Skýrsluágrip Rannsóknastofnunar fiskiðnaðarins
Icelandic Fisheries Laboratories Report Summary

Titill:	**Shelf Life of Chilled Ocean Perch Fillets**
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Ágrip á íslensku:	Aukinn áhugi er á flakaflutningi þar sem flökin eru kæld með lofti. Þar sem ekki er alltaf hægt að tryggja stöðugan hita né hita nálægt 0°C vakna spurningar um geymsluþol slíkra flaka. Tilgangur þessarar tilraunar var að kanna geymsluþol karfaflaka sem geymd eru við mismunandi hitastig, kanna hvort hægt sé að reikna út geymsluþolið með geymsluþolslíkönum og athuga fjölgun baktería á flökum við vinnslu þeirra. Niðurstöðurnar sýndu að geymsluþol karfaflakanna var 4-5 daga (8°C), 8 d. (0°C), og 14 daga við -2°C. Gott samræmi var milli geymsluþols ákvarðað með skynmati og þegar gerlafjöldi var um og yfir 10.000.000/g. Hægt var að segja fyrir um geymsluþol með geymsluþolsspám við 8 og 0°C með nokkurri nákvæmni en síður við -2°C sem e.t.v. má skýra með mismunandi gerlaflóru. Umtalsverð aukning (100-500x) varð á gerlafjölda við framleiðslu karfaflakanna. Slík aukning getur stytt geymsluþol flakanna um marga daga (5 til 8 daga við 0°C). Því er m.a. sú ályktun dregin að gæta verði ýtrasta hreinlætis og sjá til þess að hitastig sé í lágmarki við framleiðslu flakanna.
Lykilorð á íslensku:	Karfi, flök, kæling, geymsluþol
Summary in English:	There is an increased interest in air chilled storage and transport of fish fillets were the storage temperature is not likely to be constant and usually not 0°C. The purpose of this study was to predict the storage time for ocean perch fillets, to follow the quality change in the fillets stored in air at different temperature -2°C, 0°C and +8°C and to check the increase of bacteria in a ocean perch processing line. The results showed that the shelf life of chilled ocean perch fillets was 4-5 (8°C), 8 (0°C), and 14 (-2°C) days. Good agreement was found between estimated shelf life as judged by sensory evaluation and the shelf life found when bacterial number of 10,000,000/g was reached. During processing there was a substantial (100-500x) increase in bacterial load in the fillets. In order to decrease quality losses, every care should be taken to lower the initial bacterial load and to keep the temperature low.
English keywords:	Ocean perch, chilled, fillets, shelf life

INTRODUCTION

Traditional storage of fresh fish is in ice at temperatures close to 0°C. The shelf life of fish stored in such conditions is fairly well known. Recently an increased interest in air chilled storage and transport of fish fillets has been noted but then the storage temperature is less likely to be constant and usually not 0°C. Although cod fillets have been studied in this respect very little information is available for other fish species. One example is ocean perch fillets. The shelf life has been studied at 0°C (Martinsdóttir et al., 1991) but not at other temperatures.

In order to have better possibilities to judge different alternatives of storage and transport of ocean perch fillets the Icelandic Freezing Plants Corporation (IFP) initiated this study.

PURPOSE

During initial discussion between Icelandic Fisheries Laboratories (IFL) and IFP the purpose of this project was defined as follows:

1. To predict the storage time for ocean perch from data obtained from experiments with cod.
2. To follow the quality change in ocean perch fillets, which were stored in air at different temperature -2°C, 0°C and +8°C.
3. To compare results from 1 and 2.
4. To find environmental factors or circumstances for transport of unfrozen ocean perch fillets to foreign markets at given conditions (e.g. temperature recordings).
5. To review the literature on "super-chilling".
6. To check the increase of bacteria in a ocean perch processing line.

MATERIALS AND METHODS

A. Estimated quality changes in ocean perch fillets from cod data

The results of Einarsson 1992 and 1994 were used. Lag-time for bacteria was set to 1 day and the growth rate from in Table 1 was used. For shelf life estimation Table 2 was used. The initial bacterial number was set 63.000 /g (then it is 4 generations to 1,000,000 and 7-8 generations to 10,000,000/g).

B. Monitoring of quality changes in ocean perch fillets

1. Raw material
Ocean perch (2 days old, stored in ice) was filleted and deskinned at Grandi Ltd. Reykjavik. The fillets were about 150-300g and in total 300-400 fillets were used. The fillets were brought to IFL within two hours from processing. There the fillets were packed into bags to minimize the dehydration. Each bag contained 6-8 fillets.

2. Storage.
The fillets were stored at three different temperatures. The temperature was recorded throughout the storage periot and found to be as follows (mean ±sd, °C):

1). +7.9 (±0.2)
2). ±0.0 (±0.4)
3). -1.7 (±0.1)

3. Sampling.
Samples were taken for microbial analyses, TMA/TVN content and also for sensory test according to the following plan.

	Storage time (days)							
	0	2	3	6	8	10	13	15
t°C								
-2			2	2	2	2	2	2
0	2		2	2	2	2		
8		2	2	2				

4. Bacterial counts.
Two fillets were minced and 25 g weighed into a stomacher bag containing 225 mL Butterfield´s buffer solution(pH 7.2). Blending was done in a Stomacher for 1 min. The bacterial counts were done by the spread(surface) plate method and with plate count agar (PCA-Difco) with 0.5% NaCl(w/v) added. The plates were incubated at 22°C for 3 days. The total count and selective counts of H_2S-producing bacteria were also done on iron agar (Gram, et al., 1987) with overlay (IA). The plates were incubated at 15°C for 5 days. Bacteria forming black colonies on this agar produce H_2S from sodium thiosulphate and/ or cysteine.

5. Sensory evaluation.
Sensory evaluation was done by a trained panel of 8-10 people on samples of cooked fillets. Fillets were cooked in a steam oven (6 min at 99°C) and the smell and taste were judged on a rating scale from 10 (highest freshness) to 3 (lowest freshness). The scale is based on the Torry scale as originally described by Shewan et al. (1953) with slight modifications. The fish was judged unfit for consumption when the mean value for sensory score was below 5.5. On every sampling day there where two replications.

7. Determination of total volatile basic nitrogen (TVB-N)
A TCA extract was steam distillated by the modified method of Malle and Tao (1987). One hundred gram of fish muscle were deproteinized as described previously and the filtrate was collected. Steam

distillation was carried out using a Struers-type distillator. Twenty-five ml of filtrate were loaded into the distillation flask followed by 10 ml of 10 % NaOH. An Erlenmeyer bottle containing 10 ml of 4% aqueous boric acid solution and 0,04 ml of methyl red and bromocresol green indicator for titration of ammonia was placed at the end of the condensor. Distillation was continued until a final volume of 90 ml was obtained in the beaker (80 ml of distillate). The boric acid solution turned green when alkalinised by the distilled TVB-N. This was titrated using a 0,1-ml graduated burette containing 0,025 N H_2SO_4 Complete neutralisation was obtained when the color turned pink on the addition of a further drop of sulfuric acid.

The quantity of TVB-N in mg was determined from the volume of sulfuric acid (n ml) added as follows:

TVB-N = (n) (4,2 mg N/100 g)

8. Determination of TMA.

A TCA extract was steam distillated by the modified method of Malle and Poumeyrol (1989). Modification of the TVB-N (TCA) assay. Twenty ml of formaldehyde was added to the distillation flask to block the primary and secondary amines. Steam distillation was then performed as for the determination of TVB-N in TCA extract. When the required amount of formaldehyde was added, only the TMA was distilled. The TMA content was calculated from the volume of 0,025 N H_2SO_4 used for titration (n ml) as follows:

TMA = (n) (4,2 mg N/100g).

RESULTS AND DISCUSSION

General observations

As stated in the introduction the purpose of this study was to examine out quality changes in fresh ocean perch fillets as affected by temperature. This was done by storing the fillets at three different temperatures and by measuring some important quality related parameters. The main results are shown in the figures and tables below while the original results are given in appendices.

Quality changes in ocean perch fillets

1. Bacterial counts.

The results of the bacteriological analyses are shown in Figures 1-3. The initial count (PCA: Plate Count Agar, inoculated at 22°C) was 40.000 to 130.000 /g indicating some variation between samples. As expected the storage temperature had a marked effect on the growth as can be seen in Figure 1.

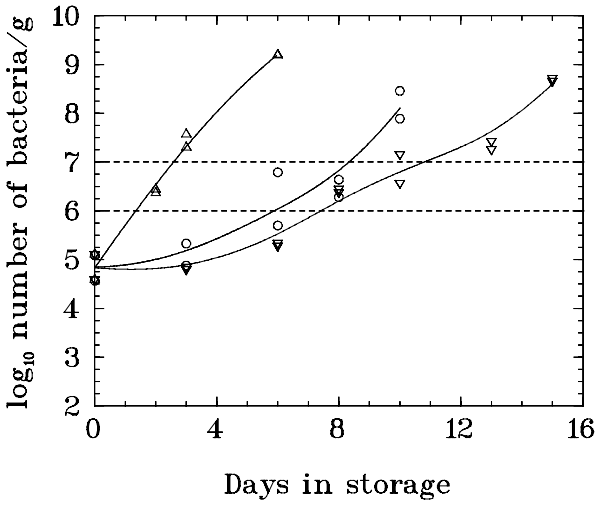

Figure 1: Growth of bacteria (PCA-22°C) in Ocean Perch fillets (skin off) stored at -2°C(∇), 0°C(•) and 8°C(Δ). Each symbol represents results from two fillets.

Inoculation of PCA at 22°C has been used at this institute (IFL) for evaluation of bacteriological quality of fish but other temperatures and media have been recommended for special purposes. Thus the use of a special agar is

recommended for detection of spoilers. Also lower temperatures have been recommended to enhance growth of more psycrophilic bacteria. In this experiment 15°C and Iron Agar was chosen but "presumptive spoilers" form black colonies on that agar. The total number of bacteria on IA was generally higher than on PCA agar but the overall growth was similar to that on the PCA agar (Figure 2).

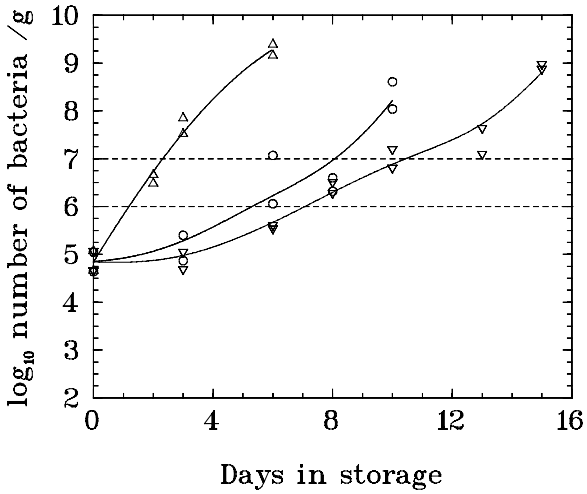

Figure 2: Growth of bacteria (Iron agar 15°C-total) in Ocean Perch fillets (skin off) stored at -2°C(∇), 0°C(•) and 8°C(Δ). Each symbol represents results from two fillets.

Initially the number of black colonies was 700/g or approximately 1% of the total number. The number of black colonies increased during storage and at a higher rate than the "white colonies" (Figure 3). At the end of storage the black colonies were 10-50% of the total which is commonly noted in similar experiments.

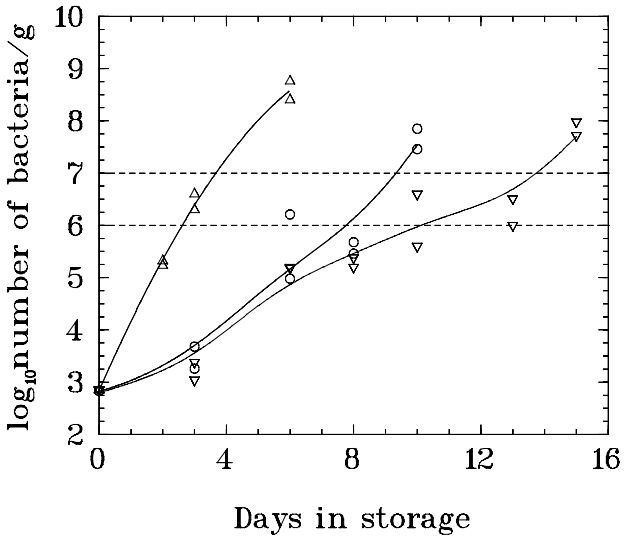

Figure 3: Growth of bacteria (Iron agar at 15°C, black colonies) in Ocean Perch fillets(skin off) stored at -2°C(∇), 0°C(•) and 8°C(Δ). Each symbol represents results from two fillets.

2. Sensory evaluation

The initial sensory quality was high (sensory score greater than 9) as can be seen on Figure 5. In general the drop in sensory quality was as expected, it decreased most rapidly at 8°C and least at -2°C. On day 3, however, the mean score was 8.1 for the 8°C sample and 7.7 for the -2°C sample but this is not significantly different. There was a great difference in the results for the -2°C sample on the 10th. day of storage and this difference can not be explained.

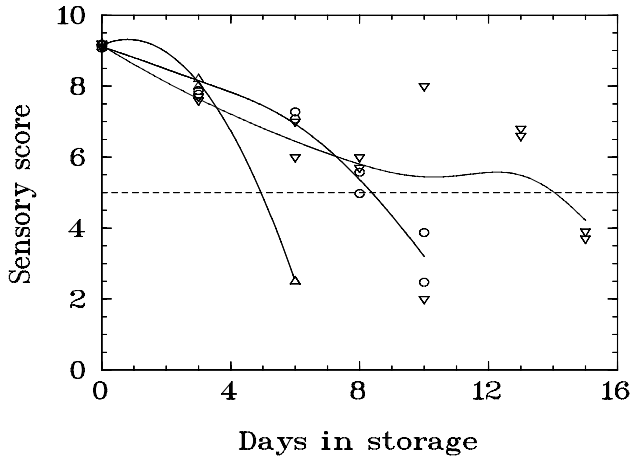

Figure 4: Sensory evaluation in Ocean Perch fillets(skin off) stored at -2°C (∇), 0°C(•) and 8°C(Δ). Each symbol represents results from two fillets.

3. Chemical analyses

The results for the TMA content is shown in Figure 6 and for TVB in Figure 7. The initial content was low, TMA was less than 1 mg N/100g fish and TVB-N approximately 10 mg N/100g, and both components showed a marked and similar "lag" period before the values increased.

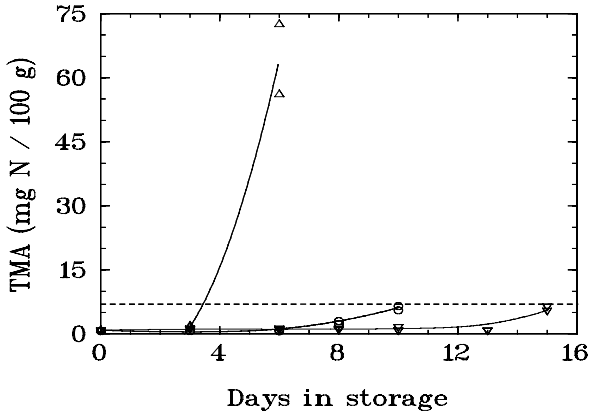

Figure 5: TMA in Ocean Perch fillets (skin off) stored at -2°C(∇), 0°C(•) and 8°C(Δ). Each symbol represents results from two fillets.

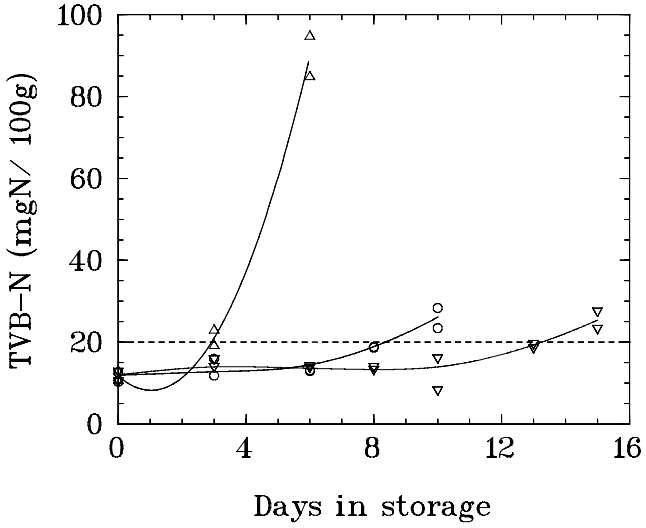

Figure 6: Total volatile basic nitrogen (TVB-N) in Ocean Perch fillets (skin off) stored at -2°C(∇), 0°C(•) and 8°C(Δ). Each symbol represents results from two fillets.

4. Shelf life

The shelf life of the ocean perch fillets was estimated and the results are presented in Table 1. The shelf life (sensory score) was estimated 4-5, 8 and 14 days, at 8, 0 and -2°C respectively. Good agreement was between estimated shelf life as judged by sensory evaluation and the shelf life estimated by the other methods except when the lower bacterial number (1,000,000 /g) was used. This limit was taken here as it is sometimes used in trade agreements.

Table 1: Shelf life of ocean perch fillets (skin off) stored at -2, 0 and 8°C.

	Shelf life (days)				
Storage temp. (°C)	Sensory evaluation	1,000,000 bacteria/g	10,000,000 bacteria/g	TMA	TVB-N
-2	14	7-8	13-14	14-15	13-14
+0	8	5-6	8-9	8-9	8-9
+8	4-5	2-3	3-4	3-4	3

[The shelf life was judged expired when the sensory score was lower than 5, when the bacterial number (black colnies on IA) was higher than 1,000,000/g and 10,000,000/g, when TMA content was higher than 5 mg N/100 g and when TVB-N was higher than 20 mg N /100g.]

Comparison of found and estimated quality changes

As stated in the introduction one purpose of this study was to see if the results from experiments with cod (Einarsson, 1992) could be used to draw conclusions for ocean perch. From the results of Einarsson (1992) following shelf life was calculated (Table 2).

Table 2: Calculated shelf life of ocean perch fillets stored at -2, 0 and 8°C from cod data (Einarsson, 1992) and shelf life found in this study.

				Shelf life (days)		
Storage	Sensory evaluation		10,000,000 bacteria/g		10,000,000 black c./g	
temp. (°C)	1992 data	This study	1992 data	This study	1992 data	This study
-2	22	14	8-9	13-14	23	13-14
+0	11-12	8	7-8	8-9	8	8-9
+8	3-4	4-5	3-4	3-4	4	3-4

The table shows that there is some agreement between results. However the opposite was also true. The shelf life (sensory evaluation) was estimated higher than found except at +8. The estimated bacterial (total count) shelf life was lower or same as found while the black colonies were estimated as found except at -2°C. This indicates that the results of Einarsson (1992) can not be used for accurate estimation of shelf life of ocean perch fillets without some further evaluation of the data.

In the study of Martinsdóttir et al. (1991) the shelf life of ocean perch fillets stored on ice was found to be 10-12 days compared to 8 days in this study. The initial bacterial count found by Martinsdóttir et al. (1991) was 1,000-3,000 which is 100x lower than in this study.
The fish used by Einarsson (1992) was, as in this study, fresh (sensory score approximately 9) but the initial count in this study was approximately 100 times higher then in the one from 1992.
The initial bacterial count was more similar between the studies of Einarsson (1992) and Martinsdóttir et al. (1991). Also the predicted shelf life 11-12 days (Table 2) is close to that found by Martinsdóttir et al. (1991) which was 10-12 days.

The growth rates and lag-times were between this study and the one of Einarsson (1991) were compared. (Table 3).

Table 3. Growth rate and lag-times for bacteria found in this study and in the study of Einarsson (1992).

	Growth rate			
Storage	Total number		Black colonies	
temp. (°C)	1992	This study	1992 data	This study
-2	0.309	0.277	0.174	0.328
+0	0.343	0.346	0.501	0.547
+8	1.100	0.726	0.970	0.944

	Lag time			
	Total number		Black colonies	
	1992	This study	1992 data	This study
-2	0-1	3-4	0-1	0-1
+0	0-1	2-3	0-1	1-2
+8	0-1	0-1	0-1	0-1

The growth rate (total number) in this study was lower and the lag-time longer and this is reflected the longer found bacterial shelf life. The growth rate and lag-time for the H_2S produsing bacteria was more alike between the two experiments, except for -2°C, and this is also reflected in much closer agreement of estimated and found shelf life.

Bacterial changes during fillet processing.

I was decided to follow the bacterial changes through out the production of the fillets used in this study. The results are shown in Table 4.

Table 4. Bacterial changes in fish during processing of samples for this study.

	bacteria/g (PCA 22°C)	bacteria/g (IA, 15°C)			
Sample		black. col.	white col.	total	pH
1. skin	112,000	15,000	230,000	245,000	
2. fish fillet	220	<10	200	200	
3. fish after filleting	6,200	140	8,000	8,140	
4. fish after skinning	1,140	20	1,400	1,420	
5. fish after packing -a	128,000	700	116,000	116,700	6.87
6. fish after packing -b	39,000	700	45,000	45,700	6.90

The table shows that initial total count was low (200-220/g) in the flesh while the skin had more than 100,000 bacteria/ cm^2. The number of black colonies was <10/g in the flesh while the skin had 15,000 bacteria/ cm^2. During processing there was a substantial (100-500x) increase in the flesh (total number) and the black colonies increased to 700/g.

CONCLUDING REMARKS

The shelf life of chilled ocean perch fillets was found to be:
4-5, 8 and 14 days, at 8, 0 and -2°C respectively.

Good agreement was between estimated shelf life as judged by sensory evaluation and the shelf life found when bacterial number of 10,000,000/g was reached. (Hveað með TMA/TVB)

The shelf life found in this study was lower (e. g. 2-4 days at 0°C) than expected when compared to earlier results from this institute.

At least some of the difference can be explained by higher initial bacterial numbers found in this study.

During processing there was a substantial (100-500x) increase in bacterial load in the fillets.

In order to decrease quality losses, every care should be taken to lower the initial bacterial load and to keep the temperature low.

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APPENDIXES

Appendix 1. Bacterial count.

Day		LT-22°C-PCA	LT-15°C-iron agar			pH
			black colonies *	white colonies**	total	
0		128.000	700	116.000	116.700	6.87
		39.000	700	45.000	45.700	6.90
2	8°C	2.330.000	170.000	2.860.000	3.030.000	6.79
		2.730.000	210.000	4.400.000	4.610.000	6.77
3	8°C	20.000.000	4.000.000	66.000.000	70.000.000	6.76
		37.000.000	2.000.000	31.000.000	33.000.000	6.85
	0°C	222.000	5.000	260.000	265.000	6.87
		79.000	1.900	75.000	76.900	6.95
	-2°C	63.000	1.100	48.000	49.100	6.75
		70.000	2.400	109.000	111.400	6.80
6	8°C	1.550.000.000	580.000.000	1.860.000.000	2.440.000.000	7.54
		1.570.000.000	250.000.000	1.190.000.000	1.440.000.000	7.44
	0°C	530.000	100.000	1.100.000	1.200.000	6.79
		6.400.000	1.700.000	10.700.000	12.400.000	6.77
	-2°C	190.000	160.000	245.000	405.000	6.74
		220.000	150.000	200.000	350.000	6.79
8	0°C	4.600.000	300.000	3.900.000	4.200.000	6.71
		2.000.000	500.000	1.700.000	2.200.000	6.81
	-2°C	2.800.000	160.000	3.100.000	3.260.000	6.74
		2.400.000	240.000	1.670.000	1.910.000	6.78
10	0°C	82.000.000	30.000.000	84.000.000	114.000.000	6.85
		300.000.000	74.000.000	357.000.000	431.000.000	6.96
	-2°C	14.300.000	4.000.000	12.000.000	16.000.000	6.80
		3.700.000	400.000	6.100.000	6.500.000	6.75
13	-2°C	18.000.000	1.000.000	11.600.000	12.600.000	6.71
		27.000.000	3.200.000	40.000.000	43.200.000	6.76
15	-2°C	460.000.000	95.000.000	870.000.000	965.000.000	7.00
		530.000.000	53.000.000	710.000.000	763.000.000	7.00

***black colonies are presumptive spoilers . **white colonies other bacteria.**

Appendix 2. TMA/TVB.

days	TVB-N(mgN/100g)	TMA(mgN/100g)
0	12.9	0.85
0	10.6	0.85
-2	16.0	1.3
-2	14.1	1.2
0	16.2	0.85
0	12.1	0.85
8	22.9	1.9
8	19.1	1.5
-2	13.9	1.1
-2	14.3	1.3
0	13.2	1.1
0	13.4	0.8
8	84.8	56.1
8	94.7	72.5
-2	13.4	1.1
-2	14.1	1.3
0	18.9	2.5
0	19.1	3.2
-2	16.2	1.7
-2	8.4	0.85
0	23.7	5.7
0	28.6	6.7
-2	18.7	0.85
-2	19.7	0.85
-2	23.4	5.5
-2	27.6	6.5

Appendix 3. Sensory evaluation. results

		temperature in storage		
days	**replication**	**-2°C**	**0°C**	**8°C**
0	**1**	**9.2**	**9.2**	**9.2**
	2	**9.1**	**9.1**	**9.1**
	mean	**9.2**	**9.2**	**9.2**
3	**1**	**7.6**	**7.8**	**8.2**
	2	**7.7**	**7.9**	**8.0**
	mean	**7.7**	**7.9**	**8.1**
6	**1**	**7.0**	**7.1**	**<3**
	2	**7.6**	**7.3**	**<3***
	mean	**7.3**	**7.2**	
8	**1**	**5.7**	**5.0**	
	2	**6.0**	**5.6**	
	mean	**5.9**	**5.3**	
10	**1**	**7.8**	**3.9**	
	2	**7.2**	**---**	
	mean	**7.1**		
13	**1**	**6.6**		
	2	**6.8**		
	mean	**6.7**		
15	**1**	**3.9**		
	2	**3.7**		
	mean	**3.8**		

***not evaluated by the taste panel**