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Report on the OnVu™ TTI Label Evaluation

CHILL-ON Wet Trial in Iceland November 2009

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December 2010

PREFACE

The performance of TTI (Time Temperature Indicators) OnVu^{TM} labels for consumer packed cod fillets was evaluated in a simulated trial for export of fresh cod fillets from Iceland to Europe. The studies were part of wet trials in the EC-funded project CHILL-ON (FP6-016333-2) entitled "Developing and integrating novel technologies to improve safety, transparency and quality assurance of the chilled/frozen food supply chain - test case fish and poultry". The main author of the report Nga Mai was a PhD student at the Faculty of Food Science and Nutrition at University of Iceland and received a scholarship from the United Nations University - Fisheries Training Programme. The ASCS research group at UoI was responsible for the implementation and validation of the technologies in field trials in the CHILL-ON project. Matís ohf participated in the project and provided facilities for the shelf life studies. University of Bayeruth participated in the trials and contributed to the validation of the TTI models. University of Bonn provided guidance and training material for the OnVu^{TM} labels. Following papers have been published based on the experiments described in this report as part of the PhD thesis of Nga Mai.

Scientific papers

Nga Mai, Hubert Audorff, Werner Reichstein, Dietrich Haarer, Gudrun Olafsdottir, Sigurdur Bogason, Judith Kreyenschmidt, and Sigurjón Arason, 2011. "Performance of a new photochromic time-temperature indicator under simulated fresh fish supply chain conditions". International Journal of Food Science and Technology 2011, 46, 297–304

Nga Mai, María Gudjónsdóttir, Hélène Lauzon, Kolbrún Sveinsdóttir, Emilía Martinsdóttir, Hubert Audorff, Werner Reichstein, Dietrich Haarer, Sigurdur Bogason, and Sigurjón Arason, 2011. "Continuous quality and shelf life monitoring of retail-packed fresh cod loins in comparison with conventional methods". *Food Control*, 22(6), 1000-1007.

PhD Thesis

Mai Thi Tuyet Nga 2010. Enhancing quality management of fresh fish supply chains through improved logistics and ensured traceability. PhD Thesis, Faculty of Food Science and Nutrition. School of Health Sciences University of Iceland . 226 p. Available at http://hdl.handle.net/1946/5934

Published report from the CHILL-ON wet trials

Lauzon, H.L., Margeirsson, B., Sveinsdóttir, K., Reynisson, E., Gudjónsdóttir, M., Martinsdóttir, E., Gospavic, R., Haque, N., Popov, V., Ólafsdóttir, G., Haflidason, T., Gudlaugsson, E., Bogason, S.G. 2010. Functionality testing of selected Chill-on technologies during a transport-simulation study of palletized cod boxes: qPCR for fish spoilage bacteria, SLP model and QMRA to evaluate pathogen growth in spiked cod. Matís report 35-10, Reykjavik, Iceland, 31 p

CHILL-ON

Titill / Title	Report on OnVu label evaluation during the wet trial in Iceland November 2009		
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Skýrsla / Report no.	ASCS-10-01	*Útgáfudagur / Date:*	December 2010
hyperlinks			
Styrktaraðilar / funding:	*European Commission Chill-on (contract FP6-016333-2)*		
Ágrip á íslensku:	Markmiðið var að sannreyna virkni ***OnVu***^{TM} TTI (time temperature indicator) merkja við raunverulegar aðstæður við geymslu á þorskhnökkum í kæli, bæði í EPS (expanded polystyrene) kössum og í neytendapakkningum. Könnuð voru áhrif mismunandi upphafshleðslu TTI merkjanna og staðsetningu þeirra á umbúðum til að ákvarða gildi sem gæfi sambærilegan líftíma (samkvæmt litabreytingu) við mismunandi hita- og tímaálag og geymsluþol vörunnar miðað við skynmat og efna – og örvermælingar og /eða spágildi um líftíma vörunnar við mismunandi umhverfisaðstæður. Þrenns konar geymslu og flutninga aðstæður voru skoðaðar fyrir fersk þorskflök/hnakka frá pökkun til neytenda i) án hitaálags; ii) sex tíma hitaálag; iii) ofurkæling (kælihermar Matís). Geymsluþol áætlað með TTI merkjum á yfirborði geymslupakkninga (bottom) var í samræmi við niðurstöður spálíkans byggt á "square root" líkani (SSSP Seafood Spoilage and Safety Predictor), þar sem notast var við skráningar á hitastigi vörunnar. TTI merki sem staðsett voru á botni neytendapakkninga með upphafs gildi 61 gáfu sambærilegar niðurstöður um geymsluþol við 0,5 °C og skynmat. Því er mælt með staðsetningu á TTI merkjum á botni pakkninga til að fylgjast með geymsluþoli. Hraðafræðilíkan Kreyenschmidt *o.fl.* (2010) reyndist vel fyrir gögn úr tilraunum við geymsluhitastig undir 2 °C, þar sem ekki var um að ræða hitaálag. Það bendir til þess að hægt sé að aðlaga líkanið að lágum geymsluhita og ákvarða upphafshleðslu merkjanna í samræmi við skilgreint geymsluþol vöru við viðkomandi aðstæður. Hins vegar reyndist geymsluþol við ofurkælingu samkvæmt TTI merkjum vera ofáætlað. Hugsanleg skýring er sú að miklar sveiflur voru á hitastigi í kælihermunum, sem gæti hafa orsakað frostskemmdir í fiskvöðva og þar af leiðandi haft áhrif á styttra geymsluþol. TTI merki henta því ekki til að spá fyrir um geymsluþol við aðstæður undir -1°C, þar sem hætta er á að frostskemmdir geti átt sér stað.		
Lykilorð á íslensku:	*TTI, Timi og hitastig, Geymsluþolsspá, SSSP, neytendapakkningar á fiski*		

CHILL-ON

Summary in English:	The objective of this study was to validate the performance of the ***OnVu***^{TM} time temperature indicator (TTI) labels and prove the use of such labels under practical conditions during storage of cod loins, in both expanded polystyrene (EPS) boxes and retail packages. This was done by finding the appropriate initial charging value of the labels in agreement with the product time temperature history, finding a suitable position of the labels on the packages and by investigating whether the shelf life, as indicated by the colour change of the TTI labels, was in agreement with sensory, chemical and/or microbiological predictions of the shelf life of cod loins after different storage treatments. Three different storage treatments were studied i) a fresh cod supply chain scenario from packaging to consumption without abuse during transportation; ii) a fresh cod supply chain scenario from packaging to consumption with six-hour abuse during transportation and iii) a fresh cod supply chain back-up scenario from packaging to consumption including superchilled storage (whole simulation at Matis laboratory). Shelf lives predicted by TTI on bottom tray surface and predicted by the SSSP (Seafood Spoilage & Safety Predictor) square-root spoilage model (based on product temperature) were in good agreement for all storage treatments. Placement of TTI labels with the initial square value of 61 on the bottom surface of the retail trays stored at 0.5 °C also gave similar shelf life as the product shelf life declared by sensory evaluation. It is therefore, suggested to stick the labels on the bottom of trays to monitor the shelf life of the product. The kinetic model of Kreyenschmidt *et al*. (2010) for TTI labels worked well with data from non-abuse storage at temperatures below 2 °C, which indicates the potential to extend their quality contour diagram to low temperatures so that a charging level can be defined to suit the shelf life of a product stored under the same conditions. However, the shelf life predicted by the TTI labels for the superchilled treatment was overestimated, due to observed temperature fluctuations in the cooling simulator, which led to freeze damage of the muscle, and thus reduction of real shelf life. TTIs should therefore not be used for temperatures below -1°C, since TTIs cannot predict shelf life reduction due to freeze damage.

| *English keywords:* | *Time Temperature Indicators, Self life prediction, SSSP, consumer packed cod fillets* |

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INTRODUCTION

The objective was to validate the performance of the ***OnVu^{TM} time temperature indicator (TTI) labels*** and prove the use of time temperature indicators (TTIs) under practical conditions. The data from the field trials was integrated in a developed TTI model and compared to the data from the laboratory investigations.

For the ***OnVu^{TM} labels*** the goal was both the integration into packaging and performance validation. The integration verified whether the tags were correctly placed for optimum performance, stability and accessibility, and whether the position of the label on the package was practical for fresh cod supply chains. The performance validation tested whether it is possible to control the cold chain of the product by using TTIs; it analysed the reproducibility of the charging process and discolouration process of the labels; the overall outcome showed whether the TTI label could reflect indirectly the freshness of the product.

The aims were as follows:

- To find the right starting/initial square value in accordance with the shelf life of product (i.e. cod loins);
- To investigate whether the colour change of TTI labels was in accordance with the storage conditions, i.e. the product time temperature history;
- To investigate the application of TTI labels for different packaging solutions (on plates in EPS boxes; on retail packs);
- To find suitable placement of the TTIs in the EPS boxes and on the retail packs;
- To validate the TTI model developed by UB at low temperatures (below 2 °C).

Three experiments were conducted:

- Fresh cod supply chain scenario from packaging to consumption without abuse during transportation;
- Fresh cod supply chain scenario from packaging to consumption with six-hour abuse during transportation;
- Fresh cod supply chain back-up scenario from packaging to consumption (whole simulation at Matis)

MATERIALS AND METHODS

1.1 MATERIALS

Three tubs (approximate weight of 1500 kg) of fresh, whole Atlantic cod (*Gadus morhua*), were obtained at the fish market in Grindavík. The fish was caught by Danish Seine by the vessel Páll Helgi IS 142 on Nov 21st 2009. The fish was gutted, iced in tubs and sorted (average weight 1.38 kg; average length 0.350 m). The fish was iced in layers interchanged with layers of flake ice. On Nov 23rd the fish arrived to the processing plant, where it was stored overnight in the factory cooling chamber until processed at noon on Nov 24th, 3 days post catch. In the storage studies this day is referred to as day 0. The ice to fish ratio was appropriate with fish temperature of 0 °C. The fish tub was emptied into a water bath for rinsing at the beginning of the processing line, followed by beheading, filleting, cooling in a water-ice mixture, trimming and cutting the fish into loins and tails. Thereafter, the fish loins were chilled in a liquid cooling medium to reach a temperature of -0.5 °C and packed into EPS boxes, each weighing 5 kg. The EPS boxes were packed with 2 absorbent pads, lined with a bag and a 250g ice mat put on top of the closed bag.

1.2 METHODS

1.1.1. EXPERIMENTAL DESIGN

The study scheme for investigating the applicability of OnVu labels for cod loins in EPS boxes and retail packs at different scenarios is shown in Figure 1.

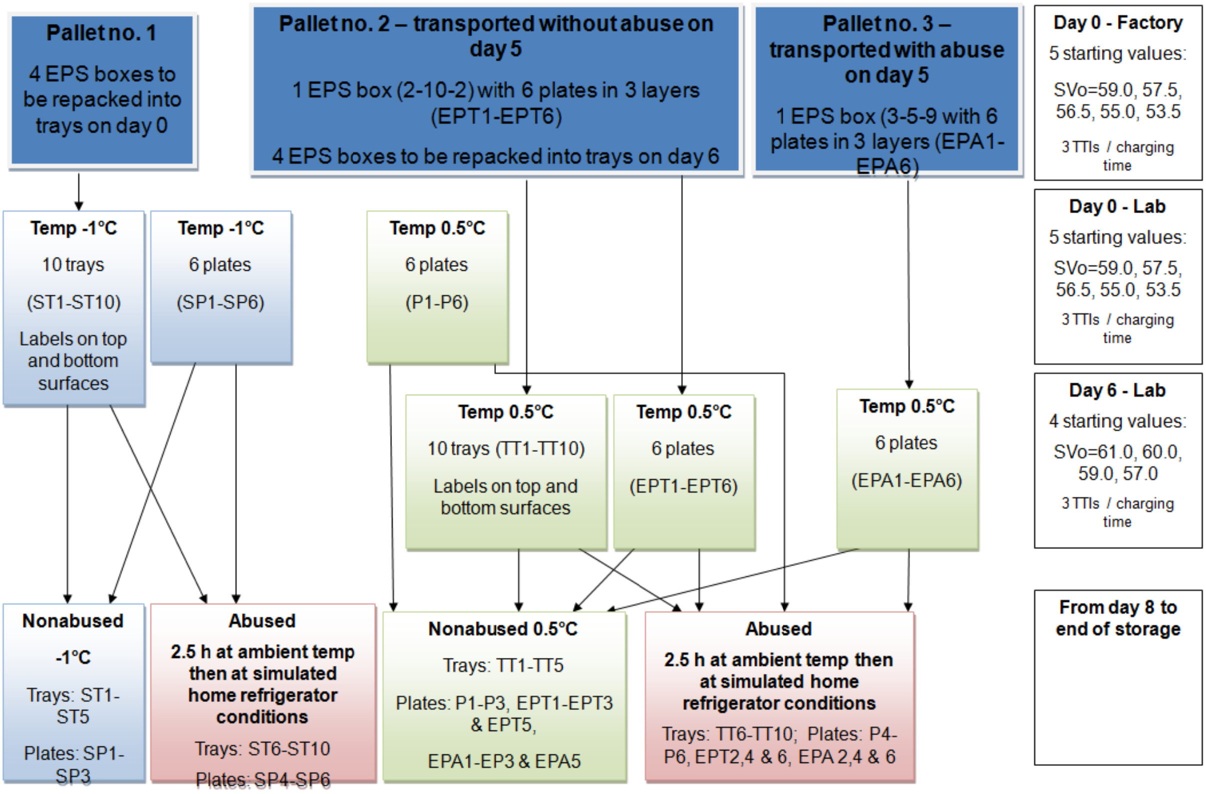


Figure 1. Study scheme of the TTI trials.

At the factory, two EPS boxes were packed with cod loins (5 kg each) in two layers. Six Plexiglas plates with a layer of white label were stuck with TTI labels of five different charging times (3 labels per charging time) (Table 2) to obtain 5 initial squared values SV (SVo) of 53.5; 55.0; 56.5; 57.5; and 59.0 ± 0.3. Two plates were put on the bottom of the box (EPT 5 & 6 for pallet No.2; EPA 5 & 6 for pallet No.3), 2 in the middle of the box between fish layers (EPT 3 & 4 for pallet No.2; EPA 3 & 4 for pallet No.3), and 2 on top of the fish (EPT 1 & 2 for pallet No.2; EPA 1 & 2 for pallet No.3) inside each box. The plates were organised in a way that those with odd numbers (i.e. 1, 3, and 5) were on the same side of the box, and evenly numbered plates were on the other side of the box. Each plate was equipped with a DS1922L iButton® temperature logger (Maxim Integrated Products, Inc., CA). One additional temperature logger Testo 174 (Testo AG, Lenzkirch, Germany; range:-30 to +70 °C; accuracy: ± 0.5 °C) was placed between the 2 plates of each height level (i.e. 3 loggers in each box). Finally, an ice mat was put on top of the fish and plates (Figure 2).

The discolourations of TTI labels (with the same initial SV) on EPT and EPA plates were then compared to see the effect of temperature abuse during the transport phase.

Figure 2. EPS box containing 5 kg of cod loins with TTI plates, iButtons, logger, and ice mat on top.

The cod loins were repacked into yellow polystyrene trays (Linstar E39-34, 225 x 175 mm) upon arrival at the laboratory with approximately 771-854 g of fish per tray (about 6 loins). The trays were then sealed in a plastic bag (250 x 350 mm, 20 PA/50 PE = 70 µm thick and 40% vacuum), stuck with 2 layers of white labels on both the top and bottom surfaces, and then with TTIs of five different charging times. One iButton temperature logger was put on each tray surface close to the TTIs. iButtons were also placed in direct contact with the fish inside some trays (On the day of processing (day 0) 12 plates, covered with two layers of white labels, were stuck with TTIs of 5 different charging times (same as for retail trays, Table 2) at the Matís facilities. Six of them were stored in a cooling simulator at -1 °C, and other six in another simulator at 0.5 °C. One iButton temperature logger was put on each plate surface. The trays were stored in a grid rack to ensure that trays were not stacked on top of each other. This was made to ensure that all plates encountered the same ambient conditions.

Table 1, Figure 3). As shown on the study scheme (Figure 1), 10 trays were packed and stuck with TTIs on day 0 (from pallet 1), and another 10 on day 6 (from pallet 2). In addition to the TTI trays, fish was also packed into trays and sealed with the same procedure for sensory, chemical, and microbiological analyses. Eighteen trays were packed for these analyses on each repacking day. On the day of processing (day 0) TTI labels of five different charging times (Table 2) or 5 initial SV (SVo), namely 53.5; 55.0; 56.5; 57.5; and 59.0 ± 0.3, were studied. On day 6 of storage TTI labels of four different charging times (Table 2) or 4 initial SV (SVo), namely 57.0; 59.0; 60.0 and 61.0 ± 0.3, were used. The repackaging was done to simulate a real supply chain situation with fresh fish, exported from Iceland, which is often repacked upon arrival to retailer in Europe 6-7 days after catch/processing. Pallets 2 and 3 were transported (to the Westman Isles and back to Reykjavik) to simulate the transport conditions (temperature and time) of fresh fish exported from Iceland to France via England.

Figure 3. Retail trays with TTI labels and ibuttons: tray with iButton inside (left); tray repacked on day 0 with TTIs of five charging times (middle); and tray repacked on day 6 with TTIs of four charging times (right).

On the day of processing (day 0) 12 plates, covered with two layers of white labels, were stuck with TTIs of 5 different charging times (same as for retail trays, Table 2) at the Matís facilities. Six of them were stored in a cooling simulator at -1 °C, and other six in another simulator at 0.5 °C. One iButton temperature logger was put on each plate surface. The trays were stored in a grid rack to ensure that trays were not stacked on top of each other. This was made to ensure that all plates encountered the same ambient conditions.

Table 1. Trays for TTI labelling. Position in the grid rack, weight of fish and relevant iButton numbers.

Temperature conditions	Tray code	Position in cooling simulator 0.5 °C	Weight (g)	iButton in bag (top)	iButton in bag (bottom)	iButton on top surface	iButton bottom surface	Origin
-1 °C constant	**ST1**	top	766	470		108	121	
	ST2	middle	787	124		63	71	
	ST3	bottom	792			437	304	
	ST4	top	729			68	373	
	ST5	middle	806			125	483	
-1 °C from day 0 + abused on day 8	**ST6**	bottom	732	456		479	161	
	ST7	top	753	482		457	444	
	ST8	middle	679			32	165	
	ST9	bottom	758			170	408	
	ST10	top	905			450	409	
	Average		771 ± 60					
0.5 °C constant from day 6	**TT1**	top	919	5b	11a	4a	12b	EPS2-10-7
	TT2	middle	872	22b	40b	13a	22c	EPS2-10-7
	TT3	bottom	841			6a	7a	EPS2-10-7
	TT4	top	834			8b	14a	EPS2-10-7
	TT5	middle	846			10a	15a	EPS2-10-7
0.5 °C from day 6 + abused on day 8	**TT6**	bottom	822	3a	31b	4b	39	EPS2-10-6
	TT7	top	861	5a	9a	12a	24b	EPS2-10-6
	TT8	middle	848			18b	33	EPS2-10-6
	TT9	bottom	863			30b	32b	EPS2-10-6
	TT10	top	837			2a	13b	EPS2-10-6
	Average		854 ± 27					

The discolourations of the TTI labels (with the same initial square values) on EPT, EPA, and P plates were then compared to find out the effect of different time-temperature history on the TTIs. The configuration of the TTI labels on the surfaces of the retails packs and plates is shown in Figure 4.

Row 1	tc1	OnVu1	OnVu2	OnVu3
Row 2	tc2	OnVu1	OnVu2	OnVu3
Row 3	tc3	OnVu1	OnVu2	OnVu3
Row 4	tc4	OnVu1	OnVu2	OnVu3
Row 5	tc5	OnVu1	OnVu2	OnVu3
Row 6		Ibutton		

Figure 4. Configuration of TTI labels on a tray or plate surface: tc = charging time; charging times decrease from tc 1 to tc 5.

OnVu TTI labels B1+090807 were activated and covered with a transfer film TTR 70QC 53141 in an automated UV light charger GT 240 Bizerba (Bizerba GmbH & Co. KG, Germany) with a speed of 10 labels/min. The charging conditions are shown in Table 2.

Table 2. On site charging conditions.

Location	Date	Start	End	Duration	Ambient temperature* (°C)	Humidity* (%)	TTR heat (%)	Charging time (ms)
Factory Plates	24.11.2009	14:28	15:23	00:55	9.4-9.8	65	-18	2400
								1570
								1200
								950
								670
Matis	24.11.2009				6.0-7.0	60	-18	2700
Retail packs		18:00	19:41	01:41				1700
Plates		19:46	20:21	00:35				1280
								950
								650
Matis Retail packs	30.11.2009	17:45	19:43	01:58	7.0-7.1	63.9	-18	1100
								700
								500
								450

* Ambient temperature and relative humidity were measured by loggers Testo 171-3 (Testo AG, Lenzkirch, Germany; temperature range: -20 to +70 °C; temperature accuracy: ± 0.5 °C; humidity range: 0-100 % rH; humidity accuracy: ± 3 %rH)

To analyse the effect of the UV charging time and the dependency of different environmental temperatures on the discolouration process, four to five different charging times; two different steady temperatures (-1 and 0.5 °C), and simulated chain temperature fluctuation were investigated.

In total 900 TTI labels (Figure 1) were investigated to analyse the influence of different intensities of activation (charging times) and the discolouration process as related to elapsed time and temperature in a similar manner as in the study of Kreyenschmidt *et al.* (2010). The charging times (initial SV) investigated were based on predefined shelf life of cod loins at temperatures of -1 and 0.5 °C, which were expected to be around 15 and 6-7 days from packing at the factory, respectively.

Half of all studied TTI plates and retail packs were temperature abused (Figure 1) for about 2.5 hours on day 8 and then stored at simulated home refrigerator conditions until end of the study (days 13-16). This was to simulate handling and storage conditions of the end consumers.

TTI colour changes were measured with the Gretag Macbeth OneEye spectrophotometer (X-Rite, Althardstrasse 70, Regensdorf, Switzerland) at D65 illumination and 2° observation angle conditions. At the factory the measurements were done under the ambient temperature of about 10 °C, while other measurements were done at the Matís laboratory at an ambient temperature around 6-7 °C.

For the test series the Square Value (SV) in CIELab space (Eq. 1) was used to characterise the TTI-charging and discolouration process:

$$SV = \sqrt{L^2 + a^2 + b^2} \qquad (1)$$

Where

L represents the lightness of the labels;

a represents their redness and greenness;

b represents their yellowness and blueness.

The reference scale corresponds to a measured SV value of 71 which is regarded as the end of a charged label's shelf life.

iButton temperature loggers were set to record the temperature at 10 minute intervals with a precision of 0.5 °C.

1.1.2. *REFERENCE LABORATORY METHODS*

1.1.2.1. Sensory evaluation

Two groups of cod fillets were evaluated with sensory evaluation. The main purpose was to study differences in shelf life according to sensory evaluation by a trained panel.

Quantitative Descriptive Analysis (QDA), as introduced by Stone and Sidel (1985), and Torry freshness score sheet were used to assess cooked samples (MA09sky114-116,118-122) of five sample groups of cod. Ten panellists all trained according to international standards (ISO 8586, 1993); including detection and recognition of tastes and odours, trained in the use of scales and in the development and use of descriptors participated in the sensory evaluation. The members of the panel were familiar and experienced in using the QDA method and Torry freshness score sheet for cod. The panel was trained in recognition of sensory characteristics of the samples and describing the intensity of each attribute for a given sample using an unstructured scale (from 0 to 100%). Most of the attributes were defined and described by the sensory panel during other projects (Sveinsdottir *et al*., 2009). The sensory attributes were 30 and are described in Table 3.

Samples weighing ca. 40 g were taken from the loins and placed in aluminium boxes coded with three-digit random numbers. The samples were cooked for 6 minutes in a pre-warmed oven (Convotherm Elektrogeräte GmbH, Eglfing, Germany) at 95-100 °C with air circulation and steam, and then served to the panel. Each panellist evaluated duplicates of each sample in a random order in nine sessions (maximum four samples per session). A computerized system (FIZZ, Version 2.0, 1994-2000, Biosystèmes) was used for data recording.

Table 3. Definition of sample groups evaluated by sensory evaluation.

Sample name	Description	Sampling days
ST	Superchilled trays	0, 6, 10, 13
TT	D06 trays (shipped)	6, 10, 13

Table 4. Sensory vocabulary for cooked samples of cod (*Gadus morhua*).

Sensory attribute	Short name	Description of attribute
Odour		
sweet	o-sweet	sweet odour
shellfish, algae	o-shellfish	shellfish, algae, characterict fresh odour
meaty	o-meat	meaty odour, reminds of boiled meat or halibut
vanilla, boiled milk	o-vanilla	vanilla, sweet boiled milk
boiled potatoes	o-potatoes	odour reminds of whole, warm, boiled potatoes
frozen storage	o-frozen	reminds of odour found in refrigerator and/or freezing compartment
table cloth	o-cloth	reminds of damp, unclean cloth (left on kitchen table for 36 h)
TMA	o-TMA	TMA odour, reminds of dried salted fish, amine
sour	o-sour	sour odour, spoilage sour, acetic acid
sulphur	o-sulphur	sulphur, matchstick, boiled kale
Appearance		
light/dark colour	a-dark	Left end: light, white colour. Right end: dark, yellowish, brownish, grey
homogenous/ heterogeneous	a-heterog.	Left end: homogenous, even colour. Right end: discoloured, heterogeneous, stains
white precipitation	a-prec.	white precipitation in the broth or on the fish
Flavour		
salt	f-salt	salt taste
metallic	f-metallic	metallic flavour
sweet	f-sweet	characteristic sweet flavour of very fresh (boiled) cod
meaty	f-meat	meaty flavour, reminds of boiled meat
frozen storage	f-frozen	reminds of food which has soaked in refrigerator/freezing odour
pungent	f-pungent	pungent flavour, bitter
sour taste	f-sour	sour taste, spoilage sour
TMA	f-TMA	TMA flavour, reminds of dried salted fish, amine
off flavour	f-off	strenght of off flavour (spoilage flavour/off-flavour)
Texture		
flakiness	t-flakes	the fish portion slides into flakes when pressed with the fork
firm/soft	t-soft	Left end: firm. Right end: soft. Evaluate how firm or soft the fish is during the first bite
dry/juicy	t-juicy	Left end: dry. Right end: Juicy. Evaluated after chewing several times: dry - pulls juice from the mouth
tough/tender	t-tender	Left end: tough. Right end: tender. Evaluated after chewing several times
mushy	t-mushy	mushy texture
meaty	t-meaty	meaty texture, meaty mouth feel, grude muscle fibers
clammy	t-clammy	clammy texture, dry red wine, tannin
rubbery	t-rubbery	rubbery texture, springy

1.1.2.2. Chemical analysis

Chemical analysis of total volatile base nitrogen (TVB-N) and trimethylamine (TMA) was performed by the methods described by Malle and Tao (1987). The TVB-N was steam distilled (Struer TVN distillatory, STRUERS, Copenhagen, Denmark) and titrated, after extraction of the fish muscle with a 7.5% aqueous trichloroacetic acid (TCA) solution. After distillation the TVB-N was collected in a boric acid solution and finally titrated with sulfuric acid solution. TMA was measured in TCA extract by adding 20 mL of 35% formaldehyde. All chemical analyses were performed in duplicate. The pH was measured in 5 grams of mince moistened with 5 mL of deionised water. The pH meter was calibrated using the buffer solutions of pH 7.00 ± 0.01 and 4.01 ± 0.01 (25°C) (Radiometer Analytical A/S, Bagsvaerd, Denmark). The salt content was measured in the raw material on day 0 with the Volhard Titrino method (AOAC 976.18, ed. 17. 2000).

1.1.2.3. Microbiological analysis

Total viable psychrotrophic counts (TVC) and counts of H_2S-producing bacteria (black colonies) were evaluated on iron agar (IA), as described by Gram *et al.* (1987), with the modification of using 1% NaCl and no overlay. Plates were incubated at 17 °C for 4-5 days. Measurements of presumptive pseudomonads (mCFC) were performed on modified Cephaloridine Fucidin Cetrimide (CFC) agar as described by Stanbridge and Board (1994). *Pseudomonas* Agar Base (Oxoid, UK) with CFC selective Agar Supplement (Oxoid, UK) was used and the plates were incubated at 22 °C for 3 days. Levels of *Photobacterium phosphoreum* were estimated by a quantitative Polymerase Chain Reaction method (qPCR) developed at Matis (E. Reynisson, unpublished data). Average counts are reported as log colony-forming units (CFU) per g.

1.1.3. VALIDATION OF TTI MODEL

The Lab-square values SV of the kinetics as a function of time of the non-abused label were fitted with the Slogistics1 function (Eq. 2), developed for isothermal conditions at 2-15 °C (Kreyenschmidt *et al.,* 2010), to test if the model worked for the temperature below 2 °C.

$$SV(t) = \frac{d}{1 + e^{-k(t-c)}} \qquad (2)$$

Where:
d is the amplitude of the colour change;
c reversal point;
k the rate constant of the colour change, which is temperature dependent;
t is the storage time.

Based on pre-test results, it was observed that the lifespan of TTI (time to reach SV 71) showed an exponential decay of charging level SVo, which is described in Eq. 3:

$$t_L = \exp\left(\frac{b_2 - SV_o}{a_2}\right) \qquad (3)$$

Where t_L: the lifespan/shelf life time of TTI (h); a_2: the decay constant; and b_2: factor.

Therefore, a charging level required to suit a shelf life of product can be recalculated using Eq. 4 with the same parameters as in Eq. 3:

$$SVo = -a_2 * \ln(t_L) + b_2 \qquad (4)$$

In this case t_L equals the shelf life of the product concerned.

1.1.4. DATA ANALYSIS

Microsoft Excel 2003 was used to calculate mean, standard deviation and to build graphs. Origin 7.5 (OriginLab, Northampton, MA, USA) was used to fit the TTI data to obtain model parameters and their standard errors.

One-way ANOVA (analysis of variance) with post hoc Tukey (if there were more than 2 groups), two-independent-samples t-test (if there were 2 groups), and non-parametric two-independent-samples Wilcoxon W test (or Mann-Whitney U test) (if number of samples in each group were even or below 6) were conducted to compare the means of temperatures or means of SV at different positions, such as on top and bottom of trays, or at different height levels inside EPS boxes during transport. Differences in average temperature of the pack surface and product were also analysed. The statistical analysis software SPSS version 16.0 (released September 2007) was used for this purpose. All tests were performed with a significance level of 0.05.

The Seafood Spoilage and Safety Predictor (SSSP) software version 3.0 (DTU Aqua, Denmark) was used to predict the effect of time-temperature combination on the RSL based on the recorded temperature profile. Recorded temperature data of cod loins from different positions inside EPS boxes and retail trays was separately fitted into a Square-root model for relative rate of spoilage (RRS) of fresh seafood from temperate water. In SSSP, RRS at T °C has been defined as the shelf life at a reference temperature T_{ref}, which normally is 0 °C, divided by the shelf life at T °C (Dalgaard 2002), where shelf life was determined by sensory evaluation. The SSSP uses the concept of accumulative effects of time and temperature. A reference shelf life of 10.5 days (from processing) stored at -0.08 °C for fresh cod loins in retail packs (based on sensory evaluation in this study) was used.

QDA data was corrected for level effects (effects caused by level differences between assessors and replicates) by the method of Thybo and Martens (2000). Principal Component Analysis (PCA) on mean level corrected values of sensory attributes and samples was performed. Analysis of variance (ANOVA) was carried out on QDA data corrected for level effects in the statistical program NCSS 2000 (NCSS, Utah, USA). The program calculates multiple comparisons using Duncan's multiple comparison test. The significance level was set at 5%, if not stated elsewhere.

RESULTS AND DISCUSSION

1.3 DISCOLOURATION PROCESS OF TTI LABELS OF DIFFERENT CHARGING TIMES AT DIFFERENT STORAGE CONDITIONS

The initial charging values for the TTIs were determined in pre-experiments to match the estimated shelf lives at the expected temperature with the discolouration of the OnVu labels. Four or five different charging times were used at each set of experiments to find the correct charging time. The charging was very homogenous and reproducible as shown in Figure 5.

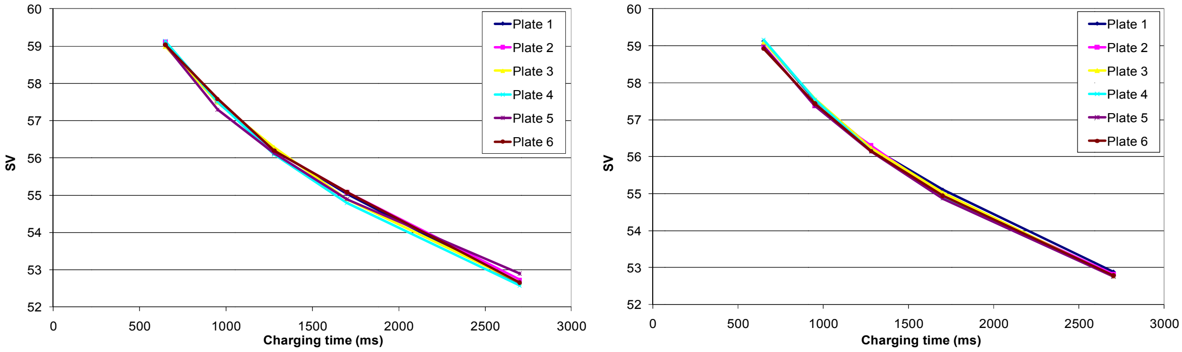


Figure 5. Charging curves of TTI labels on plates stored at Matis at -1 °C (left) and 0.5 °C (right).

As expected, the discolouration of the labels was obvious as time elapsed, sooner with those labels of shorter charging times (i.e. higher initial SV) (Figure 6-Figure 11).

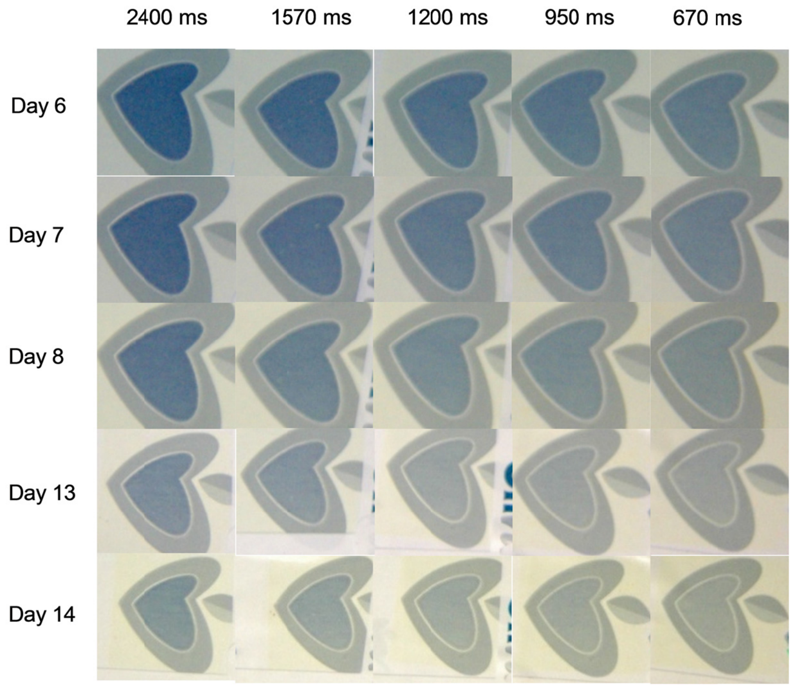


Figure 6. Discolouration process of TTI labels of different charging times on plate EPT1, which had undergone transport without abuse.

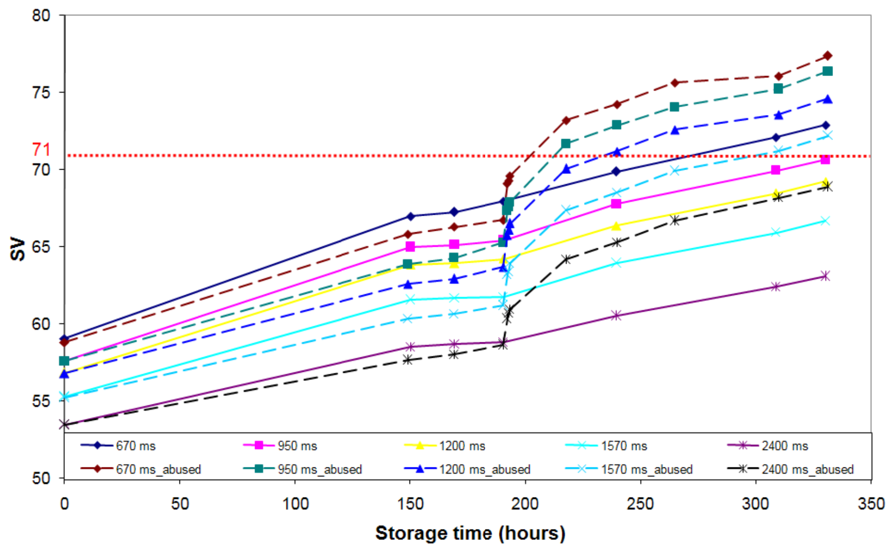


Figure 7. Discolouration process of TTI labels on plates of chain scenario without temperature abuse during transportation (Group TB, plates configured with EPT). Three of the plates were temperature abused on storage day 8).

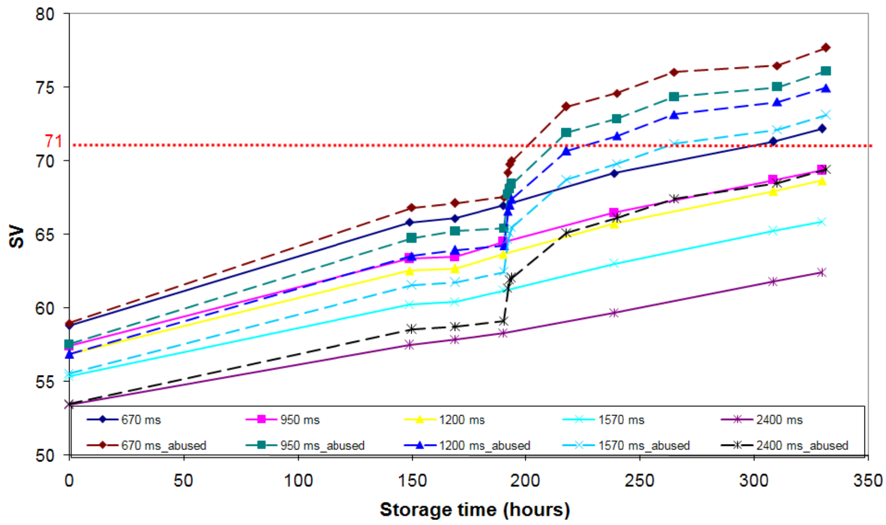


Figure 8. Discolouration process of TTI labels on plates of chain scenario with six-hour temperature abuse during transportation (Group AB, plates configured with EPA).

The effect of the temperature abuse during transport on the labels from experiment EPA (Figure 8) compared to EPT (Figure 7) was not visible (no significant difference, i.e. $p > 0.05$, in means of SV over time) despite the fact that the mean temperature of the EPT samples was significantly lower ($p < 0.05$) than their EPA counterparts during the transport period (days 0-6, see also Figure 17), as well as through the whole storage study. This indicates that the TTIs were not sensitive to the abuse at the early storage stage.

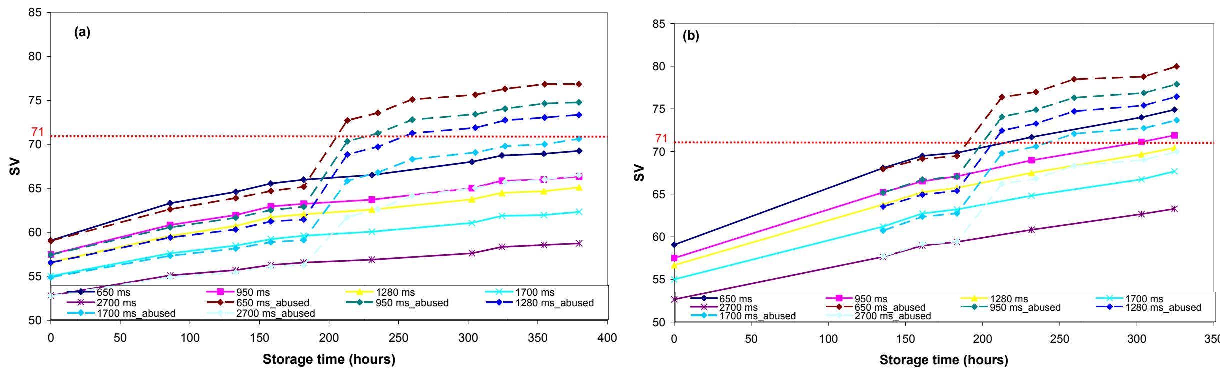


Figure 9. Discolouration process of TTI labels on plates stored at Matis at -1 °C (SP plates) (a) and 0.5 °C (P plates) (b), with and without temperature abuse on day 8.

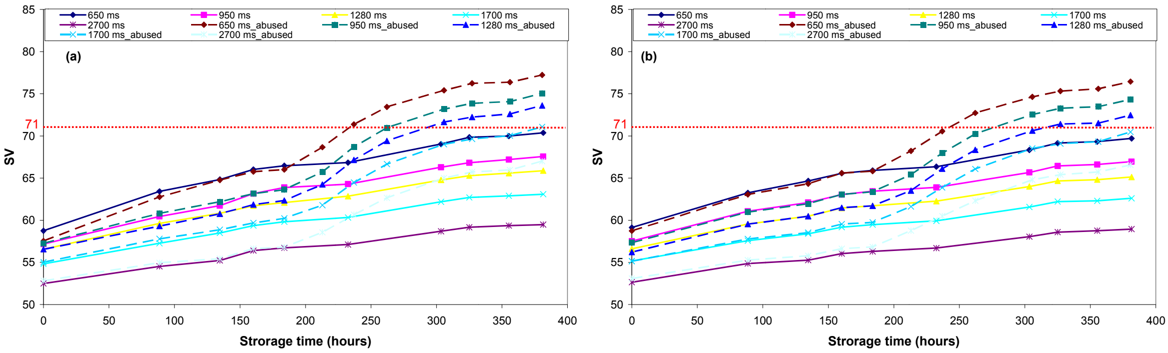


Figure 10. Discolouration process of TTI labels on (a) top surface and (b) bottom surface of retail packs stored at Matis at -1 °C from day 0 (ST trays) with and without temperature abuse on day 8.

Figure 10 and Table 5 show that there is no clear difference in the discolouration of the TTI labels on the top and bottom of the trays stored at -1 °C without abuse. However, the labels on top of the abused trays discoloured faster than those on the bottom to some extent. These differences are clearer for abused ST trays with shorter charging times (650, 950, and 1280 ms). The difference in the rate of discolouration of labels of the same charging time, in case of abuse, may be caused by the difference in temperature distribution on the top and bottom surfaces of the ST trays during storage. The tray bottom was in close contact with the table surface during abuse on day 8, i.e. with limited exposure to ambient air, while the top was more exposed to the surroundings. This resulted in a lower temperature (2.6 °C) and smaller fluctuation of temperature (± 3.3 °C) on the bottom of the abused trays than on the top (2.8 and ± 3.5, respectively) during the abuse period and some time after (i.e. period 180-213 hours) (Table 5). However, no significant difference ($p > 0.05$) was found in the average temperatures between the top and bottom surfaces for the whole studied period in both non-abused and abused cases.

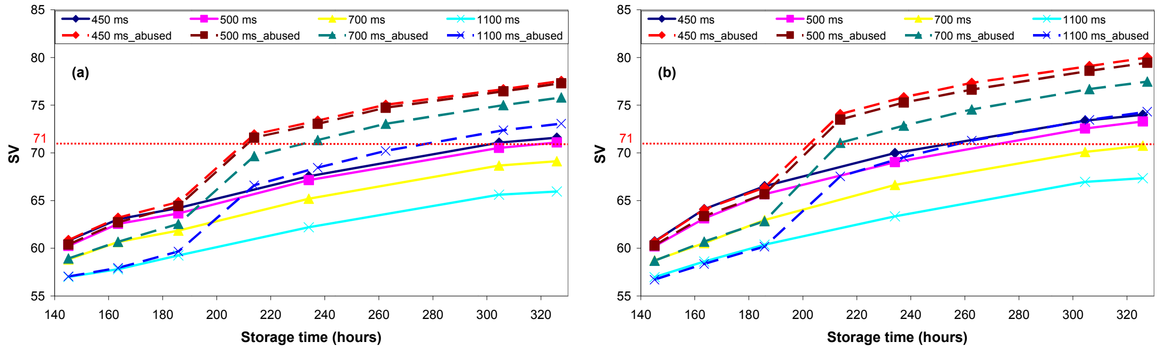


Figure 11. Discolouration process of TTI labels on (a) top surface and (b) bottom surface of retail packs stored at Matis at 0.5 °C from day6 (TT trays), with and without temperature abuse on day 8.

Figure 11 shows that the discolouration of the TTI labels on the bottom surface of the trays stored at 0.5 °C was faster than that on the top surface. These differences in the discolouration rates were observed for both abused and non abused TT groups. These differences were around ΔSV = 2 at the end of the storage period.

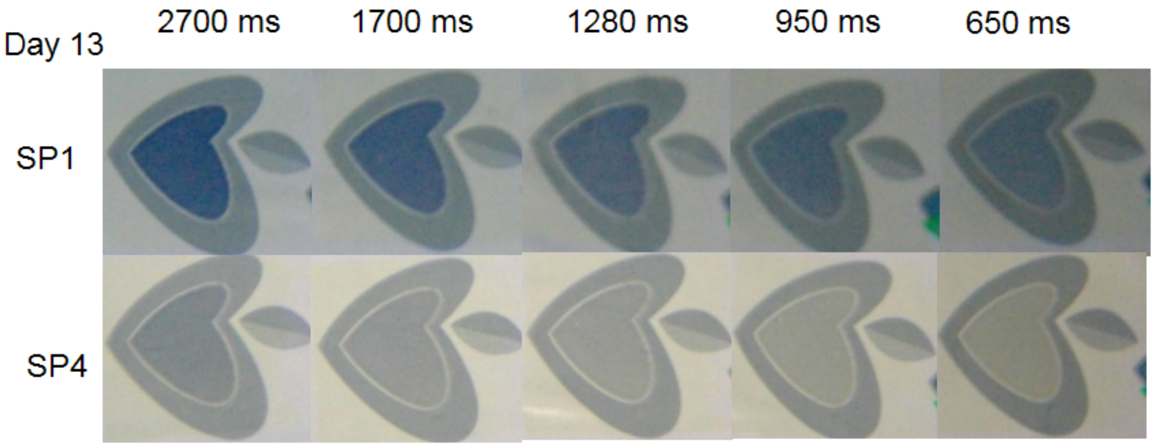


Figure 12. Colour of TTI labels with five different charging time on superchilled plates (SP plates) at day 13: non-abused (upper) versus abused from day 8 (lower).

Directly after the charging, the standard deviation of the labels of the same set of experiments and with the same charging time was compared. The standard deviation between plates was around 0.1, but never higher than 0.2. For the consumer packs the standard deviation was higher due to the inhomogeneous background/uneven surfaces of the trays. At the bottom it was around 0.3 and up to 0.6 and for the top also around 0.3 with a maximum of 0.8. In general, the standard deviation of the newly charged labels had a minimum at moderate charging times, but increased at the highest and lowest charging times. During the kinetics, the standard deviation was higher than directly after the charging, but no trend, as a function of time, could be observed. Table 11 shows the average standard deviations during the kinetics. Relatively, standard deviation was less than 3% of the dynamic range of the label SV.

Another measure of the quality of the homogeneity of the charging and the kinetic is the time difference between the first and last label to reach the reference colour. It is shown in the last row of Table 11. As already stated above, many labels did not reach the reference colour after 300 h, because the temperature in the refrigerator at Matis was lower than expected.

The trays/plates, which had undergone 2.5 hours of temperature abuse on day 8 followed by storage at refrigerated conditions, discoloured faster than those without abuse (Figure 7- Figure 12). The difference between the abused and non-abused groups could be clearly observed from the day of abuse. At the abuse, a sudden large increase in the SV values is visible and afterwards, the discolouration happens faster due the increased temperature. In all experiments, the simulation of inappropriate handling of the fish by consumers can be clearly seen in the kinetics. All these indicate great potential to use TTI labels to monitor the time temperature history of the product.

For the EPT plates, and in some extend the SP plates, it was observed that the labels for the abuse experiment discoloured slower than the labels for the non-abuse, even before the abuse. It might be due to the fact that the initial SV of non-abused samples were somewhat lower than those of the abused samples (see Figure 7 and Figure 9 (a)). That also explains why the abused labels on EPT discoloured faster than their non-abused counterparts even before the abuse on day 8 (see Figure 8). Meanwhile, the non-abused and abused P plates and ST and TT trays had the same kinetics until the abuse started (Figure 9 (b) and Figure 10).

1.4 COMPARISON OF TEMPERATURE HISTORY BETWEEN TTI ACQUISITION AND TEMPERATURE LOGGING

The temperature in the simulator that was set to -1 °C was not stable, causing high fluctuations in a wide range (from -8.8 to +2.0 °C) of the temperatures of the tray/plate surfaces and product (Figure 13 and Figure 14a). It might be because of some malfunction of the simulator and also because of opening and closing the simulator for loading of the pallets and for sampling. The product was therefore partially frozen at this condition. This temperature history explains why the TTI labels on non-abused plates/trays did not reach the reference colour after 14-16 days of storage (Figure 7) as expected. Temperatures of the product in the range of -1 to -10 °C might cause significantly negative effect (freeze-concentration effects) on the product quality, due to phase changes of the water in the product (Taoukis *et al.*, 1997). In this temperature range, non-enzymatic reactions are expected to be notably accelerated (Taoukis *et al.*, 1997; Fennema *et al.*, 1973). Enzymatic reactions also deviate from the Arrhenius behaviour in the immediate subfreezing range (Taoukis *et al.*, 1997). There is no significant difference ($p > 0.05$) in means of the top surface temperature and the top product temperature for neither the non-abused nor the abused cases. This indicates that the TTI labels on the surfaces of the retail packs and the product inside the packs have undergone similar temperature conditions.

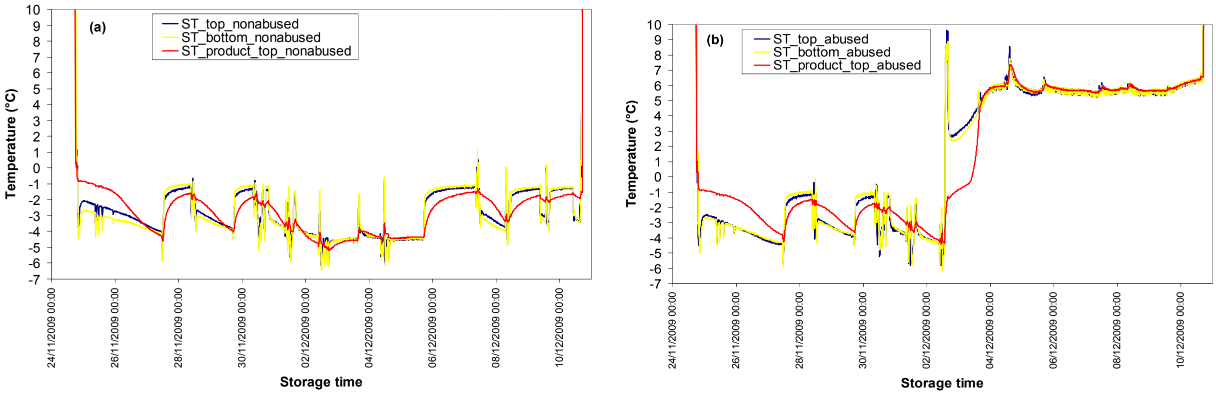


Figure 13. Temperature conditions at the top surface, bottom surface, and top product of retail packs stored at -1 °C without abuse (a) and with abuse on day 8 (b).

A clear difference between the response of the SP plates and ST trays to temperature variations could be observed. The SP plates have a low heat capacity and therefore respond fast to the temperature change. The temperature of the SP plates as a function of time looks like a step function (Figure 14), whereas for the ST trays, which have a high heat capacity, the temperature looks like an exponential change of temperature (Figure 13).

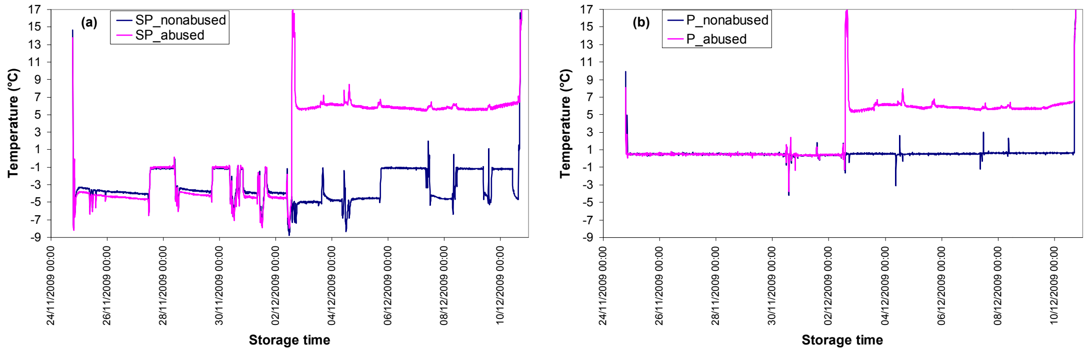


Figure 14. Temperature conditions of TTI plates stored at -1 °C (a) and 0.5 °C (b) with and without abuse on day 8.

Figure 14 shows clear differences in the temperature history of the plates stored at -1 °C chamber (SP plates) and at 0.5 °C chamber (P plates). This explains the faster discolouration rate of TTI labels on P plates compared to SP labels (Figure 9).

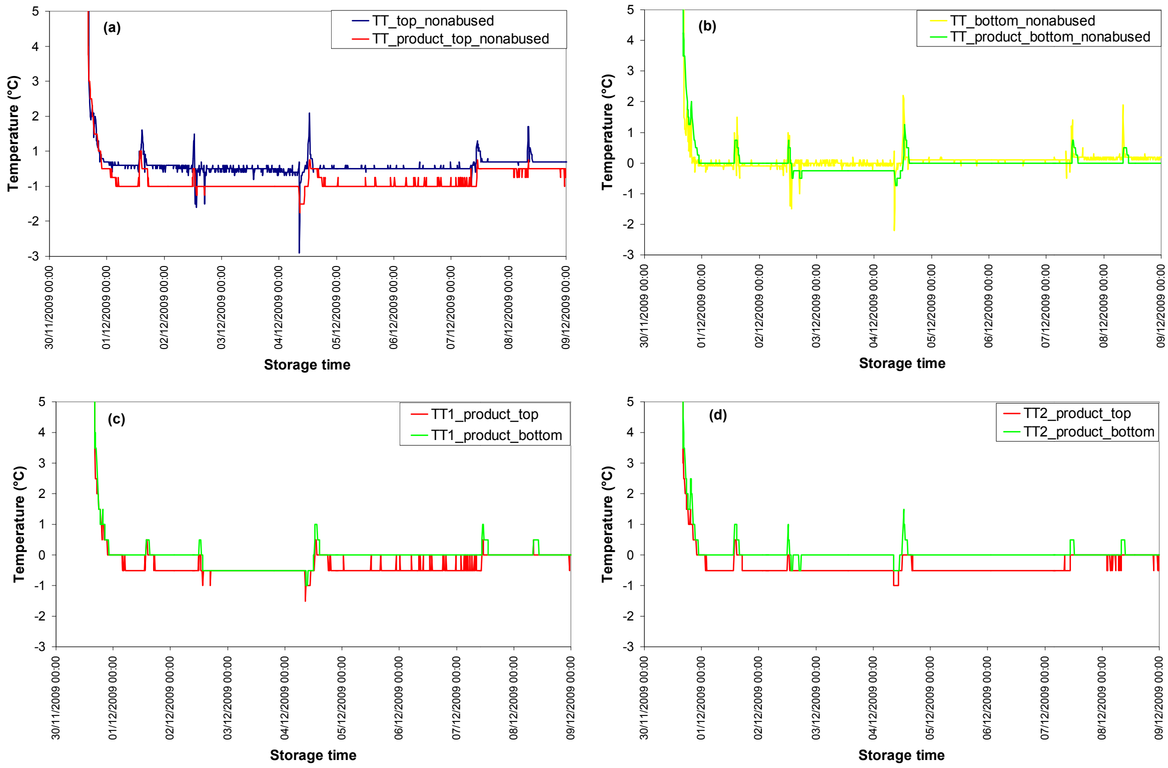


Figure 15. Temperature conditions on the top product and surface (a), at bottom product and surface (b), and at top and bottom of product in retail pack 1 (c) and pack 2 (d) stored at 0.5 °C without abuse on day 8.

For non-abused retail packs at 0.5 °C, it was observed that there was a significant difference ($p < 0.001$) in the temperatures of the top product and the bottom product. Furthermore, average temperatures on the top surfaces significantly differed ($p < 0.001$; most of the time higher) from average temperature of the top product (Figure 15a). This indicates that the TTI labels on the top surface would underestimate the product shelf life. Figure 15 (c) and (d) show that, in general, the product temperature on the top was lower than the product temperature on the bottom, with a difference of about 0.5 °C. No significant difference ($p > 0.05$) was found in the temperatures on the bottom surface and of the product on the bottom (Figure 15b). This indicates that TTI labels on the bottom surface and the bottom product have undergone similar temperature conditions. There was no significant difference ($p > 0.05$) in the temperature means of the top and bottom surfaces.

Concerning the packs stored at 0.5 °C with temperature abuse from day 8 (

Figure **16**), no significant differences ($p > 0.05$) were found between any of the top or bottom surface or product temperatures.

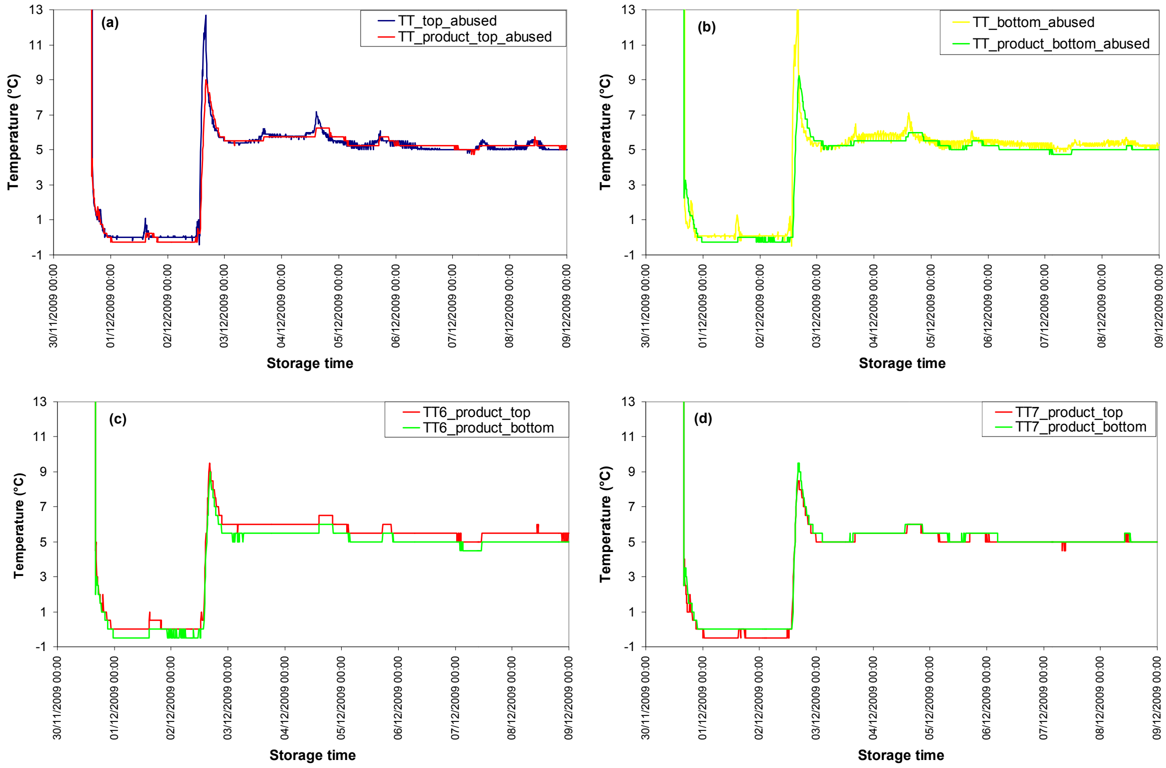


Figure 16. Temperature conditions on the top product and surface (a), at the bottom product and surface (b), and at the top and bottom of product in retail pack 6 (c) and pack 7 (d) stored at 0.5 °C with 6-hour abuse on day 8 and followed by a storage at refrigerated condition (TT trays).

The temperature history of the plate/tray surfaces (Figure 13-

Figure *16*) are in good correlation with the discolouration rate of the TTI labels as seen in Figure 9-Figure 11.

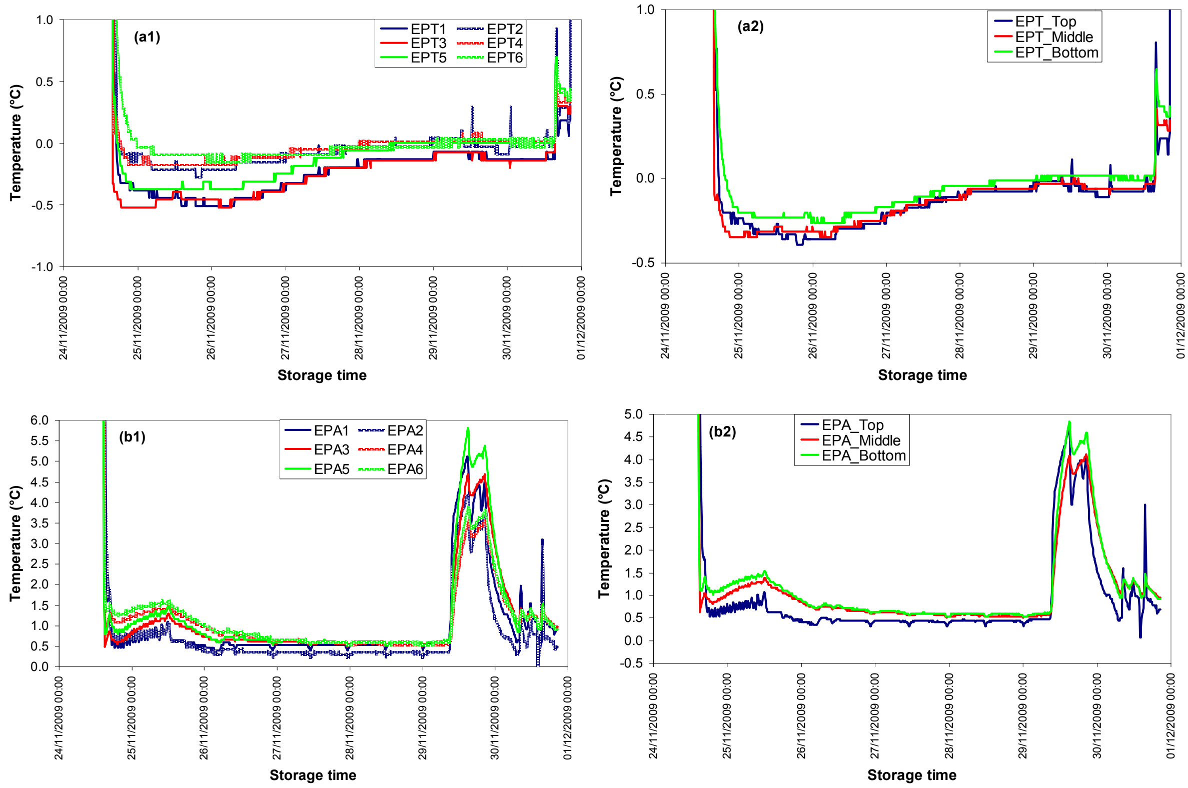


Figure 17. Temperature conditions of the TTI plates during transportation without abuse (a1, a2) and with 6-hour abuse on day 5 (b1, b2).

Regarding the TTI plates in the EPS boxes which were transported, from it can be seen from Figure 17 that the top plates had the lowest temperature most of the time during transportation. It is because they were located close to the ice mats. Temperature of the middle plates were the most stable, since the middle part of a box was better isolated from the outside environment than the top and the bottom. The abuse during transport phase shows a clear effect on the temperature of the plates/product, causing an increase up to 5.8 °C for more than 22 hours (Figure 17b). It should be noted that the temperature means of the plates on the same top and middle height levels are significantly differed ($p < 0.05$) from each other, e.g. EP1 vs. EPT 2; EPT3 vs. EPT4; and EPA1 vs. EPT2. This reveals the effect of different exposure levels to the surrounding environment of different box corners on a pallet, for instance in case of the TTI box on pallet No.2, the corner with plates EPT 2, 4, and 6 were placed at the outer part of the pallet, resulting in higher temperatures inside the box. The discolouration process of the TTIs, however, does not reflect the effect of these differences in temperature history due to heterogeneity in the initial SVs of the same charging time in the transport cases with and without abuse (Figure 7-Figure 8).

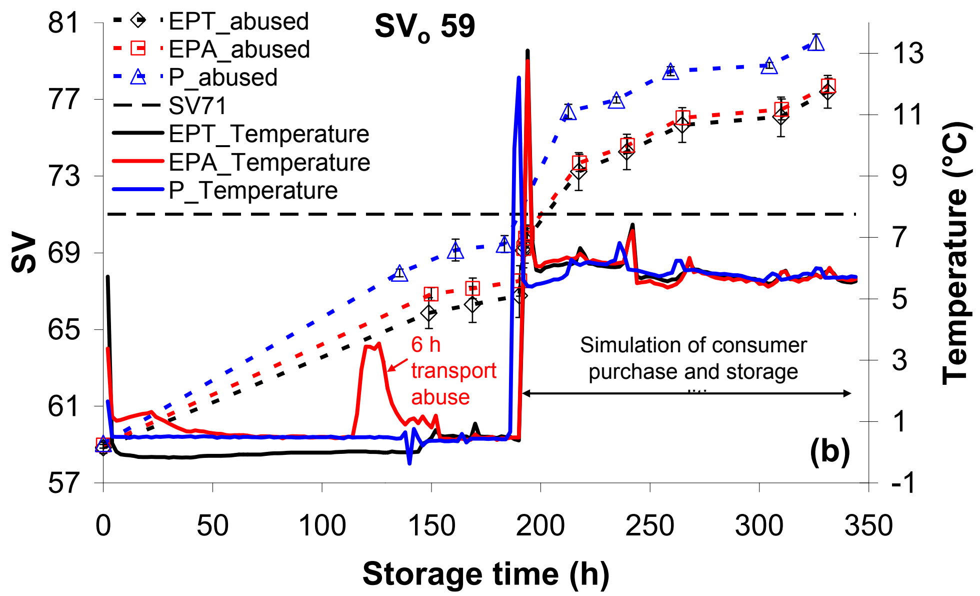


Figure 18. Comparison of discolourations of TTI labels with average initial square value SVo 59 at different temperature condition during transport phase. The EPT curve shows the mean SV from 3 plates (i.e. 9 labels) transported without 6-h abuse; the EPA curve shows the mean SV from 3 plates transported with 6-h abuse; and the P curve shows the mean SV from 3 plates stored at the laboratory.

Figure 18 shows the effect of temperature on the discolouration process of TTI labels. EPT labels discoloured at the slowest rate compared to EPA (though insignificant as stated before) and P counterparts since the temperature of EPT plates was the lowest during the first/transport phase. Despite of the exposure to lower temperature condition of the P plates compared to the EPA plates during the early phase, P labels discoloured faster than EPA labels. This reveals the effect of charging conditions, such as ambient temperature and relative humidity (see Table 2), on the discolouration process of TTI. The result is in accordance with the findings of Kreyenschmidt *et al.* (2010).

Table 5. The average square values (SV) and the average temperature of the TTIs placed on the top and bottom of retail packs during storage at -1 °C with and without abuse on day 8 (ST trays).

Duration (hours)	Temperature (°C)						Storage time (hours)	SV									
	Top			Bottom				650 ms		950 ms		1280 ms		1700 ms		2700 ms	
	Mean	±	Stdev	Mean	±	Stdev		Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
Non-abused ST trays																	
0	20.7			15.3			0	58.7	59.1	57.2	57.5	56.6	56.6	54.8	55.2	52.5	52.7
0-89	-2.5	±	1.5	-2.8	±	1.5	89	63.4	63.3	60.5	61.1	59.6	59.5	57.3	57.6	54.5	54.9
89-135	-2.5	±	1.1	-2.5	±	1.3	135	64.8	64.7	61.7	62.1	60.8	60.5	58.5	58.4	55.2	55.3
135-160	-3.0	±	1.2	-3.1	±	1.3	160	66.0	65.6	63.2	63.1	61.7	61.4	59.4	59.2	56.4	56.0
160-184	-3.9	±	1.1	-3.9	±	1.2	184	66.5	65.9	63.9	63.4	62.1	61.7	59.8	59.5	56.7	56.3
184-232	-4.5	±	0.9	-4.5	±	0.9	232	66.8	66.4	64.3	63.9	62.9	62.3	60.3	59.9	57.1	56.7
232-303	-2.7	±	1.7	-2.6	±	1.7	303	69.0	68.3	66.3	65.7	64.8	64.0	62.2	61.6	58.7	58.0
303-325	-3.1	±	0.9	-3.4	±	1.0	325	69.8	69.2	66.8	66.4	65.3	64.7	62.7	62.2	59.2	58.6
325-355	-1.8	±	0.8	-1.7	±	1.0	355	70.0	69.3	67.2	66.6	65.6	64.8	62.9	62.3	59.4	58.8
355-381	-1.9	±	1.0	-1.8	±	1.0	381	70.4	69.7	67.6	66.9	65.9	65.1	63.1	62.6	59.5	58.9
Abused ST trays																	
0	17.7			11.9			0	57.5	58.7	57.3	57.3	56.5	56.2	55.0	55.1	52.8	53.1
0-89	-2.9	±	1.7	-2.9	±	1.5	89	62.8	63.1	60.8	61.0	59.3	59.5	57.8	57.8	55.0	55.3
89-135	-2.6	±	1.3	-2.5	±	1.4	135	64.8	64.3	62.2	62.0	60.8	60.5	58.9	58.5	55.4	55.8
135-160	-3.1	±	1.3	-3.0	±	1.3	160	65.8	65.6	63.2	63.1	61.9	61.5	59.7	59.5	56.6	56.6
160-184	-4.0	±	1.1	-3.9	±	1.2	184	66.0	65.8	63.6	63.4	62.3	61.7	60.2	59.8	56.8	56.8
184-213	2.8	±	3.5	2.6	±	3.3	213	68.7	68.2	65.7	65.4	64.2	63.5	61.8	61.6	58.6	58.8
213-237	5.9	±	0.8	5.8	±	0.8	237	71.4	70.6	68.7	68.0	67.2	66.1	64.5	63.9	60.7	60.5
237-262	5.9	±	0.4	5.9	±	0.3	262	73.4	72.7	71.0	70.2	69.4	68.3	66.7	66.1	62.7	62.3
262-306	5.7	±	0.2	5.7	±	0.2	306	75.4	74.6	73.2	72.5	71.7	70.6	69.0	68.5	65.0	64.8
306-327	5.7	±	0.2	5.7	±	0.2	327	76.2	75.3	73.9	73.3	72.2	71.4	69.6	69.1	65.7	65.4
327-356	5.6	±	0.2	5.6	±	0.2	356	76.4	75.6	74.1	73.5	72.6	71.5	70.0	69.3	66.0	65.7
356-381	6.1	±	0.3	6.1	±	0.3	381	77.2	76.4	75.1	74.3	73.6	72.5	71.1	70.5	67.0	66.6

Table 6. The average square values (SV) and the average temperature of the TTIs placed on the top and bottom of retail packs during storage at 0.5 °C with and without abuse on day 8 (TT trays).

Duration (hours from charging)	Temperature (°C)						Storage time (hours)	SV							
	Top			Bottom				450 ms		500 ms		700 ms		1100 ms	
	Mean	±	Stdev	Mean	±	Stdev		Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
Non-abused TT trays															
0	19.9	±		20.0			0	60.8	60.7	60.2	60.2	58.8	58.7	57.0	57.0
0-18	1.0	±	3.3	0.7	±	3.2	18	63.0	64.1	62.6	63.1	60.7	60.5	57.8	58.6
18-41	0.1	±	0.3	0.0	±	0.5	41	64.2	66.5	63.7	65.7	61.9	63.0	59.2	60.4
41-89	0.0	±	0.4	0.0	±	0.4	89	67.5	70.0	67.2	69.0	65.2	66.6	62.2	63.4
89-159	0.0	±	0.3	0.1	±	0.3	159	71.1	73.4	70.5	72.6	68.7	70.1	65.6	67.0
159-181	0.2	±	0.3	0.2	±	0.3	181	71.6	74.0	71.1	73.3	69.1	70.8	66.0	67.4
Abused TT trays															
0	19.2	±		19.5			0	60.9	60.7	60.4	60.3	58.9	58.7	57.1	56.7
0-18	0.9	±	3.1	0.8	±	3.1	18	63.2	64.1	62.7	63.4	60.7	60.7	57.9	58.3
18-41	0.1	±	0.2	0.1	±	0.4	41	64.8	66.4	64.4	65.7	62.5	62.8	59.7	60.2
41-69	5.2	±	2.8	5.0	±	3.0	69	71.9	74.1	71.6	73.5	69.7	71.1	66.6	67.5
69-92	5.8	±	0.3	5.8	±	0.4	92	73.4	75.8	73.0	75.3	71.3	72.9	68.5	69.5
92-117	5.6	±	0.5	5.6	±	0.4	117	75.0	77.3	74.7	76.6	73.0	74.6	70.2	71.3
117-161	5.2	±	0.3	5.4	±	0.3	161	76.7	79.1	76.5	78.6	75.0	76.7	72.4	73.5
161-182	5.1	±	0.2	5.3	±	0.3	182	77.6	80.0	77.3	79.4	75.8	77.5	73.1	74.3

Table 6 summarises the temperature conditions (step mean and standard deviation) and SV of the TTI labels on the top and bottom of the TT trays. It can be seen that the step average temperatures of the TT tray bottom surface, in both non-abused and abuse storage, are very similar. However, the colour of the labels on the bottom faded faster than their top counterpart. This might be due to the fact that the initial temperatures on the bottom surfaces were higher than those on the top. The real reasons for the difference in discolouration rates between top and bottom labels, however, need further investigation to clarify.

1.5 COMPARISON OF THE SHELF LIFE BASED ON THE TTI RESPONSE AND REFERENCE LABORATORY RESULTS

Sensory results

End of shelf life is usually determined when sensory attributes related to spoilage become evident. When the average Torry score is around 5.5 most of the sensory panellists detect spoilage attributes, and these limits have been used as the limits for consumption at Matis (see e.g. Olafsdottir *et al.,* 2006). According to this criterion, sensory evaluation with Torry scale showed that ST and TT reached end of shelf life after 10-11 days (Figure 19).

When the average QDA score for those attributes is above the value 20 (on the scale 0 to 100) most panellists detect them (Bonilla *et al.,* 2005; Magnússon *et al*., 2006). ST had not reached the end of shelf life after 15 days, but hints of spoilage attributes were detected on days 13 and 15. TT was close to end of shelf life on day 10, with obvious table cloth odour, with hints of TMA odour and off-odour. A summary of sensory shelf life estimation is shown in Table 7.

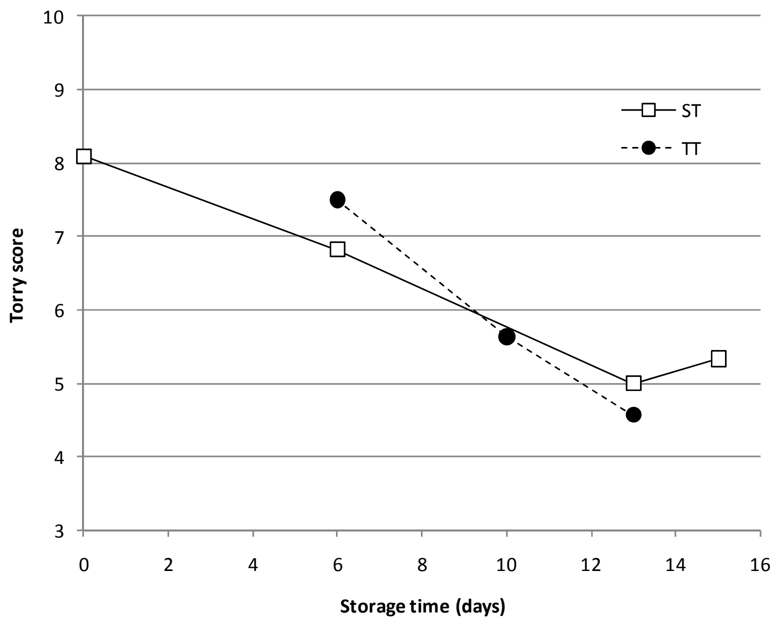
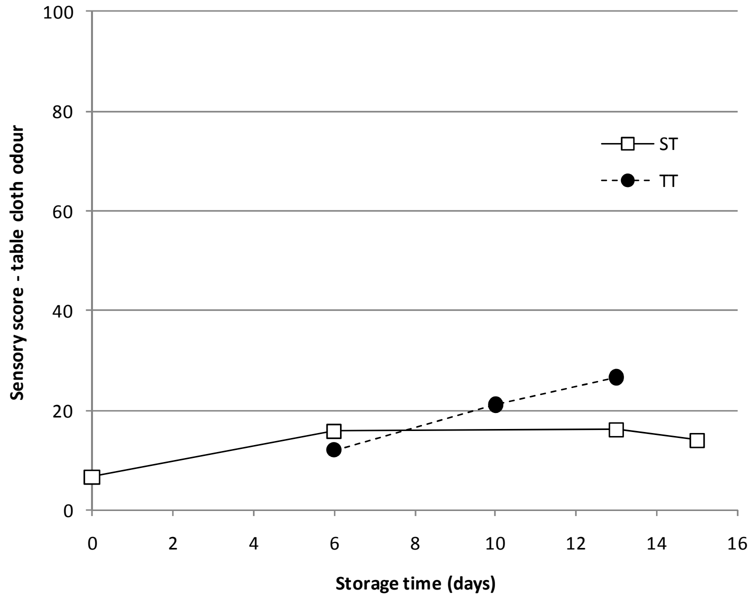


Figure 19. Average Torry freshness scores.



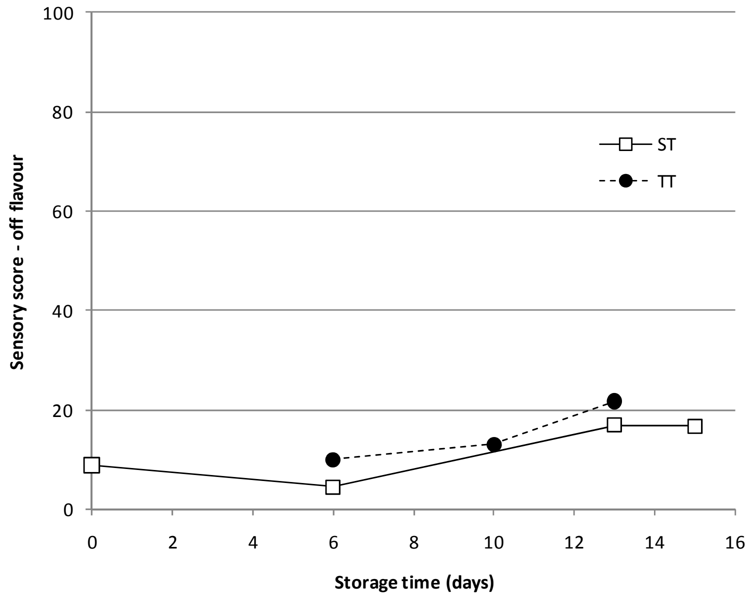


Figure 20. Average sensory scores (on the scale 0-100) for table cloth odour and off-flavour.

Table 7. Estimation of shelf life according to sensory evaluation with Torry freshness score sheet and QDA.

Sample name	Shelf life (Torry)	Shelf life (QDA)
ST	10-11	15+
TT	10-11	10-11

After 13 days of storage, the ST group had a less flaky texture, a darker colour, discoloured appearance and more white precipitation than the TT group. Further, the ST group had a less soft and a less juicy texture than the TT group (Table 8).

Table 8. Appearance and texture attributes.

	a-dark	a-discol.	a-prec.	t-flaky	t-soft	t-juicy	t-tender	t-mushy	t-meaty	t-clammy	t-rubbery
p-value	0.0001	0.0000	0.0000	0.0000	0.0000	0.0006	0.0001	0.0001	0.2741	0.0036	0.0095
ST-D0	23^{b}	20^{b}	20^{d}	64^{a}	81^{a}	64^{a}	77^{a}	70^{a}	12	6	4^{b}
ST-D06	23^{b}	32^{b}	45^{ab}	60^{a}	56^{d}	53^{b}	60^{bc}	43^{b}	24	10^{c}	7^{b}
ST-D13	44^{a}	52^{a}	53^{a}	41^{b}	53^{cd}	49^{b}	54^{bc}	47^{bc}	24	13	14
ST-D15	35	38^{b}	46^{ab}	37^{b}	50^{d}	41^{b}	50^{c}	33^{c}	19	20^{a}	16^{a}
TT-D06	20^{c}	25^{b}	33^{cd}	60^{a}	73^{ab}	63^{ab}	73^{ab}	56^{ab}	12	9^{c}	4^{b}
TT-D10	21^{b}	29^{b}	36^{bc}	55^{a}	67^{bc}	54^{b}	60^{bc}	50^{b}	25	16^{ab}	10
TT-D13	27^{b}	33^{b}	36^{bc}	58^{a}	64^{b}	51^{b}	66^{ab}	50^{b}	19	10^{bc}	6^{b}

Summary

The ST group (superchilled trays) had a longer shelf life according to sensory evaluation, more than 15 days based on QDA attributes, which was at least 4 days longer than TT group. However, according to Torry, the shelf life of the ST and TT packed fish was around the same or 10-11 days. The ST group was more described with dark colour, precipitation and discolouration, and less flaky, juicy and soft texture.

The ST group (superchilled trays) had the longest shelf life according to sensory evaluation of flavour and odour. However, because of the negative influence of too low temperature on the appearance and texture of this group, the shelf life was concluded to be 11 days. Based on these results, the total shelf life of the ST group did not differ from other groups in shelf life which had a shelf life of 10-11 days according to Torry and QDA.

The ST group differed from other groups in appearance and texture attributes, as it was more described with dark colour, precipitation and discolouration, more rubbery texture and less juicy, tender, flaky, soft and mushy texture than other groups. This indicates chemical and enzymatic spoilage rather than bacterial spoilage.

Chemical results

The amount of total volatile base nitrogen (TVB-N), trimethylamine (TMA) and pH was measured in tray samples on days 0, 6, 10, 13 and 15 of storage. Three trays from each group were used on each sampling day. Salt concentrations were also evaluated in the samples on day 0. The results can be viewed in Table 9.

Table 9. Chemical results of the analysed trays during the storage period.

Storage time (days)	Storage time (h)	Group	TVB-N [mgN/100g]	TMA [mgN/100g]	pH	salt [%]
0	0	Raw material	11.3 ± 1.0	0	6.97 ± 0.06	0.4 ± 0.2
6	135	ST:d0-tray	11.3 ± 0.5	0	6.83 ± 0.06	
6	143	TB 2-9-1/TT:d6-tray:	12.7 ± 0.6	0	6.97 ± 0.06	
10	233	TT:d6-tray	15.0 ± 0.9	1.6 ± 0.2	6.90 ± 0.00	
13	303	ST:d0-tray	15.0 ± 2.5	1.1 ± 1.2	7.00 ± 0.00	
13	305	TT:d6-tray	41.9 ± 14.9	24.5 ± 9.0	7.20 ± 0.00	
15	351	ST:d0-tray	14.7 ± 1.1	2.1 ± 1.0	6.97 ± 0.15	

***Same raw material was used for TT trays repacked on day 6 as for group TB.**

A salt concentration of 0.4% was measured in the fish on day 0 in the trial, but untreated fish muscle normally contains approximately 0.2% salt. It is therefore evident that the muscle has taken up some salt during the liquid ice immersion before packaging at the factory. Table 9 also shows that the total volatile base nitrogen (TVB-N) and trimethylamine (TMA) forms slowly in the samples and does not become evident until between day 10 and 13 in the trays stored at 0.5 °C (TT trays). No significant changes are found in these parameters in the superchilled samples (ST trays) during the storage period studied. No significant changes were observed in the pH in the fish during storage time, except in the TT trays on day 13, where a sudden increase in the pH was observed. This increase is in correlation to the higher amount of TVB-N and TMA observed in this sample.

Microbial results

Evaluation of total psychrotrophic viable counts (TVC), black colonies (H_2S-producing bacteria), *Pseudomonas* spp. and *Photobacterium phosphoreum* (Pp) was performed throughout storage and the results can be viewed in Figure 21.

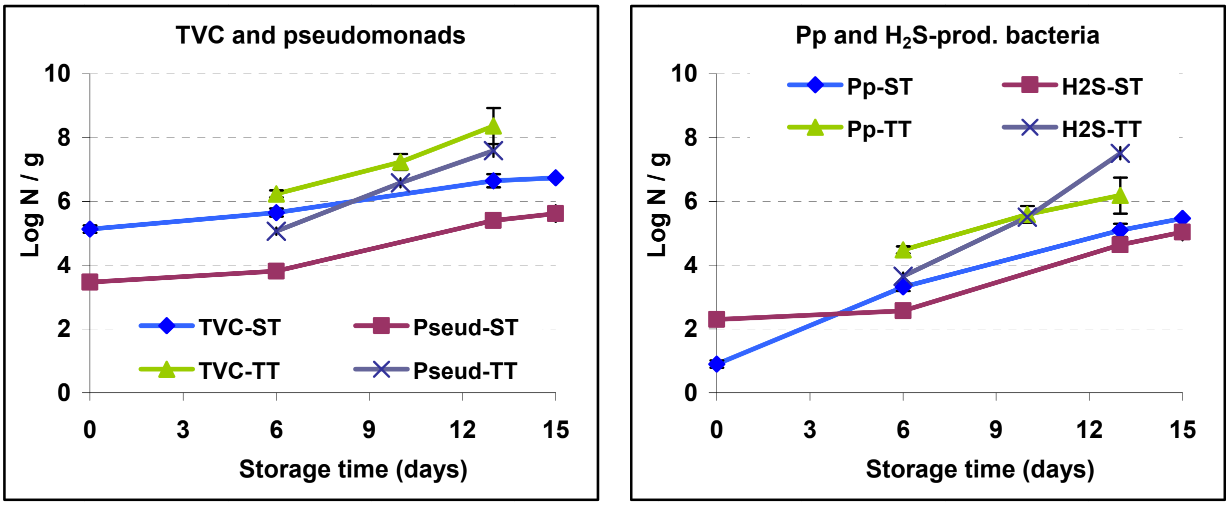


Figure 21. Bacterial growth in ST and TT groups studied. TVC, total viable psychrotrophic counts; Pp, Photobacterium phosphoreum; H2S, H_2S-producing bacteria; ST, superchilled trays packaged on d0; TT, trays packaged on d6 from fish boxes originating from pallet 2.

TVC of the raw material amounted to log 5.1 ± 0.2 CFU/g, while H_2S-producing bacteria were found to be log 2.3 ± 1.0 CFU/g, *Pseudomonas* spp. log 3.5 ± 0.1 CFU/g and *Photobacterium phosphoreum* log 0.9 ± 0.1 CFU/g. Figure 21 shows that ST samples stored at superchilled conditions showed slower growth for all bacterial species studied during the trial period compared to the TT samples , i.e. trays repacked on day 6 after transport of EPS boxes. On day 13, counts were significantly higher in TT than ST samples, TVC (almost 100-fold), H_2S-producing bacteria (almost 1000-fold), pseudomonads (about 100-fold) and Pp (about 10-fold). Pseudomonads reached the highest levels in both groups but were also present in higher numbers initially.

Table 10. Comparison of product shelf life (days from processing) given by sensory evaluation, SLP model (WIT), TTI discolouration, and square-root spoilage model in SSSP.

		ST		TT	
		Non-abused	Abused	Non-abused	Abused
Sensory shelf life (days)		11		10-11	
Predicted shelf life (days) based on SSO = 7 $\log_{10}$(CFU/g)	*Photobacterium phosphoreum* (Pp)	17.5		14.5	
	Pseudomonas spp (Ps)	18.4		11.1	
	H_2S producer counts	18.4		13.5	
TTI_top (days)*	Measured	> 15.9	9.8	12.7	8.7
	Simulated	15.9**		12.7**	
TTI_bottom (days)*	Measured	> 15.9	10.1	10.7	8.5
	Simulated	> 15.9**		10.7**	
SSSP, RRS model (SL) (days)***		> 15.3	10.8	10.2	8.8

* Charging time of TTI labels at 7 °C were 650 ms on day 0 (packaging day, i.e. 3 days from catch) for ST samples and 450 ms on day 6 for TT samples; ** Simulated using Eq. 2

Table 10 shows that a charging time of 450 ms at 7 °C (or initial SV = 61 ± 0.3) of the TTI labels on the bottom tray surface suited best for the monitoring of shelf life of the fresh cod loins in retail polystyrene trays repacked on day 6 after processing and stored thereafter at 0.5 °C. According to the table the TTI shelf life of the retail trays stored at 0.5 °C (TT-P2) showed a significant difference between the labels on the top and bottom of the trays. The labels on the bottom showed a colour fading indicating a shelf life comparable to the sensory shelf life of the group (10-11 d) while the labels on top of the trays show a longer estimated shelf life of 12-13 d. This indicates that placing the TTI labels on the bottom of the trays is a better estimate of the shelf life than placing them on the top surface of the trays. Further, it can be seen that the shelf life prediction based on *Pseudomonas spp*. is also in accordance with the sensory estimation for the TT trays at 0.5 °C. The TTIs also showed that the abuse on day 8 resulted in a reduction of about 2 days in the product shelf life. TTIs seem to have potential in monitoring the quality and shelf life of fish products stored under chilled conditions.

Regarding the ST trays at the set temperature of -1 °C, the sensory shelf life was shorter than the shelf life given by the SLP model, RRS in SSSP, and TTI evaluation. It is due to the fact that partial freezing of the samples, due to the temperature fluctuations observed in the cooling chamber, caused negative changes in appearance and texture of the product. It is worth noting that the RRS model in SSSP work well with the data set of the temperature range from -3 °C to 15 °C. Therefore, the prediction for ST samples might not be very accurate.

1.6 FITTING OF DATA FROM NON-ABUSED STORAGE

The data of the non-abused label shown in Figure 7-Figure 11 was fitted with the Eq.2. Table 11 shows the fit parameters for data from superchilled plates SP and chilled plates P. As seen from Table 11, the fits converge quite well with a high correlation coefficient (R^2), 0.996 in average and 0.993 as the lowest. The general trend is that, with increasing charging time, parameters d and k decreased and the absolute value of the parameter c increased. This is what one would expect as with increasing charging time the label discolouration develops more slowly (Kreyenschmidt *et al*., 2010). Lowering the storage temperature, such as in case of P (0.5 °C) and SP (-0.1 °C), results in smaller d values and higher c absolute values, and therefore, a slower discolouration of the labels with the same charging times. The fitting results show that the kinetic model of Kreyenschmidt *et al*. (2010), which was developed for the temperature range of 2-15 °C, could also be applied for lower temperature conditions. This indicates the potential to extend their quality contour diagram to a lower temperature such as 0.5 °C, so that a charging level can be defined to suit the shelf life of a product stored at the same temperature.

Table 11. Fit parameters of the non-abused labels and other sample data

Charging time (ms)	SVo ± 0.3	d	Standard error	k (h^{-1})	Standard error (h^{-1} x 10^{-5})	c (h)	Standard error (h)	R^2
P samples at set 0.5 °C (P_non-abused)								
650	59.0	79.199	1.125	0.00528	0.00049	-204.330	12.211	0.997
950	57.5	77.740	0.768	0.00443	0.00025	-235.770	7.084	0.999
1280	56.5	76.962	1.038	0.00410	0.00029	-250.900	8.746	0.999
SP samples at set -1 °C (SP_non-abused)								
650	59.0	70.983	0.573	0.00529	0.00054	-305.260	26.399	0.994
950	57.5	68.595	0.781	0.00442	0.00059	-374.210	38.898	0.993
1280	56.5	67.578	0.752	0.00412	0.00051	-400.030	37.295	0.994

Alternatively, the choice of the right charging level(s) to suit the shelf life of fish product(s) of the same storage conditions can be done using the correlation of TTI lifespan (t_L) and charging level (SVo) at specific temperature conditions as described in Eq. 3 by using Eq. 4 and the parameters estimated from Eq. 3. For the case of storage at 0.5 °C, the parameters a_2 and b_2 are estimated equal $a_2 = 3.205 \pm 0.226$; $b_2 = 75.755 \pm 1.128$; and the coefficient of correlation

(R^2) is 0.980. This was based on the results of this study and a pre-test investigation. The correlation is shown in Figure 22.

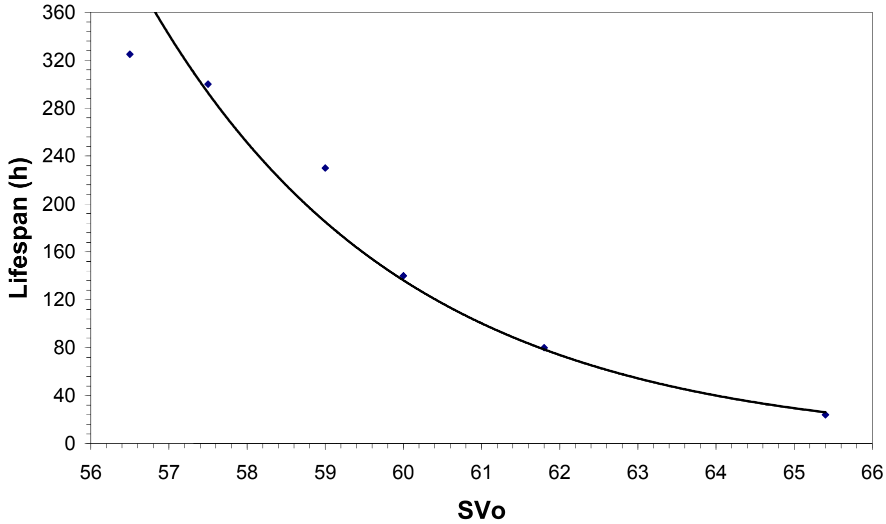


Figure 22. Lifespan of the TTI with different charging levels at a storage temperature of 0.5 °C. Experimental data (each of the first three points shows mean of data from 9 labels of P-plates in this study; each of the last three points shows mean of data from 5 labels of pre-test study) and fitted curve.

CONCLUSIONS AND SUGGESTIONS

- Standard operating procedures developed in the Chill-on project were applied as guidelines for the implementation of the TTIs (see Annex1).
  - Procedure 4 (SOP-ITENE-UB-NPS-03.doc) was followed in the wet trial without any changes.
  - Procedure 8 (SOP-ITENE-UB-NPS-03.doc): Pictures were taken at each sampling day, but set up difficulties with the camera were observed. Setting up of the camera should be of special concern for the next trial
- Operation of the climatic chamber at -1 °C was unstable, causing temperatures as low as -9 °C. The TTI discolouration of superchilled plates and trays therefore did not reach the reference colour by the end of the storage time. Suggestions for improvements:
  - check the climatic chamber temperature control before trial
  - adjust (decrease) the charging times of TTI labels for the storage at -1 °C.
- Placement of TTI labels with the initial SV of 61 on the bottom surface of the retail trays stored at 0.5 °C gave similar shelf life as the product shelf life declared by sensory evaluation. It is therefore, suggested to stick the labels on the bottom of trays to monitor the shelf life of the product.
- No significant effect was observed with the TTI on plates at different height levels within the EPS boxes during transportation with pallets No.2 and No.3 despite some difference in temperatures of the TTI plates during this period. The TTI labels did not significantly reflect the short time abuse during transport phase, i.e. during early step of the chain. The best position to place the TTI labels inside an EPS box needs to be clarified in the next trial. Suggestions for improvements in next trial so that the data from the plates can be used to compare with the shelf life of product in EPS boxes:
  - Have 2 boxes with TTI plates on each pallet;
  - The frequent measurements of TTI discolouration on plates will be carried out with only one box;
  - The TII from the other box will be measured just by the end of the product shelf life.
  - The plates are put back to the EPS boxes after measurements.

- Charging conditions such as ambient temperature and relative humidity influence the discolouration process of the TTI labels.
- Shelf lives given by TTI on bottom tray surface and predicted by SSSP square-root spoilage model (based on product temperature) are in good agreement for both ST and TT trays with and without simulated consumer abuse.
- The studied TTI could be used to monitor the abuse and fluctuation in temperatures during storage of fresh cod loins in retail packs.
- The kinetic model of Kreyenschmidt *et al*. (2010) worked well with data from non-abuse storage at temperatures below 2 °C, which indicates the potential to extend their quality contour diagram to low temperatures so that a charging level can be defined to suit the shelf life of a product stored under the same conditions. The charging levels can also be chosen based on the correlation between the charging levels and lifespan of the TTI found in this study.

AKNOWLEDGEMENT

The CHILL-ON Integrated Project (FP6-016333-2) "Developing and integrating novel technologies to improve safety, transparency and quality assurance of the chilled/frozen food supply chain – test case fish and poultry" was funded by the European Commission within the Sixth Framework Programme Thematic Priority: Food Quality and Safety. Project website: www.chill-on.com

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ANNEX 1 STANDARD OPERATING PROCEDURES FOR ONVU^{TM} LABEL

From: SOP-ITENE-UB-NPS-03; **CHILL-ON WP5 – Field Trial Standard Operating Procedure (SOP) New Packaging Solutions with integrated RFID and TTIs. (Selected chapters relevant to OnVuTM label)**

Editors Nuria Herranz Solana (ITENE) and Judith Kreyenschmidt (UB)

Particpating Chill-on partners: ITENE, UB, FRESHPOINT, ACTVALUE and TTZ

Information about the Chill-on project and the partners are on the website: www.chill-on.com

OnVu^{TM} labels will be used to control temperature conditions in the different selected cold chains and to control indirect the shelf life of a product. They can be used in 3 different ways:
1. Simple stand alone technology: By controlling visual the colour change of the label the TTI can be used to control the cold chain from production to consumption in an easy way (consumer oriented use).
2. Stand alone technology: By measuring the colour change of the TTI by a spectrophotometer at different steps of the chain, a more precise decision about the temperature history of the product and about interruption of the cold chain can be made. The measured square value (SV= ($L^{1/2}$+$a^{1/2}$+$b^{1/2}$) can be integrated in quality control system resp. HACCP system to document the right handling of the product with regard to temperature. The measured data will be integrated in a TTI model. There is also the possibility to implement these data in the Tracechill server.
3. Integrated technology: The square values can be combined with the shelf life model and integrated in the Tracechill server which allows the calculation of the remaining shelf life of a product. Requirement is that the shelf life kinetic correlates with the kinetic of the TTI and the most relevant temperatures. Above 7°C this kind of TTI reacts very fast to temperature conditions and does in most cases not correlate with the shelf life kinetics of fresh products. Hence, the discoloration process at these temperatures is more conservative than the actual shelf life of the product and temperature abuse can be detected.
For the named solution 1 and 2 the square value will be the input parameter. Output parameter will also be the square value. This parameter has to be transferred to statements which deliver information if the product is still acceptable respectively fresh based on its temperature history or if an interruption of the cold chain has taken place.
If the square value will be combined with a shelf life model (Integrated technology, no 2), estimation of the microbiological status could be the output.

Typical materials for the Onvu integration and validation test

- Temperature Dataloggers (number depend on placement in packages with OnVu TTIs.)
- 1 Special adhesive tape for cold temperatures
- Adhesive white paper for a homogeneous background
- Plastic waterproof bags
- 1 permanent pen
- TTIs (Quantity depending on experimental design)
- filter (can vary depending on the number and placement of packages with OnVu TTIs.)
- 1 charger
- 1 Eye One

- 1 measurement equipment for the TTIs: Eye-One Xright / Spectrophotometer Konica Minolta
- 1 incubator
- 1 fridge
- 1 digital camera
- Selected packaging solutions with ice and fish

Procedure 4 – Integration and Configuration of *OnVu™ label*

1. Power-on the charger one hour before the sampling should take place (2°C – 15°C)

   Since the environmental temperature is influencing the activation process and also the lamination process of the filter (TTR), following points should be taken into account**:**
   - Temperature shifts and huge air circulation during charging must be avoided.
   - In- or decrease of temperature during the charging will influence the start values
   - The ventilator has to be switched on during the experiment
   - Store the label 2 hours before the trial takes places under cold conditions (same temperature at which charging will take place.
2. -Configure dataloggers with a sampling rate of 5 sample/minute.
3. Introduce the datalogger inside a waterproof plastic bag if they are used inside a package and they are in contact with water
4. Place the dataloggers close to the place where the TTIs will be stucked
5. For measuring the label a spectrophotometer (EyeOne, Xright) will be used Calibrate the EyeOne and measure ones the colour scheme. Appropriate measurement settings: illumination D65, observation angle 2: The **EyeOne** has to be stored **1-2 hours** before the investigation start, at cold temperature otherwise condensate can be generated
6. Charge blind labels as a reference (without UV activation), please throw always away the first 1-2 m from a new role of TTIs
7. Charge the label to a defined value depending on the product characteristics; Please check the saturation level.
8. Write a number on the label with a permanent pen to identify it
9. . Write the label identification and the measured value down.
10. The background of the package material has to be straight, concavities are influencing the measuring process. The measuring adapter of the Eye one has to kept in the same direction for all measurements
11. Stick the labels in defined places on the units (cardboard box, MAPs, etc), on two layers with white labels. Put the units on different places at one pallet including the upper corner boxes. Since the placement depends from the packaging material, the placement will be defined when the partners deliver all necessary information to UB and ITENE
12. Repeat until all labels are charged
13. If not all boxes are label on a palette, mark in the outside of the boxes where the labels are placed on
14. Please write down comments in each step of the procedure.

15. Please make also a picture from the label at each measuring point. Same light conditions are important for the picture
16. Please use for all experiments labels from the same printing process (same batch number)

Before starting the final trial the mean value and the standard deviation of the 30 label has to be checked. The standard deviation should be lower as 0.2.
If it is higher, please control:
- the saturation level
- the print head
- if the ventilator is switched on
- if there is air circulation or huge temperature fluctuation
- if you use the EyeOne in a correct way (as described)

During the experiment the start values and the lamination of the filter should be controlled from time to time if it is still in range.
It is advisable to check the lamination of the filter by bringing UV light on the laminated labels. There should be not recharging and no visible dark spots on the labels.

Please check also the SOPs presented from UB during the meeting in Bremerhaven 2008

Procedure 8 – Validation of *OnVu*^{TM} *label*

1. Check optically the colour of the labels (take pictures of the labels).
2. Check colour of the labels by measuring - Values will be integrated automatically in the excel file delivered by UB and Freshpoint.
3. Introduce the value in the Tracechill system
4. Please write down comments in each step of the procedure.

The figure below shows an example how the measuring point could be look like in a chain.
The points will be defined in each chain together with the responsible partners of the chain, the companies and UB, ITENE and Freshpoint

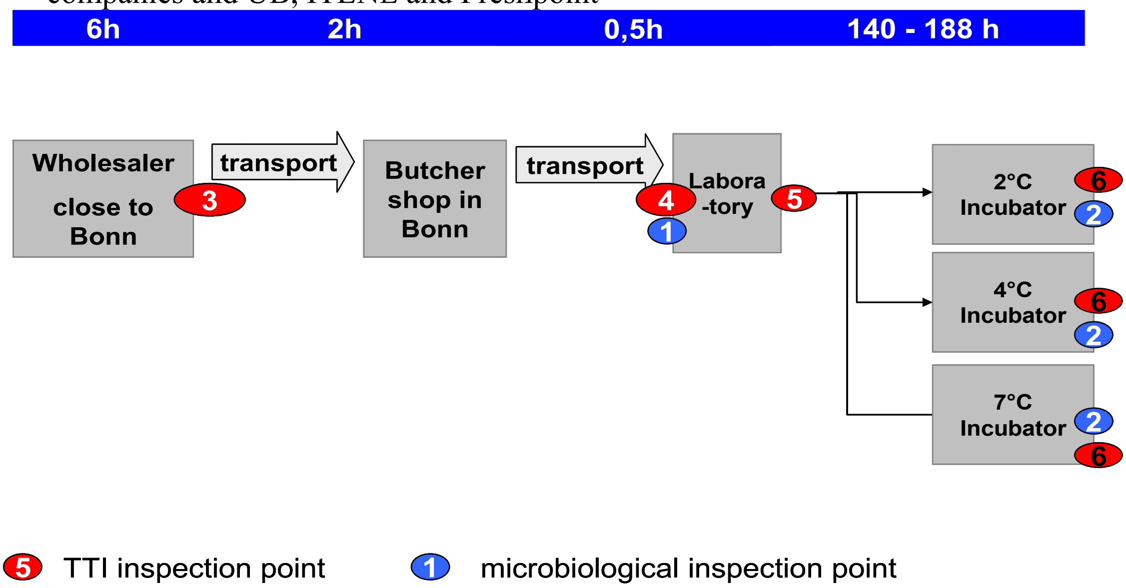

Figure 1. Example for measuring points at different steps of the chain (University Bonn)

CHILL-ON

SOPs for the implementation of TTIs in different pilot chains (draft)

www.chill-on.com

University of Bonn (UB)

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Contents of theSOPs

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1. The charger: GLP 240
   The Xright : EyeOne
2. Approach for the implementation of TTIs in pilot studies

   2.1 General aspects before starting the labeling process

   2.2 Measuring approach

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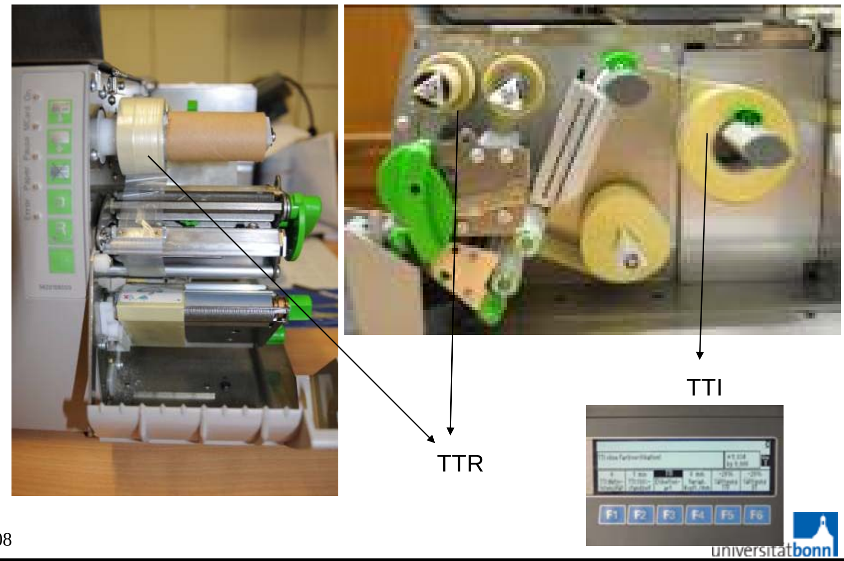
1. The charger GLP 240



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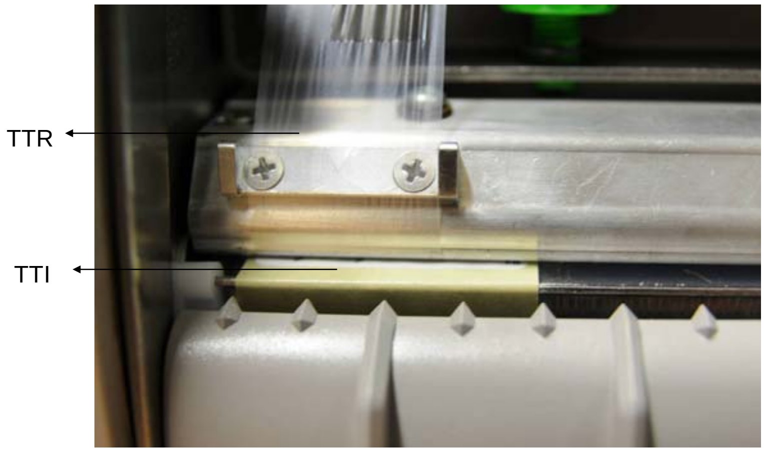
1. The charger GLP 240



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1. The Xright : EyeOne

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2.1 General aspects before starting the labeling process

Since the environmental temperature is influencing the activation process and also the lamination process of the filter (TTR), following points should be taken into account:

- Charging should be conducted at a constant temperature **below 15°C and above 2°C**
- **Temperature shifts and huge air circulation during charging must be avoided**. In- or decrease of temperature during the charging will influence the start values
- the environmental temperature and the **temperature of the charger** has to be the same. That means if you will start a charging process at low temperature, the charger has to be stored at low temperature **for 1-2 h** before the experiment will start (important for the pilot studies in the companies!!!)
- The **ventilator** has to be switched on during the experiment
- If the charging will be conducted at low temperature the **EyeOne** has to be stored **1-2 hours** before the investigation start, otherwise condensate can be generated

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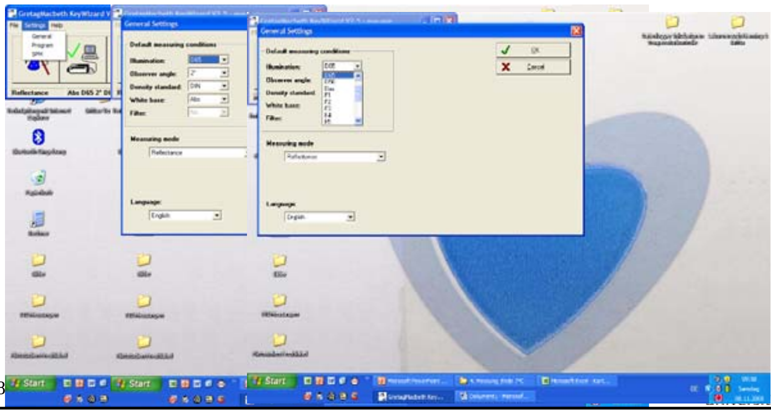
2.1 General aspects before starting the labeling process

1. To compare data between the samples the same measuring equipment should be used: Xright: Eye one

- appropriate measurement settings: illumination D65, observation angle 2°



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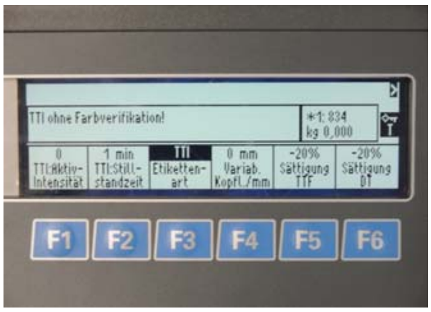
2.2 Measuring approach

1. **Program the right activation time** for the start value which is needed for the experiment

2. **Set the saturation level:**

- Temperature is influencing the lamination of the filter (TTF), that means for different temperature, different saturations have to be use.
- if the saturation is to high: you will see small blisters on your label,
- if the saturation is to small, the UV filter will not be transferred completely to the label which leads to a recharging.



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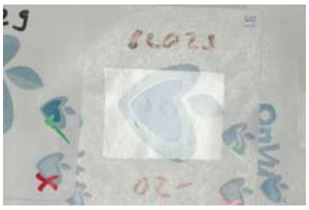
2.2 Measuring approach

TTR transfer temperature

to low
area not
completly covered



good
sharp edges&area
completly covered

to high
edges smudged

→ setting with the lowest TTR transfer temperature which shows good transfer has to be found

The print speed should be set on 120mm/s

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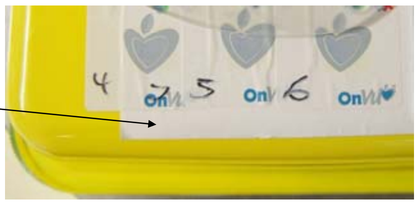
2.2 Measuring approach

4. If the saturation level had been changed, the start value and its range has to be checked again. It might be that the activation time has been adapted.
   The first 3 labels has to be threw away!!!

5. To get a reproducible measuring process it is recommended to measure nearly 30 labels with the set parameters before starting the final experiment.

6. To measure the label it is important to have a homogenous background.
   This can be reached by putting two layers of white paper behind the TTIs.

White paper



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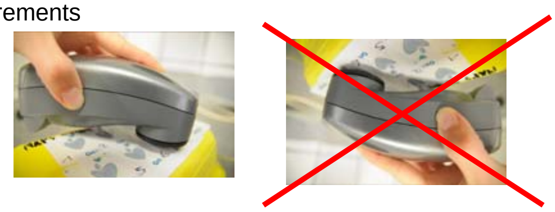
2.2 Measuring approach

7. The background of the package material has to be straight, concavities are influencing the measuring process

8. The measuring adapter of the Eye one has to kept in the same direction for all measurements



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2.2 Measuring approach

9. Before starting the final experiment the mean value and the standard deviation of the 30 label has to be checked. The standard deviation should be lower as 0.2. If it is higher, please control:
   - the saturation level
   - the print head
   - if the ventilator is switched on
   - if there is air circulation or huge temperature fluctuation
   - if you use the EyeOne in a correct way (as described)

10. During the experiment the start values and the lamination of the filter should be controlled from time to time if it is still in range.
    It is advisable to check the lamination of the filter by bringing UV light on the laminated labels. There should be not recharging and no visible dark spots on the labels.

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2.2 Measuring approach

11. During the experiment the environmental temperature and the temperature inside the GLP should be controlled by data logger.

12. Please throw always away the first 1-2 m from a new role of TTIs

13. Please document always the batch number of the TTIs and the TTR you used. Example for complete documentation for each measurement:

X174 cinetics:
stored in dark at 4°C
3 labels each charged with GLP
B1+080409
20 labels/min
with and without TTR transfer: 70QC
51423 -45%

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Thanks for your attention

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