

Validation Results and Final Selection of Solutions for Rice Infestation Prevention

December 2024



FID

TRACE-RICE with Grant nº 1934, is part of the PRIMA Programme supported under Horizon 2020, the European Union's Framework Programme for Research and Innovation



http://trace-rice.eu



TECHNICAL REFERENCES

Project Acronym	TRACE-RICE
Project Title	Tracing rice and valorizing side streams along
	Mediterranean blockchain
Project Coordinator	Carla Moita Brites
	<u>carla.brites@iniav.pt</u>
Project Duration	September 2020 – October 2024 (50 months)
Deliverable No.	2.5
Dissemination level*	CONFIDENTIAL
Work Package	2
Task	2.3
Lead beneficiary	EM
Contributing beneficiaries	INIAV
Due date of deliverable	31 August 2023
Actual submission date	20 December 2024





Written by: Inês Sousa, Jorge Oliveira, Carla Brites

HISTORY OF CHANGES								
Date	Beneficiary	Version	Change					
31/10/2024	INIAV	V1	Draft version sent to the coordinator					
07/11/2024	EM	V2	Text revision and addition of an executive summary					
20/12/2024	INIAV	V3	Final Version approved by project coordinator					

TABLE OF CONTENTS

EXEC	UTI	/E SUMMARY	5
1. Int	rodu	uction	6
2.	Met	hodology	7
2.1	1.	Materials	7
2.2	2.	Incorporation of Essential Oil in Rice	7
2.3	3.	Sensory Properties of Rice Impregnated with Essential Oils	8
2.4	1.	Disinfection of Rice with Ultraviolet Light	8
3.	Resu	ults	9
3.1	1.	Performance of the Essential Oils	9
3.1	1.1.	Effect of Essential Oil Compounds on Rice Quality1	0
3.2	2.	Performance of UV-C Light treatment in Sitophilus spp. Development	4
4.	Impl	lementation Plan1	5
5.	Con	clusion1	6
6.	Reco	ommendations for Future Studies1	6
7.	Refe	erences1	6

EXECUTIVE SUMMARY

The urgency of finding innovative solutions to prevent insect infestations in rice storage led to the need for this work package. Current mitigating measures are increasingly unsatisfactory for two main reasons: some insects have developed resistance to the most common chemicals and the chemicals themselves may pose a health risk to humans.

A previous deliverable (2.1) mapped the alternative solutions that have been proposed in the literature. A first choice stemmed from considerations of the feasibility of implementing the solution. It was considered that technologies that imply a cost per unit of product sold were not desirable. This is the case with modified atmospheres. Even though the cost of a Modified Atmosphere Package, or a fully hermetic package (as also proposed in literature) is small, the current market fights on the basis of price to the last cent. On the other hand, capital investments in processing equipment, although more significant as a one-time measure, can be considered to off-set to the cost of returns, recalls and the various problems that occur when whole shipments become affected by insect infestation.

Thus, two processing solutions were tested: oil polishing and treatment with electromagnetic waves. Oil polishing is the cheapest solution, as many companies in Southern Europe already export to North African and Middle Eastern markets oil polished rice, which is appreciated by those consumers. The currently used oil is a mineral oil (high-grade paraffin), which in spite of its high grade runs the risk of excess concentration of MOAH compounds, which in Europe would now be falling under very tight legislation. Thus, an oil that would mitigate insect development and offer comparable sensory properties to paraffin would indeed by a fantastic opportunity. Alas, in spite of literature claims to the contrary, real-life testing with infested rice showed that the eggs are sufficiently protected and growth to larvae and adult insects was not affected at all by the oil polishing with any of the oils tested.

However, a positive side results was obtained, the sensory properties with essential oils showed no statistically significant differences to paraffin. This is an opportunity worth exploring in case the markets in question became as restrictive to MOAH content as the EU currently is.

The application of electromagnetic waves could only be tested in the UV range (280 nm). The treatment was effective when applied on larvae, as it is if applied to adult insects, it this has the same capacity to disinfect rice as the current chemical phosphine does. However, it did not deter in any way the development of eggs to full-grown adult. Thus, UV treatment is indeed an alternative to phosphine of the same quality but does not offer the added benefit of preventing further investigation ideally sought.

Notwithstanding, this results is most likely due to the incipient penetration depth of electromagnetic waves longer than visible. It is therefore considered that radiofrequency or microwaves (the former having also been proposed in the literature for this purpose) are worthy of further investigation. It was not possible to do so in the lifetime of this project, as prototypes do not exist commercially yet. There is some concern as to the potential impact of such treatments on sensory and cooking quality (as they may cause hotspots of temperature), so the recommendation is for researcher to engage in a rigorous real-life assessment as done here for the essential oils and UV treatments.

1. Introduction

The urgency of finding innovative solutions to prevent insect infestations in rice storage led to the need for this work package. Current mitigating measures are increasingly unsatisfactory for two main reasons: some insects have developed resistance to the most common chemicals and the chemicals themselves may pose a health risk to humans (see deliverable D2.1 and project output De Sousa et al., 2023). *Sitophilus* species was selected for trials and validation, being major pests responsible for rice infestations. These insects not only destroy grains but also lay eggs within them, with larvae developing inside the grains until they reach the adult stage. Since the majority of the *Sitophilus* life cycle occurs hidden within the grains, detecting these hidden infestations is crucial for effective prevention.

Trials presented in Deliverable D2.2 (i) showed that carbon dioxide (CO_2) levels increase significantly during the insect's life cycle - a change not observed in non-infested rice. This suggests that CO_2 monitoring was established as a reliable marker for identifying hidden infestations. Additionally, the quantitative real-time polymerase chain reaction (qRT-PCR) also reported previously demonstrated potential for infestation detection, although further testing with blank samples and optimization is needed to confirm its applicability on an industrial scale.

A work plan to test the possible innovative solutions to control infestation by means other than the use of chemicals was established followed the outcome of deliverable D2.1. The methodology needed for a rigorous validation of the solutions is described next, having consisted in the actual development of selected insects so as to ensure with certainty not only insect presence, but also the specific type of insect and time in its lifecycle. This created a difficulty in liaising samples between the location of the insect growth (INIAV) and the EM company (200 km away), so the experimental work was undertaken at INIAV and the results analysed by EM.

The selection of methods identified in the previous Deliverable D2.1 to be assessed for this Deliverable was based on cost implications, and how they are met in an industrial setting. The current market conditions are extremely tight and therefore solutions using packaging technologies that would imply a permanent additional cost per unit sold were not considered priority by the company. These included vacuum packaging, packaging in hermetic films, modified atmosphere packaging. Priority was given to 2 types of potential solutions:

- Use of oils. Although using an extra ingredient implies a higher cost per unit, oil encapsulation is already used for exports from Europe to North Africa and Middle East, where the oil covering is appreciated (and in effect demanded) by those consumers. This important market is also where more serious problems of infestation tend to occur (which is likely due to the local handling of the products exposing them to very high ambient temperatures). Paraffin oils are currently used for the product appeal. However, there is new EU legislation in this regard that prevents the use of some mineral oils if high values of MOAH (aromatics) are reached. Thus, oils that would have the dual benefit of meeting sensory targets for the export market and be free or MOAH while mitigating insect infestation problems would have a very high interest. Thus, the sensory impact of oil impregnation also needed to be tested.
- Use of new processing technologies. Although capital investment needs to be recovered over the typical reintegration of equipment costs (10 years), it is a simpler decision to invest in equipment that has a fixed one-time cost compared to an increased cost per unit that will be added to the product "for ever", which is the case with packaging solutions. Therefore, assessment of whether electromagnetic waves can successfully sterilize products from egg infestation would also be a priority option to be considered.

The following options were tested within the lifetime of the project:

- Essential oils: impregnation with S-Limonene, Eugenol, Thymol and L-Menthol. These were chosen based on their repellent activity from previous work (see deliverable D2.2)
- Electromagnetic waves: Treatment exposure in the Ultra-violet wave range (UV-C)

The primary objective of solution assessment in Task 2.3 is to validate the effectiveness of selected treatments in a real-world setting. For this trial, rice from the Ariete variety, harvested in 2023 and previously used in the pilot study (D5.3), was chosen. This rice was intentionally contaminated with adult insects prior to controlled storage and packaging to evaluate the selected infestation prevention methods in a post-harvest context.

Food-grade compounds derived from essential oils were applied to prevent weevil infestations in stored rice, and the effectiveness of UV-C light in controlling infestations was also tested. Additionally, the impact of these treatments on the physicochemical and sensory qualities of the rice was assessed to ensure that product quality is maintained.

2. Methodology

2.1. Materials

Rice from the Ariete variety, harvested in 2023 and previously used in the pilot study (see deliverable D5.3), was chosen. The rice was de-husked and milled, and then intentionally contaminated by exposure to adult insects especially grown in-house at INIAV for this purpose. Each sample of 10 g was infested by contacting with 10 insects for 10 days (these numbers come from the previous work, see Deliverable 2.2).

2.2. Incorporation of Essential Oil in Rice

After the 10-day exposure, the live insects were removed. (S)-(-)-Limonene and Eugenol were applied as liquids (10 μ L and 20 μ L/100 g), while Thymol and L-Menthol were applied as solids (0.1 mg and 0.2 mg/100 g), using the mixing equipment represented in Figure 1. Coating with paraffin was chosen for the control sample (0.4 mL/100 g). Samples were placed in hermetically sealed containers and daily measurements of atmosphere composition inside the containers began, continuing until new adult insects were detected. Oxygen (O₂) and CO₂ levels were monitored using a CheckMate 9900 portable sensor (PBI Dansensor).



Figure 1 - Mixing Equipment (Ernesto Morgado, S.A.) to incorporate essential oil compounds in rice.

2.3. Sensory Properties of Rice Impregnated with Essential Oils

Rice quality was assessed (n=2) and compared with the control samples using methods detailed in Table 1.

Analysis	Method				
Basic chemical composition	Near Infrared Spectroscopy (NIR)				
Desting properties	Rapid Viscoanalyser (RVA)				
Pasting properties	AACC 61-02.01				
Colour	Colourimeter Minolta CR300				
Moisture	ISO 712:2009				
Amylose	ISO 6647-2:2020				
Resistance to extrusion	ISO 11747:2012				

Table 1 – Analyses for Evaluating Rice Physicochemical Quality and Corresponding Methods.

The basic chemical composition (ash, fibre, starch, protein, and lipid contents) was determined using Near Infrared Spectroscopy (NIR). Rice quality analysis also included colour (Colourimeter Minolta CR300), evaluated according to CIELAB system, resistance to extrusion via a compression test (ISO 11747:2012) and pasting properties using a Rapid Viscoanalyser (RVA) according to AACC 61-02.01 method.

Additionally, sensory acceptance tests were conducted with a trained panel with 13 tasters, who evaluated the cooked rice impregnated with essential oil compounds using a 1-to-5 hedonic scale.

2.4. Disinfection of Rice with Ultraviolet Light

Ultraviolet (UV) light is a non-ionizing form of electromagnetic radiation in the 100-400 nm wavelength range, next to visible (380 to 700 nm). It has demonstrated germicidal properties and offers a non-toxic alternative for food disinfection (Dittgen et al., 2021).

Collaborative trials with iBET partner were undertaken to investigate the impact of UV-C diodes (emitting light at 280 nm) on rice infested with *Sitophilus* spp. The infested samples were exposed to UV-C light for durations of 15, 30, and 40 minutes (Figure 2). Infestation progression was monitored through daily CO₂ measurements using a portable sensor.

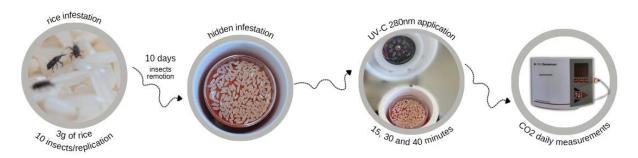


Figure 2 - UV Treatment Protocol: Application of UV-C diodes emitting light at 280 nm, with exposure durations of 15, 30, and 40 minutes.

3. Results

3.1. Performance of the Essential Oils

Preliminary results for Thymol and L-Menthol were negative. The lack of effect may have to do with them having to be used in powder form. This may explain a poor repellent effect.

After the 10 days of contact for infestation, the development of the next generation (F1) in rice impregnated with paraffin, limonene and eugenol (20 μ L/100 g) was assessed. There was a very significant variability among the 6 repeats. The variability can be due to the specific mix of 10 insects that originated the population, as it affects the number of eggs inserted in the grains. Sex ration is a factor, as ensuring an equal number of male and female insects is challenging. This balance is crucial for optimal reproduction but can only be confirmed at the end of the experiment by examining the insects' genitalia. Also, the reproductive capacity of *Sitophilus* can vary with age, as older insects tend to produce fewer eggs. Figure 3a to 3c show the worst case of CO₂ growth in each set, the best case, and the average values.

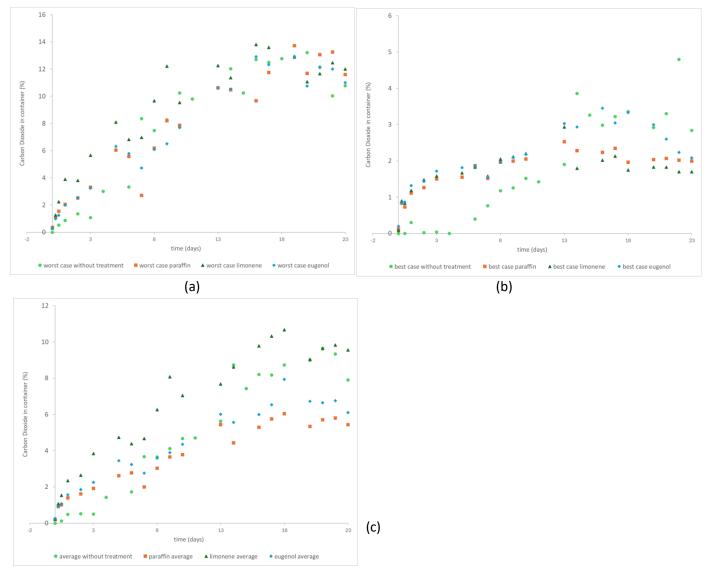


Figure 3 - CO_2 levels (%) in hermetic containers with pre-infested rice \bullet untreated, and impregnated with \blacksquare paraffin, \blacktriangle limonene or \blacklozenge eugenol for 6 replicates in each. (a) worst replicate; (b) best replicate, (c) average of all 6 replicates.

Т

In practical terms, what matters are the worst cases, as we would need all rice to be resistant to the development of a new breed of insects. The results with paraffin, limonene and eugenol were compared with the previous results with untreated rice.

It is evident that none of the oils offered significant protection, the CO₂ growth that indicates the presence of developing eggs, larvae up to new insects, stabilises at very comparable levels regardless of oil, or of having no oil, whether one considers worst case, best case, or average. The results are therefore very disappointing and do not validate the earlier promise that oils have a detrimental effect on infestation development. In a real-life scenario of a significant infestation (10 insects per 10g of rice) it takes just 20 days of incubation or so for new insects to pop up.

However, there is an interesting difference which is that rice impregnated with oil seems to stimulate egg growth, as CO_2 starts building up faster, which is particularly noteworthy in the best cases (Figure 3b). High CO_2 is actually detrimental to adult insect development, which is the reason why modified atmosphere packaging with high CO_2 is a recommended way of mitigating infestation problems (see previous deliverable 2.1). Thus, it can only be speculated that oils facilitate the growth of CO_2 and for this reason may appear to have a protective effect. However, in all cases tested new adult insects were observed, meaning that even at 12% CO_2 this build-up is not sufficient to ensure no growth of live insects. These results also contradict expectations that hermetic packages could improve the problem, as suggested in the literature (see deliverable 2.1).

Furter controlled studies could be done and may elucidate better on the reasons for this failure, but there is no evidence at present that oil polishing offers significant protection against infestation, nor that natural CO₂ build-up is a successful way of avoiding the development of new generations of adult insects from infested rice.

3.1.1. Effect of Essential Oil Compounds on Rice Quality

Colour

In spite of oils having no proven effect in real-life conditions to mitigate infestation problems, the results on quality factors are still very relevant at present, as regardless of the infestation problem, there is a good market in the Middle East and North Africa for oil polished rice. High grade paraffin is typically used, but in spite of its high grade there have been reports of excessive concentrations of MOAH compounds (mineral oil aromatic compounds). European legislation has recently been updated in relation to these components, which are deemed potential carcinogens, and for products such as rice the minimum amount allowed is just 0.5 mg/kg of rice. MOAH can find their way to rice from a variety of sources, but evidently when limits are so low the last thing one would wish to have is some more coming from the use of paraffin in these products. Therefore, we wish to ascertain if the essential oils, even though they will not offer advantages in terms of infestation control, might be appropriate replacements for paraffin.

The critical sensory quality appreciated by these consumers is the appearance, brightness and shiny look that the oil gives to the rice.

The colour of the milled rice was evaluated using CIELAB coordinates, with the following parameters:

- L*: Lightness (values range from 0 to 100%).
- a*: Greenness to redness (ranging from -60 to 60).
- b*: Blueness to yellowness (ranging from -60 to 60).

A ΔE^* value can be used to quantify the colour difference between the control and treated samples with a single number, being the Euclidean distance defined by the differences between each sample and a standard. In our case, the standard of interest is paraffin, the il that we would like to replace.

$$\Delta E = \sqrt{\left(L_{sample} - L_{standard}\right)^{2} + \left(a_{sample} - a_{standard}\right)^{2} + \left(b_{sample} - b_{standard}\right)^{2}}$$

The colour was measured 6 times in all samples and the results are shown in Table 2.

Table 2 – Averages of colour parameters or untreated, phosphine treated and oil impregnated rice, according to each concentration (1-10 μ L/100 g and 0.1 mg/100 g; 2- 20 μ L/100 g and 0.2 mg/100 g).

sample	L	а	b
untreated	94,49	-0,34	1,40
phosphine	96,49	0,09	0,64
paraffin	81,58	-0,90	2,84
limonene1	84,75	-0,76	2,70
limonene2	84,91	-0,62	2,69
eugenol1	82,81	-0,72	3,03
eugenol2	82 <i>,</i> 39	-0 <i>,</i> 85	4,91
menthol1	82,23	-0,75	3,03
menthol2	83,41	-0,72	3,34
thymol1 81,72		-1,00	2,89
thymol2	83,74	-0,86	2,97

It can be seen in Table 2 that untreated rice and phosphine treated (to prevent infestation) differ only slightly in colour (phosphine treated is significantly more reddish in colour in the green-red axis), although the difference is not noticeable to the naked eye. It can also be seen that there are no significant differences between the 4 essential oils and paraffin.

The integrated measure ΔE thus showed more differences between paraffin and the higher concentration of essential oils compounds (Table 3). The Euclidean distance is calculated in relation to the average ΔE for the 6 measures with paraffin. Differences between any of the oils and paraffin are within the variability of different measurements in paraffin itself.

It can be concluded that in terms of the most important sensory parameter, aspect, any of the oils can successfully replace paraffin, with thymol and menthol giving the closest results overall (although differences between any of the 4 are within the margin of error).

Table 3 - Average Euclidean distances of the 3 colour parameters in the CIELAB scale in samples impregnated with different oils (1- 10 μ L/100 g and 0.1 mg/100 g; 2- 20 μ L/100 g and 0.2 mg/100 g), in relation to the average of the paraffin measurements.

sample	ΔΕ
limonene1	3,179659
limonene2	3,346941
eugenol1	1,25942
eugenol2	2,218988
menthol1	0,695312
menthol2	1,906445
thymol1	0,180285
thymol2	2,172695

Texture

In appearance, as measured instrumentally with colour, the essential oils tested can replace the mineral oil paraffin. However, it is also necessary to ensure that on eating the cooked rice there is no difference in feel. In order to obtain an objective quantitative measurement, the extrusion force required to extrude a sample of rice was measured, as defined by ISO 11747:2012. Two samples were measured for untreated rice, rice treated with phosphine and impregnated with paraffin and the 4 essential oils. In Table 4 it can be seen that untreated and phosphine-treated rice have no differences with the paraffin-impregnated rice once cooked. Menthol, and especially eugenol and limonene give higher hardness, therefore, it will be necessary to test with actual sensory/organoleptic taste with a trained panel to verify if these differences are noticeable to the eating experience.

sample	mean extrusion force (kg/cm ²)
untreated	0,60 ± 0,04
phosphine	0,67 ± 0,01
paraffin	0,65 ± 0,01
limonene 1	0,85 ± 0,14
limonene 2	0,85 ± 0,03
eugenol 1	0,82 ± 0,04
eugenol 2	0,85 ± 0,06
menthol 1	0,80 ± 0,02
menthol 2	0,78 ± 0,03
thymol 1	0,84 ± 0,02
thymol 2	0,77 ± 0,01

Table 4 - Averages of hardness of cooked grains measured by extrusion force (kg/cm²) (1- 10 μ L/100 g and 0.1 mg/100 g; 2- 20 μ L/100 g and 0.2 mg/100 g).

The quality of the gumminess of the rice is also relevant for japonica varieties, which is the case for this market. Therefore, pasting properties were also measured. However, in this case, there was no difference between any of the samples, as summarised in Table 5.

sample	peak viscosity	through viscosity	breakdown	final viscosity	setback	peak time	pasting temperature
untreated	1269,5	1206,5	63	2563	1293,5	6,14	94,68
phosphine	1330,5	1235,5	95	3070	1739,5	5,87	93,8
paraffin	1239,5	1117	122,5	2630	1390,5	5,94	94,33
limonene1	1410	1292,5	117,5	2964	1554	5,94	80,23
limonene2	1173,5	1103,5	70	1485,5	312	5,97	94,73
eugenol1	1247,5	1138	109,5	2321	1073,5	5,97	94,23
eugenol2	1208,5	1117,5	91	2227,5	1019	6	94,23
menthol1	1236	1133,5	102,5	2002,5	766,5	6,07	94,65
menthol2	1281	1155	126	2040	759	6	94,25
thymol1	1318,5	1175,5	143	2561,5	1243	5,97	93,93
thymol2	1177	1103	74	2385,5	1208,5	6,04	94,65

Table 5 - Averages of pasting properties of cooked grains with different treatments (1- 10 μ L/100 g and 0.1 mg/100 g; 2- 20 μ L/100 g and 0.2 mg/100 g).

Sensory panel assessment

Sensory acceptance tests were performed with 13 trained tasters who evaluated the cooked rice samples based on their preference in a scale of 1 (worst) to 5 (best). Only samples impregnated with paraffin, limonene and eugenol ($10 \mu L/100 g$) were tested, as they were the only oils being used at the time. In addition to 5 sensory descriptors, panellists were also asked if they would buy that product (+1 score), were indifferent to it (0 score) or would not buy it (-1 score). The results are shown in Table 6Table 6. Statistically significant differences were established with the Fischer LSD method at 95% confidence level.

Table 6 - Averages of 5 sensory descriptors and buying intention for cooked rice from oil-impregnated samples. In each column numbers with the same superscript letter are not significantly different at a 95% confidence level according to the Fischer LSD test.

sample	odour intensity	sample	flavour intensity	sample	appearance	sample	odour	sample	flavour	sample	buying intention
limonene	2.23ª	paraffin	2.23ª	eugenol	3.77ª	eugenol	3.31ª	eugenol	3.15ª	eugenol	0.077ª
paraffin	2.23ª	limonene	2.46ª	paraffin	4.08ª	limonene	3.85 ^{ab}	limonene	3.61 ^{ab}	limonene	0.462 ^{ab}
eugenol	4.15 ^b	eugenol	3.92 ^b	limonene	4.15ª	paraffin	4.00 ^b	paraffin	4.07 ^b	paraffin	0.769 ^b

It can be seen in Table 6Table 6 that limonene was not significantly different from paraffin in any of the descriptors, whereas eugenol-impregnated samples were judged significantly different from paraffin (and eugenol) in most cases. Thus, of these 2 oils limonene would be the most suitable to replace the mineral oil paraffin. However, in terms of colour and texture, the parameters measured instrumentally, limonene was actually the most different of the oils compared to paraffin, even though the differences in those cases were not statistically significant. Notwithstanding, as eugenol and limonene did not prove to have any advantage in terms of insect infestation, it is recommended that further work with menthol and especially thymol should also be carried out, as they may prove advantageous in terms of replacing the paraffin.

It is however noted that the trained panel was not constituted by typical consumers of the oil polished rice. Those are North African and Middle East markets, and therefore, an attempt to switch from paraffin to essential oils as a solution to overcome MOAH limitations is suggested, with sensory tests recommended to be done by representative buyers/consumers of these products.

3.2. Performance of UV-C Light treatment in Sitophilus spp. Development

The second alternative to conventional phosphine treatment selected was the use of electromagnetic waves. During the lifetime of the project, it was only possible to test in the UV range, more specifically, a wavelength precisely at the border between UV-B (280-315 nm) and UV-C (100-280 nm). UV exposure is a known disinfectant and therefore can be relied on to replace phosphine in terms of controlling living (adult) insects. However, it's effect on eggs and larvae, that is, further development of new generation of insects, had not been tested. Hopes were modest given that UV range is quite short (shorter wavelengths than visible) and therefore has poor penetration capabilities. This means that it might not be able to reach sufficiently inside to act on the eggs. An alternative discussed in a previous Deliverable (2.1), EMW in the radiofrequency range (way longer than visible), could not be tried as it is so far experimental and no prototypes available to handle rice were found. Microwaves and radiofrequency waves have some penetration capability, although they can also cause run-away heating, so if UV would work, it would definitely be preferable.

The first tests consisted in placing actual larvae in rice to ascertain the disinfection power of UV in larvae themselves. Visible larvae (similar in size to small rice grains) were placed in samples and exposed to 280 nm UV for 15 and for 30 min. The CO_2 concentration in the atmosphere was taken as an indicator of insect infestation (Figure 4).

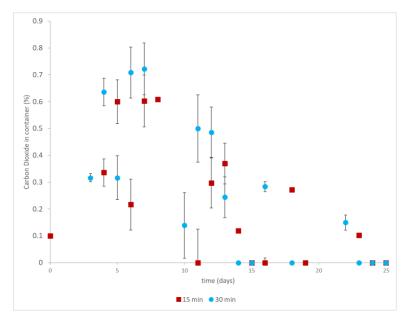
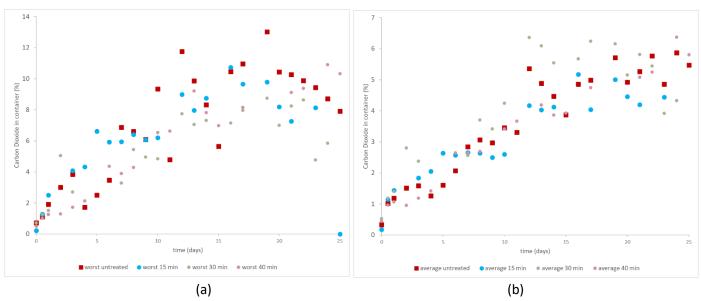


Figure 4 - Average (of 6 samples) carbon dioxide concentration in hermetic samples infested with larvae after treatment with 280 nm UV for 15 or 30 min. Bars denote the standard deviations.

Results show that the effect of the treatment is not immediate, larvae are still alive and thus the CO_2 content grows, until reaching a peak at around 5-8 days after the treatment. The lethal effect then causes their demise within an additional 10 days or so, with no activity visible past day 25. There is no evidence that 30 min treatment was any better than 15 min.

There was now some hope that UV might also be helpful to limit egg development, or in the very least eliminates larva development to adult. Samples with 10g of rice were infested with 10 insects, similarly to the work with essential oils, and then treated with UV for 15, 30 and 40 min, whereas other samples



were left untreated, as controls. The results are shown in Figure 6a (worst case) and b (average of all samples).

Figure 5 - CO₂ levels (%) in hermetic containers with pre-infested rice ■ untreated, and exposed to 280 nm UV for ● 15 min, ● 30 min and ● 40 min. (a) worst replicate; (b) average of all 6 replicates.

The results are very similar to those of using essential oils: the UV treatment is ineffective in preventing eggs to mature all the way to adult insects (which were visible after 25 to 42 days).

These disappointing results may be due to the lack of penetration depth, concluding that eggs are not sensitive to the UV treatment. As such, new infestation will develop. Thus, UV is an effective replacement for phosphine use, it kills larvae and adult insects. However, it does not offer the desired extra effect of also preventing eggs from developing all the way to mature insects. but given that larvae when exposed do end up dying instead of growing all the way to adult, it is also possible that exposing 10g of rice in one go in the device utilised would have been ineffective.

4. Implementation Plan

A key objective of the TRACE-RICE project is to identify cost-effective, sustainable solutions to prevent egg hatching in stored, packaged, and shipped rice without relying on chemical products. The urgency to find innovative methods stems from environmental and health concerns, coupled with the limited efficacy of chemical treatments in completely eradicating infestations.

The incorporation of essential oils and UV radiation were chosen for having the greatest likelihood of being feasible to implement in a real-life environment and had shown potential. However, the validation tests were negative in both cases. It is recommended that the technology using much lower wavelengths with greater penetration power, radiofrequency (or eventually also microwaves) be tested, but so far there are doubts on the ability to prevent eggs to hatch into larvae and then adults (which even chemicals currently used cannot do).

On the incorporation of essential oil compounds, there are however some promising results with the possibility of replacing the currently used mineral oil (paraffin) by essential oils. There are many

sensory similarities, and in this case, the recommendation is to further test the replacement with sensory tests done by the North African and Middle Eastern markets that consumer the oil-polished rice.

5. Conclusion

Based on the findings, the application of essential oil compounds did not significantly influence insect development or prevent infestation. Additionally, these compounds had minimal effects on rice quality characteristics.

In the case of UV-C treatment, higher doses and intensities showed promise for a lethal impact on larvae. However, the limited penetration of UV-C rays in solid foods remains a challenge (as also identified by Fan et al., 2017). Higher wavelengths with greater penetration warrant further study as they would be the most likely technologies of all solutions considered in this project to be implemented in a real company. It is again reminded that solutions that increase the unit price (e.g. packaging solutions) are less likely to be implemented than those that require a capital investment – as the latter can be off-set against cost of returns and recalls, without influencing the unit price proposed to the clients, which is a critical element of market success in the current climate.

6. Recommendations for Future Studies

Efforts to control and prevent infestations in stored rice must remain a priority for industry stakeholders. Some solutions proposed in the literature, such as the use of some essential oils and UV treatment, did not stood up to real-life testing. Once rice is infested with eggs that the adult insect manages to "drill" inside the rice, this seems to offer a protection that ensures its survival to the treatments tried, so a new generation of insects will emerge nonetheless.

Further exploration of electromagnetic treatments with higher wavelengths than visible (microwave or radiofrequency) is considered the most promising approach at the moment, requiring further testing and rigorous validation, as developed here for the technologies tested.

7. References

- De Sousa, I. G., Oliveira, J., Mexia, A., Barros, G., Almeida, C., Brazinha, C., Vega, A., & Brites, C. (2023).
 Advances in Environmentally Friendly Techniques and Circular Economy Approaches for Insect
 Infestation Management in Stored Rice Grains. *Foods*, 12(3), 511.
 https://doi.org/10.3390/foods12030511
- Dittgen, C. L., Silveira, M. M. D., Kröning, D. P., Ziegler, V., Oliveira, M. D., & Ferreira, C. D. (2021). Chemical, physical, and sensory changes in rice subjected to UV-C radiation and its acceptability to rice weevil *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and humans. *Journal of Stored Products Research*, 90, 101760. https://doi.org/10.1016/j.jspr.2020.101760

Fan, X., Huang, R., & Chen, H. (2017). Application of ultraviolet C technology for surface decontamination of fresh produce. *Trends in Food Science & Technology*, 70, 9–19. https://doi.org/10.1016/j.tifs.2017.10.004





TRACE-RICE Consortium

