PlasmaMade[®] filter in Seatrade Reefer containers

Effects on banana quality, ethylene and microbial air quality

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Report 1670

Colophon

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Summary

Seatrade Reefer Chartering NV from Antwerp (Seatrade), represented by Dale Rolfe (operations manager), indicated that Seatrade is considering to equip all its (new) Reefer containers with an additional air purification system. PlasmaMade, located at Staphorst, Netherlands, has informed Seatrade, that their patented PlasmaMade® filter system could be a good system for improved fruit transport conditions, although PlasmaMade has no experience with respect to this specific application. Therefore Seatrade decided that an independent expert view is necessary to support Seatrade in making the right decisions about the potential of this novel and patented filter technology in Reefer container transport. Wageningen UR institute Food & Biobased Research (FBR) was asked to provide independent support on this, with the specific questions:

- Does the PlasmaMade unit oxidize/remove ethylene effectively?
- Does the PlasmaMade unit destroy airborne fungi and reduce the overall fungal load inside a container?

To this end, the focus of this research report is on effects on air purity and the resulting benefits of clean air on fruit quality. Economic considerations are not included in this study.

The project consisted of two main topics:

1. Pilot banana shipment using PlasmaMade filter in Seatrade Reefer Containers
2. Technical testing of effects on ethylene reduction and fungal load by PlasmaMade in Wageningen under controlled conditions

Ad 1) Pilot banana shipment using PlasmaMade filter in Seatrade Reefer Containers

Goal test: To verify the influence of PlasmaMade filters (combined with a minimal fresh air exchange setting) on banana quality during a sea container transport.

Test results of air sampling upon arrival: ethylene concentration in the reference container was somewhat lower than in the PlasmaMade container: resp. 13 and 69 ppb.

Petri-dishes for airborne fungi testing were fully overgrown in both containers, showing no differences, same goes for the spore content in the air samples taken upon arrival.

Results of the quality check before and after ripening:

No statistically significant differences in both colour and degree of fungal infection at stem/flower end were observed between the two test pallets.

Ad 2) Technical testing of effects on ethylene reduction and fungal load by PlasmaMade in Wageningen under controlled conditions

Goal test: To verify the influence of PlasmaMade filters on ethylene removal and fungal air quality under controlled conditions

Ethylene was injected in the empty PlasmaMade equipped container. No effect of PlasmaMade on reduction of ethylene concentrations was observed.

Additionally, the effects PlasmaMade on airborne fungi was also tested under these controlled conditions. No significant effect of PlasmaMade on reduction of airborne spore viability was observed.

An extra test was performed with settings optimized to show effects of PlasmaMade by creating a very high PlasmaMade-unit surface to treatment volume. Here some ethylene reduction was observed (0.08 ml ethylene reduction per hour per PlasmaMade unit). This would translate in >20 units required per banana reefer.

Recommendations

- Stop testing and trials (intended to find effects on produce quality and ethylene levels) with PlasmaMade unless hardware is further developed for the intended application.
- Evaluate this type of equipment by testing under controlled conditions before investing in pilot shipments

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Introduction

Seatrade Reefer Chartering NV from Antwerp (Seatrade), represented by Dale Rolfe (operations manager), indicated that Seatrade is considering to equip all its (new) Reefer containers with an additional air purification system. This was discussed in a meeting at Wageningen UR's Food & Biobased Research (FBR) d.d. 11-02-2016. PlasmaMade, located at Staphorst, has informed Seatrade, that their patented PlasmaMade® filter system could be a good system for improved fruit transport conditions, although PlasmaMade has no experience with respect to this specific application. Therefore an independent expert view is necessary to support Seatrade in making the right decisions about the potential of this novel and patented filter technology in Reefer container transport.

FBR is an independent research institute and having in-depth knowledge about both marine fruit transport and Reefer container technology. For this reasons FBR is asked by Seatrade to come up with a project proposal describing a test series with the PlasmaMade® filter system.

1.1 Background

Seatrade is managing (and owning) a fleet of vessels including specialised reefer vessels and multi-purpose carriers. In the second category vessels can stow (on deck) a limited amount of Reefer containers. This dual transport system enables Seatrade to offer its clients better services and more flexible “cold chain” solutions. Part of this service package could be the addition of extra (functional) equipment in the Seatrade Reefer containers. The idea is that an additional air purification filter will create added value, hence will create a competitive lead in comparison with other shipping lines services.

PlasmaMade® is a self-cleaning filter with 4 functions inside: an ionisation part (producing active oxide radicals), an electrostatic loaded glass fibre filter for filtering of bigger polluting particles, an electrostatic discharge part for inactivation of specific particles and an active carbon filter to bind organic compounds and to avoid release of ozone in the surrounding. The filter has no moving parts like ventilators, valves etc.; the settings of the filter can be adjusted such that air borne spores are targeted or to target specific gas components as for example the ripening hormone ethylene (C_2H_4). The latter will harm fruits when the concentrations become too high in a reefer container.

FBR is one of Wageningen UR's research institutes and has a long history in applied research in food chains. A spearhead programme is “sustainable fresh chains” where junior and senior postharvest

specialists are employed. FBR can recruit for projects: plant physiologists (fruit experts), microbiologists, ethylene specialists, refrigeration & ventilation specialists, reefer transport specialists. For field and laboratory tests special equipment is available; at FBR a special designed Reefer container test room can be used for controlled environment (on land) testing.

1.2 Intended results

The intended result of this project is that Seatrade will have a good knowledge base that will enable Seatrade to come to the decision whether installing of an air purification filter i.e. a PlasmaMade® air filter in a standard Starcool 40ft. hi-cube Reefer container (or AV+ with auto air disabled) has added value and does what the manufacturer claims.

That is:

- Does the unit oxidize/remove ethylene effectively?
- Does the unit destroy airborne pathogens and reduce the overall fungal load inside a container?

The research is focussing on effects on air purity and as a result of that the benefits of clean air on fruit quality. Economic considerations are not included in this study.

1.3 Intrinsic approach

To validate the current PlasmaMade® filter performance in fruit transport and to speed up the evaluation process, Seatrade has decided to start the evaluation with a real-world test, where the performance of a standard Starcool 40ft. hi-cube Reefer container and equipped with a PlasmaMade® filter and a reference container was compared.

FBR supervised this pilot and prepared special product samples at location (in Costa Rica), measured air and product samples (at arrival) and performed ripening and shelf life tests in Wageningen. Both Reefer containers were loaded with an identical batch of green bananas. After this first pilot/inventory phase a more thorough investigation was executed on land at FBR's premises in Wageningen. This location allows to do an in-depth (technical) testing in a controlled environment and the on-land situation enables continuous monitoring of the filter performance and reliable product quality evaluations.

The chronology of these technical testing activities in Wageningen was: measurement of filter performance and capacities by simulating a full load with artificial production of ethylene and micro-organisms. PlasmaMade® was allowed to adjust/optimize filters settings for this fruit transport application.

NB

The technical test was to be followed by a product (green bananas) transport simulation, where the effect of the filter on air purification and on product quality (green bananas) would be observed. Due to conclusions drawn after transport trial and ethylene absorption tests, it was decided not to do this last intended product (green bananas) transport simulation.

2 Pilot shipment of green bananas Costa Rica – Netherlands

2.1 Banana quality trial

To validate the current PlasmaMade® filter performance in fruit transport and to speed up the evaluation process, Seatrade has decided to start the evaluation with a real-world test, comparing the performance of a standard Starcool 40ft. hi-cube Reefer container and equipped with a PlasmaMade® filter and a reference container.

FBR supervised this pilot and prepared special product samples at location (in Costa Rica), measured air and product samples (at arrival) and performed shelf life tests in Wageningen. Both reefer containers were be loaded with an identical batch of green bananas.

2.1.1 Plantation location

Location of plantation in Costa Rica: Platanera Rio Sixaola SA; 160 ha.

2.1.2 Used reefers

Type of containers:	StarCool CAI	
Containers numbers:	556330 7	556384 2
Settings: temperature	13.3	13.3
Fresh air exchange (FAE)	40 cmh	10 cmh
PlasmaMade	No	Yes

STAR*COOL

型号 Type S4BCF-5.2Y-25D

编号 S.No. 1988405099

活塞压缩机

RECIPROCATING COMPRESSOR

额定电压 Nominal Voltage		频率 Frequ.	最大工作电流 Max. Operat. C.		启动电流 Locked Rotor C.		排气量 Displacem.	转速 Speed
V Δ 3Ph~	V Y 3Ph~	Hz	A (Δ)	A (Y)	A (Δ)	A (Y)	m³/h	RPM
210	367	50	19,0	11,0	117	67	19,0	1450
254	440	60	19,0	11,0	117	67	23,0	1750
防护等级 IP		重量 Weight	63,00 kg	制冷剂 Refrigerant	HFC		日期 Date	04/2015
低压/高压 LPHP max.		19 / 28	bar	37802883

Refrigerators and gensets used

2.1.3 Selection of bananas, harvest and handling

Three different types of bananas: Cavendish, Gros Michel, Datil (baby bananas)

Bananas harvested at weeks after flowering: 12/ 13/14/15/ >15

Selected from seven locations the banana plantation: see ★

1. Selected banana bunches on field transport

2. Bunches measured at arrival for handling

3. Bunches sorted in right line and safety pads are taken

1

2

3

Selected bananas: 1. Right row and taking anti-damage transport pads, 2. Taking flowers for bleeding, 3. Cutting from stem and in first bath.

4

5

6

Selected bananas: 4. Second cutting to customer size, 5. On trays for one box, cleaning and marking, 6. Packed into boxes.

Date of handling and sorting:

May 20th 2016

2.1.4 Marking and palletizing test pallets

Two test pallets were created in which various banana cultivars, ripeness stages etc were distributed randomly. Dataloggers and agarplates for microbial testing were distributed over various boxes.

20-5-2016 Selection of bananas on pallets					reference	PlasmaMade
Cultivar	Ripeness	Plastic liner	Weeks after full bloom	Field Sector	#pallet 1	#pallet2
Cavendish	Normal	yes	13	28	3	2
Cavendish	Normal	yes	13	41	2	3
Cavendish	Normal	yes	12	41	2	3
Cavendish	Normal	no	12	28	3	2
Cavendish	Normal	yes	14	43	3	3
Cavendish	Normal	no	14	43	2	1
Cavendish	Normal	yes	15	39	3	3
Cavendish	Normal	no	15	39	0	1
Cavendish	Sigatoca	yes	-	36	4	5
Cavendish	Ripe	yes	-	-	5	5
Gros Michel	Ripe	yes	-	-	3	3
Gros Michel	Ripe	no	-	-	2	2
Gros Michel	Normal	yes	-	-	4	4
Gros Michel	Normal	no	-	-	1	2
Baby banana	Normal	yes	-	-	7	6
Cavendish	Normal	yes	-	-	4	3

The test pallets were placed in the two reefers on the same position in the middle of the reefer. Both reefers were filled up with 23 pallets bananas from normal commercial operation.

2.1.5 Installation PlasmaMade equipment

Photo above:

Installation of red coloured PlasmaMade system in Reefer container by Ludo Tissingh of Aersmash BV.

Photo right:

Connection of PlasmaMade to electrical system of the Reefer container by Sander van Gameren.

2.1.6 Results of reefer conditioning

PlasmaMade reefer downloads

Reference reefer downloads

Temperatures and relative humidity graphs show that conditioning in both reefers was rather comparable. fresh air exchange settings were 40 and 10 CMH (cubic meter per hour) for the Reference and PlasmaMade reefers. Last setting was realized only 48h after closing the PlasmaMade reefer.

Although fresh air exchange in graphed download of reference container shows 0 cmh for long periods, the fresh air exchange was never actually closed, but this coincided with low circulation fan speed. This revealed however, that circulation fan speed in the PlasmaMade reefer had operated with full fan speed continuously and the Reference container did not. Besides difference in fresh air exchange opening between reefers, circulation fan speed likely also affected actual fresh air exchange, making fresh air exchange overall less in the Reference reefer than the 40 cmh opening suggested, e.g. the intended difference in fresh air exchange was probably lower than expected.

The temperature/RH loggers placed in the test pallets did not show relevant differences between test pallet conditions in both reefers.

2.1.7 *Quality evaluation of bananas in Wageningen*

At unloading in Vlissingen the bulk load of bananas looked fine and green, despite the presence of the test pallets containing some boxes of ripe and yellow bananas, and restricted fresh air exchange settings. No problems were reported regarding the quality of the bulk load bananas later on.

Both test pallets were transported to Wageningen in the Reefer containing the PlasmaMade unit for quality evaluation and ripening.

After arrival in Wageningen the test pallets were evaluated for banana quality June 9th and conditioned at 13.3°C until starting the ripening protocol. After ripening, quality was checked again June 20th. The boxes with clearly yellow bananas upon arrival (specific ripe harvested bananas) were discarded before ripening to prevent infectious decay spreading.

Ripening was induced by adding 100 ppm ethylene for 24 hours using the following ripening schedule, bananas were warmed up to 18°C pulp temperature prior to start ethylene treatment:

Ripening schedule	Fruit pulp temperature (°C)						
	Days in the ripening room						
	1 st	2 nd	3 rd	4 th	5 th	6 th	7 th
4 days	18 ethylene	18	16.5	15.5	store at 14.5		

Ripening was performed in the reefer itself.

2.1.7.1 Quality evaluations

Colour

A standard banana colour scale was used to determine peel colour:

Stem end rot, flower end rot and specific other damage was recorded in scales from 0-3:

- 0 none
- 1 light, just visible
- 2 clear
- 3 severe

2.1.7.2 Statistical analysis

Data were analysed statistically without transformation. Different treatments were analysed for significant differences by analysis of variance (ANOVA) with the statistical package Genstat. When significant differences were found, comparisons between pairs of data were made using the least significant differences between means (LSD) at a significance level of 95%.

Some pictures of banana quality upon arrival June 9th

Results peel Colour

Average of color measurement	Cultivar	Ripeness	liner	weeks AF	reefer PlasmaMade	Reference
on arrival	Baby banana	Normal	yes	n.d.	2.6	2.0
on arrival	Cavendish	Normal	No	12	2.0	2.0
on arrival	Cavendish	Normal	No	14	2.0	2.5
on arrival	Cavendish	Normal	No	15	2.0	
on arrival	Cavendish	Normal	yes	12	2.0	2.0
on arrival	Cavendish	Normal	yes	13	2.0	2.0
on arrival	Cavendish	Normal	yes	14	2.0	2.0
on arrival	Cavendish	Normal	yes	15	2.2	2.0
on arrival	Cavendish	Normal	yes	n.d.	2.0	2.0
on arrival	Cavendish	Ripe	yes	n.d.	6.0	7.0
on arrival	Cavendish	Sigatoca	yes	n.d.	2.1	2.4
on arrival	Gros Michel	Normal	No	n.d.	2.0	2.0
on arrival	Gros Michel	Normal	yes	n.d.	2.0	2.0
on arrival	Gros Michel	Ripe	No	n.d.	4.0	4.0
on arrival	Gros Michel	Ripe	yes	n.d.	5.7	6.3
on arrival Total					2.8	2.9
after ripening	Baby banana	Normal	yes	n.d.	6.0	5.9
after ripening	Cavendish	Normal	No	12	3.5	3.3
after ripening	Cavendish	Normal	No	14	5.0	4.5
after ripening	Cavendish	Normal	No	15	5.0	
after ripening	Cavendish	Normal	yes	12	3.7	3.0
after ripening	Cavendish	Normal	yes	13	4.0	3.4
after ripening	Cavendish	Normal	yes	14	4.7	4.3
after ripening	Cavendish	Normal	yes	15	5.7	5.3
after ripening	Cavendish	Normal	yes	n.d.	3.0	3.3
after ripening	Cavendish	Sigatoca	yes	n.d.	3.4	4.8
after ripening	Gros Michel	Normal	No	n.d.	5.0	5.0
after ripening	Gros Michel	Normal	yes	n.d.	5.0	5.5
after ripening Total					4.5	4.5
Grand Total					3.6	3.6

No statistical significant differences were found between the colour of bananas transported with or without PlasmaMade, both on arrival and after ripening.

Also no significant differences were observed if only specific cultivars, ripening stages at harvest, or boxes with/without plastic liner were compared between both reefers. Significant differences in colour only occurred when comparing cultivars and ripening stages itself.

Results Stem end rot

Average of Stem rot measurement	Cultivar	Ripeness	liner	weeks AF	reefer PlasmaMade	Reference
on arrival	Baby banana	Normal	yes	n.d.	0.00	0.00
on arrival	Cavendish	Normal	No	12	2.00	1.00
on arrival	Cavendish	Normal	No	14	2.00	1.00
on arrival	Cavendish	Normal	No	15	1.00	
on arrival	Cavendish	Normal	yes	12	0.00	0.00
on arrival	Cavendish	Normal	yes	13	0.00	0.00
on arrival	Cavendish	Normal	yes	14	0.00	0.00
on arrival	Cavendish	Normal	yes	15	0.00	0.00
on arrival	Cavendish	Normal	yes	n.d.	0.00	0.00
on arrival	Cavendish	Ripe	yes	n.d.	0.00	0.40
on arrival	Cavendish	Sigatoca	yes	n.d.	0.00	0.00
on arrival	Gros Michel	Normal	No	n.d.	0.00	0.00
on arrival	Gros Michel	Normal	yes	n.d.	0.00	0.00
on arrival	Gros Michel	Ripe	No	n.d.	2.00	2.00
on arrival	Gros Michel	Ripe	yes	n.d.	0.00	0.00
on arrival Total					0.23	0.23
after ripening	Baby banana	Normal	yes	n.d.	0.33	0.86
after ripening	Cavendish	Normal	No	12	1.00	1.33
after ripening	Cavendish	Normal	No	14	2.00	1.00
after ripening	Cavendish	Normal	No	15	2.00	
after ripening	Cavendish	Normal	yes	12	0.67	0.00
after ripening	Cavendish	Normal	yes	13	0.20	0.00
after ripening	Cavendish	Normal	yes	14	0.00	0.00
after ripening	Cavendish	Normal	yes	15	0.33	0.33
after ripening	Cavendish	Normal	yes	n.d.	0.33	1.00
after ripening	Cavendish	Sigatoca	yes	n.d.	0.60	0.00
after ripening	Gros Michel	Normal	No	n.d.	1.00	1.00
after ripening	Gros Michel	Normal	yes	n.d.	0.25	0.75
after ripening Total					0.50	0.55
Grand Total					0.35	0.37

Upon arrival some stem end rot was observed in many boxes. Stem end rot increased somewhat after ripening, but:

No statistical significant differences were found between stem end rot of bananas transported with or without PlasmaMade, both on arrival and after ripening.

Flower end rot

Average of Flower rot measurement					reefer	
	Cultivar	Ripene	liner	weeks AF	PlasmaMade	Reference
on arrival	Baby banana	Normal	yes	n.d.	0.00	0.00
on arrival	Cavendish	Normal	No	12	0.00	0.00
on arrival	Cavendish	Normal	No	14	0.00	1.00
on arrival	Cavendish	Normal	No	15	0.00	
on arrival	Cavendish	Normal	yes	12	0.00	0.00
on arrival	Cavendish	Normal	yes	13	0.80	0.20
on arrival	Cavendish	Normal	yes	14	0.67	0.00
on arrival	Cavendish	Normal	yes	15	0.33	0.33
on arrival	Cavendish	Normal	yes	n.d.	1.00	0.75
on arrival	Cavendish	Ripe	yes	n.d.	1.20	1.60
on arrival	Cavendish	Sigatoca	yes	n.d.	0.40	0.00
on arrival	Gros Michel	Normal	No	n.d.	0.00	0.00
on arrival	Gros Michel	Normal	yes	n.d.	0.00	0.75
on arrival	Gros Michel	Ripe	No	n.d.	0.00	0.00
on arrival	Gros Michel	Ripe	yes	n.d.	0.67	1.00
on arrival Total					0.42	0.44
after ripening	Baby banana	Normal	yes	n.d.	0.00	0.00
after ripening	Cavendish	Normal	No	12	0.00	0.33
after ripening	Cavendish	Normal	No	14	0.00	0.00
after ripening	Cavendish	Normal	No	15	0.00	
after ripening	Cavendish	Normal	yes	12	0.00	0.00
after ripening	Cavendish	Normal	yes	13	0.60	0.00
after ripening	Cavendish	Normal	yes	14	0.33	0.00
after ripening	Cavendish	Normal	yes	15	0.00	0.33
after ripening	Cavendish	Normal	yes	n.d.	0.67	0.75
after ripening	Cavendish	Sigatoca	yes	n.d.	0.60	0.00
after ripening	Gros Michel	Normal	No	n.d.	0.00	0.00
after ripening	Gros Michel	Normal	yes	n.d.	0.50	0.25
after ripening Total					0.29	0.16
Grand Total					0.36	0.31

Upon arrival some flower end rot was observed in some objects. Flower end rot seemed to decrease somewhat after ripening, probably by becoming less visible because of slight drying of the flower ends and darkening of the initially whitish fungi. But:

No statistical significant differences were found between flower end rot of bananas transported with or without PlasmaMade, both on arrival and after ripening.

Other damages (e.g. latex burns, bruising) were observed in low numbers and no connection to reefer type was observed.

2.2 Air sampling for ethylene and microbial quality

After the trip Costa Rica – Netherlands both reefers were sampled for ethylene concentration and microbial air quality in order to observe effects on these factors of the PlasmaMade unit.

After sampling both reefers were opened and emptied. Agar plates placed in the test pallets were retrieved, and test pallets from both reefers were combined in the PlasmaMade equipped reefer and transported to Wageningen next day for quality evaluation.

Present at Vlissingen seaport on Monday 6th of June 2015

- Ludo Tissingh (AirSmash/PlasmaMade)
- Sander van Gameren (AirSmash/PlasmaMade)
- Martin van der Sluis (AirSmash/PlasmaMade)
- Jan Verhage (BMT Surveys)
- Dale Rolfe (Seatrade)
- Jouke Spriensma (FBR)
- Mariette Helmond (FBR)

2.2.1 Sampling procedure

Container 556384.2 contained the PlasmaMade unit and was sampled first.

Setpoint 13.3°C, Supply 12.6, Vent open 4%/10 CMH (see pictures)

Ethylene was sampled in triplicate in 125 ml glass gas sample tubes by drawing air from the reefers through the fresh air exchange vent using a pump.

After taking the Ethylene samples the amount of fungal spores in the reefer air was sampled in an identical manner. Airsamples were taken with the adapted Merck MAS-100 Eco airsampler (see picture)

- Plastic funnel and water hoses were prior to departure from Wageningen disinfected with 0.25% Chlorine, on site device and hoses were disinfected with 70% ethanol.
- Merck MAS-100 Eco airsampler was flushed 5 min with 100L/min.
- 500, 100 and 10 L samples were taken at a flow of 100l/min from the vent, identical to the way ethylene samples were taken.
- Two types of microbiological growth medium were used (MEA and DCBC)

Reference Container 556330.7 was sampled after finishing the air samples from the PlasmaMade container.

Setpoint 13.3 °C Supply 13.4, 15%/40CMH (see pictures)

Air samples for ethylene and microbial air quality were taken in an identical manner for both reefers. After sampling ethylene gas samples and agar plates were taken to Wageningen for analysis.

2.2.2 Visual analysis of agar plates obtained from the reefers

The agar plates placed in the test pallets were visually judged on microbial growth that had occurred during the trip. Below schedule of various objects and pictures to match.

Test container			
no in picture	code agarplate	media	remarks
1	25 cav15N39 FP OAT	Oatmeal agar	dehydrated
2	25cav15N39 FP DPA	Dextrose potato agar	dehydrated

3	45cavsig36 fp in		
4	45 cavsig36 fp in pda	Dextrose potato agar	
5	test 70 cav rp fp pdi	Dextrose potato agar	
6	test 70 cav rp fp oat	Oatmeal agar	
7	testc 65 gmrp oat	Oatmeal agar	
8	test 65 gmrp pda	Dextrose potato agar	dehydrated
9	52 gav 13 n41 fp in		
10	52 cav13n41 fpin pda	Dextrose potato agar	
11	38 gmn fpin pda	Dextrose potato agar	
12	38 gmn fp in oat	Oatmeal agar	
Reference container			
no in picture	code agarplaat	media	remarks
13	30cav15n39 fpont oat	Oatmeal agar	dehydrated
14	30cav15n39 fp outer pda	Dextrose potato agar	dehydrated
15	34gmnfpin pda	Dextrose potato agar	
16	34gm n fp in		
17	55cav13n41 fpin pda	Dextrose potato agar	
18	55 cav13n41 fp in oat	Oatmeal agar	
19	63 gm rp ref		dehydrated
20	63 gmrp ref		dehydrated
21	50cavsig36 fpin pda	Dextrose potato agar	
22	50cavsig36 fp in oat	Oatmeal agar

Agar plates that were placed in test-boxes during transport, numbers correspond with description tables above.

Plates were visually analysed on amount of growth and diversity of fungal species by microbiologists Masja Nierop Groot and Mariette Helmond.

2.2.3 Results and conclusions of air-samples ethylene after trip

Ethylene air samples from both reefers before opening after the trip were analysed using a gas chromatograph (Shimadzu) equipped with FID and calibrated prior to measurements using certified calibration gas in the 100 ppb range.

reefer ethylene concentration (ppb) after trip		
	Reference	PlasmaMade
average	13	69
stdev	3	14

Test results of air sampling upon arrival: ethylene concentration in the reference container was lower than in the PlasmaMade container: resp. 13 and 69 ppb.

fresh air exchange settings were different in the PlasmaMade and Reference reefer (resp. 10 and 40 cmh) and also circulation fan speed was not comparable. Reference container regularly had low fan speed while PlasmaMade had high air speed continuously, which impacts fresh air exchange also. (In the reefer log downloads, at low fan speed fresh air exchange seems to be recorded as 0 CMH).

These last factors make it hard to attribute the relatively small difference in ethylene concentrations in both reefers to the effect of differences in fresh air exchange or to the PlasmaMade unit. In the technical testing (see Chapter 3, Technical testing in Wageningen) effects of differences in fresh air exchange were excluded, giving better information on the effects of PlasmaMade on ethylene breakdown.

2.2.4 Results and conclusions of air-samples microbial air quality after trip

After 1 week incubation at 21 °C the air-sample agarplates were analysed (see pictures).

Colonies count:

Volume	556330.7 reference		556384.2 (PlasmaMade)	
	MEA	DRBC	MEA	DRBC
10 L	2	2	0	3
100 L	13	18	11	8
500 L	57	65	31	18

In order to show significant difference in microbial air quality, colony counts should differ in order of magnitude. In this case there is **no significant difference** in growth and/or amount of fungi between both containers.

3 Technical testing in Wageningen

3.1 Effects of PlasmaMade® on ethylene removal

Reefer StarCool CAI nr 5563842 was equipped with PlasmaMade units as described in the previous test. An external power switch was installed to operate the PlasmaMade unit during tests. Tests were performed in empty reefer at setpoint 13.3°C with fresh air exchange vent setting at 5%, ~12.5 CMH (cubic meter per hour).

Ethylene was monitored using a photoacoustic laser detector (ETD300, SensorSense)(Cristescu, Persijn et al. 2008).

This is a very accurate and ethylene specific device measuring every 5-10 seconds, enabling to monitor ethylene concentrations in time very accurately (resolution 0.3ppb). The detector was placed just outside the reefer and gas was drawn continuously with a sample pump (approx. 3 l/h). Pure ethylene was injected into the reefer using tubing under the door rubber (approx. 700-1000 ppb).

After several tests no clear ethylene reducing activity of the PlasmaMade filters inside the reefer unit could be observed, while the technical aspects of the tests all seemed OK.

PlasmaMade was contacted (Ludo Tissingh) to check the PlasmaMade unit and to optimise performance.

Optimizing performance of the PlasmaMade unit proved not to be an option, to increase performance a key component required redesign which was not feasible for testing on short notice.

It was made sure the PlasmaMade unit was functioning properly before running a final test, resulting in the next graph:

After injection of ethylene the concentration inside the reefer increased to 700 ppb (blue line). After this, ethylene concentration slowly decreased (brown line). Ethylene reduction was plotted against a 3rd power polynomial function (dotted black line), which modelled ethylene reduction with high accuracy ($R^2 > 0.998$). The on or off switching of the PlasmaMade units during this test (pink line on top of the graph) did not result in significant changes of the ethylene reduction speed, e.g., the ethylene reduction observed could be fully explained by fresh air exchange, and **no added value of PlasmaMade to reduce ethylene levels could be observed.**

In order to maximize efforts to show ethylene breakdown efficacy of PlasmaMade, it was decided to test classic PlasmaMade units with their own fan system inside a gastight 550l container with water lock, to have maximum exchange between the air and the units. Ethylene was measured here by injecting syringe drawn 2.5 ml samples in the gas chromatograph. Starting ethylene level was increased to approx. 2 ppm to facilitate accurate GC measurements.

After two PlasmaMade units were installed in this 550l container, effect of PlasmaMade could be shown:

After stabilisation of the ethylene concentration after injection to 2.3 ppm (during the first 4 hours), the two PlasmaMade units were switched on and during 4 hours a linear decrease of the ethylene concentration was observed from which ethylene removal capacity could be calculated.

(trendline function $y = -0.2939x + 3.2979$)

ethylene decrease	0.294	ppm/h
per PlasmaMade unit	0.147	ppm/h
Volume	550	liter
ethylene breakdown	0.081	ml ethylene/h
Estimated production in bananas at 13°C	0.1	($\mu\text{l/kg}\cdot\text{h}$) ethylene production
Estimated ethylene production in banana reefer	1.8	ml ethylene/h
PlasmaMade units required per reefer to neutralize ethylene production	22.3	units

3.1.1.1 Conclusions ethylene

- No added value of PlasmaMade to reduce ethylene levels could be observed in a reefer
- In an extra test with settings optimized to show effects of PlasmaMade by creating a very high Plasma-unit surface to treatment volume, some ethylene reduction was observed (0.081 ml ethylene reduction per hour per PlasmaMade unit. This would translate in >20 units required per banana reefer.

3.2 Effects of PlasmaMade® on microbial air quality

3.2.1 Desk research on fungi species relevant for kiwi and banana fruits storage and transport

Desk research resulted in two tables of relevant fungi strains, fungi strains that are available at FBR are mentioned in the right column

Table 1 Kiwifruits (*Actinidia deliciosa*)

Disease	Fungi strain (asexual state)	Occurrence	Reference	-80°C FBR
Grey mould rot	Botryotinia fuckeliana (Botrytis cinerea)	All growing area's	(Snowdon 1990)	Botrytis cinerea
Blue mould rot	Penicillium expansum	New-Zealand		Penicillium expansum Link, industrial isolate
Phomopsis rot	Diaporthe perniciosia Diaporthe actinidiae Phomopsis actinidiae			
Ripe rot	Botryosphaeria, Colletotrichum, Phoma and Phomopsis spp.		(Snowdon 1990, Peres, Kuramae et al. 2002, Elena 2009)	Phoma foveata 10192 B (IPO'96 PdL)
Fruit spoilage	Aspergillus foetidus		(Al-Hindi, Al-Najada et al. 2011)	

Table 2 Bananas (*Musa*)

Disease	Fungi strain (asexual state)	Occurrence	Reference	-80°C FBR
Anthraxnose	Colletotrichum musae	All producing countries	(Snowdon 1990, Bhutia, Zhimo et al. 2016)	
Cigar-end rot	Trachysphaera fructigena, Verticillium theobromae	West and Central Africa, South* and Central America*	(Snowdon 1990, *personal communication Mr. D. Rolfe)	
Crown mould, crown rot and Finger-stalk rot	Acremonium spp., Botryodiplodia theobromae, Ceratocyctis paradoxa, Colletotrichum musae, Fusarium pallidroseum, Verticillium theobromae	All growing area's		
Finger rot	Botryodiplodia theobromae	Most growing area's		
Johnston spot, Pitting disease	Magnaporthe grisea (Pyricularia grisea)			
Sigatoka	Mycosphaerella spp.	Most producing		

disease		area's		
Other diseases	Fusarium, Gibberella, Deightonella, Cercospora, Botryosphaeria, Guignardia, Phyllosticta, Phytophthora, Trichothecium, Rhizopus, Cladosporium, Nigrospora, Sclerotinia			Cladosporium cucumera CBS173.54
Fruit spoilage	Fusarium oxysporum		(Al-Hindi, Al-Najada et al. 2011, Raza, Ling et al. 2016)	Fusarium oxysporum f.sp. tulipae CBS242.59
Panama-disease	Fusarium oxysporum f. sp. cubense	plantage	Wikipedia.com	
Black Sigatoka disease	<i>Mycosphaerella fijiensis</i>	plantage	Wikipedia.com	

3.2.2 Selection of fungi

The following fungi strains were inoculated on Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA) and incubated at 21 °C for two weeks until sporulation occurred.

1. Botrytis cinerea, cause of grey mould and fast growing on PDA
2. Penicillium expansum Link , common mould, grows on MEA at
3. Phoma foveata grows on MEA under daylight
4. Cladosporium cucumera grows on MEA
5. Fusarium oxysporum, grows on PDA

3.2.3 Experimental setup

Experiments were carried out according to the time line depicted below.

Two plates overgrown with fungi were statically placed on the T-bar floor of the container, see picture. Spores produced by the fungi on the agar plates will become airborne by the force of the airstream produced by the ventilator. It was estimated that after 10 minutes all spores on the agar plates would be released into the air of the reefer container.

The PlasmaMade unit was turned off and the amount of viable spores in the air was measured after 10, 60 and 120 minutes. Subsequently the agar plates were replaced by fresh ones, the PlasmaMade device was turned on and the effect of the device on the amount of viable spores in the air will be measured after 10, 60 and 120 minutes.

Malt Extract Agar (MEA) was used for air sampling, all measurements were performed in duplo. 10,100 and 500 Litre of air was sampled. Prior to the technical testing an ozone odour was detected inside the reefer container. To prevent interference with the test the air in the reefer contained was refreshed until the ozone odour was no longer detectable.

3.2.3.1 Air sampling experimental procedure

- The MAS-100 Eco air sampler (Merck) was adapted with a sterile funnel and water hose to be able to take air samples from the exhaust (see picture below)
- The sampling head with funnel and hose attached was opened and a sterile fresh malt extract agar plate (MEA) was inserted.
- After replacing the sampling head the air sampler was switched on and an air sample was taken.
- A new agar plate was placed in the sampling head as previous and another air sample was taken
- In total three predefined volumes of air (10-100 and 500 litre) were taken in duplo at a speed of 100l/min
- After sampling all agar plates were incubated at 22 °C for one week

The MAS-100 Eco air sampler (Merck)

Setup artificial infection in reefer using overgrown agar plates taped to T-bars

3.2.4 Results

3.2.4.1 Growth on agar

All 5 fungi strains were in duplo inoculated on MEA agar and incubated for 14 days at 20 °C. Only *Fusarium oxysporum* and *Botrytis cinerea* grew well enough on both plates and were used in the technical testing.

3.2.4.2 Technical testing

Two agar plates, one overgrown with *Botrytis* and one overgrown with *Fusarium* (see picture) were placed inside the container, taped on the T-bar.

The air sampling fan was turned on and after 10, 60 and 120 minutes air samples were taken.

After one week of incubation at 20 °C the amount of colony forming units (CFU) per agar plate was determined.

Table 3 CFU/agar plate after 1 week incubation

Sampling time	PlasmaMade device ON						PlasmaMade device OFF					
	10 L		100 L		500 L		10 L		100 L		500 L	
10 min	3	1	2	1	9	15	4	16	18	6	25	nc
60 min	0	0	13	2	5	15	1	3	5	4	5	10
120 min	0	0	1	1	6	14	1	0	5	0	11	10

nc not countable, overgrown

The difference in CFU/agar plate between the PlasmaMade device switched on or off is too small to conclude that there is any effect on the amount of airborne fungi or inactivation of spores in this test.

3.2.5 Discussion

A clear ozone-like odour was noticeable after the second test but the amount of ozone produced by the PlasmaMade device during the test was not measured.

3.2.6 Conclusions

There is no measurable effect of the PlasmaMade device on the amount of airborne fungi spores in the reefer container within the tested period of two hours.

4 Conclusions

1) Pilot banana shipment Costa Rica-NL using PlasmaMade filter in Seatrade Reefer Containers

No significant effects of PlasmaMade were found:

- On banana quality (colour, fungal decay) after pilot shipment of a range of bananas with different quality backgrounds, both on arrival and after ripening
- On reduction of ethylene levels in reefers upon arrival (13 ppb reference vs. 69 ppb PlasmaMade)
- On reduction of fungal growth on selective agar plates transported with the bananas during Pilot shipment Costa Rica-Netherlands
- On viable airborne fungi sampled from the reefers after Pilot shipment Costa Rica-Netherlands

2) Technical testing of effects on ethylene reduction and fungal load by PlasmaMade in Wageningen under controlled conditions

No significant effects of PlasmaMade were found:

- On reduction of ethylene levels in a reefer
- On viable airborne fungi sampled from the reefers after up to two hours

Ethylene reducing capacity by PlasmaMade could only be detected in an extra test with settings optimized by creating a very high Plasma-unit surface to treatment volume (in a small volume). Here ethylene reduction was observed (0.08 ml ethylene reduction per hour per PlasmaMade unit) that would translate in >20 PlasmaMade units required per banana reefer to compensate expected ethylene production.

5 Recommendations

- Stop testing and trials (intended to find effects on produce quality and ethylene levels) with PlasmaMade unless hardware is further developed for the intended application.
- Evaluate this type of equipment by testing under controlled conditions before investing in pilot shipments

Abstract

Seatrade Reefer Chartering NV from Antwerp (Seatrade), represented by Dale Rolfe (operations manager), indicated that Seatrade is considering to equip all its (new) Reefer containers with an additional air purification system. PlasmaMade, located at Staphorst, Netherlands, has informed Seatrade, that their patented PlasmaMade® filter system could be a good system for improved fruit transport conditions, although PlasmaMade has no experience with respect to this specific application. Therefore Seatrade decided that an independent expert view is necessary to support Seatrade in making the right decisions about the potential of this novel and patented filter technology in Reefer container transport. Wageningen UR institute Food & Biobased Research (FBR) was asked to provide independent support on this, with the specific questions:

- Does the PlasmaMade unit oxidize/remove ethylene effectively?
- Does the PlasmaMade unit destroy airborne pathogens and reduce the overall fungal load inside a container?

To this end, the focus of this research report is on effects on air purity and the resulting benefits of clean air on fruit quality. Economic considerations are not included in this study.

The project consisted of two main topics:

3. Pilot banana shipment using PlasmaMade filter in Seatrade Reefer Containers
4. Technical testing of effects on ethylene reduction and fungal load by PlasmaMade in Wageningen under controlled conditions

Ad 1) Pilot banana shipment using PlasmaMade filter in Seatrade Reefer Containers

Goal test: To verify the influence of PlasmaMade filters (combined with a minimal fresh air exchange setting) on banana quality during a sea container transport.

Two 40ft StarCool CAI containers supplied with a genset were delivered by Seatrade at banana farm Platanera Rio Sixaola SA in Costa Rica 20-06-2016. At that date two PlasmaMade filters were installed in one of the reefers by Aersmash. The filters were placed in the airstream behind the evaporators. As the fan for air circulation starts the PlasmaMade filters were operational.

For each container identical test pallets with 48 boxes of bananas were prepared by Wageningen UR.

Test pallet boxes contained:

- Three cultivars of bananas: Cavendish, Gros Michel and Datil
- Bananas harvested at various ripening stages (12/13/14/15 or >15 weeks after flowering)
- Selected from eight locations within the banana plantation
- Boxes with or without box liner
- From healthy and Sigatoka infected trees.

Temperature and humidity loggers were placed in boxes in both pallets. Also open Petri dishes with two types of fungi-selective growth agars were placed on both pallets in order to test airborne fungi.

Both containers were loaded with 23 normal pallets with green bananas and a test pallet. Both test pallets were placed on the same location inside the containers, more or less in the centre of the container.

After loading and closing of the containers the refrigeration started, Temperature set points were 13.3°C for both reefers. Air exchange setting for the reference container was the usual 40 cmh, and 10 cmh for the PlasmaMade equipped container. This last setting was realized after the fresh air exchange was closed the first 48h by accident.

The transport of the containers was organized by Seatrade. Both containers were located next to each other on the deck of the Seatrade vessel “Emerald”. Loading was done at Limon in Costa Rica on 22-06-2016. Arrival in the harbour of Vlissingen in the Netherlands at 06-06-2016. Before custom clearance Wageningen UR took air samples from the closed containers in order to measure ethylene and microbial load in the air.

Test results of air sampling upon arrival: ethylene concentration in the reference container was somewhat lower than in the PlasmaMade container: resp. 13 and 69 ppb.

Petri-dishes for airborne fungi testing were fully overgrown in both containers, showing no differences, same goes for the spore content in the air samples taken upon arrival.

After unloading both containers, the 2 test pallets were transported to Wageningen for quality check before and after ripening. Quality attributes scored in all boxes: colour, stem end rot and flower end rot. Some boxes contained very yellow bananas and were only checked on arrival. The green bananas were ripened using 100 ppm ethylene for 24h at 18° C, followed by a standard temperature protocol of ripening in four days. Afterwards the banana quality was evaluated again.

No statistically significant differences in both colour and degree of fungal infection at stem/flower end were observed between the two test pallets.

Ad 2) Technical testing of effects on ethylene reduction and fungal load by PlasmaMade in Wageningen under controlled conditions

Goal test: To verify the influence of PlasmaMade filters on ethylene removal and fungal air quality under controlled conditions

Ethylene was injected in the empty PlasmaMade equipped container (see previous test) with usual settings for banana transport (temperature SP 13.3°C), but with fresh air exchange set to 5 cmh. No effect of PlasmaMade on reduction of ethylene concentrations was observed.

Aersmash was given opportunity to check the PlasmaMade units for good operation and improve capacity. This last proved not possible during the test period since it required redesign of a ceramic element.

Additionally, the effects PlasmaMade on airborne fungal spores (*Fusarium oxysporum* and *Botrytis cinerea*) was also tested under these controlled conditions. Spores were released and after 10, 60 and

120 minutes air samples were taken and checked for fungal growth, once with PlasmaMade switched off and once while on.

No significant effect of PlasmaMade on reduction of airborne spore viability was observed.

An extra test was performed with settings optimized to show effects of PlasmaMade by creating a very high PlasmaMade-unit surface to treatment volume. In this test two PlasmaMade units with their own fan system were put in a gas-tight 550l container. Here some ethylene reduction was observed (0.08 ml ethylene reduction per hour per PlasmaMade unit). This would translate in >20 units required per banana reefer.

Overall conclusions

1) Pilot banana shipment Costa Rica-NL using PlasmaMade filter in Seatrade Reefer Containers

No significant effects of PlasmaMade were found:

- On banana quality (colour, fungal decay) after pilot shipment of a range of bananas with different quality backgrounds, both on arrival and after ripening
- On reduction of ethylene levels in reefers upon arrival (13 ppb reference vs. 69 ppb PlasmaMade)
- On reduction of fungal growth on selective agar plates transported with the bananas during Pilot shipment Costa Rica-Netherlands
- On viable airborne microorganisms sampled from the reefers after Pilot shipment Costa Rica-Netherlands

2) Technical testing of effects on ethylene reduction and fungal load by PlasmaMade in Wageningen under controlled conditions

No significant effects of PlasmaMade were found:

- On reduction of ethylene levels in a reefer
- On viable airborne microorganisms sampled from the reefers after up to two hours

Ethylene reducing capacity by PlasmaMade could only be detected in an extra test with settings optimized by creating a very high Plasma-unit surface to treatment volume (in a small volume). Here ethylene reduction was observed (0.08 ml ethylene reduction per hour per PlasmaMade unit) that would translate in >20 PlasmaMade units required per banana reefer to compensate expected ethylene production.

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