# 1 The potential of UVC decontamination to prolong shelf-life of par-baked bread

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# 10 ABSTRACT

11 The effect of UVC (254 nm) treatment on the mould-free shelf-life of par-baked wholemeal, rye and six-12 grain bread was examined. Currently, these breads are par-baked, wrapped in HDPE-foil and transported 13 or stored at room temperature for a couple of days before being full-baked and sold/consumed. Generally, 14 after five days, these breads show signs of mould spoilage. A shelf-life increase of one or more days would 15 already offer immense economical and logistic benefits for the baker or retailer. In this study, the 16 parameters fluence rate (irradiation intensity), fluence (UV dose), distance to the UV-lamp (DTL) and 17 number of layers of a common wrapping HDPE-foil (20 µm) were diversified. The breads were subjected 18 to a UVC treatment (0 – 2502 mJ/cm<sup>2</sup>), packed and stored at room temperature for a period of 15 days 19  $(21.5 \pm 0.8^{\circ}C)$ . Similar as for the breads, agar plates with mould spores of Aspergillus niger, Aspergillus 20 montevidensis and Penicillium roqueforti were UVC treated (0 - 1664 mJ/cm<sup>2</sup>) and checked daily for visible 21 mould growth during 15 days (25°C). A. niger showed the strongest resistance towards UVC, a fluence of 22 800 mJ/cm<sup>2</sup> was needed to inhibit growth during 15 days of storage, whereas for *P. roqueforti* and *A.* 23 montevidensis respectively UV levels of 291 and 133 mJ/cm<sup>2</sup> were found sufficient. Furthermore, the shelf-24 life of wholemeal, rye and six-grain bread can be prolonged from 5 to respectively 6, 8 and 9 days using 25 2502 mJ/cm<sup>2</sup>. The effect of higher UVC dosage and longer shelf-lives was strongly impacted by the wide 26 variability on mould growth. Main factors influencing the potential of UV decontamination were the rough 27 bread surface, differences in DTL, the possibility of post-contamination and UV permeability of packaging 28 materials.

- 29 **Keywords:** UVC; par-baked bread; *Aspergillus niger*; *Penicillium roqueforti*; *Aspergillus montevidensis*
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#### 31 **1. Introduction**

Par-baking contributes to the extension of the microbiological and technological shelf-life of bread 32 33 (Almeida et al., 2015; Debonne et al., 2018). One of the major benefits of par-baking is flexibility provided 34 for bakers, restaurants and consumers who can easily bake off products according to demand (Decock & 35 Cappelle, 2005; Lambert et al., 2009). Usually, par-baked (PB) bread products for business-to-business 36 purposes are baked for 95%. However, this can vary from 50 up to 95% depending on the type of product 37 and the offset market (Eveline Lopes Almeida & Chang, 2013; Fik & Surwka, 2002). PB breads can be stored under either frozen, ambient or chilled temperatures. The market share of frozen bread is large and is 38 39 increasingly growing in Europe (Decock & Cappelle, 2005). Distribution of frozen PB bread is aiming at 40 hotels and catering industries (eating outdoors), while for unfrozen PB breads, bake-off and consumption 41 are mainly intended to take place indoors. The market share of unfrozen PB bread is smaller, nevertheless 42 important. These breads are mostly stored at room temperature and are packaged under modified 43 atmosphere (MAP). They often comprise packages of small amounts of products for individual use. On the 44 contrary, bakers and restaurants have a higher demand of products which renders small packaging an 45 expensive and inefficient strategy to store products. For small par-baked bread buns and rolls, frozen storage is currently the most important strategy. However, freezing can speed up retrogradation of bread 46 47 after full-baking, thereby shortening the physico-chemical shelf-life of bread (Bárcenas & Rosell, 2006; Fik 48 & Surwka, 2002). In larger breads which are not consumed immediately but over a period of time, freezing 49 has a much more pronounced effect on the physico-chemical quality (Debonne et al., 2017) and is 50 therefore to be avoided. PB breads are characterized by a high aw ( $\geq 0.94$ ), depending on the degree of 51 par-baking and the type of product (Debonne et al., 2018). Therefore, unfrozen PB bread products are 52 highly sensitive to post-baking contamination. Mould growth is by far the most important shelf-life limiting 53 factor of PB bread, with Penicillium spp., Cladosporium spp. and Aspergillus spp. being the most dominant 54 species (Saranraj, 2012). The shelf-life of frozen PB bread can be up to 12 months (Vulicevic et al., 2004), 55 whereas that of clean label MAP PB-bread is 3 weeks (Deschuyffeleer et al., 2011) and without MAP 4 to 56 6 days (Debonne et al., 2018). Main factors influencing microbiological and physico-chemical properties of 57 bread are relative humidity (RH) of the packaging environment and storage temperature. RH plays an 58 important role in moisture migration during storage. When the RH of the environment is lower than the 59 aw of bread, the environment will take up water from the moist bread. This can result in harder bread 60 crumb properties after full-baking (Baik & Chinachoti, 2000), but also in a bread which is potentially more 61 resistant towards microbiological spoilage due to the lower moisture content and reduced aw. A similar 62 trade-off exists for storage temperature.

63 Besides chilled and frozen storage, MAP and (chemical) preservatives, UVC decontamination (254 nm) of 64 air and surfaces can also be a promising strategy to prolong mould-free shelf-life of par-baked bread. In a 65 study of Debonne et al. (2020), the use of a Flexbaker-UV storage unit with built in UVC air 66 decontamination was tested. Storage of PB-bread in a flexbaker-UV with 90% RH and 3°C extended mould 67 free shelf-life to up to 3 weeks and showed similar antifungal effect as MAP. Begum et al. (2009) investigated the effectiveness of UVC surface treatment against Aspergillus flavus, Aspergillus niger, 68 69 Aspergillus ruber (formerly known as Eurotium rubrum) and Penicillium coryphilum and found that UVC 70 irradiation can effectively inactive spores, but the efficacy varies significantly according to methods of 71 exposure and among genera. Spores of A. niger showed the strongest resistance compared to the other 72 moulds tested. Kawaguchi et al. (2019) found that 70 mJ/cm<sup>2</sup> is effective in delaying mould growth of 73 Penicillium spp. from 4 to 6 days without having to compromise on taste. They also stated that to improve 74 the use of UV irradiation in bread production lines, it is necessary to decrease the amount of mould on 75 bread. By doing so, UV will be more effective. One of the limitations of UVC decontamination is the small 76 penetration depth of UVC light. The presence of surface cracks or small particles on bread (e.g. flour, 77 grains) reduces the effectiveness of UV as some fungal spores might not be exposed to UV light directly 78 (Begum et al., 2009; Kawaguchi et al., 2019). The fact that this technology does not emit heat nor causes 79 condensation on the packaging is a positive aspect. Moreover, it is considered a highly efficient green 80 technology which reduces the need of any anti-microbial and comes with a lower operational cost 81 compared to other food processing techniques. The data on the disinfection of surfaces of solid foods, 82 meat, and fresh products are rather limited (Singh et al., 2021).

The aim of this study was to evaluate the effect of UVC surface decontamination on agar plates against three bread moulds and on three bread varieties of par-baked bread which were prone to natural contamination. In order to mimic a real situation in the bakery, the effect of a wrapping foil (HDPE-foil) was also studied. On the agar plates, fluence was varied between 0 and 1664 mJ/cm<sup>2</sup>, whereas on bread the range was 0 to 2502 mJ/cm<sup>2</sup>.

#### 88 2. Materials and methods

# 89 2.1. Experimental design

90 This study consisted of three main research questions (Figure 1). First, what influence does the distance 91 to the lamp (DTL) and layers of HDPE-foil have on the fluence? Second, how sensitive are bread moulds 92 towards UVC? Third, can UVC decontamination be used as a tool to prolong mould-free shelf-life of par-93 baked bread? In the first part, the effect of DTL (12.5, 17.0 and 21.5 cm) and HDPE-foil (0 – 4 layers) on the 94 UVC transmission was studied. In the second part, three moulds were selected. A. niger was selected based 95 on its increased resistance towards UVC (Begum et al., 2009) and has been isolated from bread products 96 (Dagnas et al., 2015; Valerio et al., 2009), A. montevidensis (formerly known as Eurotium amstelodami) is 97 known for its growth on intermediate moisture foods and has been isolated from bakery products (Greco 98 et al., 2021; Guynot et al., 2005; Vytřasová et al., 2002), and P. roqueforti based on its high tolerance against weak organic acid preservatives (Kalai et al., 2017) and its occurrence as early bread spoilage 99 100 organism (Garcia et al., 2019; Moro et al., 2022). Their sensitivity against UVC was tested on agar plates to 101 reduce all other factors influencing the outcome. In the third part, par-baked wholemeal, rye and six-grain 102 breads were subjected to UVC decontamination and mould-free shelf-life was recorded during a period of 103 15 days.

# 104 **2.2. Materials and methods**

# 105 2.2.1. Fungal isolates and growth medium

Aspergillus niger van Tieghem (DTO 359-C5), Aspergillus montevidensis (formerly known as Eurotium amstelodami (Chen et al., 2017)) (MUCL 15640) and Penicillium roqueforti (MUCL 046746) were kept active
 on malt extract agar (MEA, Thermo Scientific<sup>™</sup> Malt Extract Agar (Dehydrated) - 500 g) or dichloran-

- 109 glycerol agar (DG18, Thermo Scientific<sup>™</sup> Dichloran-Glycerol 18% (DG18) (ISO) Agar Base (Dehydrated) 500
- 110 g) + chloramphenicol supplement; for *Eurotium* spp.) (Oxoid). They are maintained in the culture collection
- of the Laboratory of Applied Mycology MYCOLAB, Department of Food Technology, Safety and Health,

Ghent University (Ghent, Belgium). One week prior to use, fungal spores were transferred to fresh 112 MEA/DG18 plates (3x) and incubated for 7 days at 26°C. Sterile Tween 80 (polyoxyethyleensorbitan mono 113 114 oleaat, Merck) - water solution (1 g Tween 80 per liter distilled water) (5 mL) was added to a full-grown petridish. All the fungal material was scraped loose from the petridish. The Tween-solution was filtered in 115 116 a sterile falcon tube using a sterile cotton filter (3x). The filter was removed and the falcon tube was centrifuged for 15 min at 8000 rpm (Relative centrifugal force (RCF) of 6654) and 4°C. After removal of the 117 supernatant, the pellet was resuspended in 25 ml Tween-PBS (1 g Tween 80 and 10 tablets of PBS per liter 118 119 of distilled water) (Phosphate buffered saline, Oxoid). The centrifugation step was repeated and the supernatant was removed. Furthermore, the pellet was resuspended in 25 ml of PBS and the latter 120 121 centrifugation step was repeated a second time. The concentration of spores present was determined by 122 a microscopic evaluation using a Thoma cell counting chamber  $(7 - 8 \log \text{ spores/mL})$ . Finally, a 123 concentration of 100 spores per 20 µL volume was obtained and used to inoculate the agar plates. Three spots of the mould spore suspension (5 x 10<sup>3</sup> spores/mL) were applied on each Petri Dish. In total there 124 125 were 12 replicate spots per condition.



126

127 **Figure 1.** Schematic overview of the experimental design of the study.

#### 128 2.2.2. Breads

129 Three bread varieties were supplied by Bakkerij BroodNodig (Hofstade, Belgium): rye bread, wholemeal 130 bread and six-grain bread (Figure 2). All breads were par-baked at the bakery and transported in crates 131 (per 12), wrapped in High-density polyethylene (HDPE)-foil (20 μm, Galoppin), to the University Campus 132 (Ghent, Belgium) where the UVC treatments were conducted. Par-baking was stopped when the core 133 temperature of the breads reached a certain temperature: e.g. 93°C for wheat bread and 97°C for rye 134 bread. These breads are considered fully-baked after 10-11 min baking at 190-200°C (reheated until core 135 temperature reaches 65°C). However, the breads were already consumable due to the advanced state of 136 par-baking. Because of logistic reasons, the par-baked breads were frozen upon arrival (-18°C), and were 137 collected from the freezer when needed for UVC treatment. UV treatment of all the breads was spread 138 out over multiple days. All breads were thawed for 1 to 3h before the decontamination treatment. The 139 intrinsic properties of the bread crust were measured max. 3h after thawing and 48h after thawing. The 140 aw was checked with a LabMaster-Aw (Novasina) whereas pH was measured with a portable pH meter (model HI 83141, Hanna Instruments) (Table 1). The pH of the crust was measured as follows: 10 g crust 141 142 was dissolved in 100 mL distilled water. After 1h soaking, pH was measured of the suspension. The height 143 of the breads is also given in Table 1, as the distance between the bread surface and the UVC lamp played 144 an important role in the final fluence reaching the bread surface. The distance to the lamp (DTL) was 145 respectively 14.0 (rye), 12.0 (wholemeal) and 13.5 (six-grain) cm. Height was measured at the highest 146 point. Storage temperature and relative humidity in the bags were logged with four Testo 174H loggers. 147 The data was retrieved using a Testo Interface 174T/174H and Software ComSoft Basic (Testo SE & Co. 148 KGaA, Germany). The temperature was recorded throughout the whole storage period of the experiment.

149 The temperature was  $21.5 \pm 0.8$  °C and relative humidity was  $77.0 \pm 7.4\%$ .



150

- 151 Figure 2. Side (left) and top (right) view of the three types of bread, from top to bottom: rye bread, wholemeal bread
- and six-grain bread.
- **Table 1:** pH,  $a_w$  and height of the breads. pH and  $a_w$  measured at the start (max. 3h after thawing) and after 48h stored at 21.5°C (n = 3)

	At the start		48h after thawing		
	рН	a <sub>w</sub>	рН	aw	Height (cm)
Rye bread	5.47 ± 0.02	$0.914 \pm 0.011$	5.50 ± 0.02	$0.939 \pm 0.001$	7.5 ± 0.5
Wholemeal bread	5.74 ± 0.03	0.883 ± 0.015	5.78 ± 0.02	0.933 ± 0.002	9.5 ± 0.5
Six-grain bread	5.88 ± 0.06	$0.870 \pm 0.007$	5.74 ± 0.04	0.925 ± 0.003	8.0 ± 0.5

#### 155 2.2.3. UVC treatment (lamp and dosimetry)

The UVC lamp (254 nm, 230V, 50/60 Hz, 0.14A, 2x15W, produced by Thermo Electron Corporation 156 157 (Massachusetts, USA) was placed in a closed box in which a measuring probe was added (XRD140T254, 158 LOT Quantum Design GmbH, Darmstadt, Germany). The probe measured the fluence rate or intensity of 159 the lamp (mJ/cm<sup>2</sup>) at a given DTL. Based on exposure time (s) and fluence rate, the UVC dose or fluence 160 (mJ/cm<sup>2</sup>) was calculated. Additionally, an experiment with HDPE-foil was conducted in order to gain 161 information on the UVC permeability of the wrapping foil, used in a bakery environment for transporting 162 the par-baked breads in crates. After par-baking, the breads are packed per 12 in crates covered with 163 HDPE-foil. On the bottom and the side of the crates, one layer of HDPE-foil is present, whereas on the top 164 there are four layers of foil present (overlaying/wrapping technique). Currently, breads in the center of 165 the crates are touching each other, no foil is present between them. The effect of 0, 1, 2, 3 and 4 layer of 166 HDPE-foil covering the probe and DTL of 21.5, 17 and 12.5 cm was studied. The fluence rate was measured 167 three times over a period of 180s. UV treatment on bread was performed without HDPE-foil.

168 The effect of UVC on spoilage moulds on agar (DTL = 12.5 cm) and the mould-free shelf-life of bread was 169 studied with respectively maximal levels of 1664 mJ/cm<sup>2</sup> and 2502 mJ/cm<sup>2</sup>. The exposure time of the agar 170 plates was varied from 0 to 600 s which correlated with the following fluence (mJ/cm<sup>2</sup>): 0 (0s), 27 (15s), 60 171 (30s), 133 (60s), 291 (120s), 458 (180s), 626 (240s), 800 (300s) and 1664 (600s). The breads were subjected 172 to longer exposure times and therefore also higher fluence: 0 (0s), 149 (60s), 481 (180s), 818 (300s), 1155 173 (420s), 1660 (600s) and 2502 mJ/cm<sup>2</sup> (900s). The fluence for the breads was calculated with the probe 174 placed at 12.5 cm DTL (matches with a height of bread of 9 cm). After UVC treatment, the agar plates were 175 sealed with parafilm and incubated at 25°C for 15 days, whereas the breads were packed in sealed plastic 176 bags (PA/PE/20/70) (PA: polyamide; PE: polyethylene) to ensure no further post-contamination. The plates 177 were checked daily for visual mould growth. The breads were treated similarly, but here we made a 178 distinction between the top, sides and bottom of the bread. The static experimental set-up only allowed 179 decontamination on the top of the breads.

# 180 2.6. Statistical analysis

To assess significant differences among samples, a multiple comparison analysis of samples was performed using SPSS. Where the results were normally distributed, either a Tukey test (homoscedasticity) or Dunnett T3 test was used to describe the means with 95% confidence (p = 0.05). A Dunn test for multiple comparisons was applied, preceded by a non-parametric Kruskal-Wallis one-way ANOVA, for non-normally distributed data. For the determination of the significant differences between the mould-free shelf-life, the Bonferroni correction was applied for multiple tests (p = 0.05).

# 187 3. Results & Discussion

# 188 **3.1. UVC transmission of HDPE-foil**

189 At a semi-industrial level, par-baked breads are often wrapped in plastic foil and placed in bread crates.

- 190 HDPE is of high interest in flexible packaging due to its high moisture barrier and strength (Bauer et al.,
- 191 2021). However, UVC cannot pass through most plastics or ordinary glass (Ha et al., 2016). In this study,
  192 the transmission of UVC irradiation through HDPE-film was studied in order to examine the potential of
- using this film for packaging of bread and consecutive UVC decontamination as to reduce the risk of post-
- contamination upon storage. Figure 3 shows the measured fluence rate in terms of the number of HDPE-
- layers  $(0 4; 20 \,\mu\text{m})$  and at distance to lamp (DTL) 12.5, 17.0 and 21.5 cm. A certain time is needed to

196 reach an equilibrium fluence rate. In Table 2, the mean values between 150 and 180s of irradiation time 197 are presented. The fluence rate strongly decreased with increasing DTL and is conversely proportional to 198 the square of the distance from the source (Jin et al., 2005) or coincide with the power trend line (fluence 199 rate x DTL) (Chadyšiene et al., 2009). These findings were validated and resulted in R<sup>2</sup> ranging from 0.995 200 (0 HDPE) to 1.000 (4 HDPE) for the power trend line; e.g. 0 layers HDPE-foil: y = fluence rate; x = DTL; eq. 201 y = 117.95x<sup>-1.314</sup>; 4 layers: eq. y = 94.274x<sup>-1.315</sup>. Four layers of HDPE-foil reduced UV transmission by 19.3 -202 22.1% compared to no layers of HDPE, whereas one layer reduced transmittance with 2.4 – 4.4%. Ha et al. 203 (2016) found that PE-film of 70 µm thick transmits 60% of UVC light, whereas PP (polypropylene) of the 204 same thickness 80%. The DTL plays a big role on fluence, but also the position of the lamp and the 205 orientation of the light influences the decontamination potential (Oms-Oliu et al., 2010).



206

207	Figure 3. Fluence rate (mW/cm <sup>2</sup> ) measured at 12.5, 17.0 and 21.5 cm Distance to Lamp (DTL) and influence of 0, 1,
208	2, 3 or 4 layers of HDPE-foil (n = 3).

209	Table 2: Fluence rate measured at different DTL (Distance to Lamp, cm) and influence of 0, 1, 2, 3 or 4 layers of
210	HDPE-foil, mean fluence rate measured between 150 and 180s (n = 3). The last column represents the relative
211	decrease of fluence rate compared to 0 layers of HDPE-foil (%) (n = 3).

DTL (cm) HDPE		fluence rate (mW/cm <sup>2</sup> )	% decrease vs 0 HDPE	
12.5	0	4.21 ± 0.03	-	
	1	$4.11 \pm 0.01$	2.4%	
	2	$3.90 \pm 0.01$	7.4%	
	3	3.62 ± 0.04	14.1%	
	4	$3.40 \pm 0.01$	19.3%	
17	0	2.94 ± 0.02	-	
	1	$2.81 \pm 0.02$	4.4%	
	2	2.66 ± 0.02	9.3%	
	3	$2.51 \pm 0.01$	14.7%	
	4	2.29 ± 0.01	22.1%	

21.5	0	2.07 ± 0.01	
	1	$2.02 \pm 0.01$	2.4%
	2	$1.92 \pm 0.00$	7.2%
	3	$1.79 \pm 0.01$	13.6%
	4	$1.67 \pm 0.01$	19.4%

#### 212 **3.2. Susceptibility of moulds to UVC**

213 Growth of all three moulds was inhibited for 15 days with the use of 800 mJ/cm<sup>2</sup> (Table 3). A. niger showed 214 the highest resistance, compared to A. montevidensis and P. roqueforti for which respectively 133 and 291 215 mJ/cm<sup>2</sup> was sufficient for growth inhibition. A. niger spores contain the pigment melanin which can 216 partially absorb UVC (Begum et al., 2009). This shows that spores with dark pigmentation are more 217 resistant towards UV. Begum et al. (2009) found that 928 mJ/cm<sup>2</sup> was enough to completely destroy A. niger on agar plates which is in line with the results obtained in this study. In a study of Trivittayasil et al. 218 219 (2015), UVC resistance of *Cladosporium cladosporoides* and *Penicillium digitatum* was compared. They 220 concluded that P. digitatum, which is a non-melanized fungus, is significantly more sensitive to UVC radiation. In order to achieve 3 log reduction on PDA agar, 500 mJ/cm<sup>2</sup> was required for C. cladosporoides 221

whereas for *P. digitatum* 60 mJ/cm<sup>2</sup> was sufficient (Trivittayasil et al., 2015).

223	Table 3: Percentage of UVC treated agar plates, containing spores of A. niger, A. montevidensis and P. roqueforti,
224	showing no growth after 15 days of storage at 25°C (n = 9)

UV dose (mJ/cm <sup>2</sup> )	A. niger	A. montevidensis	P. roqueforti
0	0%	0%	0%
27	0%	22%	33%
60	0%	78%	56%
133	0%	100%	78%
291	0%	100%	100%
458	33%	100%	100%
626	78%	100%	100%
800	100%	100%	100%
1664	100%	100%	100%

#### 225 3.3. Mould-free shelf-life of par-baked bread treated with UVC

226 Values for pH of crust, aw of crust and height of par-baked bread after 1-3h after thawing and 48h after storage at room temperature are given in Table 1. The pH of rye bread (5.47 ± 0.02) was slightly lower 227 228 compared to whole meal  $(5.74 \pm 0.03)$  and six-grain bread  $(5.88 \pm 0.06)$ . The pH did not change significantly 229 over time. The  $a_w$  was the lowest for wholemeal and six-grain bread crust (resp. 0.883  $\pm$  0.015 and 0.870 230  $\pm$  0.007), compared to rye bread at the start (0.914  $\pm$  0.011). However, after 48h of storage in closed plastic 231 bags, aw increased for all breads and ranged from 0.925 – 0.939. We assume that pH and aw will not impact 232 potential differences observed for mould-free shelf-life as the values are more or less similar for all three 233 bread types. Initial microbiological load, presence of cracks (irregular surface) and small particles on the 234 bread crust such as flour and grains will be more important. The effectiveness of UVC on natural spoilage 235 on bread was studied (Figure 4). Mould-free shelf-life was recorded daily during a period of 15 days. 236 Without UV treatment, (median) shelf-life of par-baked bread was 5 to 6 days. A significant increase in 237 shelf-life was already observed for rye and six-grain bread at 149 mJ/cm<sup>2</sup>. The median value increased with 238 respectively 2.5 days and 1 day. At higher fluence, the median shelf-life showed an increase compared to 239 previous UV dosage for wholemeal bread at 481 mJ/cm<sup>2</sup> (+ 2 days), 1155 mJ/cm<sup>2</sup> (+ 3 days) and 2502 240 mJ/cm<sup>2</sup> (+ 3.5 days). However, due to the increase in interquartile range and the Bonferroni correction 241 applied on the data, further increase in shelf-life was not proven significant. Similar trend was observed for rye bread (+ 5 days at 1155 mJ/cm<sup>2</sup>; + 7.5 days at 1660 mJ/cm<sup>2</sup>) and six-grain bread (+ 3 days at 481 242 mJ/cm<sup>2</sup>; + 4 days at 1660 mJ/cm<sup>2</sup>). These values which are referred to are median values. A wide 243 244 interguartile range is often observed and is inherent to mould-free shelf-life. This is due to potential post-245 contamination after UVC treatment, the shadowing effect and the irregular surface of the bread crust 246 (Kawaguchi et al., 2019; Raeiszadeh & Adeli, 2020). When considering the highest dosage tested (2502 247 mJ/cm<sup>2</sup>) and the minimum shelf-life, fungal spoilage on wholemeal bread can be prolonged from 5 to 6 248 days, for rye bread from 5 to 8 days and for six-grain bread from 5 to 9 days. The values for the coefficient of determination ( $R^2$ ) between UV dosage and mould-free shelf-life were respectively  $R^2 = 0.31$ ,  $R^2 = 0.66$ 249 250 and  $R^2 = 0.53$  for wholemeal bread, rye bread and six-grain bread. This means that the shelf-life can be 251 partly assigned to UV dosage, whereas the rest will be a results of post-contamination, shadowing effect 252 and irregular bread crust.

253 Kawaguchi et al. (2019) found that within *Penicillium* spp. UVC resistance varies among species or strains. 254 For example, P. hispanicum was far more resistant than P. chrysogenum, whereas P. paneum and P. 255 chermesinum were the most sensitive. They also studied the effectiveness of UVC decontamination on 256 white bread and concluded that the mould-free shelf-life could be prolonged with one day using 70 mJ/cm<sup>2</sup> 257 UVC radiation. Higher fluence was not tested due to the concern of the risk of oxidized flavor in white 258 bread. However, the formation of off-flavors and aromas is likely dependent on the wavelength and dose 259 and will be different for different food products. Consequently, in order to determine the best way to use 260 UV, it is necessary to test each product for its spectral response to UV (Koutchma, 2009). Sensory tests 261 were not conducted in this study, because the UVC treatment was only applied on the crust of the par-262 baked bread, whereas the share of UVC treated bread crust is minimal upon consumption of a bread slice. 263 Moreover, there are only few studies mentioning the sensory impact of UVC treatment of bread.







#### 267 3.4. Industrial application

268 Prolonging mould-free shelf-life of par-baked bread with a few days can provide various economical and 269 logistic benefits for industrial bakers. In research papers, the use of static UVC systems for solid foods is 270 often mentioned, either with lamps on one side, two sides, or lamps placed longitudinally around the 271 chamber's inner surface (Lázaro et al., 2014; Manzocco et al., 2011; Manzocco & Nicoli, 2015; Rosario et 272 al., 2021). In static systems with one side exposure, the food product must be turned to obtain an even 273 level of decontamination. Whereas with two or more side exposure the food product must be placed on a food support in the form of a UV-permeable net or film. The static approach in this study showed the 274 275 potential of UVC decontamination on the top of the breads, meaning in direct contact with UV-light. 276 Moreover, the possibility of using HDPE-foil as an extra hurdle for preventing post-contamination was 277 highlighted. The UVC permeability of the foil reduced with max. 22.1% using 4 layers of HDPE-foil. This 278 showed that the combination of UVC treatment and wrapping of the breads in HDPE-foil can be a 279 promising strategy to prolong shelf-life of par-baked bread with a couple of days. An engineering approach 280 can further elucidate on how to implement mechanical rotating systems which can be used for UVC 281 decontamination, preferably of par-baked breads packaged in UVC permeable foil and bread crates which 282 enables easy storage and transport of these breads. However, stacking in bread crates introduces an extra 283 problem for which a solution must be found: blind spots between two adjacent breads. There the bread 284 crust has no direct contact with UVC radiation and is therefore not UVC treated. Further research is needed 285 solving this problem as well, without having to increase the amount of packaging foil or excessive energy 286 needs.

# 287 4. Conclusion

288 UVC treatment and HDPE-foil packaging can be promising alternatives to prolong mould-free shelf-life of 289 par-baked bread. Shelf-life of wholemeal, rye and six-grain bread was extended with respectively one day 290 (from 5 to 6), three days and four days. Moreover, the HDPE-foil showed promising UVC transmittance 291 and can be used as an extra hurdle to prevent early mould spoilage on par-baked bread. This study 292 encourages future studies approaching the mould-free shelf-life of bread using UVC decontamination.

# 293 Acknowledgements

The authors wish to thank Marc Van Eeckhout (Broodnodig, Bakkerij Van Eeckhout, Tervuursesteenweg 233, 1981 Hofstade, Belgium) for providing fresh bread that made it possible to perform this research.

Furthermore, our thanks also goes to the students for their assistance in the laboratories at Campus

297 Schoonmeersen (Lisa Krikilion, Nele Van Schepdael, Charlotte Velghe and Isabel Vernaillen).

# 298 Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or notfor-profit sectors.

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