Inactivation of *Salmonella spp.* in Wheat Flour by 395 nm Pulsed Light Emitting Diode (LED) Treatment and the Related Functional and Structural Changes of Gluten

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Abstract

Salmonella spp. is one of the top foodborne pathogens associated with low-moisture foods and they exhibit significant resistance to conventional thermal treatments. UV light pulses emitted from light emitting diode (LED) has shown antimicrobial potential in high-moisture foods and water. However, limited information is available about the antimicrobial potential of UV light with different wavelengths, including 395 nm in low-moisture foods. The objectives of this study were to investigate the antimicrobial potential of 395 nm pulsed LED light in wheat flour and the resulting quality changes. This study demonstrated a maximum 2.91 log reduction of *Salmonella* cocktail in wheat flour treated with 395 nm pulsed LED for 60 min in a semi-closed system. Oxidation occurred in wheat flour after 30 and 60 min exposure to the 395 nm LED, which subsequently led to bleaching, and polymerization of gluten components through disulphide linkage. The water holding capacity of gluten was reduced by oxidation, and the contents of secondary structures were altered significantly after pulsed LED treatment, but the rheological properties were not deteriorated. The disulfide bond formation naturally happens during dough formation and the oxidation triggered by pulsed LED treatment may play a role on accelerating this process. The 395 nm pulsed LED treatment could be a promising decontamination technology for wheat flour with an additional benefit of bleaching of the flour without chemicals.

Industrial relevance

A number of foodborne outbreaks and recalls have been related to low-moisture foods in these decades and recently several outbreaks were reported due to the occurrence of *Salmonella* in wheat flour. However, it is difficult to solve this problem through conventional thermal approaches because of the increased thermal resistance of *Salmonella* at low water activity environment. The emerging LED light source can produce light with monochromatic wavelengths without the use of mercury vapor lamps. It also has high durability, low heat generation, and is relatively easy to be adapted in an existing production line. Therefore, there is a great potential of using certain UV wavelengths emitted from LED to disinfect the low-moisture foods in food industries. To the best of our knowledge, no research was conducted on decontamination of wheat flour by using LEDs and only limited studies are available on the influence of pulsed LED treatment on food quality. The aim of this study was to explore the possibility of using 395 nm pulsed LED treatment as a novel tool for decontamination of *Salmonella* in a low-moisture food product (wheat flour) with industrial feasibility, and investigate the influence of the pulsed LED treatment on quality changes in the product.

Highlights

- The 395 nm pulsed LED resulted 2.9 log reduction of *Salmonella spp.* in wheat flour
- Bleaching and oxidation occurred in wheat flour with 395 nm LED treatment
- LED treatment induced polymerization of gluten proteins through disulphide linkage
- The elastic property of gluten was enhanced by LED
- Contents of intermolecular β-sheet and β-turn structures were increased

Keywords

Low-moisture foods, wheat flour, *Salmonella*, light emitting diode (LED), UV-A, oxidation

1. Introduction

According to the Government of Canada (2016), about 4 million Canadians are affected by foodborne illnesses every year, and 5% of the cases are attributable to *Salmonella enterica*. In the annual report of CDC (2015), 7719 *Salmonella* infections were confirmed in US and among which 535 infections were outbreak-associated. Compared with 2015–2017, the incidence of infection significantly raised 9% for *Salmonella* in 2018 (Tack et al., 2019). Recalls and foodborne illnesses have been increasingly linked to low-moisture foods with water activity (a_w), with a_w <0.85, such as wheat flour, milk powder, dry nuts, spices, and pet foods (Beuchat et al., 2013; CDC 2015; Syamaladevi et al., 2016a; Syamaladevi et al., 2016b). Low-moisture foods are often considered as long shelf-life products with negligible risk to deteriorate, for example, wheat flour usually have a shelf-life from 3 months to 12 months. Contamination of *Salmonella spp.* is one of the top hazards in low-moisture foods, because they can survive for a period of time under low-moisture conditions. In addition, the thermal resistance of *Salmonella* is greatly increased at lower a_w (Archer, Jervis, Bird and Gaze, 1998; Bari et al., 2009), therefore, *Salmonella* in low-moisture foods is difficult to control by conventional thermal treatments.

Light emitting diodes (LED) are semiconductors that emit light in response to electric current (Schubert, 2003). LEDs emitting UV light pulses inactivate microorganisms; in comparison to mercury vapor lamps, LEDs generate less heat, and the light intensity remains constant over the lifetime of the lamp (Shin et al., 2016). UV-C light with wavelength between 200-280 nm has been generally used for disinfection purpose. It exhibits high antimicrobial potential and mainly targets on the DNA/RNA damage of bacteria (Beck et al., 2015; Dai et al., 2012). UV-A (315-400 nm) also has photo-bactericidal activity, but acts by different mechanisms because it is not directly absorbed by native DNA (Rastogi, et al., 2010). Notably, the exposure to

the UV-B (280-315 nm) and UV-C light can induce sunburn and skin cell damage, while the UV-A is much less hazardous to human eyes and skin (Shirai, Watanabe and Matsuki, 2016).

Microbial inactivation by UV-A light is mainly attributed to oxidation of unsaturated membrane fatty acids and the interactions between the UV photons and endogenous photosensitizers of bacteria (Bintsis, Tzanetaki and Robinson, 2000). The photosensitizer in excited state transfers energy to generate reactive oxygen species (ROS) such as peroxides, superoxide, hydroxyl radical and singlet oxygen, and induce oxidative stress, which further results in DNA damage and cell death of microorganisms (Karran and Brem, 2016). The ROS released during this process, however, may also interact with biomolecules in the treated food matrix. Oxidation of the food matrix may affect the functional properties, nutritional and sensory attributes of food products. Therefore, it is necessary to determine whether decontamination treatment with UV-A also changes the quality of the food. Only few studies employed LED sourced UV-A light for inactivation of foodborne pathogens (Shirai, Watanabe and Matsuki, 2016; Haughton et al., 2012). No previous study reported the use of UV-A LED for *Salmonella* inactivation in low-moisture foods or oxidation of the food products after UV-A LED treatments.

In recent years, there were several *Salmonella* outbreaks associated with wheat flour and its related products around the world (CDC, 1998; McCallum et al., 2013; Forghani et al., 2019; FDA, 2019). A current study pointed the prevalence of *Salmonella* in wheat flour as well (Myoda et al., 2019). The FDA alerted the public that consuming raw flour and its derivatives showed health risks due to the possibility of *Salmonella* contamination (FDA, 2017). Therefore, it is worthy to investigate the effect of UV-A LED on *Salmonella* contaminated wheat flour. ROS released in the food matrix during the UV-A treatment may oxidize flour components including gluten (consists of gliadin and glutenin proteins), which is the most important functional protein

component in wheat flour. The polymerization of glutenin by intermolecular disulfide bonds forms the glutenin macropolymer, a three-dimensional protein network that is the major determinant of the baking quality of wheat (Wieser, 2007). Oxidation of gluten proteins increases the number of inter-molecular disulfide bonds and improve gluten functionality in baking applications (Wieser, 2007).

The aim of this study was to explore the possibility of using 395 nm pulsed LED treatment as a novel tool for decontamination of the *Salmonella* in wheat flour with industrial feasibility. The functional and structural changes of the gluten proteins in the wheat flour following the 395 nm pulsed LED treatment due to possible oxidation were also investigated in this study.

2. Materials and Methods

2.1 Materials

Unbleached enriched wheat flour (moisture 10.4%, ash 0.62%, protein 10.82%, carbohydrates 78.16%) was obtained from P&H Milling Group (Lethbridge, AB, Canada). The initial a_w of the wheat flour was 0.20, measured at 25 °C using a water activity meter (Meter group, Pullman, WA). Five strains of *Salmonella spp.* (ATCC 13311, ATCC 43845 from American Type Culture Collection , FUA 1934, FUA 1946 and FUA 1955) were stored in tryptic soy broth (TSB) and 70% (v/v) glycerol at -80 °C. The *Salmonella enterica* ATCC 13311 and ATCC 43845 were selected to include strains with average and exceptionally high resistance to wet heat (Ng et al., 1969; Mercer et al., 2017). The *Salmonella* FUA 1934, FUA 1946 and FUA 1955 are waste water isolates showing resistance to drying and to dry heat but not to wet heat (cite Bina's thesis). The tryptic soy agar (TSA), TSB, yeast extract and peptone were purchased from Fisher Scientific (Hampton, U.S.). All solvents used in liquid chromatography were high performance liquid

chromatography (HPLC) grade, and all other chemicals were of analytical grade. All buffers were prepared with Milli-Q purified water (Millipore, Bedford, MA).

2.2 Bacterial culture conditions and cocktail

Each frozen *Salmonella* stock culture was streaked on a TSA plate supplemented with 0.6 % (w/v) yeast extract (TSAYE) and incubated for 24 h at 37 °C. A single colony from each plate was picked and cultured in 5 mL of TSB for 24 h at 37 °C, followed by transferring 100 µL into 5 mL TSB (20 h at 37 °C). Then 100 µL of the culture was evenly spread onto a TSAYE plate and the bacterial lawn was harvested with 1.5 mL 0.1% (w/v) peptone water after 24 h incubation at 37 °C (Danyluk, Uesugi and Harris, 2005**).** Bacterial cells were harvested by centrifugation (9,632 × g, 5 min) and resuspended with 1 mL of peptone water and subjected to another centrifugation. After removing the supernatant, the pellet was suspended again with peptone water to make up to 1 mL total volume. *Salmonella* cocktail (10^{10} CFU/mL) was obtained by mixing all the five 1 ml suspensions and used immediately to inoculate the wheat flour sample.

2.3 Inoculation and equilibration of wheat flour

The inoculation and equilibration procedures were followed according to Liu et al. (2018) with some modifications. The *Salmonella* cocktail (1.25 mL) was added into 10 g of wheat flour in a sterile polyethylene sampling bag, and then the wheat flour was hand massaged for 3 minutes. The inoculated wheat flour was air-dried for 2 h and then mixed well. Six 0.3 g samples were randomly selected, plated and enumerated on TSAYE plates to confirm the uniformity of inoculum distribution that reached to a final concentration of 10^8 CFU/g. For enumeration, 0.3 g of the sample were mixed with 100 mL of 0.1% (w/v) peptone water and homogenized at 230 rpm for 5 min in a Seward Stomacher (Seward, London, UK). Ten milliliters of the suspension were collected, serial diluted, and plated on TSAYE plates in triplicate. The plates were incubated

aerobically at 37 °C for 24 h and the number of colonies was counted. Enumeration of background microflora in non-inoculated wheat flour from three random 0.3 g samples was also performed. Before treatment, the a_w of the inoculated wheat flour was equilibrated to 0.75±0.02 in a humidity box filled with saturated sodium chloride solution ($a_{w,\ 25\ °C}$=0.75) for 3 days.

2.4 LED systems and inactivation

An LED head (111x70x128 mm, JL-3 series, Clearstone, Minnesota, U.S.) consisting of 6 high intensity pulsed LEDs emitting 395 nm light with a built-in cooling fan was connected to a high power pulsed UV LED system (CF3000, Clearstone, Minnesota, U.S.) (Fig. 1A). 0.3 gram of inoculated and equilibrated wheat flour sample was evenly spread over the bottom of the sample cup with a diameter of 3.7 cm and 0.45 mm depth. The sample cup (AquaLab, Pullman, WA, U.S.) was placed under the center of the LED head with a gap of 2 cm. The pulse frequency was 100 Hz, which is the number of pulse periods per unit of time. For inactivation, the power level was selected to 60% with the LED irradiance of 0.45 W/cm^2, where the ON (t_{ON}) and OFF (t_{OFF}) times of LEDs during pulse period were 6 and 4 milliseconds respectively (Fig. 1B). The emission spectra from the LEDs were determined by StellarNet Inc. spectrometer (Black Comet C-25) (Fig. 1C). The pulsed LED treatments of wheat flour were performed for 0 min, 10 min, 30 min and 60 min. An external fan was used to reduce any temperature increase in the sample during the treatments. The UV dosage values corresponding to the treatment times were determined using a laser energy meter (7Z01580, Starbright, Ophir Photonics, USA). The sample temperature was monitored by attaching a thermocouple in contact with the sample (under LED light) and read at 10 min, 30 min and 60 min. The weight loss was also calculated after treatments.

To explore the industrial feasibility of the LED set-up, two conditions were used to perform the LED inactivation. In an open system, the LED set-up was placed in a plastic box that was placed

in a biosafety cabinet at ambient temperature and relative humidity. In a semi-closed LED system, the LED set-up was positioned in a controlled humidity chamber with 75% relative humidity and 25 °C. The surviving bacterial cells after different LED treatment times were enumerated and compared between both the systems.

2.5 Color changes of wheat flour after pulsed LED treatment

Color was measured in the wheat flour samples after 0 min, 30 min and 60 min pulsed LED treatments in the semi-closed LED system. The lightness (L^*), redness or greenness (a^*), and yellowness or blueness (b^*) values were obtained using a Minolta CR-400 colorimeter (Konica Minolta Sensing Americas Inc., Ramsey, NJ).

2.6 Gluten extraction

Wet gluten was extracted from the wheat flour samples based on the method of Kieffer et al. (2007). Two grams of untreated or treated wheat flour sample was mixed with 1 mL of 0.4 M NaCl solution. After resting for 5 min, the dough was washed manually with 20 mL 0.4 M NaCl solution and this step was repeated 5 times until a cohesive matrix was obtained. The gluten was then washed with distilled water to remove the salt, followed by centrifugation at 5,320 × g for 10 min. Dry gluten powder was obtained after lyophilization (Labconco Corporation, Kansas City, MO, USA) and used for the following analyses.

2.7 Size-exclusion HPLC

The molecular weight profiles of the gluten proteins obtained from untreated and treated wheat flour samples were studied by a size-exclusion high performance liquid chromatography (HPLC) according to the method of Morel et al. (2000) with some modifications. Five milligrams of freeze-dried gluten sample were mixed in 150 µL of 0.1 M sodium phosphate buffer (pH 6.9) containing 1% (w/v) SDS at 60 °C for 80 min to dissolve the soluble gluten proteins followed by 5 min

sonication. After centrifugation at 5000 × *g* for 5 min, an aliquot of the supernatant was collected for HPLC analysis. The size-exclusion HPLC was conducted on a Varian Prostar system connected with a Superdex 75 10/300 GL column. To observe the size distribution, 25 μL of the sample was loaded on the column and separated by an isocratic elution at a flow rate of 0.5 mL/min with 0.1 M sodium phosphate buffer (pH 6.9, 1.1% SDS). The detection was performed at a wavelength of 214 nm. The total SDS extractable protein and protein content in different molecular weight ranges were calculated from the fraction areas in the chromatogram. The standard calibration curve was obtained by using a series of pure standards from a gel filtration markers kit (MW 12,000-200,000 Da).

2.8 Free sulfhydryl groups (-SH)

Sulfhydryl groups represents one of the most vital functional groups in native proteins and it is a crucial indicator for protein oxidation and crosslinking. To evaluate the protein oxidation level of the wheat flour after pulsed LED treatment, the content of free -SH was evaluated as described in the method by Wang et al. (2014). Briefly, 40 mg of each gluten sample was incubated with 4 mL of tris-glycine buffer (pH 8.0) containing 5 mM EDTA and 2.5% (w/v) SDS with intermittent vortex for 30 min, and then the -SH content was determined after the addition of Ellman's reagent (5,5-dithio-bis-(2-nitrobenzoic acid). The absorbance was measured at 412 nm against the blank and the calibration curve was plotted with cysteine.

2.9 Surface hydrophobicity

The surface hydrophobicity of gluten proteins from untreated and treated wheat flour samples was determined as the method illustrated by Kato and Nakai (1980), using 8 mM 1-anilinonaphthalene-8-sulfonic acid (ANS) in 0.1 M phosphate buffer (pH 7.0) as a fluorescent probe. The gluten suspension (2 mg/ml) was prepared in 0.01 M phosphate buffer (pH 7.0) with stirring for 1.5 h at

20 °C, and the supernatant obtained after centrifugation (8000 × g for 20 min) was diluted with the same buffer to 0.005, 0.01, 0.05, 0.1 and 0.5 mg/mL for the test. The protein concentration of the supernatant was determined by the BCA assay. For the test, 20 μL of ANS solution was added into 1.5 mL of each diluted sample and the fluorescence intensity measured with an excitation wavelength at 365 nm and an emission wavelength at 484 nm was recorded after subtracting the blank with corresponding protein solution. The initial slope of the linear regression plot obtained from the fluorescence intensity versus protein concentration was used as the surface hydrophobicity index.

2.10 Water holding capacity (WHC)

The water holding capacity of gluten from different LED-treated (0, 30 and 60 min) wheat flour was determined according to Raghavendra et al. (2004). The WHC is defined as the amount of water that per gram of gluten can hold without an external force. It is an important functional property of proteins, that is related to protein structures and texture of foods. Half a gram of freeze-dried gluten powder was hydrated by incrementally adding distilled water while stirring. In total 15 mL of water was added and the gluten matrix was hydrated for 18 h at ambient temperature. Then the supernatant was filtrated off on a paper towel, and the gluten residue was weighed (G_1) and dried at 105 °C for 2 h (G_2). The WHC was calculated using the following equation:

$$\text{WHC}\left(\frac{g_{water}}{g_{dry\ gluten}}\right)=\frac{G_1-G_2}{G_2} \qquad (1)$$

2.11 Rheological properties

The gluten samples were rehydrated by distilled water and kneaded with a spatula until uniform, cohesive matrices formed. The gluten gels were then rested in sealed tubes at 25 °C for 2 h prior to rheological analyses. The rheological analyses were performed on the Physica MCR Rheometer (Anton Paar GmbH, Virginia, USA) with a 2.5 cm parallel plate at 25 °C. The dynamic rheological

studies were conducted in the modes of strain (γ) and frequency (ω) sweeps. The linear viscoelastic region (LVR) was first determined by a strain sweep in a range of deformation from 0.01% to 100% at $\omega = 10$ Hz, and then frequency sweep was operated at γ=5% from 0.1 to 100 Hz. The gap between the parallel plate and the Peltier plate was set at 1 mm. Silicone oil was coated on the edge of the sample and used to prevent the moisture change during the measurements. The changes of storage modulus (elasticity, G´) and loss modulus (viscosity, G´´) of the samples were plotted during the measurements. The ratio of these two values (tan δ, G´´/ G´) was also recorded.

2.12 Protein secondary structure changes

The secondary structure changes of the gluten from wheat flour before and after pulsed LED treatments were evaluated in hydrated gluten samples by Fourier transform infrared spectroscopy (FTIR), according to the method by Nawrocka et al. (2018). The gluten samples were treated by deuterium dioxide (D_2O) for 18 h at 4 °C before the analysis to avoid the interference of water bands in amide I band (1600 -1700 cm^{-1}), representing protein secondary structures. The spectra were recorded by a Thermo Nicolet 8700 FTIR spectrometer equipped with a diamond attenuated total reflectance (ATR) attachment. Spectra were scanned between 4000 and 600 cm^{-1} at 4 cm^{-1} intervals and signal was averaged from 128 scans.

The amount of different secondary structures was estimated from relative peak areas obtained in the second derivative spectra of the amide I band by using Peakfit v4.12. The peak frequencies that correspond to the protein secondary structures were cited from literature (Dhaka and Khatkar, 2016; Kong and Yu, 2007; Wellner et al., 2005).

2.13 Microstructure of gluten matrices

The gluten matrices were prepared as described in Section 2.11, and freeze-dried using Thermo SuperModulyo freeze dryer. Fractured samples were mounted on the sample holder and then the

microstructure of gluten matrices was visualized under a helium-ion microscope (Zeiss Orion NanoFab, Germany). The images were magnified and captured at 50 µm field of view and 10 µm field of view. Eight representative pictures were captured for each sample.

2.14. Statistical analysis

The entire experiment was repeated three times and each analysis was conducted in triplicate. Data were analyzed using one-way analysis of variance (ANOVA), and comparison among means was assessed by conducting a studentized range test (Tukey HSD Test) at the 0.05 significance level. All statistical analyses were done using SPSS statistical software (version 20.0, SPSS Inc., Chicago, USA).

3. Results

3.1 Inactivation of *Salmonella* cocktail by 395 nm pulsed LED treatment

The disinfection effect of the 395 nm pulsed LED on *Salmonella* cocktail was examined in both open and semi-closed systems (Table 1). A significant decrease in the survival of *Salmonella* was noticed with the increase of the LED dosage in both open and semi-closed systems. In the open system, the cell counts were reduced by 2.42±0.13 log CFU/g after 60 min treatment; in the semi-closed system, the cell counts were reduced by 2.91±0.14 log CFU/g after 60 min. The temperature increased during treatment in both systems; this increase was more pronounced in the open system. Conversely, the a_w was reduced after treatment in both systems but more pronounced in the open system. The reduction of the a_w corresponded to weight losses of the samples in both systems but more pronounced in the open system. Although the controlled humidity (75%) and temperature (25 ^{o}C) inside the semi-closed system was not able to prevent dehydration of wheat flour during pulsed LED treatments, the more controlled environment inside the semi-closed system reduced LED-induced drying of the flour.

3.2 Color changes of wheat flour after pulsed LED treatment

Because the bactericidal effect of treatments in the semi-closed system were higher when compared to the open system, subsequent investigations on the quality of wheat flour focused on samples treated inside the semi-closed system. Also, since the decontamination effect of the 10 min treatment was less than 2 log, only 30 min and 60 min treatments were selected for the following tests. Color changes in the wheat flour before and after the pulsed LED treatments at 0, 30 and 60 min in the semi-closed system were evaluated by the L^*, a^* and b^* parameters (Table 2). A greater ($P<0.05$) L^* value was noted in the wheat flour sample after 60 min treatment compared to the sample without pulsed LED treatment. The a^* values of wheat flour samples increased significantly with 30 and 60 min treatments whereas the b^* value presented an opposite trend, reducing significantly with extended treatment times. The results reflect that wheat flour was bleached after pulsed LED treatment and showed markedly whiter, less greenness (more redness) and less yellowness in contrast with the original wheat flour. The picture in supplementary material showed the visual differences between the doughs made with control and the LED treated samples.

3.3 Molecular weight distribution of gluten proteins

The polymerization of gluten proteins plays a key role for the baking quality of wheat flour. Therefore, changes in gluten proteins after the pulsed LED treatments were evaluated by determination of the molecular weight of SDS-soluble gluten proteins, by quantification of sulfhydryl groups in the samples, by determination of rheological properties of wheat doughs, and by FTIR. Figure 2 shows the molecular weight profiles of the SDS-soluble gluten proteins extracted from wheat flour before and after the pulsed LED treatments. The molecular weight of gluten proteins (Fig. 2A) was divided into four fractions: F1, from 100-350 kDa, F2, from 65-100

kDa, F3, from 12-65 kDa and F4, less than 12kDa. Notably, LED treatments reduced the amount but increased the molecular weight of SDS-soluble gluten proteins (Fig. 2A and 2B), indicating that LED treatment induced formation of larger polymers.

3.4 Chemical and functional properties of the gluten after pulsed LED treatment

3.4.1 Free sulfhydryl groups (-SH)

To determine whether the LED-induced polymerization of gluten proteins is mediated by oxidation of sulfhydryl groups in flour, free -SH were measured in the SDS-soluble gluten proteins obtained from wheat flour before and after 395 nm-LED treatments (Table 3). The content of free sulfhydryl groups in gluten proteins was significantly reduced after LED treatment, with the lowest value of 1.66 ±0.01μmol/g protein in the sample treated for 60 min.

3.4.2 Surface hydrophobicity

The alteration of protein surface hydrophobicity is highly correlated with protein conformational and functional changes, which usually points to certain level of protein denaturation and exposure of hydrophobic amino acids hidden in the core of the native protein to the protein surface. Variations of surface hydrophobicity of LED treated gluten samples with different treatment lengths (0, 30 and 60 min) are shown in Table 3. The surface hydrophobicity was significantly elevated in the gluten samples after LED treatment for 30 and 60 min, with surface hydrophobicity index 11.58±0.44 and 11.96±0.38, respectively.

3.4.3 Water holding capacity

Regarding to the water holding capacity (Table 3), significant differences were observed among the gluten samples with and without LED treatment. The untreated gluten sample was capable of absorbing and retaining more water (1.66 ±0.03 g/g) in its three-dimensional networks when compared to the samples treated with 30 and 60 min.

3.4.4 Rheological properties

To determine whether LED-induced changes in gluten quality impact their rheological properties, the elastic response (storage modulus, *G'*) and viscous response (loss modulus, *G''*) of SDS-soluble gluten proteins were recorded in a frequency range from 0.1 to 100 Hz (Fig. 3). In general, the matrix formed with gluten proteins was more solid-like, verified by higher values of *G'* (Fig. 3A) than *G''* (Fig. 3B), and both of the *G'* and *G''* were dependent on the frequency. With respect to elasticity, the *G'* of the gluten with LED treatments showed higher values than the original gluten. The *G''* values, which are indicative of viscous properties, were not changed by LED treatments.

3.5 Secondary structural changes of the treated gluten

The functional changes of proteins are usually ascribed to their structural changes. Therefore, it was worthy to investigate the structural changes of the gluten proteins after exposure to the LED, which promotes a better understanding of the effect of 395 nm pulsed LED treatment on the gluten proteins. The secondary structural modifications of rehydrated gluten samples were analyzed by FTIR and the obtained spectra are presented in Figure 4. Amide I bands with frequency between 1600 and 1700 cm^{-1} in FTIR spectrum are the most sensitive vibrational bands of protein backbone that reflect the protein secondary structures (Yang et al., 2015). Based on the calculations from the second derivative peaks (Table 4), the major secondary components of all the gluten samples were β-sheets, accounting for over 50 % among the secondary structures, followed by α-helix and random coil, and then β-turns. Similar results were reported by Li et al. (2006), a great amount of β-sheet structure was found in hydrated gluten. Conformational changes were observed among the hydrated gluten samples before and after the LED treatments. The percentage of intermolecular β-sheet structures was significantly increased with the elevated LED treatment times, from 19.7±0.3%

in the untreated sample to 24.0±0.3% in the 60 min treated gluten. Significant reduction in antiparallel β-sheet and α-helix and random coil structures were caused after 30 and 60 min pulsed LED treatments.

3.6 Microstructure changes

To visualize the effect of the 395 nm LED on the gluten proteins, the microstructures of gluten dough obtained from the 0, 30 and 60 min treatments were examined under the helium-ion microscope (Fig. 5). A three-dimensional network was obtained in all the hydrated gluten samples without and with different LED exposures (Fig. 5, 50 μm and 10 μm field of view). This interconnected system formed by intermolecular disulphide bonds contains numerous pores that can entrap water molecules and ultimately results in a viscoelastic gluten framework.

4. Discussion

The results from the inactivation study suggest the potential of using 395 nm pulsed LED treatment to decontaminate *Salmonella* in low-moisture foods, particularly in a semi-closed system. Although the sample temperature increased (less than 50 °C) when the LED light was applied, there was no significant temperature change from 10 min to 60 min treatment time in the semi-closed system. In contrast, cell counts of *Salmonella* cocktail significantly reduced during this period and reached ~3 log reduction at 60 min with 395 nm LED treatment. It indicated the LED effect was dominant than thermal effect in our study. This reduction of cell counts provides proof of concept that LED at 395 nm achieves inactivation of resistant pathogens in low-moisture foods, but the limited reduction of cell counts and the long treatment times necessitates further optimization by change of a_w and temperature of treatment. In comparison to heat treatment, which also has only a limited effect on *Salmonella* in dry foods (Finn et al., 2013), LED technology could still be a potential alternative. Long treatment times between 60-240 min were required to

significantly reduce *Salmonella* in wheat flour at 55, 60, 65, and 70°C. For instance, 60 ºC heat treatment of all-purpose wheat flour for 60 min resulted in only 1.14 log CFU/g reduction in *Salmonella* (Forghani et al. 2019). However, an absolute comparison in log reductions during LED and heat treatments is not possible due to the different product and process conditions followed and strains used in different studies. From our recent experiments, it was shown that the three *Salmonella* strains used in this study i.e., FUA 1934, FUA 1946 and FUA 1955 exhibited high resistance to heat treatments (Cite Bina's M.Sc. thesis – it is online already).

To assess the influence of the 395 nm LED on the quality of wheat flour, color changes of wheat flour in the semi-closed system were evaluated after the treatments. Bleaching occurred in the wheat flour under 395 nm light exposure, which may be due to the degradation of the carotenoid compounds existing in wheat flour. Carotenoids are yellow-red pigments with antioxidant properties (Fiedor and Burda, 2014). Lutein and zeaxanthin are the major carotenoids in wheat flour (Hussain et al., 2015) and according to literature, the degradation of carotenoids was mainly attributed to oxidation (Ficco et al., 2014). The possible mechanism for the bleaching of wheat flour during 395 nm LED treatments could be related to the generation of ROS with the aid of energy produced from photons, which interacted with the carotenoid compounds and resulted in carotenoid degradation. From the picture (supplementary material), the color change after LED treatments was very evident and the treated flour doughs were much less yellow compared to the control flour dough. This indicated the whiter color could be visually identified by consumer. Wheat flour is commonly bleached by chemicals, e.g. benzoyl peroxide, to remove yellow xanthophyll and other pigments in flour to produce whiter flour and to oxidize flour proteins. The result may be a good sign for industry since it means the LED could bleach the wheat

flour without chemicals meanwhile inactivating the microbial pathogens. However, this is also an indication that oxidation happens in wheat flour during the pulsed LED treatments.

The oxidation occurred during the LED treatments was verified by the molecular weight migration from low to high molecular weight gluten proteins in the treated samples as presented in the section 3.3. The formation of bigger polymers was also observed by the total areas and the fraction areas (Fig. 2B). Crosslinking of gluten proteins by disulfide-bonds decreases the SDS-extractable proteins in wheat (Pomeranz, 2012). Accordingly, the size distribution and the amount of SDS-soluble proteins from untreated and LED-treated wheat flour imply that the LED-induced oxidation occurred in the wheat gluten with 30 and 60 min treatment, which accelerated the generation of the disulfide bonds inter or intra the gluten proteins, therefore caused the higher degree of crosslinking and large polymers formation.

The results about the chemical and functional properties of the gluten after 395 nm LED treatment provided more evidence for oxidation. The reduced amount of -SH presented in section 3.4.1 implicated more free -SH groups were oxidized into S-S bonds to form crosslinking with the extended period of LED treatment. The phenomenon of increased surface hydrophobicity after the LED exposure also implies the oxidative modifications occurred in gluten proteins. The native proteins were unfolded, exposed the hydrophobic amino acids and further formed covalently cross-linked protein aggregates. Gluten proteins are rich in hydrophobic amino acids, for instance, glycine, proline and glutamine, the greater exposure of these side chains will lead to more hydrophobic interactions, which is considered to play an important role in stabilizing gluten structure as well as baking and rheological properties of dough (Ponte et al., 2000). The development of gluten matrix involves protein-protein and protein-water interactions. Hydrated glutenins and gliadins tend to align and form crosslinks (disulfide bonds) among glutenin

molecules and between glutenin and gliadin molecules during mixing, leading to the generation of the interconnected network in which the water molecules are entrapped (MaCann and Day, 2013). However, prior to mixing, the pre-developed crosslinking structures resulted from the LED light may interfere the interaction of protein with water during kneading, thus less uniform network could be formed, and lower amount of water were retained in the protein structure (section 3.4.3). Besides, the increase in surface hydrophobicity caused certain degree of protein aggregation that may also make the proteins less accessible for water molecules.

The increase in the mechanical strength of the gluten formed gels (described in the section 3.4.4) may be the consequences of the addition of crosslinked structures after LED-induced oxidation, which provided greater level of gel elasticity. Monomeric low molecular weight (LMW) gliadins only have intrachain disulfide bonds while the polymeric high molecular weight (HMW) glutenin subunits have both intra- and interchain bonds, therefore the polymeric glutenin subunits own higher chances to enter the polymerization reactions and act as “chain extender”, to produce more cross-linked conformations. The gliadins and glutathione are found to be terminators of the chains (Wieser, 2007). The molecular weight distribution of the gluten samples (section 3.3) has illustrated a shift from the monomers to polymers and HMW subunits during LED treatment. The presence of larger amounts of the HMW subunits may subsequently involve in more polymerization reactions during mixing and form more crosslinked networks that eventually work for a stronger matrix in the LED treated samples. In terms of the viscosity, there was no evident changes among the different gluten samples, indicating the LED-induced oxidation had dominant effects on the elastic properties rather than viscous properties of the gluten matrix. Moreover, the higher surface hydrophobicity found in the LED treated samples may stimulate more hydrophobic interactions that consequently provide support to the gluten framework. In the case of dough

making or bread making, oxidation is favored during mixing for the formation of large glutenin polymers to obtain an elastic dough. The results indicate that 395 nm LED light can accelerate the mixing process and assist to produce a dough with higher resilience that would positively impact on the quality of the final products (i.e. baking products).

The structural changes of the gluten proteins are highly associated with the functional changes after the LED treatments. The α-helix has been linked as a characteristic for gliadins (Wang et al., 2014), which may be diminished due to the involvement of gliadins into forming the large polymers after LED oxidation. Since β-turns are mostly exposed on protein surface, the dramatic increase of the β-turn structures in the gluten samples after 60 min LED exposure may be correlated to the alteration of the surface hydrophobicity by LED as shown in section 3.4.2 Greater occurrence of intermolecular β-sheet structures in the gluten samples has been related to the interactions between glutenin subunits (Popineau et al., 1994). The increased amounts of β-turns and β-sheets in gluten after the LED treatments played important roles on elasticity and stability in gluten network, which is an essential property of gluten for good bread making (Dhaka and Khatkar 2016). This is in agreement with the result that large polymers formed in gluten after exposure to the LED through disulfide linking between glutenin subunits and led to higher elastic gluten matrixes.

In conclusion, the 395 nm pulsed LED treatment is a promising decontamination approach for wheat flour, since it brings multiple benefits. A maximum 2.91 log reduction of *Salmonella* cocktail was obtained in wheat flour treated with 395 nm pulsed LED for 60 min in a semi-closed system. The 395 nm LED treatments also led to oxidation in wheat flour which subsequently resulted in bleaching, and polymerization of gluten components through disulphide linkage. The contents of protein secondary structures were altered significantly after pulsed LED treatment, but

the rheological properties were not deteriorated. The LED-induced oxidation bleached the flour components without chemicals, and cross-linked gluten polymers, which may promote the efficiency of the dough mixing process and lead to better viscoelastic baking products. However, commercial application of the pulsed LED technology requires process time reduction and LED disinfection efficacy maximization (maybe through a better a_w control and a treatment system with vibratory platform for better light exposure of wheat flour), and improvement in the methods for controlling the extent of crosslinking formation to get required wheat flour product quality. By addressing these challenges, novel food decontamination and drying processes using pulsed LEDs for variety of foods can be possibly developed in the future.

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Table 1
Inactivation of the *Salmonella* cocktail after different LED treatment times at 395 nm in both open and semi-closed systems (RH=75%, T=25 °C), as well as the changes in sample temperature, weight loss and water activity.

Treatment time (min)	Dosage (J/cm^2)	Reduction of viable cell counts (log CFU/g)		Sample Temperature (°C)		Weight Loss (%)		Water activity	
		open	semi-closed	open	semi-closed	open	semi-closed	open	semi-closed
10	270	0.89±0.07^{c}	1.49±0.17^{c*}	51.3±0.6^{b*}	47.7±0.6^{a}	1.39±0.03^{b*}	1.10±0.10^{a}	0.11±0.00^{a}	0.22±0.00^{a*}
30	810	1.95±0.04^{b}	2.23±0.18^{b}	52.3±0.6^{ab*}	48±1.0^{a}	1.53±0.18^{b}	1.17±0.06^{a}	0.11±0.00^{ab}	0.21±0.00^{b*}
60	1620	2.42±0.13^{a}	2.91±0.14^{a*}	53.0±0.0^{a*}	48.7±0.6^{a}	1.88±0.03^{a*}	1.20±0.00^{a}	0.10±0.00^{b}	0.20±0.00^{b*}

Values are given as mean ± standard deviation (n = 3).
Different letters in the same column indicate significant ($p < 0.05$) differences between the means.
Asterisk (*) indicates a significant difference between the open and the semi-closed systems.

Table 2
Color changes of the wheat flour before and after different LED treatment times at 395 nm in the semi-closed system (RH=75%, T=25 °C).

Treatment time (min)	L* (lightness)	a * (greenness and redness)	b* (blueness and yellowness)
0 min	92.37±0.67[b]	-1.48±0.03[c]	13.4±0.70[a]
30 min	93.32±1.29[ab]	-0.69±0.09[b]	7.8±0.87[b]
60 min	93.76±0.40[a]	-0.47±0.03[a]	6.4±0.18[c]

Values are given as mean ± standard deviation (n = 3).
Different letters in the same column indicate significant ($p < 0.05$) differences between the means.

Table 3
Functional properties of gluten obtained from wheat flour before and after different LED treatment times at 395 nm in the semi-closed system (RH=75%, T=25 °C).

Treatment time (min)	Free –SH (μmol/g protein)	Surface hydrophobicity index	Water holding capacity (g/g)
0 min	2.88±0.17[a]	7.43±0.45[b]	1.66±0.03[a]
30 min	2.22±0.02[b]	11.58±0.44[a]	1.50±0.04[b]
60 min	1.66±0.01[c]	11.96±0.38[a]	1.48±0.04[b]

Values are given as mean ± standard deviation (n = 3).
Different letters in the same column indicate significant ($p < 0.05$) differences between the means.

Table 4
Secondary structures of hydrated gluten obtained from wheat flour before and after different LED treatment times at 395 nm in the semi-closed system (RH=75%, T=25 °C).

Treatment time (min)	Gluten Secondary Structures (%)				
	Intermolecular **β**-sheet (%) (1615±2 cm^{-1})	Antiparallel **β**-sheet (%) (1633±2 cm^{-1})	**α**-helix and random coil (%) (1650±2 cm^{-1})	**β**-turns (%) (1666±3 cm^{-1})	**β**-turns (%) (1683±3 cm^{-1})
0	$19.7±0.3^{c}$	$35.6±0.6^{a}$	$28.1±0.4^{a}$	$11.3±0.1^{b}$	$5.3±0.1^{b}$
30	$22.3±0.3^{b}$	$31.7±0.6^{b}$	$26.0±0.4^{b}$	$14.4±0.3^{a}$	$5.5±0.1^{b}$
60	$24.0±0.3^{a}$	$28.4±0.5^{c}$	$24.3±0.4^{c}$	$15.6±0.3^{a}$	$7.6±0.1^{a}$

Values are estimated from the second derivative peak areas of the FTIR spectra from 1600 to 1700 cm^{-1}.
Values are given as mean ± standard deviation (n = 3).
Different letters in the same column indicate significant ($p < 0.05$) differences between the means

Figure captions

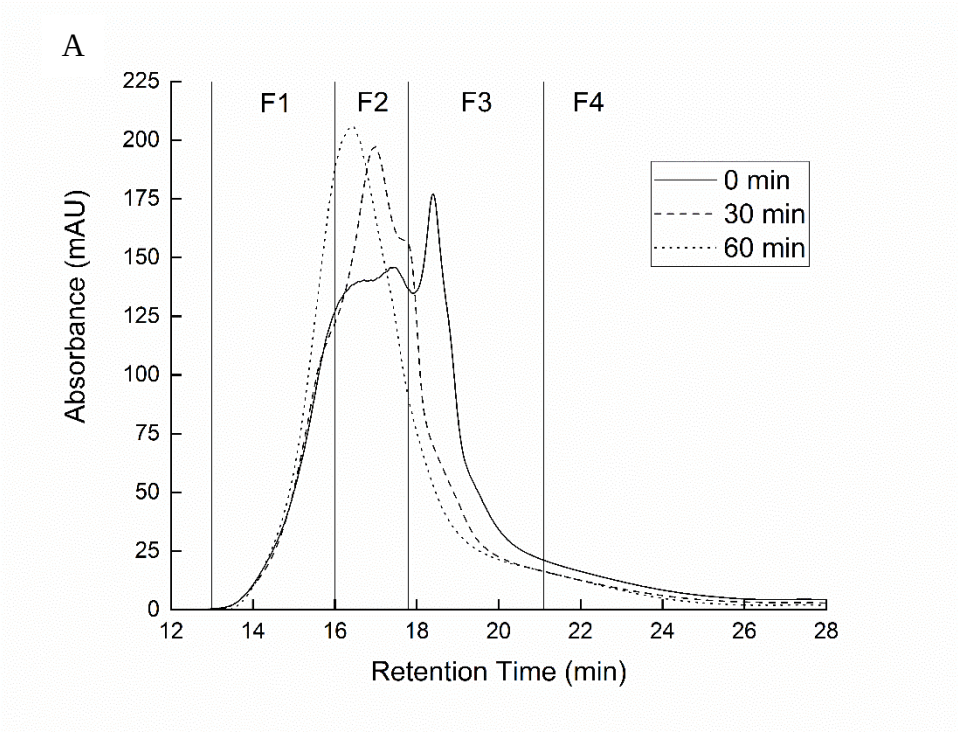
Figure 1. Molecular weight profiles of the gluten obtained from wheat flour before and after different LED treatment times at 395 nm in the semi-closed system (RH=75%, T=25 °C). A: Size distribution chromatograph of the gluten samples and the peak fractions (F1: 100-350 kDa; F2: 65-100 kDa; F3: 12-65 kDa; F4: <12kDa). B: Total areas and fraction areas of the peaks eluted.

Figure 2. Frequency sweeps of storage modulus $G^{'}$ (A) and loss modulus $G^{''}$ (B) for gluten samples obtained from wheat flour before and after different LED treatment times at 395 nm in the semi-closed system (RH=75%, T=25 °C).

Figure 3. FTIR spectra of hydrated gluten samples obtained from wheat flour before and after different LED treatment times at 395 nm in the semi-closed system (RH=75%, T=25 °C).

Figure 4. Microstructural images of hydrated gluten samples obtained from wheat flour before and after different 395 nm-LED treatments in the semi-closed system (RH=75%, T=25 °C) at different magnifications.

Figure 1


A
F1
F2
F3
F4
0 min
30 min
60 min
Absorbance (mAU)
Retention Time (min)
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200
175
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100
75
50
25
0
12
14
16
18
20
22
24
26
28

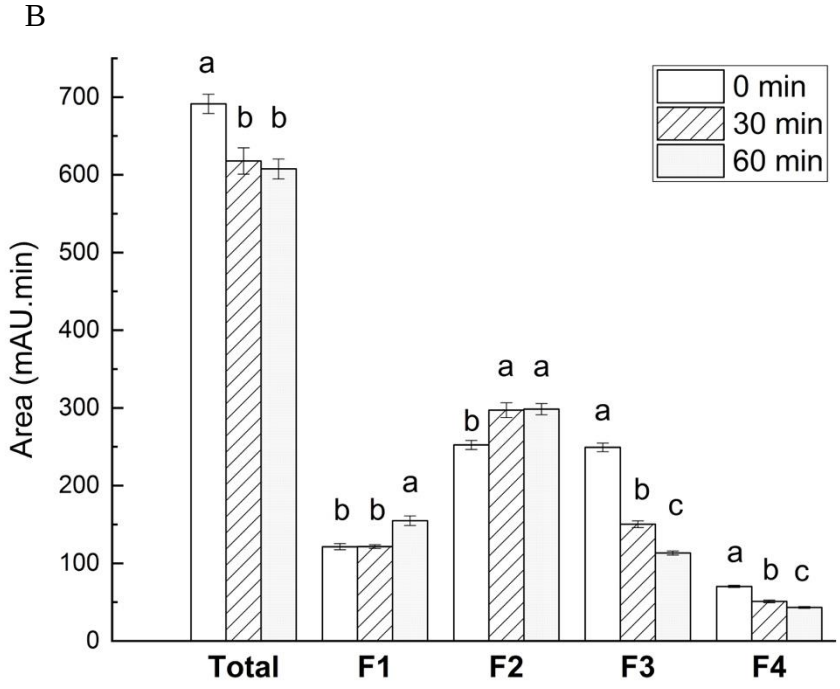

B
0 min
30 min
60 min
Area (mAU.min)
700
600
500
400
300
200
100
0
a
b b
b b a
b a a
a b c
a b c
Total
F1
F2
F3
F4

Figure 2

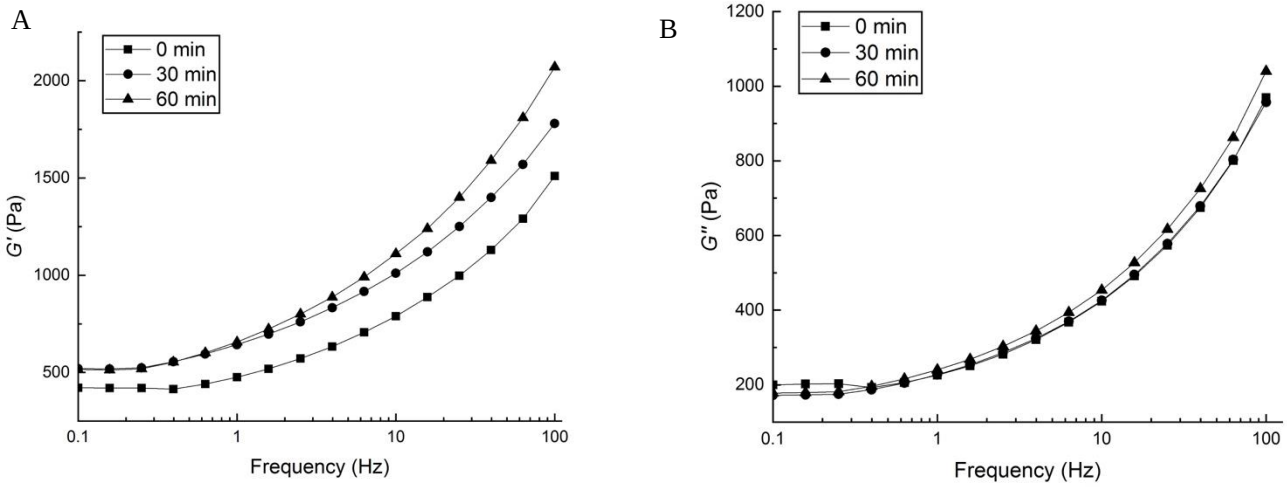


Figure 3

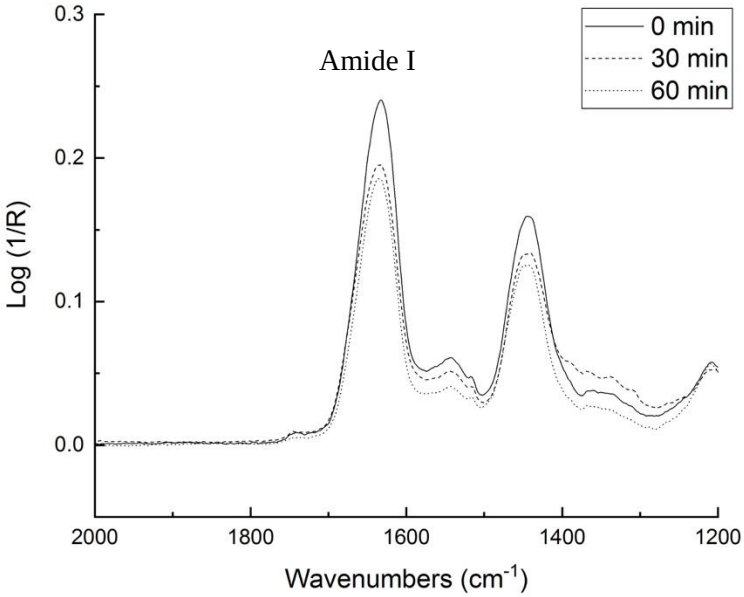
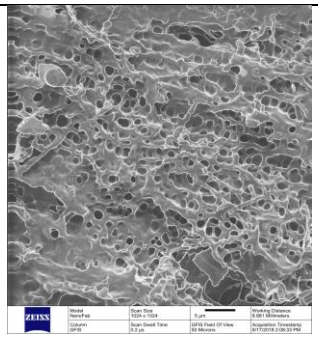
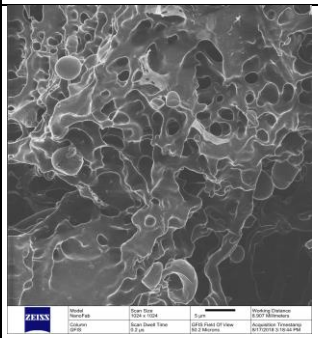
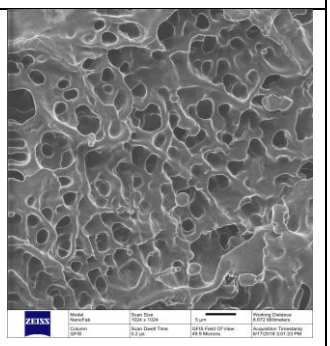


Figure 4

	0 min	**30 min**	**60 min**
50 μm × **50** μm			
10 μm × 1**0** μm