum level was not reached on day 13.

Among the bacteria responsible for the spoilage of chilled cod at atmospheric conditions is the facultative anaerobic, psychrotrophic *Shewanella putrefaciens*. *S. putrefaciens* is unable to develop to high numbers in case of CO_2 concentrations of more than 50% (Boskou and Debevere, 1997). A *S.putrefaciens* level of log 8 CFU/g is required to produce TMA at a concentration of 30 mg N / 100 g and thereby have predictable spoilage (Jørgensen & Huss, 1989; Dalgaard, 1995; Dalgaard, Mejlholm, & Huss, 1996). On day 11, the air-packaged cod was at an APC level of app. log 6 and log 7 CFU/g after two additional storage days.

The initial gas composition in the MA packages were, on average 59.5 ± 0.1% CO_2 and 0.01 ± 0.0% O_2 (Table 1). The proportions of CO_2 decreased to about 35% after 7 days of storage. The CO_2 decrease is assumed to be caused by diffusion into the fish muscle (Debevere et al., 1996). As CO_2 is a weak acid, it dissolves in the product's water, involving a change in pH and water holding capacity. In the case of a lower CO_2 level, a lower drip loss could thereby have been expected (Sivertsvik, 2007).

The low levels of O_2 on days 13 and 16 indicate that the packaging material was sufficient for its purpose and showed to be an excellent O_2 barrier. Alternatively, a low O_2 concentration is also a clear indication of microbial growth (Rotabakk, Birkeland, Jeksrud & Sivertsvik, 2006), which aligns with our microbial results on days 13 and 16 (Table 1). A health hazard from toxins produced by psychrotrophic non-proteolytic *Clostridium botulinum* is not probable using a temperature of 4 °C and a maximum shelf life of 10 days (Skinner & Larkin, 1998). No significant differences between the MAP categories on day 13 nor day 16 were observed.

A significant difference ($p < 0.05$) in L* between fillet and cutlets was detected on day 16. This difference might be related to how this product was cut, where cutlets are a cross-section of the fillet, while fillets are cut parallel to the muscle fibers. It is, therefore, likely to assume that the cross-section of the fibers (cutlet) reflects less light compared to the cut along the fibers (fillet). A significant ($p < 0.05$) but ignorable difference in the Hue values was detected on day 16 between portions filleted at day one and cutlets. A difference in 0.1 in Hue might be statistically significant but have no practical effect. The color values observed are generally comparable to previous observations (Rotabakk, Skipnes, Akse & Birkeland, 2011).

3.3. Main effects

To identify any differentiation among the product groups along the simulated e-commerce cold chain and explore possible correlations between the response variables, the data were subjected to two PCAs with correlation loading and score plots. One PCA analysis was run with variables common for all the four product categories (Fig. 3) and one PCA with the three MA product categories (Fig. 4). In the last PCA, additional variables were included.

The correlation loading plot for the four product categories included TVB-N, TMA, APC, TVC, SPB, and drip loss (Fig. 3A), while the following score plots show the distribution according to the product categories (Fig. 3B) and storage time (Fig. 3C). As expected, variables related to spoilage, TVC, SPB, APC, TVB-N, and TMA were clustered. These variables were explained along PC1, with a distinct grouping to the right (Fig. 3A). The grouping was clearly dependent on the storage time (Fig. 3C). By comparing the loading plot (Fig. 3A) with the score plot (Fig. 3B), a small grouping of the air-packaged cod was observed to the bottom right, which corresponded to the location of TVC and SPB (Fig. 3A). For the MA products, a corresponding grouping was not observed. The drip loss is explained along the PC-2 (Fig. 3A). Compared with the product categories (Fig. 3B), the drip loss was clearly associated with the MA packaging.

For the MA product category, the correlation loading plot with the variables TVB-N, TMA, odor, APC, TVC, SPB, and color (L*, Hue, C) is presented (Fig. 4A). The corresponding score plots showed the distribution of the product groups (Fig. 4B) and storage time (Fig. 4C). As for Fig. 3A, similar grouping of variables showing the deterioration processes were observed to the right. The grouping was coherent with the storage time (Fig. 4C). TVB-N, TMA, APC, SPB, and odor are all appropriate indicators for the quality deterioration, while TVC is a less suitable indicator as less than 50% of the variation was explained. The color variables were explained along PC2, where L* and Hue correlated negatively with the C. This is related to the equations applied (Eqs. 1 and 2). The color values were not related to the different MA product categories, as no grouping was observed (Fig. 4B). However, the storage time, i.e., 13 days, may indicate that the color changes observed were correlated to the storage time.

3.4. Fresh cod in e-commerce

In the simulated e-commerce cold chain for fresh cod, the benefit in keeping the fish as gutted until day 6 before further processing is marginal compared to starting with the processing one day *post-mortem*. However, this outcome is assumed to be strictly coherent with the low storage temperature in the B2B interval (Fig. 2). On day 13, the MA-packaged products were considered acceptable due to the odor. However, on day 16, the products were no longer acceptable, leading to a shelf life between 13 and 16 days. For the air-packaged fish, it was sensory acceptable on day 11 and not acceptable on day 13. Hence, the

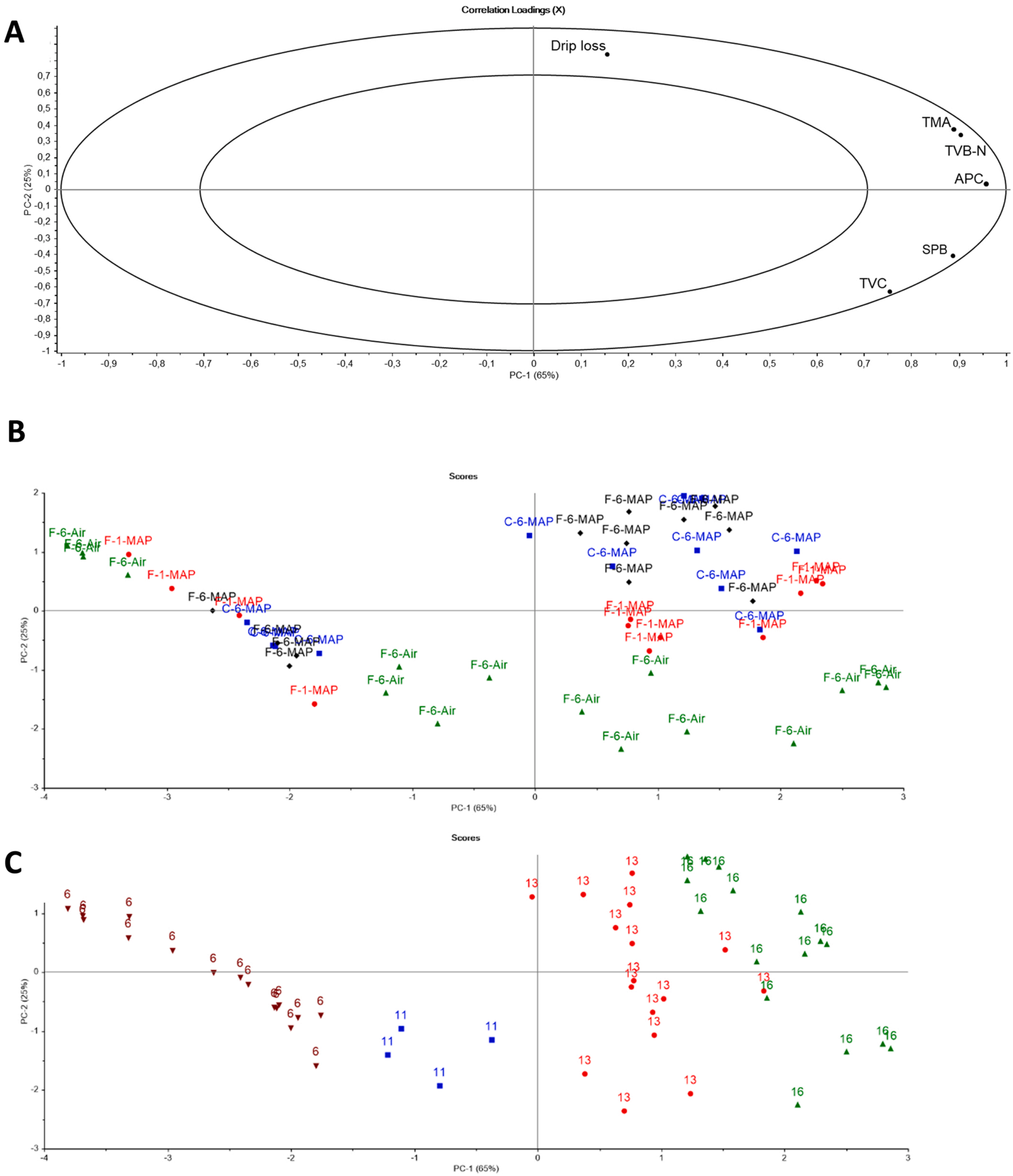

Fig. 3. Correlation loading plot (A), and score plots with observations grouped by product category (B) and days, *post-mortem* (C). The outer and inner ellipse indicate 100% and 50% explained variance, respectively (A).

shelf was considered to be app. 12 days.

Depending on the storage temperature, quality of the fish, handling, gas to product volume ratio, and the gas mixture, MA packaging can extend the shelf life with app. 50% (Sivertsvik, 2007). In an e-commerce context, the shelf life should preferably be as long as possible from the point of the package, i.e., in the B2C interval. This simulated cold chain study shows that MA packaging 6 days *post-mortem* allows for shelf life in the range of 7–10 days. For the air-packaged products, shelf life of 5–7 days was observed. Storage at a low temperature with ice in excess in the B2B interval (Fig. 2) is beneficial for both package categories.

To prolong the shelf life of fresh cod after packaging, i.e., in the B2C interval, a temperature below 4 °C is advantageous. In fact, shelf life of

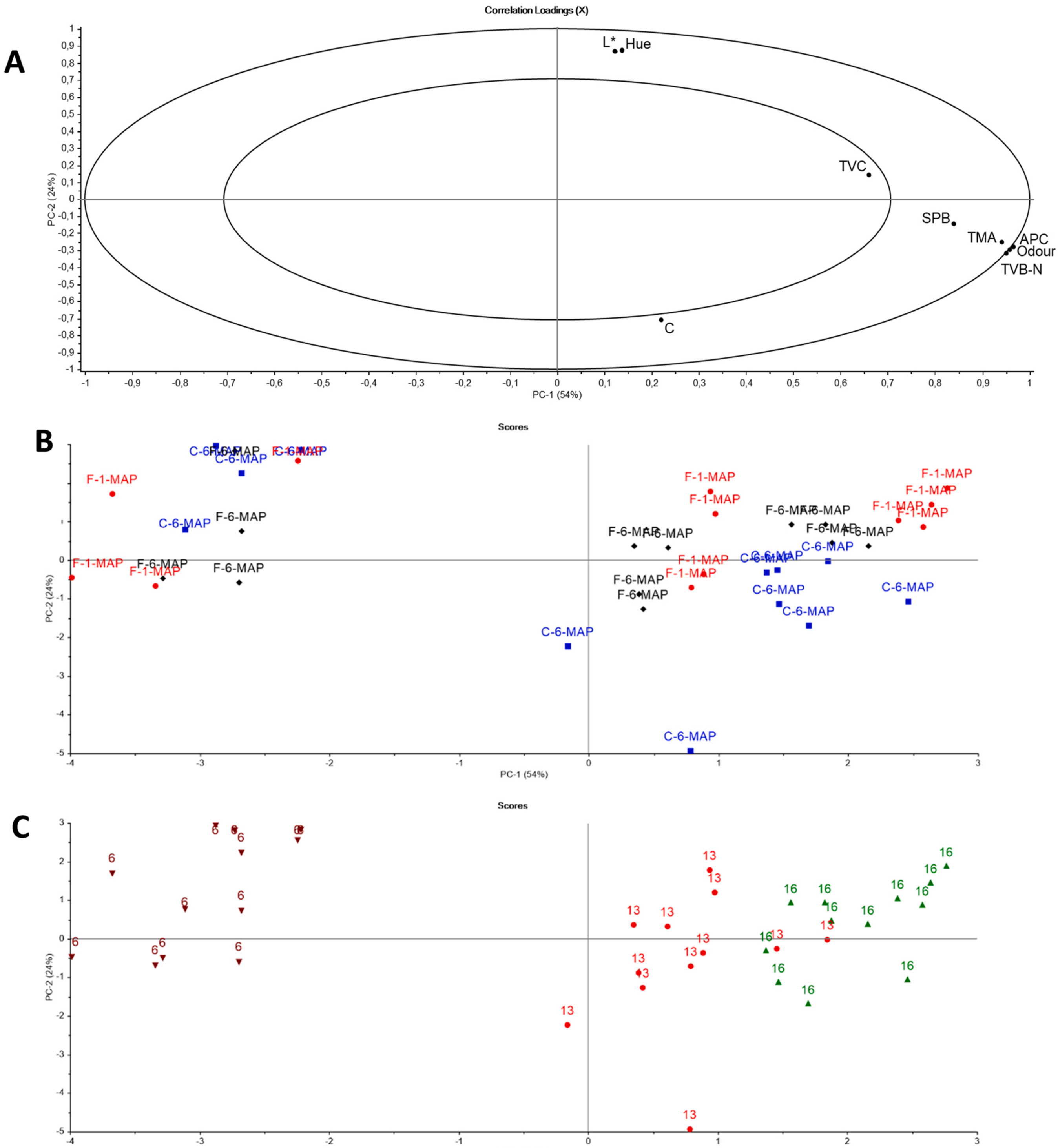

Fig. 4. Correlation loading plot (A), and score plots with observations grouped by product category (B) and days, *post-mortem* (C). The outer and inner ellipse indicate 100% and 50% explained variance, respectively (A).

MA packaged cod has been reported to be from 10 to 20 days when stored at 0–3 °C (Jensen, Petersen, Røge & Jepsen, 1980; Cann, Smith, Houston, 1983; Woyewoda, Bligh, & Shaw, 1984; Guldager, Bøknæs, Østerberg, Nielsen & Dalgaard, 1998).

E-commerce of fresh seafood challenges the transfer of information along the value chain. To solve this, TTIs can be beneficial. A TTI should enable the consumer to perform a thorough evaluation of the product upon arrival. Especially for perishable food products, knowledge about the time and temperature history is essential. Beyond the technical information generated by the TTI, the consumer should be able to trust the product, particularly the food safety. Technical devices ensuring thorough information to the consumer is assumed to be necessary, especially in succeeding with the e-commerce of fresh fish products.

4. Conclusion

The results of this work showed that in the B2B, the fish, either gutted or gutted and filleted, stored on ice, had a core temperature of app. −0.5 °C in the first 6 days. After cutting and packaging (modified atmosphere (MA) or air) at day 6, shelf life of 7–10 and 5–7 days for MA packaged and air-packaged products, respectively, was obtained when stored at 4 °C. The intensity of off-odor determined the shelf life. Time for filleting, one- or 6-days *post-mortem* for the MA products, did not influence the shelf life. This is assumed to be ascribed to the low storage temperature in the B2B interval. The TTI recording the temperature showed a good coherence with the reference temperature, while the enzymatic based TTI showed a remaining shelf life of 3.8 days for the MA packaged cod when the product was considered as non-acceptable. This inconsistency demonstrates that the TTI should have been activated earlier. Apart from the TTIs, TVB-N and TMA are appropriate and objective spoilage indicators for both MA- and air-packaged cod when stored at 4 °C. It is noteworthy that the acceptance threshold for both parameters is higher for the MA than for their air-packaged counterparts.

CRediT authorship contribution statement

Grete Lorentzen: Conceptualization, Project administration, Supervision, Methodology, Validation, Investigation, Resources, Visualization, Writing – original draft, Writing – review & editing. **Jan Thomas Rosnes:** Conceptualization, Project administration, Supervision, Methodology, Validation, Investigation, Resources, Visualization, Writing – original draft, Writing – review & editing. **Bjørn Tore Rotabakk:** Conceptualization, Project administration, Supervision, Methodology, Validation, Investigation, Resources, Visualization, Writing – original draft, Writing – review & editing. **Aase Vorre Skuland:** Conceptualization, Methodology, Validation, Investigation, Resources, Writing – original draft. **Jorunn S. Hansen**: Methodology, Investigation. **Tatiana N. Ageeva:** Conceptualization, Methodology, Validation, Investigation, Resources, Visualization, Writing – original draft, Writing – review & editing.

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