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Research Article

Effect of ultraviolet irradiation on immunoreactivity and other protein characteristics of wheat gliadin in flour and flatbread

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Abstract

Pulsed light (PL) was investigated for reducing the immunoreactivity of gliadin of wheat flour, however, the obstacle with using PL light was that the food temperature was highly increased with a short treatment time. Therefore, ultraviolet (UV) irradiation was used to study its effects on immunoreactivity of gliadin, free sulfhydryl groups, gluten, and molecular weight of wheat proteins in wheat flour and flatbread samples. Also, the effect of heat treatment combined with UV light treatment on some protein features were studied. The immunoreactivity of gliadin was highly reduced, 60%, of flour treated with only UV, while the reduction was not obvious for flour treated with infrared light along with UV light and for flatbread samples. The free sulfhydryl groups were 3030, 3878, 4372, and 4784 $\mu\text{mol.g}^{-1}$ after zero, 30, 60, and 120 min of UV light respectively. While the free sulfhydryl groups were reduced for flatbread samples treated by UV light. SDS-PAGE results showed that there was a clear reduction of the gliadin bands of flour treated with UV light compared to other treatments. In conclusion, thermal treatment along with or before UV light treatment reduced the effect of UV light on the immunoreactivity of wheat gliadin.

Keywords

ultraviolet, immunoreactivity of gliadin, wheat flour, flatbread, protein characteristics

Abbreviations

PL – pulsed light; UV – ultra violet

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Introduction

Gluten is a major storage protein present in wheat, and similar prolamins were observed in the rye, barley, and probably oats (Gessendorfer et al. 2009; Palamthodi et al. 2021). Gluten protein consists of three main groups, which are high molecular weight glutenin (HMWG) with 70-90 kDa, low molecular weight glutenin (LMWG) with 20- 45 kDa (D'Ovidio and Masci 2004), and gliadins (van den Broeck et al. 2009). Glutenin is a polymeric fraction protein as a result of disulfide cross-linked, while gliadin is a monomeric fraction protein (Panozzo et al. 2016). Gliadins consist of different subunits, which are α , β , γ with 30-40 kDa, and ω -gliadin with 60-80kDa (Heat resistance subunit) (Bietz and Wall 1980; Leszczynska et al. 2003). Allergic properties of gliadins are well recognized, whereas allergy of glutenin remains uncertain (Leszczynska et al. 2003). Avoiding a gluten diet is the only means for patients that suffer from gluten disorder. Therefore, many products have been developed to fulfill the patients' demands, which generally have poor quality and low acceptability due to the lack of wheat gluten (Cabrera-Chávez et al. 2010).

Several food processing methods have been tried to reduce gliadin allergy either in wheat flour or in wheat flour suspension as an attempt to solve the issue. In the Leszczynska et al. (2003) study, microwave processing was used to conduct the effect of microwave treatments on the immunoreactivity of wheat flour, and they found that there was no reduction effect. Panozzo et al. (2016) used pulsed light (PL) technology to reduce gluten immunoreactivity in gluten powder and suspension gluten, and the results showed that there was only a significant reduction in immunoreactivity for gluten suspension. Alhendi (2016) found that protein bands of gluten suspension were reduced due to PL treatment. The problem with a relatively long time, about 150 s PL treatment was highly raising the temperature of the treated samples (Alhendi et al. 2018). Ultraviolet (UV) wavelength, which is a part of PL wavelength, is responsible for the chemical reactions, photo-chemical effect, while the infrared wavelength is mostly responsible for the photo-thermal effect (Elmnasser et al. 2007). UV wavelength can be absorbed by several bonds of proteins, for example, the peptide bands absorb 180-230 nm, aromatic residues absorb a range of 258 to 280 nm, and the S-S band absorbs 260 nm (Schmid 2001; Teresa et al. 2012). In addition, the effect of UV-C light on

protein modification and quality change of wheat flour was investigated (Kumar et al. 2020). Therefore, this study aimed to determine the effect of using a continuous UV light on the immunoreactivity of wheat gliadin, free sulfhydryl groups, gluten and gluten index, and protein molecular weight without an increase in the sample temperature. Also, the effect of heat treatment, infrared, with UV light simultaneously or individually on some protein characteristics was conducted.

Materials and Methods

Hard red American wheat was obtained from the Quality Control Department, Grain Board of Iraq. Ammonium peroxodisulfate (Uni-chem. Inc, North Kingss Road, Greenville, SC, U.S.A., 29605), Acrylamide, NN'-Methylenebisacrylamide, Sodium phosphate dibasic heptahydrate, Sodium phosphate monobasic monohydrate, Brilliant Blue Stain Reagent, HCl (BDH Chemicals, Ltd, Poole, England), and 2-Mercaptoethanol (Lobachemie, Colaba, Mumbai, India) were purchased. Yeast powder and salt were obtained from a local market of Baghdad, Iraq.

Sample preparation. Flour was produced after hard red American wheat was cleaned from impurities, moisturized to 15% for 20-24 h, and milled by using a Quadermet mill (Brabender GmbH Co. KG, Duisburg, Germany). The extraction rate of the mill was 70% and it was raised to 80%, which is the extraction rate used in Iraq mills (Ahmed et al. 2021; Alhendi et al. 2022) by adding sieved bran.

Homemade Iraqi flatbread, Tanoor bread, was prepared by mixing the produced flour with 0.5% yeast, 1.5% salt, and a suitable amount of water to produce a consistent dough. The dough was left for about an hour for fermentation. Then the dough was divided into about 160-170 g, molded manually, and left for 10 min for rest before shaping to round

flatbread, with about 30 cm of diameter and 2 mm thickness. Iraqi flatbread was baked vertically on a round hot plate, Tanoor, using a liquid gas source.

Ultraviolet treatments. Ultraviolet treatment was conducted by Ultraviolet lamp, 120 cm, (Gaguar Lighting, China) for 30 g flour, distributed above 35 cm x 60 cm area, and flatbread samples. The UV lamp was set at 15 cm distance from the flour and

flatbread samples, and the treatment times was for zero, 30, 60, and 120 min. The flour and flatbread samples were fluctuated at the half time of each treatment.

Ultraviolet irradiation combined with infrared treatment. The temperature of 30 g flour distributed above 35 cm x 60 cm tray was adjusted

to 75°C by placing the tray inside an oven at 80°C. Then the flour was treated with UV and infrared lamp simultaneously at the same distance and times mentioned above. Table 1 expressed the flour temperature during and after the combined treatment. The flour temperature was measured by using an infrared thermometer.

Table 1. Flour temperature during combined treatment, UV and infrared

Treatments time, min	Control (zero)	30	60	120
Temperature, °C	75 (Initial temp)	84	84	88

Flatbread samples preparation. Flatbread fine particles were made by drying flatbread at 40°C overnight and milled to fine particles (Ribotta et al. 2005). The fine particles of flatbread were used to extract protein for immunoreactivity of gliadin, free sulfhydryl groups, and SDS-PAGE analyses.

Moisture and wet gluten measurement. The moisture content of flour samples was determined based on AACC (2000) Method (44-10), and wet gluten and gluten index of flour samples were measured based on AACC (2000) Method (38-12).

Immunoreactivity of gliadin. The detection and quantification of immunoreactivity of gliadin was conducted by using GlutenTox Rapid G12 sandwich kit (Hygiena LCC, California, USA). The analyses were done according to the manufacturer's recommendations on treated and untreated wheat flour and flatbread fine particles samples. One gram of samples was extracted by 10 mL of extraction solution that provided with the kit before diluting the samples by using dilution buffer. Then 100 µL of each sample, standard, positive, and negative control were added to the kit wells. The wells were incubated for an hour before washing them by wash solution. Later 100 µL of antibody was added and left for an hour. After washing the wells, substrate solution, 100 µL, was added and left for 15 min. Finally, 100 µL of stop solution was added, and the absorbance was read at 450 nm by using a microplate reader (BioTek, EIX 800, BioTek Instrument, Inc, Winooski, Vermont, USA). The concentration of immunoreactivity of gliadin was determined according to the calibration curve obtained by using the standard solutions provided with the immunoassay kit (Panozzo et al. 2016).

Free sulfhydryl bond content. The free sulfhydryl bond (SH) content of samples was determined based on the Mahroug et al. (2019) study as follow: Flour or fine particles of bread, 30 mg, was suspended in 1 mL of 8 mol.L⁻¹ urea, 3 mmol.L⁻¹ EDTA, 1% SDS, and 0.2 mol.L⁻¹ Tris-HCl, pH 8.0 buffer. The samples were vortexed for 30 s and shaken at room temperature for 60 min. Then 0.1 mL of the second buffer (10 mmol.L⁻¹ DTNB in 0.2 mol.L⁻¹ Tris-HCl, pH 8.0 buffer) was added, and shaken for a further 60 min. The samples were centrifuged (Ohaus, FC5515R, NJ 07054, USA) at 13600 xg for 15 min at room temperature. The absorbance of the supernatant was measured (PG Instruments, T60 UV-Visible Spectrophotometer, Alma park, United Kingdom) at 412 nm against the blank that contains only the two buffers. The content of SH bond was calculated based on the equation:

$$\text{Free SH} = \frac{A \times D}{\epsilon \times C} \quad (1)$$

Where A is the absorbance, D is the dilution factor, C the sample weight, 0.03 g, and ϵ is the extinction coefficient of DTNB, 13600 M⁻¹.cm⁻¹.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Gliadin and glutenin were extracted depending on Tosi et al. (2005) and van den Broeck (2010). Gliadin of flour and fine particles of flatbread was extracted by using 50% (v/v) propan-1-ol in a 1:10 (mg flour: µl solvent ratio). The mixture was mixed continuously for 30 min at room temperature before centrifugation at 10000 g for 10 min at room temperature. The supernatant was gliadin extract and the residue was used to extract glutenin. The glutenin was extracted by using a buffer containing

0.07 M Tris-HCl, pH 6.8, 2% (w/v) SDS, 10% (w/v) glycerol, 0.02% (w/v) pyronine Y, and 1% (w/v) DTT in the same ratio (1:10 w/v) and left for 30 min at 60°C with mixing every 5 min followed by centrifugation at 14000 rpm for 10 min at room temperature. Because the molecular weight of gliadin and glutenin are overlapped, SDS-PAGE for only gliadin extract was done, in addition to the mixture of the gliadin and glutenin. Gliadin extract, 0.5 ml, or gliadin and glutenin extract, 0.25 ml of each, were added to an equal amount of Laemmli sample buffer containing 0.05% 2-mercaptoethanol. The mixture was heated in a 100°C water bath for 10 min, then the samples were loaded into 12.5% polyacrylamide gels for about 1 h on 120 V. The gel was stained with Brilliant Blue Stain Reagent. Dual-color standards were used as a marker protein.

Statistical analysis. One-way analysis of variance (ANOVA) was performed for statistical analysis of data. Least significant difference (LSD) for

comparison of means was implemented by using IBM SPSS Statistics – Version 23.0. Significant differences were considered at P-value = 0.05.

Results

The effect of UV light treatment on wet gluten and gluten index are presented in Table 2. The quantity of wet gluten was reduced significantly after 30 min of UV treatment, while the gluten index changed insignificantly for the same treatment time. The quality and quantity of wet gluten reduced dramatically after 60 and 120 min treatment time. The difficulties in eluting gluten with the Glutomatic System method was observed for the longest treatment times, and the gluten was gone with the wash solution.

The immunoreactivity of gliadin of UV-treated flour reduced by 64%, 63%, and 58% after 30, 60, and 120 min treatment time, respectively compared to the control (Fig. 1).

Table 2. Gluten and gluten index of flour treated with UV irradiation for different times

Attributes	Flour treated with UV light, min			
	Control (zero)	30	60	120
Gluten% at 14%mb*	32.9 ± 0.71	25.8 ± 1.4	Not formed**	Not formed **
Gluten index%	100 ± 0.0	99.8 ± 0.07	NA	NA

* Moisture basis.

** Gluten was crumbling and there was not network formed.

NA – not applicable.

While the immunoreactive of gliadin of flour treated with UV and infrared and for flatbread treated by UV was fluctuated. The highest reduction was after 30 min for the combined treatment, which was 50%, and after 60 min for flatbread samples, which was 60% compared to the controls.

Free sulfhydryl groups of flour treated with UV at different times are shown in Fig. 2. They were increased significantly for all the treatment times compared to the control, and the increment was higher with longer treatment times. For the combined treatments, the free sulfhydryl groups were reduced significantly after 30 and 60 min, while it was slightly increased, but not significant, after 120 min compared to the control. All the flatbread samples treated with UV light had a significant reduction of free sulfhydryl groups

(Fig. 2). Gliadin extract and the mixture of gliadin and glutenin extract profile of flour treated with UV light were shown in Fig. 3A. The bands of gliadin were reduced with increasing the treatment time, while the reduction appeared slightly for the gliadin and glutenin mixture. For UV and infrared treatments (Fig. 3B), the gliadin bands were highly reduced after 30 min treatment time compared to the control, while the gliadin bands became thicker after 60 and 120 min treatments. The protein bands of gliadin and glutenin extract had no change for 30 and 60 min treatment times, but the bands were reduced slightly after 120 min treatment time (Fig. 3B). The gliadin profile of flatbread after UV treatment had no change compared to the control as well as for the gliadin and glutenin mixture profile (Fig. 4).

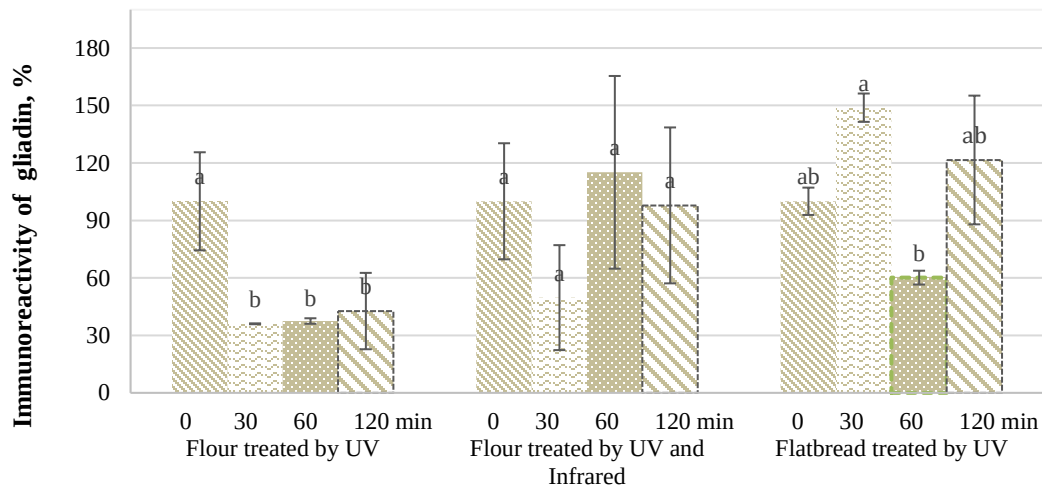


Figure 1. Immunoreactivity of wheat flour gliadin treated by only UV irradiation, by UV irradiation and infrared, and flatbread treated by UV at 0, 30, 60, 120 min treatment time. Means indicated by the same letter are not statistically different ($p > 0.05$)

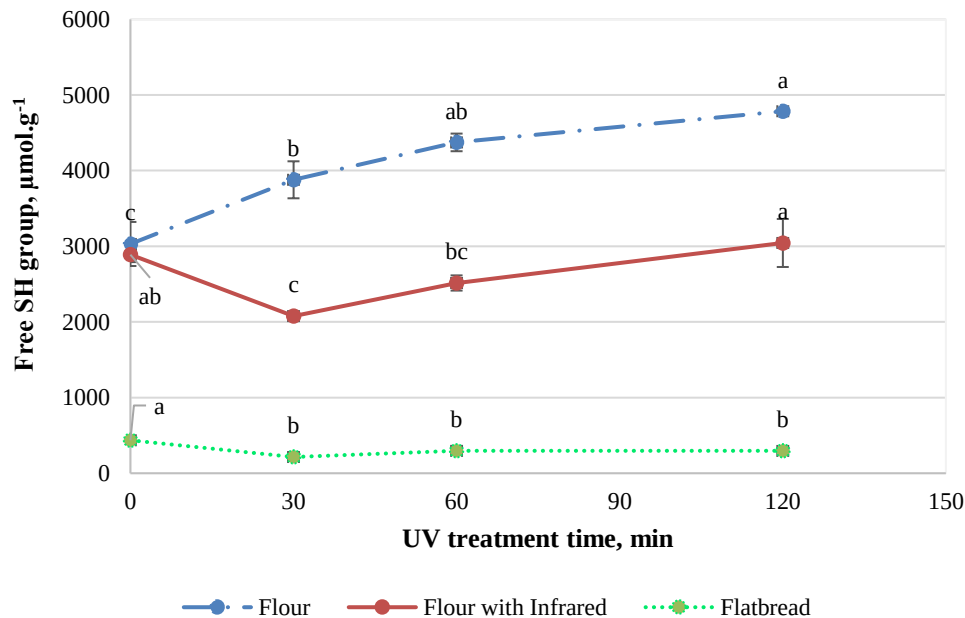


Figure 2. Free sulfhydryl groups in flour treated by only UV irradiation, by UV irradiation and infrared, and flatbread treated by UV at 0, 30, 60, 120 min treatment time. Means indicated by the same letter are not statistically different ($p > 0.05$)

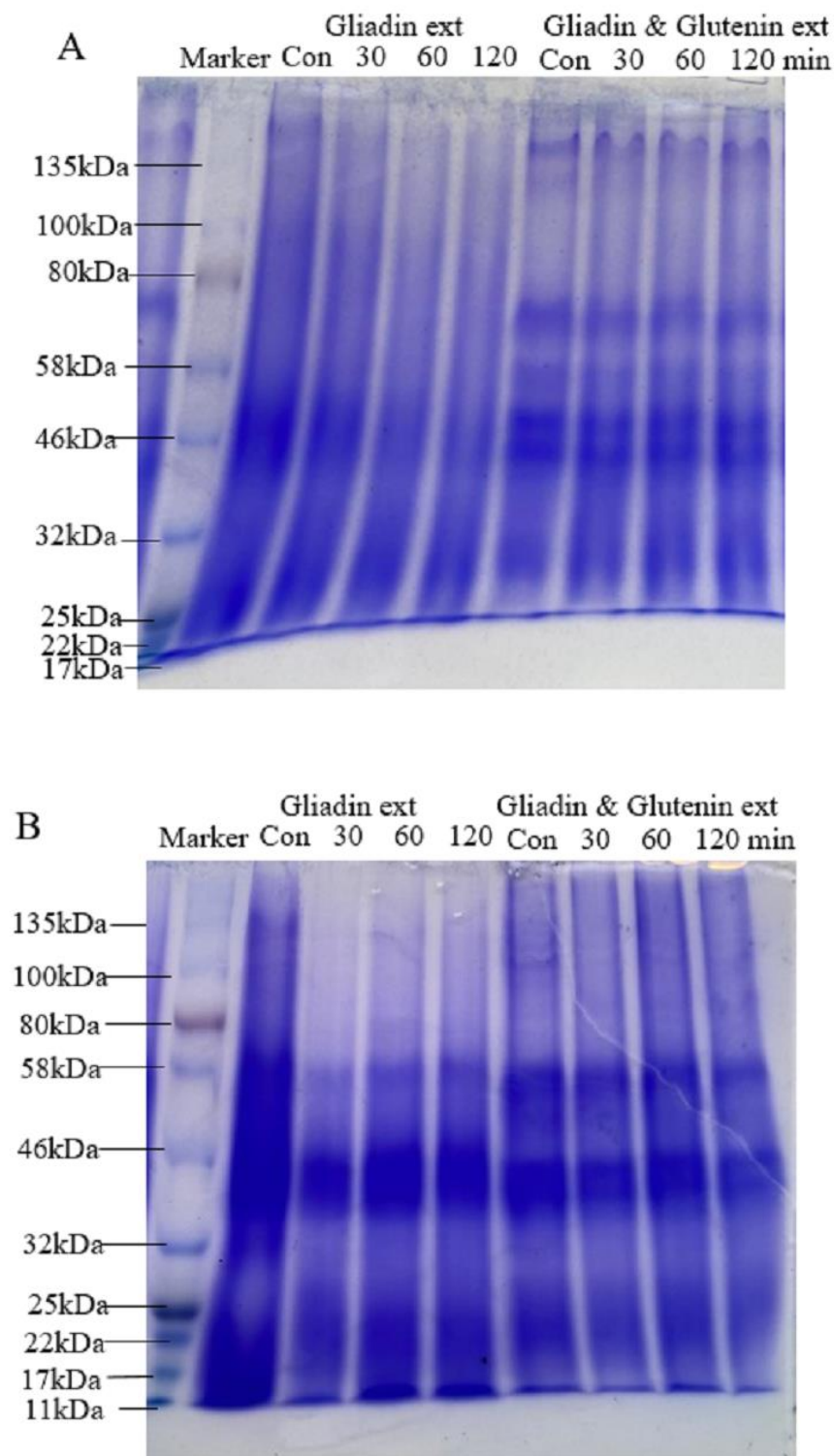


Figure 3. A: SDS-PAGE of gliadin extract and the mixture of gliadin and glutenin for of the flour samples treated by UV at different times (0, 30, 60, and 120 min); B: Flour treated with UV and infrared simultaneously. *ext = Extract.

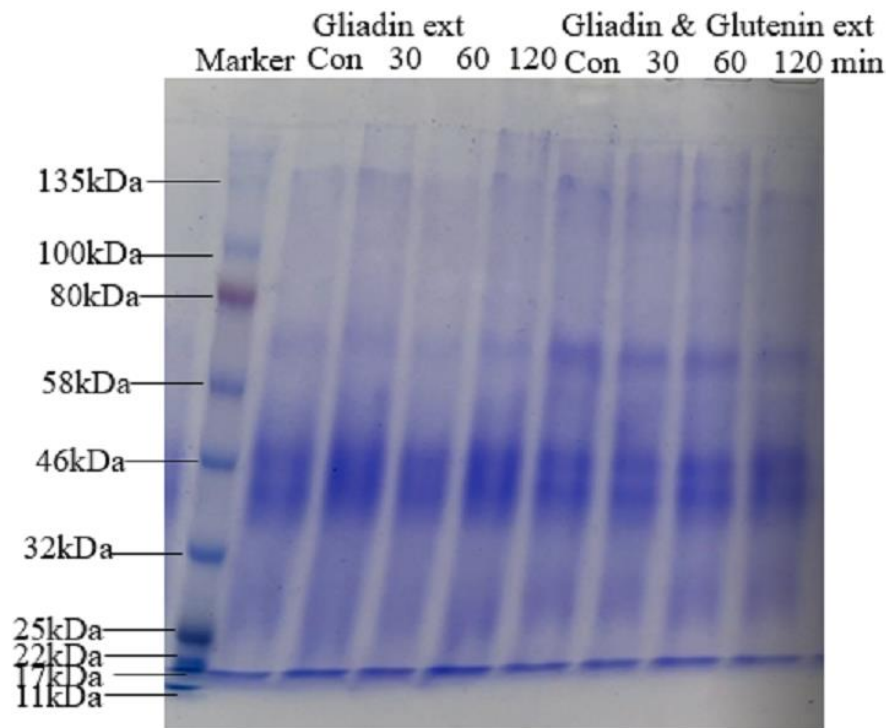


Figure 4. SDS-PAGE of only gliadin, gliadin and glutenin for the flatbread samples treated by UV at different times (0, 30, 60, and 120 min). *ext = Extract.

Discussion

Wheat protein involves of gluten and nongluten proteins. Gluten consists of about 80 to 85% of wheat proteins. Nongluten proteins are mostly with a MW lower than 25 kDa, however, some are with 60-70 kDa MW (Veraverbeke and Delcour 2002). Gluten is divided into two main proteins, which are gliadins, a monomeric protein with MW between 30 to 80kDa determined by SDS-PAGE, and glutenin, polymers with 80 KDa to several million dalton (Veraverbeke and Delcour 2002). Glutenin protein gives elastic property of gluten (Manu and Prasada Rao 2011). Gluten determined after 60 and 120 min UV treatment was crumbly compared to the control, which was chewy, elastic, and simply forming a transparent film. This result agrees with the Kumar et al. (2020) study, which they mentioned that gluten content was reduced and loss its elasticity after treating wheat flour with UV irradiation. Reducing gluten amount and subsequently gluten index after UV treatment was probably because of degrading polymeric proteins (glutenin) to monomeric proteins. Generally soft wheat products, such biscuit, require flour with less gluten strength

(Labuschagne et al. 2009), therefore, treating strong wheat flour with UV light could help in producing flour suitable for such products.

The highest reduction of immunoreactivity of gliadin happened to the flour samples treated with UV irradiation compared to the flour samples treated with UV irradiation and infrared light simultaneously or to flatbread samples, which was exposed to heat during baked process. This result refers to the negative effect of heat or photo-thermal on the reduction of immunoreactivity of gliadin. The reduction of immunoreactivity of gliadin of flour treated with only UV meet the significant increase of the free sulfhydryl groups for the same samples. UV irradiation led to increase the free sulfhydryl groups of wheat flour (Kumar et al. 2020), which agrees with the results of the current study. The temperature raising of flour during UV and infrared treatments or before UV treatment for flatbread samples led to reduce the free sulfhydryl groups. Increasing the free sulfhydryl groups indicated the photo-oxidation of disulphide (S-S)

bonds that led to form a different product having free sulfhydryl groups (Pattison et al. 2012). Panozzo et al. (2016) mentioned that PL slightly reduced immunoreactivity of gliadin in the wheat flour powder, less than 20%. Manzocco et al. (2009) mentioned that UV-C light aggregated polyphenoloxidase, while Manzocco et al. (2013) reported that PL degraded polyphenoloxidase. These last two studies gave an indication that PL and UV-C light affect differently on the same protein. The results also showed that some unexpected and insignificant increment of immunoreactivity of gliadin was happened with flour treated with UV and infrared and for flatbread samples treated with UV compared with the control (Figure 1). However, such raising occurred when gluten treated with microwave (Mahroug et al. 2019), and egg white protein treated with PL (Manzocco et al. 2013). Increasing immunoreactivity of gliadin was probably due to the reduction of free sulfhydryl content and increasing disulfide bond (S-S) (Mahroug et al. 2019; Panozzo et al. 2016).

In the current study, gliadin bands, about 50 KDa MW, were highly reduced, and the reduction increased with a longer UV light treatment. The reduction of bands of flour treated with UV was along with the reduction of the immunoreactivity of gliadin and the increment of SH bond. Therefore, all these analyses strengthen each other that UV light affected the immunoreactivity of wheat gliadin. Compared to other studies, pulsed light reduced gluten and bovine serum albumin (BSA) bands, while heat treatment of them caused no reduction, therefore, the reduction has been considered due to the photo-chemical effects (UV portion) of PL (Alhendi 2016). The negative or no effect of heat treatment agreed with the current study results, that wheat protein bands had no clear reduction for samples treated with heat (Fig. 3B and Fig. 4). Panozzo et al. (2016) found that gluten bands were degraded after suspension gluten was treated with PL, while there was no such degradation for gluten powder. The band reduction was less obvious for gluten extract, gliadin and glutenin extraction, compared to gliadin extract for flour samples treated with UV. This result might refer to that UV treatment affected different types of proteins differently.

Conclusion

The effect of UV irradiation on wet gluten, immunoreactivity of gliadin, free sulfhydryl groups, and molecular weight of wheat gluten was studied in wheat flour and flatbread samples. Ultraviolet irradiation had an obvious effect on the quality and quantity of wet gluten, immunoreactivity of gliadin, free sulfhydryl groups, and molecular weight of gliadin. While UV along with infrared light had no clear effect on immunoreactivity of gliadin, free sulfhydryl groups, and molecular weight of gliadin. Also, UV light had no reduction effect on immunoreactive of gliadin of flatbread and of MW of gliadin. While, UV irradiation reduced free sulfhydryl groups significantly of flatbread samples. In conclusion, thermal treatment of flatbread and flour samples before or along with UV treatment reduced the effect of UV light on the immunoreactivity of gliadin.

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