1 **Quality of fresh Saithe (*Pollachius virens*) in modified atmosphere packages as affected by the gas**
2 **composition**

3 Jørgen Lerfall[1,*], Gunn Merethe Bjørge Thomassen[1], Anita Nordeng Jakobsen[1]

4

5 [1)] Norwegian University of Science and Technology, Department of Biotechnology and Food Science,
6 NO-7491 Trondheim, Norway

7

8 Corresponding author: Jørgen Lerfall, Norwegian University of Science and Technology, Department
9 of Biotechnology and Food Science, NO-7491 Trondheim, Norway, e-mail: Jorgen.lerfall@ntnu.no,
10 phone: +47 73 55 97 49

11

12

13

14 **Abstract**

15 The experimental design was set up to study the effect of different modified atmospheres (CO_2 (67 or
16 33%) balanced with either O_2 or N_2) on autolytic- and microbiological deterioration of chilled saithe
17 (*Pollachius virens*). As controls, vacuum packaged saithe was used. The results showed a positive
18 effect of gas mixtures containing O_2 on physiochemical and microbial product quality. Discriminating
19 factors were; lower psychrotrophic count, slower breakdown of ATP, lower contents of certain
20 biogenic amines (*e.g.* cadaverine) and reduced drip loss during storage. A high CO_2 concentration
21 (67%) in the packaging atmosphere was moreover found to inhibit microbial proliferation. Vacuum-
22 packaged saithe stand out negatively with highest DL and reduced sensory shelf life and
23 physiochemical and microbial quality. It was moreover found that increased drip loss, and higher
24 contents of hypoxanthine and cadaverine in the muscle tissue was related to the microbiological
25 ecology (*i.e.* increased growth of *Shewanella* spp. and *Photobacterium* spp.).

26

27

28

29

30

31

32

33

34 Keywords: Saithe; modified atmosphere packaging; ATP-degradation; biogenic amines;
35 microbiological ecology

36 **1. Introduction**

37 Fresh saithe caught by local fishermen's along the Norwegian cost line is traditionally traded on ice as
38 head off gutted products or as vacuumed packaged fillets. Fresh fish are perishable and in order to
39 reduce deterioration during storage, action to preserve the fish must be taken. One technology for
40 preserving fresh fish is modified atmosphere packaging (MAP). MAP is a well-studied technology with
41 several benefits such as the possibility to design a specific atmosphere to inhibit growth of specific
42 spoilage organisms (SSO`s) (Sivertsvik, Jeksrud, & Rosnes, 2002). Seafood stored under MAP is a
43 growing segment that attract the consumers' vision of portion packaged convenient fresh food with
44 low contents of additives and a high nutritional value (Carlucci et al., 2015). In addition, extended
45 shelf life and increased quality gained by MAP is known to attract both the processing industry and
46 trendy consumers (Carlucci et al., 2015).

47 Quality changes during storage of MAP seafood are related to microbial deterioration or autolytic
48 reactions (DeWitt and Oliveira, 2016; Sivertsvik, et al., 2002). To determine the shelf life of MAP
49 products, different quality parameters, both microbial chemical, physical and sensorial changes must
50 be considered. Sensory rejection may be due to changes in colour, odour, taste, texture, or a
51 combination of these parameters (DeWitt and Oliveira, 2016).

52 Autolytic reactions, included a catabolically deterioration of adenosine triphosphate (ATP),
53 (Kassemsarn, Perez, Murray, & Jones, 1963) will start at the point of death whereas SSO`s in most
54 cases will metabolize the fish spoilage (Gram & Dalgaard, 2002). A subsequently increase of spoilage
55 metabolites in the fish muscle, *e.g.* hypoxanthine (Hx) and biogenic amines (Bulushi, Poole, Deeth, &
56 Dykes, 2009; Dalgaard, 2000) will thereafter affect the impression of the product. Degradation of ATP
57 normally results in a fast accumulation of inosine monophosphate (IMP), which is known to
58 contribute to the pleasant, fresh flavour of meat products (Howgate, 2005). Further denaturation of
59 IMP to form inosine (HxR) and finally Hx occurs at slower rates than denaturation of ATP to IMP

60 (Surette, Gill, & LeBlanc, 1988). An accumulation of Hx contribute to the progressive off-flavour and
61 bitter off-taste related to deteriorated seafood products (Dalgaard, 2000).

62 The spoilage microbiota of fresh chilled and aerobic stored cold-water fish is dominated by Gram-
63 negative, psychrotrophic, aerobic or facultative anaerobic bacteria such as *Pseudomonas* spp. or
64 *Shewanella* spp.. The specific microbiota depends on species and is affected by fishing ground, and
65 the specific packaging and storage conditions used, e.g. is *Photobacterium phosphoreum* often
66 becoming the dominant spoilage organism when MAP with high concentrations of CO_2 (>60%) is used
67 (Gram & Dalgaard, 2002; Macé et al., 2013; Powell & Tamplin, 2012; Rudi, Maugesten, Hannevik, &
68 Nissen, 2004; Sivertsvik et al., 2002).

69 Both *Shewanella* spp. and *Photabacterium phosphoreum* are facultative anaerobic microorganisms.
70 When oxygen levels are depleted, trimethylamine-N-oxide (TMAO) which is a characteristic part of
71 the non-protein nitrogen fraction (NPN) of the marine fish muscle serves as a terminal electron
72 acceptor for anaerobic respiration (Dalgaard, 1995; Easter, Gibson, & Ward, 1983) and is reduced to
73 trimethylamine (TMA) (Debevere & Boskou, 1996). It is therefore important to use oxygen as part of
74 the atmosphere to increase the product quality and shelf life of lean marine species such as cod
75 (*Gadus morhua*) (Debevere & Boskou, 1996; Guldager, Boknaes, Osterberg, Nielsen, & Dalgaard,
76 1998; A. Å Hansen, Mørkøre, Rudi, Olsen, & Eie, 2007). Sivertsvik (2007) concluded that the optimal
77 modified atmosphere for packaging of pre-rigor filleted farmed cod was 63% O_2 and 37% CO_2. To
78 increase the shelf-life and quality of fish products to meet the expectations of both the consumers
79 and the processing industries, exact knowledge of each species is important. The effect of MAP on
80 the shelf life of saithe (*Pollachius virens*), is as far as we know, limited to Dalgaard, Mejlholm,
81 Christiansen, and Huss (1997) who reported the growth and occurrence of *P. phosphoreum* affected
82 by different modified atmospheres. Studied atmospheres was however limited to air, CO_2/N_2 (60/40)
83 and $CO_2/O_2/N_2$ (45/5/50). The aim of the present study was to gain knowledge of MA-packaging of
84 saithe, and to document effects of the gas mixture (high or low contents of CO_2 (67 or 33%) balanced

85 with either O_2 or N_2 on autolytic- and microbiological deterioration during refrigerated storage (4 °C).

86 In addition, the microbiological ecology was studied at point of packaging and sensory rejection. As

87 controls, saithe packaged in vacuum was used.

88

89 **2. Material and methods**

90 *2.1. Fish material and experimental design*

91 The saithe used in the present study was caught 4th April 2016 by local fishermen's using nets at the

92 west coast of Norway (approximately 63.5°N, 9°S). All fish were instantly killed by a blow to the head

93 before the fish were gill cut and transferred to a bleeding tank. Thereafter, all fish were decapitated,

94 gutted and transported in ice slurry to Dolmøy Seafood AS, Dolmøy Norway. At Dolmøy Seafood, the

95 fish was packaged immediately in expanded polystyrene boxes and transported on wet ice to the

96 Norwegian University of Science and Technology (NTNU, Trondheim, Norway). At arrival NTNU

97 (approximately 6 hours post mortem and 4 hours post slaughtering), the muscle pH was measured to

98 be 7.0 ± 0.2.

99 Approximately 24 hours post mortem a selection of 36 saithe with average weight of 1.8 ± 0.5 kg

100 with a head off gutted condition factor equal of 1.6 ± 0.1 (pH = 6.8 ± 0.3) were filleted. The head off

101 gutted condition factor was calculated according to Equation 1.

102 Equation 1:

103 Head off gutted condition factor = (head off gutted body weight (g)* fish length (cm)$^{-3}$) *100

104 Each fillet was thereafter divided into pieces of 90.0 ± 15.9 g (n=200, each piece was tagged with the

105 original fish ID) and randomly distributed between five groups (*high* CO_2*/low* N_2; *low* CO_2*/high* N_2;

106 *high* CO_2*/low* O_2; *low* CO_2*/high* O_2 and *vacuum*).The packaging process was carried out on a

107 Webomatic Supermax-C (Webomatic, Germany) connected to a gas mixer (MAP Mix 9000,

108 Dansensor, Ringsted, Denmark)). The gasses used were food grade qualities of O_2, CO_2 and N_2. Each

109 piece of saithe was individually packaged in semi-rigid crystalline polyethylene terephthalate (CPET)

110 trays (C2125-1B, Færch Plast, Holstebro, Denmark). The air was evacuated to an end pressure of 30

111 mbar before the trays were filled with the pre-set packaging gas mixtures and sealed in polyamide

112 (PA)/polyethylene (PE) vacuum bags (135 × 180 PA/PE 20/70µm) at atmospheric pressure to obtain a

113 product to gas ratio of approximately 1:2. The oxygen transmission rate (OTR) of the bags were ~50

114 $cm^3/m^2 \times 24^1 \times bar^1$ at 23 °C and 0% relative humidity. To ensure correct gas mixture, the gas

115 composition was measured in five dummies of each group with a PBI Dansensor, Checkmate 9900.

116 The initial packaging gas composition of the respective groups conducted to MAP were; *high* CO_2*/low*

117 N_2 (CO_2 = 67.2 ± 0.2%, O_2 = 0.0 ± 0.0% and N_2 = 32.8 ± 0.2%), *low* CO_2*/high* N_2 (CO_2 = 31.8 ± 0.2%, O_2 =

118 0.1 ± 0.1% and N_2 = 68.2 ± 0.3%), *high* CO_2*/low* O_2 (CO_2 = 66.4 ± 0.4%, O_2 = 32.2 ± 0.0% and N_2 = 1.3 ±

119 0.3%) and *low* CO_2*/high* O_2 (CO_2 = 31.3 ± 0.2%, O_2 = 66.0 ± 0.1% and N_2 = 2.7 ± 0.3%). The content of

120 N_2 is presented as the balance of O_2 and CO_2.

121 After packaging, the samples were stored in a refrigerated room (4 °C) until three packages of each

122 group were found not acceptable by a sensory panel judging fish odour (*i.e.* day 10 for vacuum

123 samples and day 13 for all MAP groups). At each sampling day (0, 3, 6, 8, 10 and 13 days post

124 packaging) samples were sampled randomly and analysed for muscle pH, drip loss, degradation

125 products of adenosine triphosphate (ATP), content of biogenic amines, aerobic plate count (APC),

126 H_2S-producing bacteria, psychrotrophic aerobic plate count (PC), lactic acid bacteria (LAB),

127 *Enterobacteriaceae*, *Pseudomonas* spp. and *Brochothrix thermosphacta* (n = 3 except for drip loss

128 where n = 6). In addition, the specific microbiota were identified at day 0 and on the day of sensory

129 rejection. To confirm correct storage atmosphere of each package, the gas composition was

130 measured in all packages with a PBI Dansensor, Checkmate 9900 at the respective sampling day.

131 *2.2. Sensory evaluation of odour*

132 Sensory analysis of odour was assessed by seven semi-trained panellists judging the fish odour

133 approximately 5 minutes after the packages were opened (n = 3). A simple three-class system based

134 on Dalgaard, Gram, & Huss (1993) with class III corresponding to rejection was used by each

135 panellist. Class I and II corresponded to no off-odours and some off-odours but still acceptable,
136 respectively. In addition, the judgers were asked to describe the first impression of the off-odour of
137 each sample. The average score of each sample was used to decide whatever the sample should be
138 rejected as not acceptable or not.

139 *2.3. Microbiological analysis*

140 A 10-g sample of fish muscle was aseptically transferred to a sterile stomacher bag and diluted 1:10
141 with sterile peptone water (1.0 g bacteriological peptone and 8.5 g/L NaCl) and homogenized
142 vigorously for 60 s in a Stomacher 400 Lab Blender (Seward Medical Ltd., UK). Appropriate serial
143 dilutions were made in sterile peptone water and spread at their respective agar plates. Aerobic
144 plate count (APC) and H_2S-producing bacteria were quantified by pour plating on Lyngby's iron agar
145 (IA) (Oxoid) supplemented with 0.04% L-cysteine (Sigma-Aldrich) as total and black colonies,
146 respectively. Plates were incubated at 22 °C for 72 h. Psychrotrophic aerobic plate count (PC) was
147 quantified by spreading on Long and Hammer agar (LH) with 1% NaCl, in order to support growth of
148 the salt requiring *P. phosphoreum* (NCFA, 2006). Plates were incubated at 15 °C for six days. Lactic
149 acid bacteria (LAB) was quantified by spreading on de Man, Rogosa and Sharp agar (MRS) (Oxoid)
150 supplemented with 10 mg/L amphotericin B (Sigma-Aldrich) that was incubated in anaerobe
151 atmosphere at 25 °C for five days. Enterobacteriaceae was quantified using violet-red-bile-glucose
152 agar (VRBGA) (Oxoid) by pour plating and incubated at 37 °C for 24 h. *Pseudomonas* spp. was
153 quantified on Pseudomonas agar base (CM0559, Oxoid) supplemented with Pseudomonas CFC
154 selective supplement SR0103 (Oxoid) by spread plating and incubated aerobically at 25 °C for 48 h.
155 *Brochotrix thermosphacta* was quantified using STAA agar base (CM0881) supplemented with STA
156 selective supplement SR0162 (Oxoid) by spread plating and incubated aerobically at 22 °C for 48 h.

157 *2.3.1 Identification of microbiota*

158 Ten colonies were picked randomly from countable, LH and Pseudomonas CFC plates on day 0 and on
159 the day of sensory rejection (day 10 and 13 for vacuum and MA-packages, respectively), and

160 repropagated minimum three times on LH and Pseudomonas CFS plates respectively. Isolates from
161 Pseudomonas CFC agar were tested for oxidase and catalase activity, and for fluorescence. To further
162 confirm that the isolates belonged to the *Pseudomonas* genus, DNA was extracted using the protocol
163 for Gram-negative bacteria in the DNeasy Blood and Tissue kit (Qiagen, Oslo, Norway), and a PCR
164 amplification was performed using the genus specific primers PA-GS-F (5'-GACGGGTGAGTAATGCCTA-
165 3') and PA-GS-R (5'-CACTGGTGTTCCTTCCTATA-3') designed by (Spilker, Coenye, Vandamme, &
166 LiPuma, 2004). PCR reactions were performed with 25 µl reactions containing 1x PCR buffer, 2.0 mM
167 $MgCl_2$, 200 µM of each deoxynucleoside triphosphates, 0.4 µM each primer, 2.5 U Taq polymerase
168 (Qiagen), and 50–100 ng template DNA. The PCR amplification cycles were as follows: Initial
169 denaturation at 95 °C for 15 min, 25 cycles of denaturation at 95 °C for 60 s, annealing for 30 s at 54
170 °C, and extension at 72 °C for 60 s, followed by a final extension at 72 °C for 5 min. PCR products
171 were run on a 1% agarosegel in 1 × Tris-acetate-EDTA buffer (Sambrook & Russel, 2001) for
172 visualizing the size of the product.

173 The isolates from LH agar were characterized by Gram staining, oxidase and catalase test, and by
174 appearance and bioluminescence ability on LH agar.

175 Sanger sequencing of the V3-V9 region of the 16S gene was performed on 24 isolates from
176 Pseudomonas CFC agar and on 54 isolates from LH agar, using the universal 16S primers 338f with
177 sequence 5'CCTACGGGAGGCAGCAG 3' (Huse et al., 2008) and 1492r with sequence
178 5'ATTACCGCGGCTGCTGG 3' (Turner, Pryer, Miao, & Palmer, 1999).

179 PCR reactions were performed with 50 µl reactions containing 1x PCR buffer (1.5 mM $MgCl_2$), 200 µM
180 of each nucleotide, 0.4 µM each primer, 2.5 U Taq polymerase (Qiagen), and 50–100 ng template
181 DNA. The PCR amplification cycles were as follows: Initial denaturation at 95 °C for 15 min, 25 cycles
182 of denaturation at 95 °C for 60 s, annealing for 30 s at 58 °C, and extension at 72 °C for 60 s, followed
183 by a final extension at 72 °C for 5 min. 10 µl of the amplified PCR reaction was run on 1% agarose gel
184 to confirm that the size of the product was 1154 bp. The remaining 40 µl of the product was purified

185 using Thermo Scientific GeneJET PCR purification Kit (Thermo Scientific, Vilnius, Lithuania). The DNA
186 concentration was measured by spectrophotometry using PowerWaveXS, BioTek® (Winooski, USA),
187 and standardised to ~50 ng/µl. The samples was prepared according to instructions for LightRun
188 Sanger sequencing from GATC Biotech. Forward and reverse sequences were assembled using
189 SeqMan ProTM application in DNASTAR® Lasergene. Identification of sequences was done by using
190 BLASTN and comparison to sequences currently available in the NCBI database
191 (www.ncbi.nlm.nih.gov/BLAST).

192

193 *2.4. pH and drip loss*

194 The pH was measured in samples used for chemical analysis at each sampling day with a Testo 206
195 pH-meter (Testo Inc., New Jersey, USA) approximately 10 minutes after the sensory judgment.

196 The drip loss (DL) throughout storage was calculated gravimetrically as the difference in sample
197 weight between the raw sample and day x (x = 3, 6, 8, 10 and 13) (Equation 2).

198 Equation 2:

199 DL = $\frac{m_0 - m_x}{m_0} \times 100$%, where

200 m_0: sample weight at t_0

201 m_x: sample weight at t_x

202 *2.5. Degradation products of adenosine triphosphate (ATP)*

203 Frozen samples (frozen after sampling to be analysed altogether, stored at – 80 °C) was shredded
204 using a kitchen grater and approximately 0.6 g (exact weight listed) was homogenized with perchloric
205 acid ($HClO_4$, 0.42M, 5 mL) for 2 min with an Ultra Turrax T25 Basic (Janke & Kunkel IKA®-
206 Labortechnik, Staufen, Germany). The sample solution was thereafter added potassium hydroxide
207 (KOH, 1M, 1.5 mL), shaken lightly and centrifuged (12000rpm, 4 °C, 10 min) in a Kubota 1700

208 centrifuge (Kubota corporation, Tokyo, Japan) before the supernatant was filtered through a nylon
209 filter (0.45µm) and transferred to HPLC vials (Agilent, 862-09-16, 2 mL) for analysis.

210 Degradation products of ATP were determined on a Phenomenex synergi 4u hydro-RP80 A
211 (150×4.6mm, 4µm) HPLC column after a method by Sellevold, Jynge, and Aarstad (1986) with slight
212 modifications. The chromatographic system used was an Agilent 1290 chromatograph (Agilent
213 technologies, Paolo Alto, CA, USA) connected to an Agilent 1260 diode array UV-VIS detector.
214 Degradation products of ATP were detected (isocratic, flow 1.0 mL/min) at 210nm (ATP and
215 adenosine diphosphate, ADP) and 260 nm (adenosine monophosphate, AMP; IMP; HxR and Hx) with
216 a mobile phase consisting of potassium hydrogen phosphate (KH_2PO_4, 0.215M) and
217 tetrabutylammonium hydrogen sulphate (0.0023M) diluted in acetonitrile (C_2H_3N, 3.5% in water).
218 The pH of the mobile phase was adjusted with KOH (0.5M) to a level of 6.25.

219 Standards of ATP (Sigma, ≥99%, CAS:34369-07-8), ADP (Sigma, ≥95%, CAS:20398-34-9), AMP (Sigma,
220 ≥99%, CAS:149022-20-8), IMP (Sigma, ≥98%, CAS:352195-40-5), HxR (Sigma, ≥99%, CAS:58-63-9) and
221 Hx (Sigma, ≥99.0% CAS:68-94-0) were prepared by dilution of crystalline powder in ion-exchanged
222 water to an end concentration of approximately 0.001M. A six point two-fold serial dilution line was
223 prepared for each compound to perform a standard curve for quantification. To identify peaks,
224 retention times (t_R) were compared with those of the standard solutions. As a final control, samples
225 were spiked with standards to ensure that they were coeluated.

226 *2.6. Biogenic amines*

227 Shredded fish muscle (as described in chapter *2.5.,* 3 g) was homogenized and extracted as described
228 by Özogul, Taylor, Quantick, and Özogul (2002) in trichloroacetic acid (TCA, 6%, 15mL) for two
229 minutes (13000rpm) with an Ultra Turax T25 Basic (Janke & Kunkel IKA®-Labortechnik, Staufen,
230 Germany). All samples were thereafter centrifuged (12000rpm, 4 °C, 10 min) in a Kubota 1700
231 centrifuge (Kubota corporation, Tokyo, Japan) before the supernatant was filtrated through a
232 cellulose acetate filter (0.45µm) and derived with benzyl-chloride (99%, Sigma-Aldrich, CAS: 98-88-4)

233 according to Özogul et al. (2002). The reaction time was set to 20 min at room temperature. Benzyl-
234 amines were thereafter extracted two times with diethyl ether. The upper organic layer was then
235 transferred to a clean tube and evaporated to dryness (N_2, 30 °C) before the residue was dissolved in
236 a mixture of acetonitrile and water (90:10).

237 Chromatographic separation of benzyl-amines where performed on an YMC triart PFP (100×2mm,
238 1.9µm) UHPLC column connected to an Agilent 1290 chromatograph (Agilent technologies, Paolo
239 Alto, CA, USA) and an Agilent 1260 diode array UV-VIS detector. All samples were detected at 254nm
240 with acetonitrile and water (90:10) as mobile phase (isocratic, flow 1.0 mL/min).

241 Standards of putrescine (putrescine dihydrochloride, Sigma, CAS: 333-93-7), cadaverine (cadaverine
242 dihydrochlorine, Sigma, CAS: 1476-39-7), spermine (spermine tetrahydrochloride, Sigma, CAS: 306-
243 67-2), sperimidine (spermidine trihydrochloride, Sigma, CAS: 334-50-9), tryptamine (tryptamine
244 hydrochloride, Aldrich, CAS: 343-94-2) and tyramine (tyramine hydrochloride, Sigma, CAS: 60-19-5)
245 were prepared according to Özogul et al. (2002) and quantified by a calibration curve prepared for
246 each of the amines in the range of 0–50 µg/mL. To identify peaks, retention times (t_R) were
247 compared with those of the standard solutions. As a final control, samples were spiked with
248 standards to ensure that they were coeluated.

249 *2.7. Statistics*

250 The data were analysed by a general linear model (GLM) with content of CO_2 (67%, 33% or 0%
251 (vacuum)), balancing gas except for those samples packaged in vacuum (O_2 or N_2), and storage time
252 (days) as fixed factors. To compare different groups, one-way ANOVA and Duncan`s comparison test
253 was used. Pearson`s correlation coefficient (r) was used to calculate the linearity dependence
254 between variables X and Y. All statistical analyses were performed using an IBM Statistical Package
255 for the Social Sciences statistics software (release 24, IBM Corporation, USA). The alpha level was set
256 to 5% ($P < 0.05$). All results are given as an average ± standard deviation (SD), unless otherwise
257 stated.

258 **3. Results and discussion**

259 Catching time and location indicated that the fish was post spawning (Olsen et al., 2010). The five
260 groups of packaging studied (*high* CO_2*/low* N_2; *low* CO_2*/high* N_2; *high* CO_2*/low* O_2; *low* CO_2*/high* O_2
261 and *vacuum*) were randomly sampled from 36 individuals and the groups did not differ in the fish to
262 gas ratio (P>0.061). Randomization of the raw material before packaging resulted in a random
263 distribution of samples from all individuals among the groups. The selection of the raw material was
264 therefore regarded as homogeneous and it is likely to believe that observed differences were caused
265 by the experimental design and not by the individual variations between individuals.

266 *3.1. Headspace gas composition*

267 The initial gas composition of the headspace were significantly altered between day zero and day
268 three (Fig. 1, P<0.001) due to a diffusion of CO_2 from the headspace to the product. CO_2 is generally
269 highly soluble in the water phase of the muscle and in fluid lipids (Gill, 1988; Sivertsvik, Rosnes, &
270 Jeksrud, 2004). In the present study, the equilibrium had occurred at the first measured point at day
271 three, which resulted in no further changes in the headspace CO_2 concentration throughout the
272 storage period (57.1 ± 3.0 and 25.1 ± 1.8% in samples packaged in high and low concentrations of
273 CO_2, respectively, Fig. 1, P>0.082). The CO_2 equilibrium was moreover found independent of the
274 balancing gas used (O_2/N_2, data not shown). Significant amounts of N_2 (1.3-4.0%) in groups packaged
275 without N_2 (*high* CO_2*/low* O_2 and *low* CO_2*/high* O_2) were probably related to an analytical artefact and
276 or to diffusion of gasses through the PA/PE surrounding film.

277 *3.2. Sensory evaluation*

278 The use of O_2 as balancing gas did not give any main improvement to the sensory quality compared
279 to nitrogen only observing off-odours. The judges did however describe the off-odours of saithe
280 packaged in a mix of CO_2 and O_2 as different compared to those packaged in CO_2 and N_2. Rejection of
281 saithe packaged in an atmosphere consisting of CO_2 and O_2 were mainly based on butter-like off-
282 odours whereas TMA-ammonium-like off-odours dominated in packages with an atmosphere of CO_2

283 and N_2, and in vacuum packages. The effect of MAP on the development of off-odours was however
284 found to be significant ($P < 0.001$) resulting in a rejection of vacuum packaged saithe at day 10 (Fig.
285 2). All other groups were rejected at day 13. Vacuum packaging of marine species is earlier reported
286 to decrease the sensory shelf-life compared to MAP (Dalgaard, Gram, & Huss, 1993; Hansen, Moen,
287 Rødbotten, Berget, & Pettersen, 2016). In both Dalgaard et al. (1993) and Hansen et al. (2016) the
288 effect of CO_2 on the shelf-life were found to be significant with an increase of shelf-life of 6-7 days
289 compared to vacuum packaging. The use of O_2 is moreover found beneficial for the shelf-life of
290 Atlantic cod (Sivertsvik, 2007) but as far as we know there is a limited amount of literature regarding
291 effects of O_2 on the overall quality of marine fish species (DeWitt & Oliveira, 2016).

292 *3.3. Microbiological activity*

293 The mean initial APC in saithe filets, enumerated on IA, was 3.1 ± 0.2 log CFU/g and was
294 approximately at the same level as reported for other fresh marine fish filets (Hoel, Mehli, Bruheim,
295 Vadstein, & Jakobsen, 2015; Rotabakk, Birkeland, Lekang, & Sivertsvik, 2008), but considerably lower
296 than Kuuliala et al. (2018) reported for Atlantic cod filets. In vacuum-packed filet, the APC increased
297 to 6.0 ± 0.9 log CFU/g at sensory rejection time on day 10 (Fig. 2A). Evolution of APC was significantly
298 inhibited in MAP-samples with high content of CO_2 compared to those packed in low CO_2 or vacuum
299 (Fig. 3A, GLM, $P < 0.001$). Compared to O_2, use of nitrogen as balancing gas significantly reduced APC
300 (Fig. 3A, GLM, $P = 0.015$). During storage, APC increased slowly (Δ log CFU/g < 0.8) the first six days
301 for samples with high CO_2 content and for samples with nitrogen as balancing gas, whereas APC in
302 samples packed in low CO_2 combined with O_2 or vacuum rapidly increased (Δ log CFU/g > 2.0). When
303 all samples where sensory rejected at day 13, APC reached 5.1 CFU/g for samples with high CO_2
304 concentration. Low CO_2 concentration resulted in APC of 5.8 log CFU/g (N_2 as balancing gas) and 6.8
305 log CFU/g (O_2 as balancing gas). There are no specific microbiological criteria's available for APC of
306 fish filets, but most guidelines states that APC between 6 and 7 log CFU/g is of borderline quality, and

307 filets with more than 7 log CFU/g should be considered as unsatisfactory. (Stannard, 1997; Food
308 Safety autority of Ireland, 2016; The Centre for Food Safety, 2014).

309 APC can be used as a general indicator for remaining shelf-life, but quantification of APC do not
310 indicate the presence or absence of specific spoilage organisms that contributes to production of off-
311 odours and spoilage metabolites (Gram and Dalgaard, 2002). Sensory rejection of MAP products can
312 occur before APC indicate spoilage (e.g. APC 5-6 log CFU/g) due to non-microbiological degradation

313 The mean initial concentration of PC in saithe filets was 3.6 ± 0.4 log CFU/g. PC was enumerated on
314 LH agar to support growth of the *Photobacterium* spp. Broekaert, Heyndrickx, Herman, Devlieghere,
315 and Vlaemynck (2011) demonstrated that LH and marine agar plates obtains the best quantitative
316 data for marine bacteria in seafood. Proliferation of PC was significantly affected by the gas
317 composition (Fig. 3B; GLM, $P < 0.001$). PC in vacuum-packaged filets increased rapidly and reached a
318 maximum level of 9.1 ± 1.0 log CFU/g at day eight (Fig. 3B). High CO_2 concentrations (independent of
319 balancing gas (O_2/N_2) significantly reduced proliferation of PC compared to low CO_2 concentrations or
320 vacuum (GLM $P < 0.001$). In samples with N_2 as balancing gas, PC increased to a maximum level of 7.9
321 ± 0.1 log CFU/g at day 10 (*low* CO_2*/high* N_2) and 7.1 ± 0.4 log CFU/g (*high* CO_2*/low* N_2) at day 13.
322 Furthermore, packages balanced with O_2 demonstrated significantly slower proliferation of PC
323 compared to those balanced with N_2 (GLM $P < 0.001$). At day 13, the lowest PC of 5.7 ± 1.1 log CFU/g
324 was observed for *high* CO_2*/low* O_2 – packages. In the present study, approximately the same counts
325 was observed on LH and IA media at day 0, but later during storage higher counts are generally
326 observed on LH than IA medium for all samples analysed (Fig. 3). Kuuliala et al. (2018) reported
327 higher counts on marine agar than IA medium for cod filets during storage due to the dominance of
328 *Photobacterium* spp .

329 Initially, H_2S-producing bacteria were only sporadically detected at levels below the quantification
330 limit (2.4 log CFU/g). This is in accordance with other studies (Gram & Dalgaard, 2002; G. Olafsdottir,
331 Lauzon, Martinsdottir, & Kristbergsson, 2006) as H_2S-producing bacteria normally constitute only a

332 minor fraction of the microbiota on newly caught fish. The evolution of H_2S-produsing bacteria was
333 significantly affected by the experimental design (GLM, $P < 0.001$) and these bacteria were only
334 detected in vacuum-samples (maximum 4.1 log CFU/g at day 10) and in *low* CO_2*/high* N_2 samples
335 (maximum 5.5 log CFU/g at dag 13). A high CO_2 concentration seems necessary to inhibit H_2S-
336 producing bacteria if N_2 is used as balancing gas. Boskou and Debevere (1998) demonstrated that the
337 H_2S-producing *S.putrefaciens* is unable to develop if CO_2 concentrations exceed 50% in MA-packed
338 fish products. However, Kuuliala et al. (2018) demonstrated growth of H_2S-producers in MA-packed
339 cod with 60% CO_2. In the present study, a positive correlation between H_2S-produsing bacteria and
340 storage time was found for saithe packaged in *vacuum* (r=0.66, P<0.01) and in *low* CO_2*/high* N_2 (r =
341 0.90, P < 0.01). A maximum level of 5.5.-5.6 log CFU/g was observed, at day eight (vacuum) and day
342 13 (*low* CO_2*/high* N_2). However, these counts are probably too low to contribute to spoilage odour
343 (Olafsdottir, Jónsdóttir, Martinsdóttir, 2006). In our study, H_2S producers were completely inhibited
344 in MAP-samples containing O_2. This is in accordance with Sivertsvik (2007), which stated that H_2S-
345 producing bacteria did not grow in farmed cod packaged under different atmospheres consisting of
346 O_2. Kuuliala et al. (2018) found that the growth rate of H_2S- producers were lower at high O_2-
347 concentrations, but the growth were not completely inhibited. In seafood packed in both vacuum
348 and CO_2-enriched modified atmosphere the quantity of LAB during storage are reported to be higher
349 than achieved during storage in air (Leroi, 2010). In this study, LAB was not detected at day zero. The
350 growth of LAB was strongly affected by the experimental design (Fig. 3C, GLM, $P < 0.001$). Packages
351 with *low* CO_2*/high* O_2 allowed LAB to evolve, resulting in LAB counts of 5.8 ± 1.2 log CFU/g at day 13.
352 In vacuum-packages LAB increased to 4.2 ± 0.5 log CFU/g at day 10. The other gas mixtures ended up
353 at LAB counts of 3.8-3.9 log CFU/g at the end of storage. MAP (with and without O_2) often favors
354 development of LAB in fresh fish, as elevated CO_2 concentrations generally favour growth of LAB
355 (Leroi, 2010). However, in our study, LAB evolved better in *low* CO_2*/high* O_2 than in *high* CO_2*/low* O_2.
356 Kuuliala et al. (2018) reported growth of LAB in MA-packed cod to be independent of O_2
357 concentration if balanced with 60% CO_2. In MA-packaged chilled beef, high O_2-concentrations (80%

358 O_2, 20% CO_2) selected for *Leuconostoc* spp., a LAB that produce a buttery off-odour caused by
359 formation of diacetyl and acetoin (Jaaskelainen, et al., 2016). The buttery off-odour observed in
360 spoiled saithe packed with CO_2 and O_2 might indicate that *Leconostoc* spp. were present. In MA-
361 packed cod, Kuulila et al. 2018 detected *Carnobacterium* spp. at both high (40%) and low O_2 (5%)
362 concentrations. At high CO_2 concentrations (above 60%), *Lactobacillus* spp. are often found to
363 dominate in MA-packaged cod fillets (Sivertsvik, et al., 2002).

364 *Pseudomonas* spp. were detected in all samples at all sampling points. The MAP conditions used in
365 the experimental design affected the evolution of *Pseudomonas* spp. (Fig. 3D, GLM, $P < 0.001$). The
366 initial level in saithe filets was to 3.2 ± 0.6 log CFU/g., Growth of *Pseudomonas* spp. were strongly
367 inhibited in *high CO_2/low N_2*-samples, as the counts did not exceed the initial level during the storage
368 period of 13 days. A positive correlation between *Pseudomonas* counts and storage time was found
369 for vacuum ($r = 0.577$, $P = 0.031$) and low CO_2/high O_2 ($r = 0.88$, $P < 0.01$) samples only. High CO_2
370 concentration independent of balancing gas (O_2/N_2) significantly reduced growth (GLM, $P = 0.001$)
371 compared to low CO_2 and vacuum. N_2 as a balancing gas (independent of the CO_2 concentration)
372 significantly reduced the growth of *Pseudomonas* compared to packages with O_2 or vacuum (GLM, P
373 = 0.013). The highest level of *Pseudomonas* spp. counts observed was 6.0 ± 0.4 log CFU/g, found in
374 *low CO_2/high O_2*-samples at day 13.

375 *Brochothrix thermosphacta* was sporadically detected, but reached counts above quantification level
376 only at day 13 in *low CO_2/high O_2*-samples where a maximum of 3.8 log ± 0.5 CFU/g was reached.
377 Enterobacteriaceae was sporadically detected, but below recommended quantification level of 2.3
378 log CFU/g (NCFA, 2005 No.144) at all times for all samples.

379 *3.3.1. Microbiological ecology*

380 Among the isolates from LH agar six genera were represented (Fig. 4). Initial microbiota (day 0)
381 consisted of *Photobacterium* spp. (40%), *Psycrobacter spp.* (30%), *Shewanella spp.* (10%),
382 *Flavobacterium spp.* (10%), and *Arthrobacter spp.* (10%). All of these, except from *Arthrobacter* spp.

383 are marine bacteria often found in fresh fish products (Kuuliala et al., 2018; Moretro et al., 2016;
384 Sivertsvik et al., 2002). The microbiota at day 0 was more diverse compared to the microbiota
385 isolated at end of the product shelf life (day 10 and 13 for vacuum and MA-packages, respectively, P
386 = 0.017). The microbiota isolated from LH agar sampled at day 13 from saithe packed in high CO_2/low
387 N_2 or low CO_2/high N_2 constituted of *Photobacterium spp.* as dominating genus (90 and 80 %
388 respectively) and *Shewanella spp.* (10 and 20 % respectively). Among *Photobacterium* spp., *P.*
389 *phosphoreum* was the most prevalent species identified with the highest score in GenBank. *P.*
390 *phosphoreum* and *Pseudomonas* spp. (40% each), and *Arthrobacter* spp. (10%) were the detected
391 genera picked from LH agars sampled from saithe packed in low CO_2/high O_2. *Pseudomonas* spp. (50
392 %). and *Photobacterium* spp. (50%) were the only two genus identified from LH plates sampled from
393 saithe packed in high CO_2/low O_2.

394 In vacuum packages, *Photobacterium* spp. (60%) and *Shewanella* spp. (30%) were the only two genus
395 detected. *P. phosphoreum* was present in all different gas mixtures as well as in the initial sample.
396 This corresponds to earlier findings of Dalgaard et al. (1997) and Kuuliala et al. (2018).
397 *Photobacterium spp*. and the *Shewanella spp*. genus were dominant in relative abundance in
398 packages with N_2 as balancing gas, while *Pseudomonas* spp. and *Photobacterium* spp. were dominant
399 with O_2 as balancing gas.

400 Among the 72 isolates from Pseudomonas CFC agar all were oxidase positive while eight (11%) were
401 catalase negative. 36 (50%) of the isolates were showing fluorescence. PCR amplification with
402 Pseudomonas genus specific primers PA-GS-F/R produced products of the correct size for 61 (85%) of
403 the isolates, while no product was detected for the other 11 isolates (15%). The eight catalase
404 negative isolates are among these. Sequence analyses of the 16S rRNA gene revealed that the
405 catalase negative isolates were *Shewanella spp.* (99% ID), which is commonly found in fresh fish
406 products, in processing plants (Moretro, Moen, Heir, Hansen, & Langsrud, 2016; Vogel,
407 Venkateswaran, Satomi, & Gram, 2005) and in MA-packaged cod (Stenstrom, 1985). Catalase positive

408 isolates that were negative in PCR with Pseudomonas specific primers appeared to be

409 *Pseudoalteromonas spp.* (100% ID), which is a common bacteria in marine waters and in association

410 with other marine organisms (Holmstrom & Kjelleberg, 1999). All the other sequenced isolates

411 proved to be *Pseudomonas spp.* (99-100% ID). Identification on species level was not possible as the

412 BLASTn search resulted in the same score for several different species.

413 *3.4. Drip loss and pH*

414 DL from muscle foods occur due to changed capacity of the muscle structure to retain its natural

415 water (Huff-Lonergan & Lonergan, 2005, 2007). The DL from codfish fillets consist mainly of water

416 and proteins and is affected by a drop in muscle pH due to anaerobic glycolysis and ultra-structural

417 changes during *rigor mortis* (Ofstad et al., 1996). A rapid drop of pH can in all likelihood, also lead to

418 denaturation of muscle and sarcoplasmic proteins (Bendall & Wismer-Pedersen, 1962). In the

419 present study, the DL and muscle pH during storage of MA-packaged saithe was significantly affected

420 by the experimental design (GLM, $P < 0.001$, and $P = 0.032$, respectively). O_2 as a balancing gas

421 (independent of the CO_2 concentration) gave lower DL than N_2 (Fig. 5A), where highest DL was

422 observed for saithe packaged in *high CO_2/low N_2* (19.4 ± 2.4 %). It was moreover found that an high

423 CO_2 concentration (independent of the balancing gas) resulted in a significant higher DL compared to

424 those packaged in low CO_2 ($P = 0.032$, Fig. 5B). Vacuum packaged saithe showed similar DL as those

425 packaged in high CO_2. The squeezing effect obtained in vacuum packages may however increase the

426 DL and make them not comparable to those packaged in MAP.

427 Significant lower muscle pH was observed in packages balanced with O_2 compared to those balanced

428 with N_2 ($P < 0.023$, on average: 6.28 ± 0.2 and 6.39 ± 0.2, respectively). The highest pH was however

429 shown in vacuum packaged saithe (on average: 6.44 ± 0.2) indicating an acidification when CO_2 is

430 used in the gas mixture. The muscle pH was however not affected by the different concentration of

431 CO_2 in the headspace (GLM, $P > 0.15$), and did not correlate significantly with the DL ($r = -0.22$, $P >$

432 0.063). It is therefore likely to believe that the observed differences in pH did not have any practical

433 effect on product.

434 The positive effect of O_2 on DL (Fig. 5A) is probably related to the effect of O_2 to inhibit

435 psychrotrophic bacteria such as *Shewanella* spp. and reduced counts of *Photobacterium spp.*

436 (especially at high O_2 concentration) (Fig. 3B and Fig. 4) that are known to catalyse the reduction of

437 TMAO to TMA (Debevere & Boskou, 1996; Gram & Dalgaard, 2002; Hovda, Lunestad, Sivertsvik, &

438 Rosnes, 2007). TMAO has a function in marine fishes as an osmolyte to control osmotic stress

439 (Yancey, Clark, Hand, Bowlus, & Somero, 1982). Slower reduction of TMAO will in all likelihood

440 remain the osmolytical capacity of muscle cells and counteract DL during storage. The significant

441 effect of O_2 on the post mortem turnover of ATP (Table 1, Fig. 6) can also be of significant importance

442 and may influence the DL during storage (discussed in detail in chapter 3.5).

443 *3.5. Degradation products of ATP*

444 A significant effect of the modified atmosphere was found on the muscle concentration of HxR and

445 Hx (GLM, $P < 0.001$, Table 1) whereas the concentration of ADP and IMP were affected by storage

446 time (GLM, $P < 0.018$) but not by the atmosphere used ($P > 0.45$, data not shown). AMP was found in

447 low concentrations (on average 0.39 ± 1.1 µmol/g) but was not affected by the experimental design

448 (GLM, $P > 0.46$, data not shown). ATP was moreover not detected in any of the samples (Gram &

449 Huss, 1996; Surette et al., 1988).

450 The highest content of HxR was on average found at day three (98.4 ± 23.2 µmol/g) whereas

451 significant differences between the groups were observed at day eight and continued throughout the

452 storage period. Fastest reduction of HxR during storage was observed in vacuum packaged saithe

453 followed by saithe packaged in a modified atmosphere consisting of CO_2, with N_2 and O_2 as balancing

454 gas, respectively. Coincidental with the reduction of HxR an increase of Hx was observed ($r = -0.71$, P

455 < 0.001), which resulted in significantly highest content of Hx in vacuum packaged samples at day 10

456 ($P < 0.001$, 246 ± 34 µmol/g). At day 10, lowest content of Hx was found in *low CO_2/high O_2* –

457 packages (69 ± 5 µmol/g) followed by *high CO_2/low O_2* – packages (78.5 ± 4 µmol/g). Saithe packaged
458 with N_2 as balancing gas independent of CO_2 concentration (Hx on average: 144 µmol/g) were placed
459 in between those packaged with O_2 as balancing gas and those packaged in vacuum. This
460 corresponded with the rejection of those samples by the sensory panel. Vacuum packaged codfish is
461 commonly traded in Norway, but as shown in the present study, that might not be the optimal
462 solution. At day 13, independent of the CO_2 concentration, significantly higher contents of Hx was
463 observed in samples packaged in N_2 as compared to those packaged in O_2 (Fig. 6, on average: 187.2 ±
464 36.8 and 86.1 ± 37.4, respectively). The content of Hx correlated significantly with the PC, H_2S-
465 producing bacteria, LAB and *Pseudomonas* counts (r = 0.79, 0.65, 0.52 and 0.29 respectively, $P < $
466 0.001). The amount of Hx at the end of shelf life (10 days for fillets packaged in vacuum and 13 days
467 of those packaged in MAP, respectively) did moreover indicate that the conversion of HxR to Hx is
468 mostly affected by the growth of *Shewanella* spp. and *Photobacterium* spp. (Fig. 4).

469 *3.6. Contents of biogenic amines*

470 Significant effects of the modified atmosphere were found on the contents of putrescine, cadaverine,
471 spermine and sperimidine (Table 2). The concentration were however found to be low, except for
472 cadaverine that is known to be a useful indicator of the initial stage of fish decomposition (Bulushi et
473 al., 2009). The high SD observed within specific groups (presented in Table 2) indicated however that
474 the formation of amines was both sample specific (affected by the raw material) and affected by the
475 experimental design. The formation of cadaverine was found to increase during storage (P = 0.029)
476 and was affected by the balancing gas (O_2 versus N_2, GLM, P = 0.001, on average: 1.9 ± 3.4 and 36.9 ±
477 32.3 µmol/g, respectively). At day 10, the lowest content of cadaverine was found saithe packaged in
478 *low CO_2/high O_2* (1.4 ± 1.2 µmol/g) whereas those packaged in high and low concentration of CO_2/N_2
479 and those in vacuum showed the highest (ranged between 21-37 µmol/g). In an earlier study by
480 Baixas-Nogueras, Bover-Cid, Veciana-Nogues, Marine-Font, and Vidal-Carou (2005) *Shewanella spp.*
481 was found to be the main contributor to the formation of cadaverine in Meditarian hake (*Merliuccius*

482 *merluccius*). In the present study, this was confirmed, with higher amounts of cadaverine found in
483 fish samples stored in vacuum or in an atmosphere consisting of CO_2 and N_2. This results coincided
484 with the microbiological ecology of those samples where considerable amounts of *Shewanella spp.*
485 was found (Fig. 4). The content of tryptamine and tyramine were moreover found to be low (< 0.6
486 µmol/g) and not affected by the experimental design (data not shown).

487 **4. Conclusion**

488 It is concluded that the specific gas mixtures used in MA-packaging of saithe fillets affected the
489 overall quality of the product where the main discriminant was the use of O_2 as part of the gas
490 mixture. The use of O_2 (33 or 67%) in combination with CO_2 (67 or 33%) resulted in reduced
491 proliferation of PC, inhibition of H_2S-producing bacteria, reduced contents of Hx and biogenic amines
492 in the muscle, and reduced DL from the muscle during storage, as compared to saithe packaged in
493 CO_2/N_2 or vacuum. It is moreover concluded that a high content of CO_2 is necessary to inhibit H_2S-
494 producing bacteria. Vacuum-packaged saithe was found to stand out negatively with highest DL,
495 reduced sensory shelf-life and physiochemical and microbial quality.

496 The different gas mixtures applied affected the microbial ecology present in the product. The main
497 spoilage bacterial genus identified on LH agar was *Photobacterium* spp. and in particular *P.*
498 *phosphoreum*. Together with *Pseudomonas* spp. the genera were found in all the different gas
499 mixtures used for the MA-packaging of saithe, while *Shewanella* spp. was not identified from the gas
500 mixture *low* CO_2*/high* O_2. It is moreover concluded that the conversion of HxR to Hx is mostly
501 affected by the growth of *Shewanella* spp. and *Photobacterium* spp.

502 **Acknowledgment**

503 This work was supported by the Regional Research Found Mid-Norway (project 248954). The authors
504 wish to thank the staff at Dolmøy Seafood AS and staff and students at Norwegian University of
505 Science and Technology (NTNU, Trondheim) for excellent technical support.

506 **References**

507 Baixas-Nogueras, S., Bover-Cid, S., Veciana-Nogues, M. T., Marine-Font, A., & Vidal-Carou, M. C.
508 (2005). Biogenic amine index for freshness evaluation in iced Mediterranean hake
509 (Merluccius merluccius). *J Food Prot, 68*(11), 2433-2438.
510 Bendall, J. R., & Wismer-Pedersen, J. (1962). Some Properties of the Fibrillar Proteins of Normal and
511 Watery Pork Muscle. *Journal of Food Science, 27*(2), 144-159. doi: 10.1111/j.1365-
512 2621.1962.tb00074.x
513 Boskou, G., & Debevere, J. (1998). In vitro study of TMAO reduction by Shewanella putrefaciens
514 isolated from cod fillets packed in modified atmosphere. *Food Addit Contam, 15*(2), 229-236.
515 doi: 10.1080/02652039809374634
516 Broekaert, K., Heyndrickx, M., Herman, L., Devlieghere, F., & Vlaemynck, G. (2011). Seafood quality
517 analysis: Molecular identification of dominant microbiota after ice storage on several general
518 growth media. *Food Microbiol, 28*(6), 1162-1169. doi: 10.1016/j.fm.2011.03.009
519 Bulushi, I. A., Poole, S., Deeth, H. C., & Dykes, G. A. (2009). Biogenic Amines in Fish: Roles in
520 Intoxication, Spoilage, and Nitrosamine Formation—A Review. *Critical Reviews in Food*
521 *Science and Nutrition, 49*(4), 369-377. doi: 10.1080/10408390802067514
522 Carlucci, D., Nocella, G., De Devitiis, B., Viscecchia, R., Bimbo, F., & Nardone, G. (2015). Consumer
523 purchasing behaviour towards fish and seafood products. Patterns and insights from a
524 sample of international studies. *Appetite, 84*(0), 212-227. doi:
525 http://dx.doi.org/10.1016/j.appet.2014.10.008
526 Dalgaard, P. (1995). Qualitative and quantitative characterization of spoilage bacteria from packed
527 fish. *Int J Food Microbiol, 26*(3), 319-333.
528 Dalgaard, P. (2000). *Freshness, quality and safety in seafoods*. Dublin, Ireland: The national food
529 center.
530 Dalgaard, P., Gram, L., & Huss, H. H. (1993). Spoilage and shelf-life of cod fillets packed in vacuum or
531 modified atmospheres. *Int J Food Microbiol, 19*(4), 283-294.
532 Dalgaard, P., Mejlholm, O., Christiansen, T. J., & Huss, H. H. (1997). Importance of Photobacterium
533 phosphoreum in relation to spoilage of modified atmosphere-packed fish products. *Letters in*
534 *Applied Microbiology, 24*(5), 373-378. doi: 10.1046/j.1472-765X.1997.00152.x
535 Debevere, J., & Boskou, G. (1996). Effect of modified atmosphere packaging on the TVB/TMA-
536 producing microflora of cod fillets. *International Journal of Food Microbiology, 31*(1–3), 221-
537 229. doi: http://dx.doi.org/10.1016/0168-1605(96)01001-X
538 DeWitt, C. A. M., & Oliveira, A. C. M. (2016). Modified Atmosphere Systems and Shelf-life Extension
539 of Fish and Fishery Products. *Foods, 5*(3), 48. doi: 10.3390/foods5030048
540 Easter, M. C., Gibson, D. M., & Ward, F. B. (1983). The Induction and Location of Trimethylamine-N-
541 oxide Reductase in Alteromonas sp. NCMB 400. *Microbiology, 129*(12), 3689-3696. doi:
542 doi:10.1099/00221287-129-12-3689
543 Food safety authority of Ireland, 2016. "Guidance note No. 3: Guidelines for the Interpretation of
544 Results of Microbiological Testing of Ready-to-Eat Foods Placed on the Market (Revision 2).
545 Guidance note No. 3: Guidelines for the Interpretationof Results of Microbiological Available
546 at: https://www.fsai.ie/food_businesses/micro_criteria/guideline_micro_criteria.html
547 Gill, C. O. (1988). The solubility of Carbon Dioxide in Meat. *Meat Science, 22*(1), 65-71.
548 Gram, L., & Dalgaard, P. (2002). Fish spoilage bacteria--problems and solutions. *Curr Opin Biotechnol,*
549 *13*(3), 262-266.
550 Gram, L., & Huss, H. H. (1996). Microbiological spoilage of fish and fish products. *Int J Food Microbiol,*
551 *33*(1), 121-137.
552 Guldager, H. S., Boknaes, N., Osterberg, C., Nielsen, J., & Dalgaard, P. (1998). Thawed cod fillets spoil
553 less rapidly than unfrozen fillets when stored under modified atmosphere at 2 degrees C. *J*
554 *Food Prot, 61*(9), 1129-1136.

555 Hansen, A. Å., Moen, B., Rødbotten, M., Berget, I., & Pettersen, M. K. (2016). Effect of vacuum or
556 modified atmosphere packaging (MAP) in combination with a CO2 emitter on quality
557 parameters of cod loins (Gadus morhua). *Food Packaging and Shelf-life, 9*, 29-37. doi:
558 http://dx.doi.org/10.1016/j.fpsl.2016.05.005
559 Hansen, A. Å., Mørkøre, T., Rudi, K., Olsen, E., & Eie, T. (2007). Quality Changes during Refrigerated
560 Storage of MA-Packaged Pre-rigor Fillets of Farmed Atlantic Cod (Gadus morhua L.) Using
561 Traditional MAP, CO2 Emitter, and Vacuum. *Journal of Food Science, 72*(9), M423-M430. doi:
562 10.1111/j.1750-3841.2007.00561.x
563 Hoel, S., Mehli, L., Bruheim, T., Vadstein, O., & Jakobsen, A. (2015). Assessment of microbiological
564 quality of retail fresh sushi from selected sources in Norway. *Journal of Food Protection (in
565 press)*.
566 Holmstrom, C., & Kjelleberg, S. (1999). Marine Pseudoalteromonas species are associated with higher
567 organisms and produce biologically active extracellular agents. *FEMS Microbiol Ecol, 30*(4),
568 285-293.
569 Hovda, M. B., Lunestad, B. T., Sivertsvik, M., & Rosnes, J. T. (2007). Characterisation of the bacterial
570 flora of modified atmosphere packaged farmed Atlantic cod (Gadus morhua) by PCR-DGGE of
571 conserved 16S rRNA gene regions. *International Journal of Food Microbiology, 117*(1), 68-75.
572 doi: https://doi.org/10.1016/j.ijfoodmicro.2007.02.022
573 Howgate, P. (2005). Kinetics of degradation of adenosine triphosphate in chill-stored rainbow trout
574 (Oncorhynchus mykiss). *International Journal of Food Science & Technology, 40*(6), 579-588.
575 doi: 10.1111/j.1365-2621.2005.00924.x
576 Huff-Lonergan, E., & Lonergan, S. M. (2005). Mechanisms of water-holding capacity of meat: The role
577 of postmortem biochemical and structural changes. *Meat Science, 71*(1), 194-204. doi:
578 http://dx.doi.org/10.1016/j.meatsci.2005.04.022
579 Huff-Lonergan, E., & Lonergan, S. M. (2007). New frontiers in understanding drip loss in pork: recent
580 insights on the role of postmortem muscle biochemistry. *Journal of Animal Breeding and
581 Genetics, 124*, 19-26. doi: 10.1111/j.1439-0388.2007.00683.x
582 Huse, S. M., Dethlefsen, L., Huber, J. A., Mark Welch, D., Relman, D. A., & Sogin, M. L. (2008).
583 Correction: Exploring Microbial Diversity and Taxonomy Using SSU rRNA Hypervariable Tag
584 Sequencing. *PLoS Genetics, 4*(12), 10.1371/annotation/1373d1378a6578-ce1356-1345aa-
585 bc1371-05078355b05078851. doi: 10.1371/annotation/3d8a6578-ce56-45aa-bc71-
586 05078355b851
587 Jaaskelainen, E., et al., 2016. Development of spoilage bacterial community and volatile compounds
588 in chilled beef under vacuum or high oxygen atmospheres. Int J Food Microbiol. 223, 25-32.
589 Kassemsarn, B.-O., Perez, B. S., Murray, J., & Jones, N. R. (1963). Nucleotide Degradation in the
590 Muscle of Iced Haddock (Gadus aeglefinus), Lemon Sole (Pleuronectes microcephalus), and
591 Plaice (Pleuronectes platessa). *Journal of Food Science, 28*(1), 28-37. doi: 10.1111/j.1365-
592 2621.1963.tb00155.x
593 Kuuliala, L., Al Hage, Y., Ioannidis, A. G., Sader, M., Kerckhof, F. M., Vanderroost, M., Boon, N., De
594 Baets, B., De Meulenaer, B., Regaert, P., Devlieghere, F. (2018). Microbiological, chemical and
595 sensory spoilage analysis of raw Atlantic cod (Gadus morhua) stored under modified
596 atmospheres. *Food Microbiology, 70*(Supplement C), 232-244. doi:
597 https://doi.org/10.1016/j.fm.2017.10.011
598 Leroi, F. (2010). Occurrence and role of lactic acid bacteria in seafood products. *Food Microbiology,
599 27*(6), 698-709. doi: http://dx.doi.org/10.1016/j.fm.2010.05.016
600 Macé, S., Joffraud, J.-J., Cardinal, M., Malcheva, M., Cornet, J., Lalanne, V., Chevalier, F., Sérot, T.,
601 Pilet, M-F., Dousset, X. (2013). Evaluation of the spoilage potential of bacteria isolated from
602 spoiled raw salmon (Salmo salar) fillets stored under modified atmosphere packaging.
603 *International Journal of Food Microbiology, 160*(3), 227-238. doi:
604 http://dx.doi.org/10.1016/j.ijfoodmicro.2012.10.013

605 Moretro, T., Moen, B., Heir, E., Hansen, A. A., & Langsrud, S. (2016). Contamination of salmon fillets
606 and processing plants with spoilage bacteria. *Int J Food Microbiol, 237*, 98-108. doi:
607 10.1016/j.ijfoodmicro.2016.08.016
608 NCFA. (2005). Enterobacteriaceae. Determination in foods and feeds., Nordic committee on food
609 analysis. No. 144.
610 NCFA. (2006). Aerobic count and specific spoilage organisms in fish and fish products. *Nordic*
611 *committee on food analysis*, No. 184.
612 Ofstad, R., Egelandsdal, B., Kidman, S., Myklebust, R., Olsen, R. L., & Hermansson, A.-M. (1996).
613 Liquid loss as effected by post mortem ultrastructural changes in fish muscle: Cod (Gadus
614 morhuaL) and salmon (Salmo salar). *Journal of the Science of Food and Agriculture, 71*(3),
615 301-312. doi: 10.1002/(SICI)1097-0010(199607)71:3<301::AID-JSFA583>3.0.CO;2-0
616 Olafsdottir, G., Lauzon, H. L., Martinsdottir, E., & Kristbergsson, K. (2006). Influence of storage
617 temperature on microbial spoilage characteristics of haddock fillets (Melanogrammus
618 aeglefinus) evaluated by multivariate quality prediction. *Int J Food Microbiol, 111*(2), 112-
619 125. doi: 10.1016/j.ijfoodmicro.2006.04.045
620 Olafsdottir, G. L., H.L.; Jónsdóttir, R.; Martinsdóttir, E. (2006, 8-9th May). *Multivariate quality*
621 *prediction of cod (gadus morhua) and haddock fillets (melanogrammus aeglefinus) stored*
622 *under superchilling and temperature abusive conditions.* Paper presented at the 2nd
623 Workshop "Cold-Chain-Management", Bonn.
624 Olsen, E., Aanes, S., Mehl, S., Holst, J. C., Aglen, A., & Gjøsæter, H. (2010). Cod, haddock, saithe,
625 herring, and capelin in the Barents Sea and adjacent waters: a review of the biological value
626 of the area. *ICES Journal of Marine Science, 67*(1), 87-101. doi: 10.1093/icesjms/fsp229
627 Powell, S. M., & Tamplin, M. L. (2012). Microbial communities on Australian modified atmosphere
628 packaged Atlantic salmon. *Food Microbiol, 30*(1), 226-232. doi: 10.1016/j.fm.2011.10.002
629 Rotabakk, B. T., Birkeland, S., Lekang, O. I., & Sivertsvik, M. (2008). Enhancement of Modified
630 Atmosphere Packaged Farmed Atlantic Halibut (Hippoglossus Hippoglossus) Fillet Quality by
631 Soluble Gas Stabilization. *Food Science and Technology International, 14*(2), 179-186. doi:
632 10.1177/1082013208092051
633 Rudi, K., Maugesten, T., Hannevik, S. E., & Nissen, H. (2004). Explorative Multivariate Analyses of 16S
634 rRNA Gene Data from Microbial Communities in Modified-Atmosphere-Packed Salmon and
635 Coalfish. *Applied and Environmental Microbiology, 70*(8), 5010-5018. doi:
636 10.1128/aem.70.8.5010-5018.2004
637 Sambrook, J., & Russel, D. W. (2001). *Molecular Cloning – A Laboratory Manual* (3rd ed.). Cold Spring
638 Harbor, New York: Cold Spring Harbor Laboratory Press.
639 Sellevold, O. F. M., Jynge, P., & Aarstad, K. (1986). High performance liquid chromatography: a rapid
640 isocratic method for determination of creatine compounds and adenine nucleotides in
641 myocardial tissue. *Journal of Molecular and Cellular Cardiology, 18*(5), 517-527. doi:
642 10.1016/s0022-2828(86)80917-8
643 Sivertsvik, M. (2007). The optimized modified atmosphere for packaging of pre-rigor filleted farmed
644 cod (Gadus morhua) is 63ml/100ml oxygen and 37ml/100ml carbon dioxide. *LWT - Food*
645 *Science and Technology, 40*(3), 430-438. doi: http://dx.doi.org/10.1016/j.lwt.2005.12.010
646 Sivertsvik, M., Jeksrud, W. K., & Rosnes, J. T. (2002). A review of modified atmosphere packaging of
647 fish and fishery products – significance of microbial growth, activities and safety.
648 *International Journal of Food Science & Technology, 37*(2), 107-127. doi: 10.1046/j.1365-
649 2621.2002.00548.x
650 Sivertsvik, M., Rosnes, J. T., & Jeksrud, W. K. (2004). Solubility and absorption rate of carbon dioxide
651 into non-respiring foods. Part 2: Raw fish fillets. *Journal of Food Engineering, 63*(4), 451-458.
652 doi: http://doi.org/10.1016/j.jfoodeng.2003.09.004
653 Spilker, T., Coenye, T., Vandamme, P., & LiPuma, J. J. (2004). PCR-based assay for differentiation of
654 Pseudomonas aeruginosa from other Pseudomonas species recovered from cystic fibrosis
655 patients. *J Clin Microbiol, 42*(5), 2074-2079.

656 Stannard, C. (1997). Development and use of microbiological criteria for foods. *Food Science and*
657 *Technology Today, 1*(3), 137-176.
658 Stenstrom, I. M. (1985). Microbial flora of cod fillets (Gadus morhua) stored at 2 degrees C in
659 different mixtures of carbon dioxide and nitrogen/oxygen. *Journal of Food Protection, 48*(7),
660 585-589.
661 Surette, M. E., Gill, T. A., & LeBlanc, P. J. (1988). Biochemical basis of postmortem nucleotide
662 catabolism in cod (Gadus morhua) and its relationship to spoilage. *Journal of Agricultural and*
663 *Food Chemistry, 36*(1), 19-22. doi: 10.1021/jf00079a005
664 The Centre for Food Safety, Food and Environmental Hygiene Department of Hong Kong, 2014.
665 "Microbiological Guidelines for Food". Available at:
666 http://www.cfs.gov.hk/english/food_leg/files/food_leg_Microbiological_Guidelines_for_Foo
667 d_e.pdf
668 Turner, S., Pryer, K. M., Miao, V. P., & Palmer, J. D. (1999). Investigating deep phylogenetic
669 relationships among cyanobacteria and plastids by small subunit rRNA sequence analysis. *J*
670 *Eukaryot Microbiol, 46*(4), 327-338.
671 Vogel, B. F., Venkateswaran, K., Satomi, M., & Gram, L. (2005). Identification of Shewanella baltica as
672 the Most Important H(2)S-Producing Species during Iced Storage of Danish Marine Fish.
673 *Applied and Environmental Microbiology, 71*(11), 6689-6697. doi: 10.1128/AEM.71.11.6689-
674 6697.2005
675 Yancey, P. H., Clark, M. E., Hand, S. C., Bowlus, R. D., & Somero, G. N. (1982). Living with water stress:
676 evolution of osmolyte systems. *Science, 217*(4566), 1214-1222.
677 Özogul, F., Taylor, K. D. A., Quantick, P., & Özogul, Y. (2002). Biogenic amines formation in Atlantic
678 herring (Clupea harengus) stored under modified atmosphere packaging using a rapid HPLC
679 method. *International Journal of Food Science & Technology, 37*(5), 515-522. doi:
680 10.1046/j.1365-2621.2002.00608.x

681

Figure captions

Figure 1. Changes in headspace concentration (%) of carbon dioxide (CO_2) as affected by storage time for saithe fillets packaged in high versus low concentration of CO_2 (stored at 4 °C)

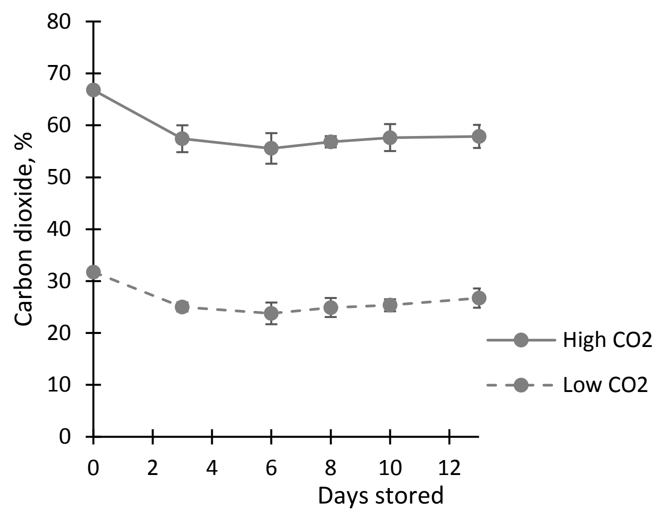
Figure 2. Main effects (GLM: $P < 0.001$) of oxygen, nitrogen and vacuum on negative fish odor from fresh saithe fillets throughout 13 days MAP storage (4 °C)

Figure 3. Evolution of (A) aerobic plate counts (APC) (GLM: $P < 0.001$), (B) psychrotrophic aerobic plate count (PC) (GLM: $P < 0.001$), (C) Lactic acid bacteria (LAB) (GLM: $P < 0{,}001$) and (D) Pseudomonas spp. (GLM: $P < 0.001$) in saithe stored at 4 °C in different atmospheres. Legends: Vacuum, ; low CO_2/high O_2, ; low CO_2/high N_2, ; high CO_2/low O_2, ; high CO_2/low N_2,

Figure 4. Relative abundance of the dominating bacterial taxa (genus level) in saithe filets at the end of fillet shelf-life (10 days for fillets packaged in vacuum and 13 days of those packaged in MAP, respectively). The initial sample was taken on day 0, before the fillets were packaged in modified atmosphere

Figure 5. Main effects (GLM: $P < 0.001$) of CO_2 ($P = 0.032$, $F = 4.7$), and oxygen versus nitrogen ($P = 0.001$, $F = 11.5$) and storage ($P > 0.001$, $F = 113.4$) on the drip loss (%) of fresh saithe fillets throughout 13 days MAP storage (4 °C)

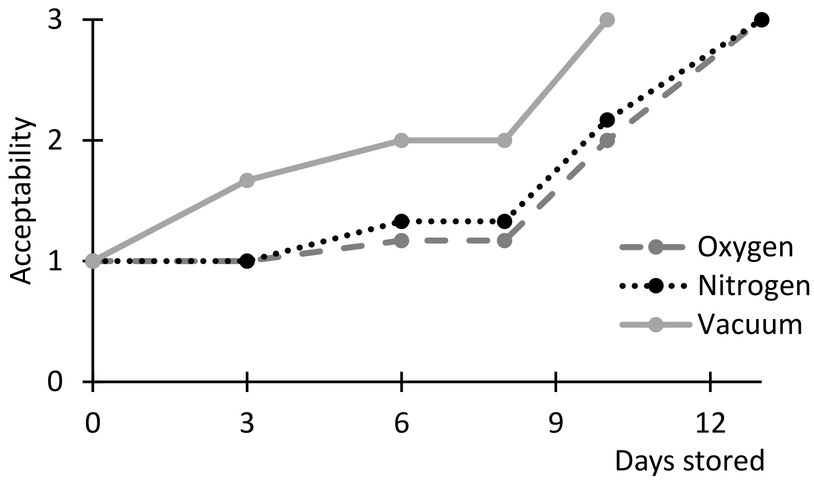
Figure 6. Main effects (GLM: $P < 0.001$) of oxygen, nitrogen and vacuum on the concentration of inosin and hypoxanthin of fresh saithe fillets throughout 13 days MAP storage (4 °C)



681

682 **Figure 1**

683

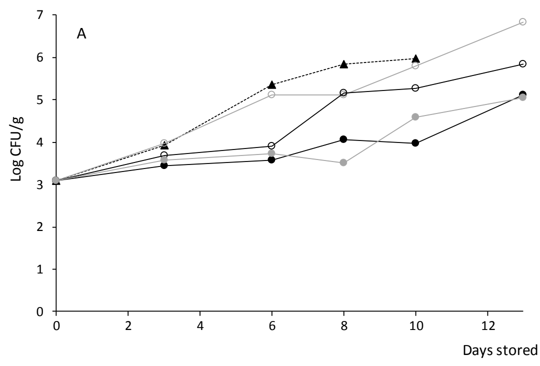


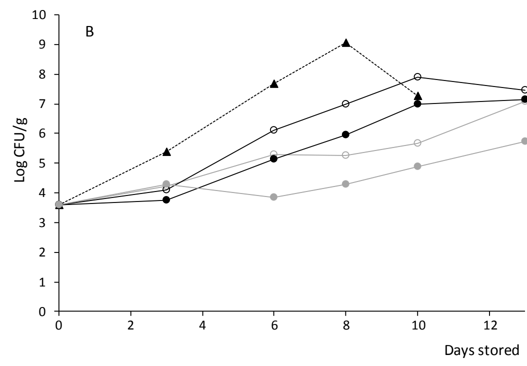
684

685 **Figure 2.**

686

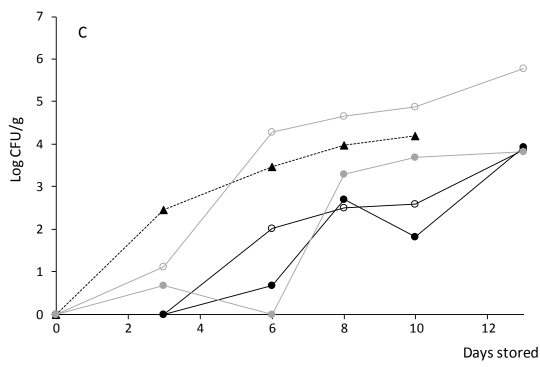
687

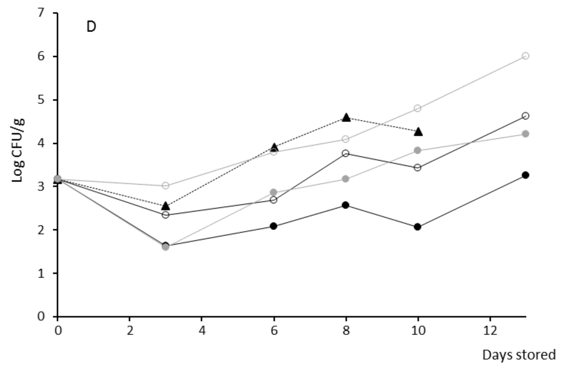




688

689

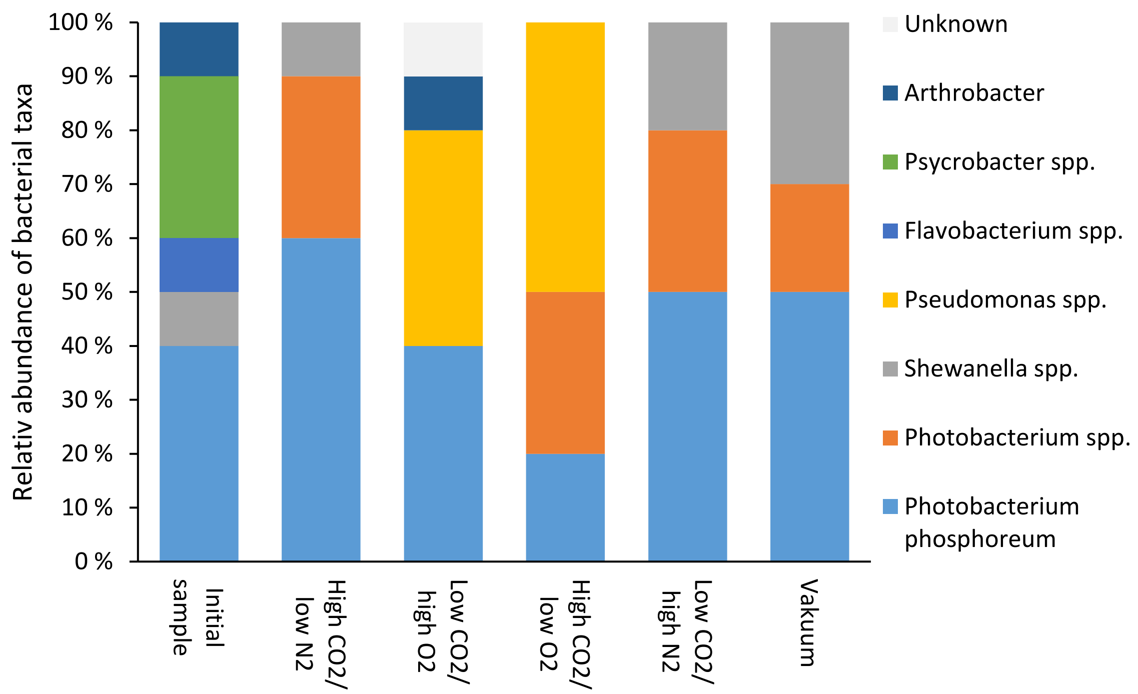




690

691 **Figure 3.**

692

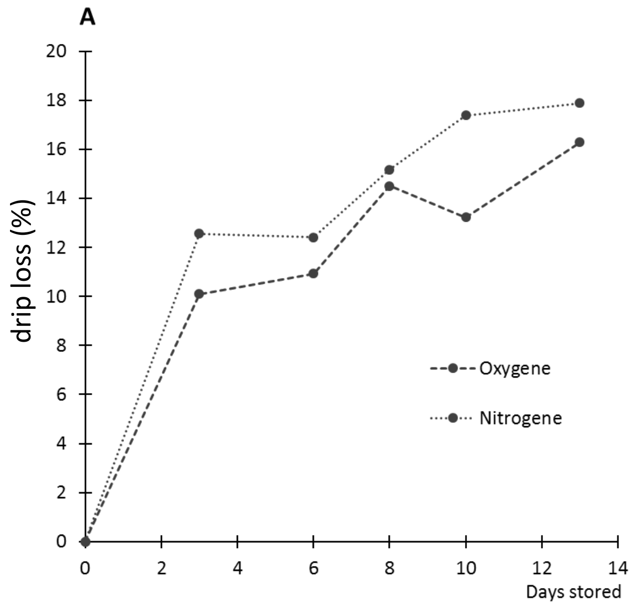


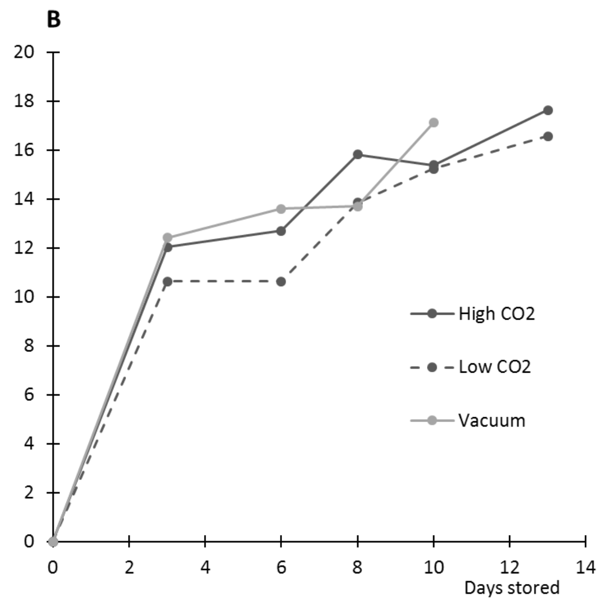
693

694 **Figure 3.**

695

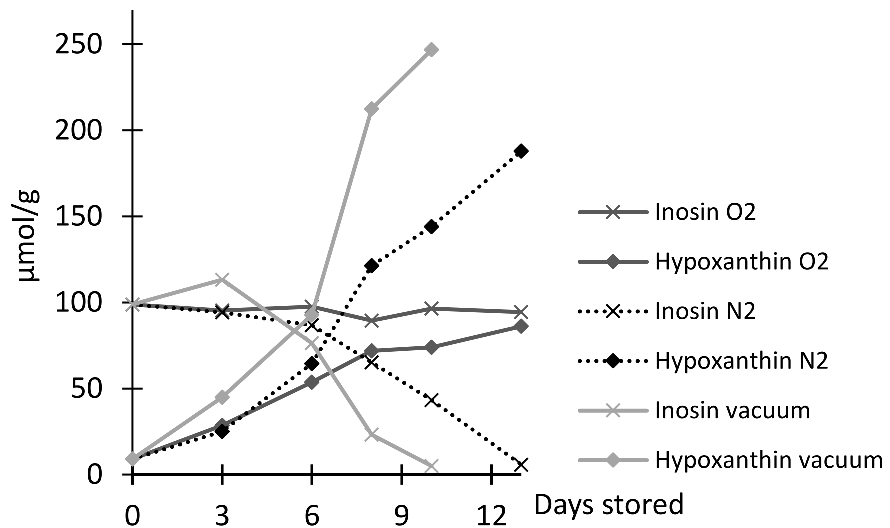
696





697 **Figure 5.**

698



699
700 **Figure 6.**
701