

Shelf life of salt-cured fillets of cod stored at 30 °C

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Nofima is a business oriented research institute working in research and development for aquaculture, fisheries and food industry in Norway.

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Report

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Summary/recommendation: <p>In 2013, the Norwegian Seafood Research Fund (FHF) initiated a project “Shelf life of salt-cured fish”. The aim of the project is to study shelf life of salt-cured cod at different storage conditions, including storage at elevated temperatures. Salt-cured fillets refer to dried salted fillets. This report presents the results of shelf life of salt-cured cod stored at 30 °C at 80 and 60% relative humidity, respectively. In this context, shelf-life is defined as the number of days until a pink / red discoloration is visible. The surface of salt-cured cod turns pink / red at a level of 10 000 000 halophiles / g salt-cured cod. This implies factors that influences the growth rate of halophiles affects the shelf life of salt-cured cod. Such factors include storage temperature, level of drying and relative humidity.</p> <p>The results show that shelf life with respect to pink or red discoloration corresponds to the initial level of halophiles and the level of humidity. In more detail, with an initial level of 10 CFU/g, the shelf life is 27 and 24 days at 30 °C when stored at 60 and 80 % relative humidity, respectively. At an initial level of 100 up to 10 000 CFU/g, the shelf life decreases successively to 13 days at both 60 and 80% relative humidity.</p> <p>It is emphasized that the results presented in this report are preliminary and indicative. Thus, corrections of suggested shelf life might occur after finalising corresponding experiments at 20, 25 and 35 °C.</p>		Client's ref.: FHF 900856 Project No.: 10478

Table of Contents

1	Introduction.....	1
2	Aim.....	1
3	Material and methods.....	2
3.1	Isolates of halophiles.....	2
3.2	Production of salted fillets	2
3.3	Preparation of inoculum.....	2
3.4	Drying programme of salted fillets.....	2
4	Results	5
5	Discussion and conclusion	7
6	References	7
	Appendixes	

1 Introduction

In 2013, the Norwegian Seafood Research Fund (FHF) initiated a project “Shelf life of salt-cured fish”. The aim of the project is to study shelf life of salt-cured cod at different storage conditions, including storage at elevated temperatures. Based on the results obtained in this project a recommendation of shelf-life at specific elevated temperatures will be suggested.

The industry and representatives from the Norwegian Export Council have requested insight in preliminary results obtained as the project itself will be finished in august 2015. Thus, this report will give a short summary of the status by March 2014.

This report refers to shelf life studies of salt-cured fish stored at 30 °C at 60 and 80 % relative humidity (RH). Salted fillets have been added cultures of halophiles at different levels, dried to “7/8” and then stored at 30 °C. A dryness of 7/8 refers to water content in the range of 45 – 48%.

Shelf life is defined as number of days until a visible discoloration is observed. A pink or red discoloration is due to growth of halophiles, such as *Halobacterium salinarum*.

2 Aim

The aim of this study was to define shelf life of salt-cured cod stored at 30 °C with respect to visible pink or red discoloration.

3 Material and methods

3.1 Isolates of halophiles

Products of salt-cured fish have been purchased from several producers in Norway. These products have been stored at elevated temperature to provoke a discoloration of the surface. Secondly, the pink and red spots were sampled and cultivated in several steps in order to obtain single strain culture of the halophile. Later on, the collection of the strains has been added to salted fillets. To maintain the strains they are kept at -80 °C.

3.2 Production of salted fillets

Red halophiles are naturally present in salt that is used in the processing of salt-cured cod. In order to avoid naturally present halophiles in the salt-cured cod in this experiment, we have produced salt-cured cod from salt without halophiles. Halophiles of the salt were eliminated using heat (120 °C for 16 hours).

Newly caught cod (*Gadus morhua* L.), 4-6 kg were filleted post rigor. After washing, the fillets were pickle salted which is a method where moisture is extracted while salt is penetrating the fish muscle. The moisture was not drained away, thus the fillets were gradually immersed in a salt pickle of extracted fluids. The fish was subsequently stored at 2 °C for three weeks in order to ripen and thereby produce its characteristic sensory properties. After three weeks of storage, the fillets were removed from the salt pickle, and then supplied with new salt when transferring them into new clean boxes with drainage. The water content of the salted fillets was 55 %.

3.3 Preparation of inoculum

Four strains of halophiles were transferred to a medium promoting growth of pink and red halophiles (NMKL no 171). The strains were incubated at 35 °C in 26 hours to obtain a level of app. Log 8 CFU/ml. Secondly, the strains were mixed to obtain a cocktail and then diluted to obtain concentrations of Log 3, Log 4, Log 5 and Log 6 CFU/ml, respectively. An inoculum of 0,1 ml from each concentration was evenly distributed to the surface of 4 salted fillets. The final level of Halophiles on the surface of the salted fish fillets were log 4, log 3, log 2 and log 1/g, respectively.

3.4 Drying programme of salted fillets

In order to obtain a salt-cured cod fillet, the salted fillet was dried. The drying process aimed to have a final level in the range of 45 to 48 % water ("7/8"). This was obtained as described in table 1.

Table 1 Drying programme of salted cod fillets

Time interval (h)	Conditions
0 – 6	20 °C, ambient RH, 30% fan speed
6 – 16	20 °C, 60% RH, 20 % fan speed
16 – 56	20 °C, 50% RH, 20 % fan speed

To control the speed of drying, the weight of each fillet was measured frequently. The drying process with respect to water content is presented in Figure 1.

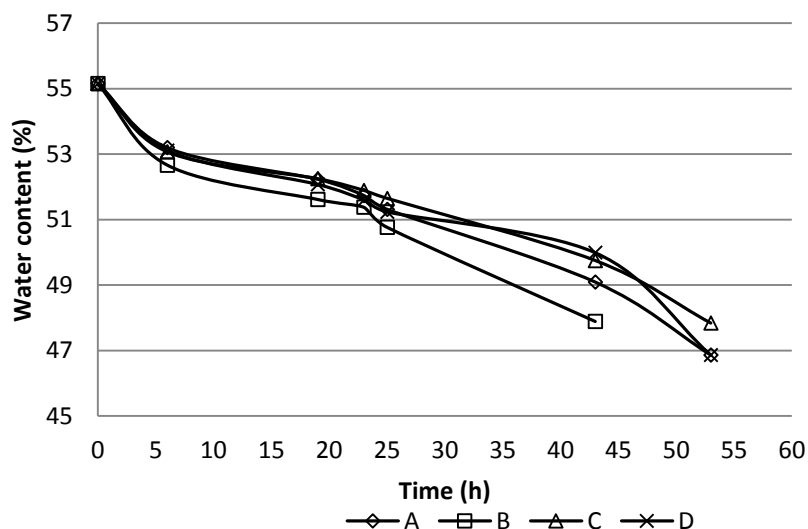


Figure 1 Drying process of salted fillets towards salt-cured fillets

The time and temperature during drying of salted fillets is presented in Figure 2.

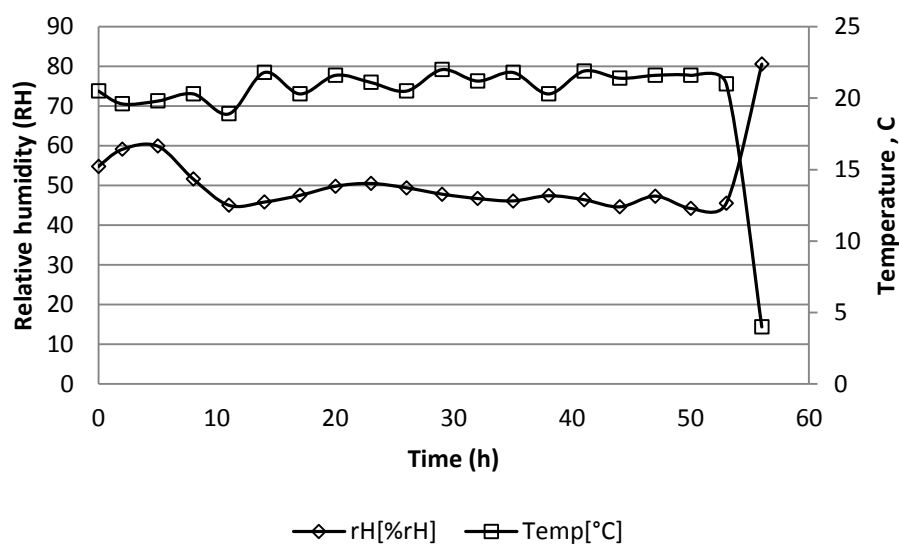


Figure 2 Relative humidity (RH) and temperature (C) during drying of salted cod fillets

At the level of the “7/8” interval, the fillets were placed at 4 °C for 48 hours. Afterwards, the fillets were cut in pieces, packed in polystyrene boxes equal to the boxes used in Brazilian markets. The boxes were wrapped with a plastic film (Baco Professional) 4 times in order to reduce drying. The boxes were then evenly distributed in two separate climate chambers set at 30 °C/80 % RH and 30 °C / 60 % RH, respectively.

During storage, each box was evaluated with respect to visible discoloration every second day. In addition, the boxes were put on a weight to record potential drying during storage. The two pictures below show the products with salt-cured fillets at the end of the storage period at 30 °C.



Picture 1 Salt-cured fish products stored at 30 °C / 80 % RH in 30 days

4 Results

Despite wrapping the boxes with plastic film 4 times, a slight decrease in weight was observed. In more detail, the weight remained app. unchanged for boxes stored at 80%, while boxes stored at 60% dried about 2 % in the total storage period of 38 days. Relative change of weight is presented in figure 3.

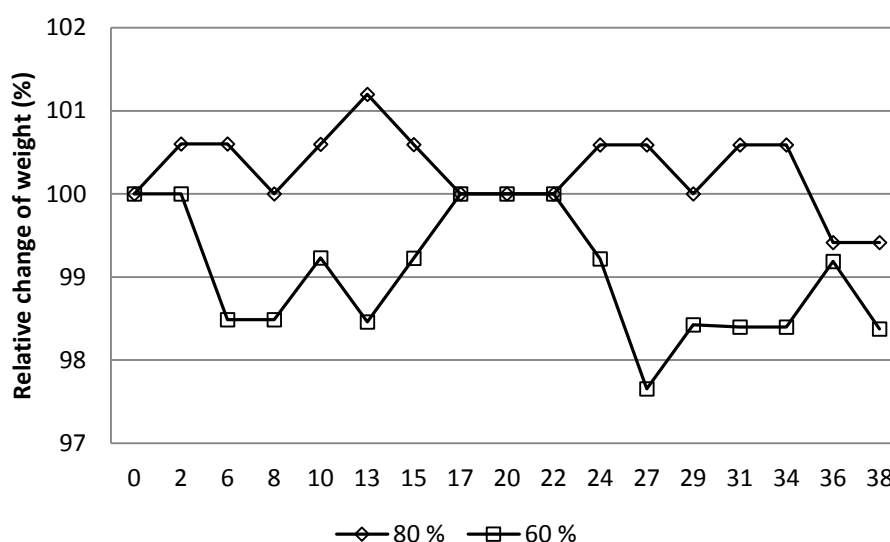


Figure 3 Relative change of weight of boxes with loins stored at 60% (□) and 80 % (◇) relative humidity

Based on visible observations, the number of days to obtain the first indications of slight pink colour was defined as shelf life. The results of shelf life of loins stored at 30 °C with an initial level in the range of log 1 – log 4, 60 and 80 % RH is presented in Figure 4.

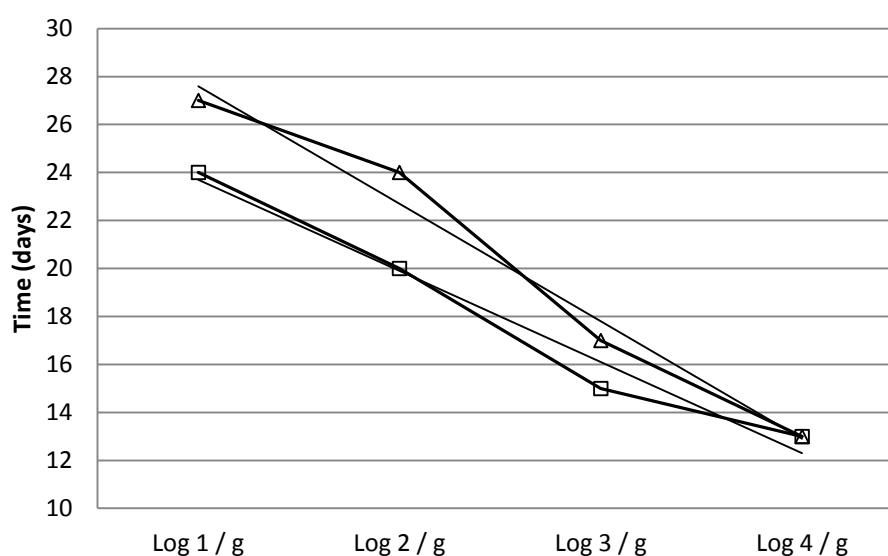


Figure 4 Shelf life of loins of salt-cured cod stored at 30 °C at 60% (Δ) and 80% (□) relative humidity with an initial level of log 1, log 2, log 3 and log 4 CFU/g, respectively. The additional straight lines indicate the coherence between initial level of halophiles, relative humidity and shelf life at 30 °C

Figure 4 indicates how the effect of relative humidity is more decisive for the shelf life at low initial levels compared to high initial levels. This indicates that the level of humidity is a relevant factor to be included and measured when estimating shelf life.

Using linear regression, the equation for estimating the shelf life at 30 °C is

$$60 \% RH: Y = 32.5 \text{ days} - 4.9X \quad (\text{Eq. 1})$$

$$80 \% RH: Y = 27.5 \text{ days} - 3.8X \quad (\text{Eq. 2})$$

Where X refers to the initial level of halophiles in the product before it is stored at 30 °C and the regression coefficient (R^2) for 60 and 80% RH is 0.978 and 0.976, respectively. Alternatively, the following equation (Eq. 3) that comprises both RH can be used.

$$Y = 10.2 + (4.55 - X) * ((-0.055 * RH) + 8.2) \quad (\text{Eq. 3})$$

Here, the RH refers to either 60 or 80. It is emphasized that Eq. 3 is to be used for storage conditions at 60 or 80 % RH as other storage conditions have not been tested.

Shelf life of salt-cured bellies and tails were more random compared to the loins (Table 2).

Table 2 Shelf life of salt-cured bellies and tails

Initial level of halophiles (log cfu/g)	30 C / 60 % RH	30 C / 80 % RH
1	*	22 (tail)
2	27 (belly)	20 (tail)
3	24 (belly), 22 (tail)	15, 20, 24 (tail)
4	15 (belly), 22 (tail)	15 (belly), 20, 24, 29 (tail)

*No visible pink or red discoloration up to 37 days of storage

Table 2 show that prediction of shelf life based on initial level of halophiles is less consistent compared to the loins. For samples of bellies stored at 80 % RH, only samples with an initial level of log 4 obtained a visible pink / red discoloration. Varying and inconsistent results of shelf life of salt-cured bellies and tails can be due to a white surface (salt crystals due to migration of soluble salt to the surface of the sample) which can have influenced the visibility of a pink / red discoloration.

5 Discussion and conclusion

As expected, the number of days to obtain a pink or red discoloration of the salt-cured cod was coherent with the initial level of halophiles added to the product. This is due to the fact that the colour turns pink / red when the level has reached app. log 7 CFU/g (10 000 000 colony forming units of halophiles / g salt-cured product).

For samples with the lowest initial level of 10 CFU/g, the shelf life is 27 and 24 days when stored at 60 and 80 % relative humidity, respectively. At an initial level of 100 up to 10 000 CFU/g, the shelf life decreases successively. Based on the two selected levels of relative humidity at 30 °C, shelf life can be calculated using an equation that comprises both levels relative humidity. It is emphasised that the results for shelf life refer to the loins. Shelf life of tails and bellies varied compared to the loins sample. It is assumed that this can be due to drying during storage.

However, to estimate shelf life at 30 °C, these results show that knowledge about the initial level of halophiles is crucial. This can either be obtained by making a representative sample and analyses according to NMKL method no 171 or by putting 3-4 salt-cured fish in a heating chamber set at 30 °C with 80 % relative humidity. The initial level of halophiles can then be calculated using figure 4 or equation no 2.

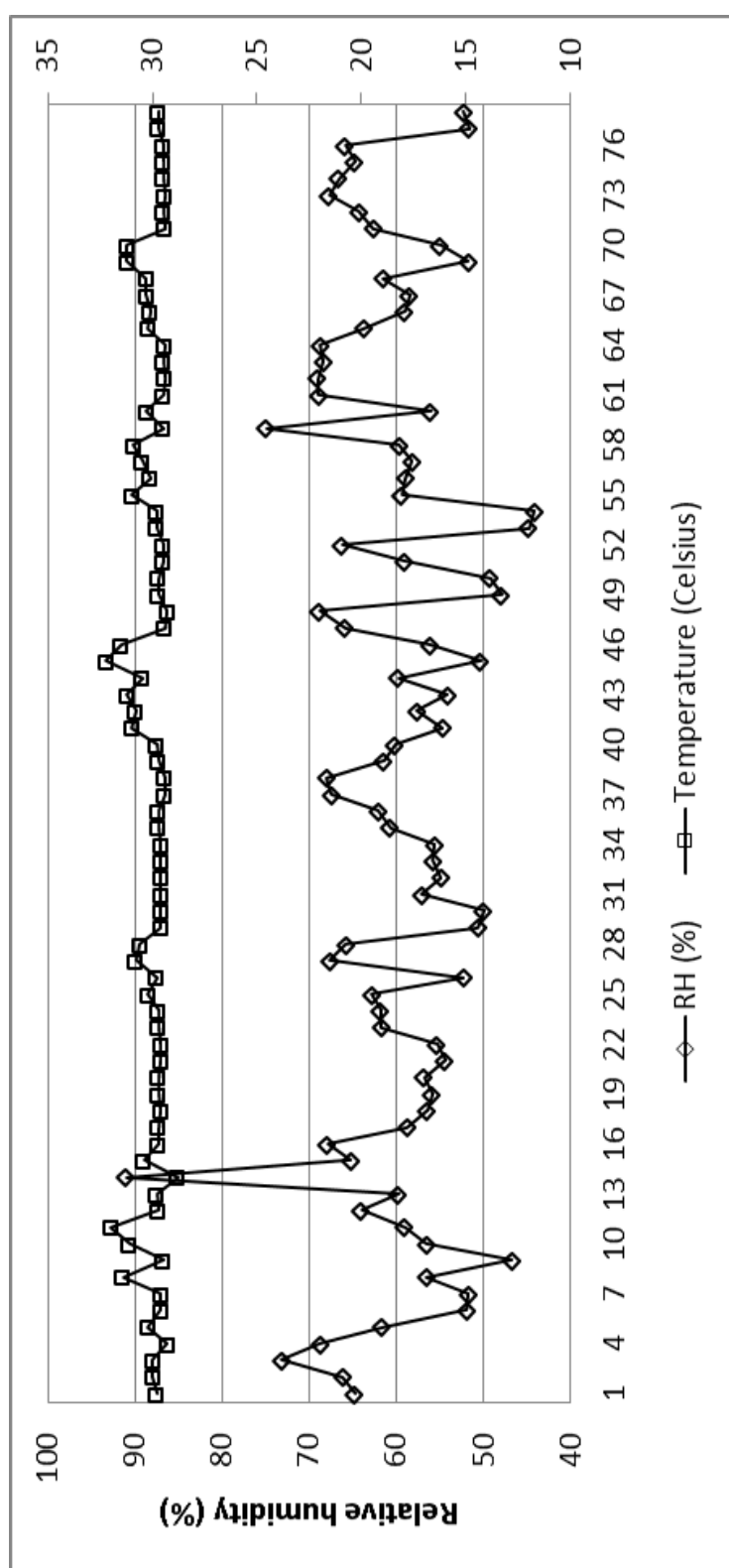
It is emphasized that the results presented in this report are preliminary and thus indicative. Thus, corrections of suggested shelf life might occur after running corresponding experiments at 20, 25 and 35 °C. Such experiments will be performed in 2014 and 2015.

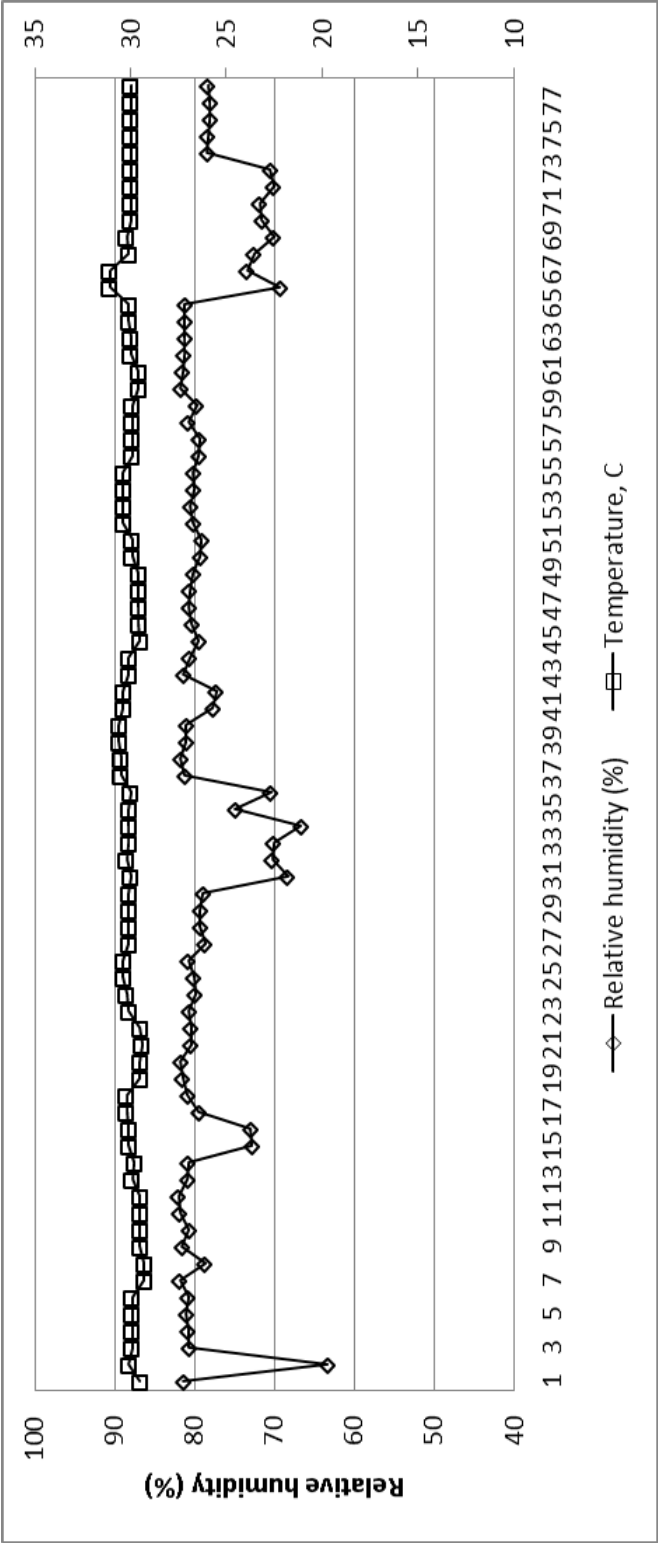
6 References

NMKL method no 171. Halophilic and osmophilic microbes ("pink" and "dun"). Determination in salt-cured fish products. 2nd ed. 2008. <http://www.nmkl.org>

Appendixes

- Appendix 1 Diagram showing relative humidity and temperature during storage at 30 °C and 60 % RH
- Appendix 2 Diagram showing relative humidity and temperature during storage at 30 °C and 80 % RH
- Appendix 3 Specification polystyrene boxes
- Appendix 4 Specification stretch film







silver plastics® GmbH & Co. KG
 Godesberger Strasse 9
 D-53842 Troisdorf

Declaration of Compliance

We are supplying you with the following material / product:

PS-trays: Vampirella
material: polystyrene, open-cell

This product complies with the following legal requirements:

1. Generally

- EU-Framework Regulation on materials and articles intended for food contact: (EC) No 1935/2004
- (the german) Lebensmittel-, Bedarfsgegenstände- und Futtermittelgesetzbuch: LFGB §§ 30 and 31
- Regulation (EC) No 2023/2006 of Dec., 22nd 2006 on good manufacturing practice (GMP)

2. Raw Materials / Composition

- Regulation (EC) No 10/2011 and following amendments
- (the german) Bedarfsgegenständeverordnung dated from April, 10th 1992 and following amendments

3. Conditions of use / Compliance with threshold values

a) Specification of the intended use:

The above mentioned product fulfils the requirements for:

- dry food (acc. 85/572/EC)
- aqueous food (solvent: 10% Ethanol)
- acid food (solvent: 3% acetic acid)
- fatty food (solvent: Isooctane)

that means for filling goods like fresh meat, poultry or fish for long term storage with temperatures up to 20°C.

b) The analysis of the total migration was carried out in consideration of the „Methoden zur Untersuchung von Bedarfsgegenständen“ according to the requirements B 80.30, 1 and 3 (EC) and the „Amtlichen Sammlung von Untersuchungsverfahren“ according § 64 LFGB and the requirements of the norm-series EN 1186, EN 13130 and prCEN/TS 14234 „Werkstoffe und Gegenstände in Kontakt mit Lebensmitteln – Kunststoffe“.

substitute solvent:	contact-time / -temperature:
10 % ethanol/water	10 days / 20°C
3 % acetic acid	10 days / 20°C
Isooctane	24 hrs/ 20°C

The critical value for the total migration measures 10mg/dm² per object resp. 60mg/kg foodstuff (substitute solvent) according to Regulation (EC) No 10/2011.

The observance of the global migration values according to the requirements of Directives 82/711/EEC and 85/572/EEC and amendments produces values below the admitted threshold values (for the abovementioned conditions of use).

The ratio of the area of the food contact material to the volume used to determine the compliance of the plastic food contact material or article measured approx. 310cm²/200cm³ resp. 410cm²/550cm³ resp. 440cm²/950cm³.

c) Information about restricted substances and / or specifications

Above mentioned products may contain substances with an SML-restriction defined in Regulation (EC) No 10/2011.

stearic acid: ref.no 24550; CAS 0000057-11-4 SML: 25mg/kg
octadecyl 3-(3,5-di-tert-butyl-...)propionate.: ref. no 68320; CAS 0002082-79-3 SML: 6mg/kg
alkyl(C8-C22)sulphonic acids: ref.no. 34230 SML: 6mg/kg
butadiene: ref. no 13630; CAS 0000106-99-0 SML: (NN)

The compliance of the limits is confirmed for the stated types of food and conditions of application.

d) silver plastics does not add Dual Use Additives to its formulations, which have acc. Regulation (EC) No 10/2011 a restriction in foodstuff.

4. Further Declarations of Conformity

The requirements of the European (packaging-) Directive 94/62/EC (dated from December 20th, 1994; including further amendments) regarding heavy metals are fulfilled.

5. Conclusion

In terms of EU-framework Directive (EC) No 1935/2004 and §§ 30 and 31 LFGB, published in the Bundesgesetzblatt (Federal Law Gazette) No 55 of 6.09.2005 there are no objections against the use of the product for the manufacture of articles intended for food contact.

This declaration is valid for the product delivered by us as specified above. The Regulation (EU) No. 10/2011 or the Directives 82/711/EEC and 85/572/EEC provide guidelines for the selection of test conditions to be used for various food products. According to that and under consideration of the food contact conditions stated, the product complies with the stipulations of these Regulations regarding the packaging of food products to be packed. The user shall verify himself that the product is suitable for the intended food to be packed beyond the stipulations of the Directives.

6. Further Information

All previous Declarations of Compliance with relation to the above mentioned products are hereby invalid.

In case of relevant changes in the characteristics of the product or in case of new relevant scientific findings silver plastics will renew this declaration.

This declaration is valid until 31st of December 2015.

Troisdorf, Aug. 12th 2013
silver plastics® GmbH & Co. KG
Godesberger Str. 9 • D-53842 Troisdorf



i.A. C. Storck
- QW/AS -

DEHA FREE (DOA) *

The ELITE SPM film is PVC extruded multi-layer, high performance stretch film. This quality film is developed for the almost all-automatic stretch wrapping packaging machines.

The ELITE SPM C films are especially adapted for the « super- and hypermarkets packaging machines» and the linear stretch wrapping machines for « difficult large products ».

All the components used in ELITE SPM C films are food contact approved and conform to European regulation (EU)

N°10/2011. This product is suitable for direct food contact with the following food categories as listed in EU directive

85/572/ CEE:

- 02.01 – 02.02 – 02.03 – 02.04 – 02.05 – 02.06A : cereals products, pastry, biscuits, cake and other bakers' wares
- 03.01 – 03.02A – 03.03A : Chocolate, sugar and products thereof confectionery products
- 04.01 – 04.02A – 04.03 – 04.04 – 04.05A : fruit, vegetable and products thereof
- 06.01 : fish - 06.03 – 06.04 : meats of all zoological species
- 07.04 : Cheeses
- 08.02 – 08.08 - 08.10 – 08.11– 08.16 - 08.17 - : miscellaneous products

It is not permitted for contact with pure fats and oils.

Property Unit Method Value

Roll length m Internal method 1500

Core size mm Internal method 113 / 76

Machine Dir. Mpa ASTM D882 41-46

Tensile Strength

Transversal dir. Mpa ASTM D882 33-38

Machine dir. % ASTM D882 260-310

Extension

Transversal dir. % ASTM D882 330-380

Machine Dir. Mpa ASTM D882 22-24

Modulus 100%

Transversal Dir. Mpa ASTM D882 12-13

Static Slippery COF - NF T 54 112 0.35-0.45

properties Cinetic COF - NF T 54 112 0.18-0.25

Optical Gloss - ASTM 2457-70 125-135

properties Haze % ASTM D1003 0.7-1.3

Oxygen transmission l/m²/24h/1atm Lissy GPM 500 11.2

Carbon dioxide transmission l/m²/24h/1atm Lissy GPM 500 69.6

Nitrogen transmission l/m²/24h/1atm Lissy GPM 500 3

Water vapour transmission rate g/m²/24h NF H 00-044 à 25°C –

75% of humidity

53.5

Figures are typical values derived from tests conducted upon actual film samples in accordance with Global Plastics International

Tests methods. Results may vary, thus this data MUST NOT form the basis of limitation specification

* Trace of DOA may be found due to contamination

Date : 23/08/2011

