



Impact of Instant Controlled Pressure Drop (DIC) Treatment on Chemical, Functional and Immunological Properties of Wheat Gluten

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Abstract

Instant controlled pressure drop DIC technology is a thermo-mechanical treatment method that combines both mechanical and heat impacts of saturated steam pressure over a short period of time. This study aims to investigate the effect of DIC (100–165 °C for 20–60 s) on chemical, functional and immunological properties of wheat gluten powder using response surface methodology. Chemical properties were studied by Fourier transform infrared spectroscopy, thiol measurement and gel electrophoresis. Protein solubility, emulsifying capacity and foaming ability were evaluated as functional properties. Immunological properties were studied by indirect ELISA. After DIC treatment, wheat gluten became rigid which was due to a significant increase in β -structures. Significant loss of α -helixes and a significant increase in intermolecular β -sheet and the rearrangement of disulfide bonds indicated the denaturation of wheat gluten protein. However, the primary structure was not affected. DIC treatment altered the functional properties. The lowest protein solubility, emulsifying capacity and foaming ability were obtained at the highest DIC treatment conditions (165 °C, 60 s). The immunoreactivity of celiac IgA antigliadin antibodies of DIC wheat gluten depended on the individual patient characteristics. However, we note that the immunoreactivity has increased overall.

Keywords DIC treatment · Wheat gluten · Functional properties · Secondary structure · Immunoreactivity

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1 Introduction

Wheat is the second most widely produced cereal in the world, known for its adaptability, high yields and nutritional value. In several parts of the world, including North Africa, wheat and its derivatives are considered staple foods and continue to rise in production and consumption with the growing population [1].

One of the key features that contribute to the widespread use of wheat is the viscoelasticity of its flour dough, a unique property that allows it to be processed into bread, baked goods, pasta and other food items. Viscoelasticity is the result of the formation of a three-dimensional network interconnected by the disulfide bonds that are present in gluten; the complex protein composed of gliadin and glutenin [2]. Numerous functional properties of wheat gluten, such as solubility, fat emulsification, gelation and foaming, have caused an enormous diversity of applications. In fact, gluten is often used in the preparation of fruit puree and soy sauce, in meat products and monosodium glutamate



manufacturing [3]. Despite the ubiquity of wheat gluten in our diet, it poses a health risk to a minority of people in the form of celiac disease, allergies food or non-celiac sensitivity [4]. Celiac disease (CD), or wheat gluten intolerance, is an immune-mediated enteropathy manifested in genetically predisposed people and induced by the consumption of the prolamin of wheat, oat, barley and rye [5]. It is characterized by malabsorption resulting from inflammatory injury to the mucosa of the small intestine [6]. CD can occur at any age and affects approximately 1% of the population [7]. The current treatment of celiac disease is a lifelong exclusion of gluten in the diet of the affected patients [8].

The gluten-free diet (GFD) is effective in relieving symptoms of CD and normalizing the antibodies in the intestinal mucosa. However, it carries severe limitations due to the risk of contamination of foods or drugs with gluten, high cost, unavailability of gluten-free products and social marginalization of celiac disease patients [9]. Furthermore, this diet is poor in micronutrients and contributes to deficiencies, including iron, folic acid, vitamin B12 and vitamin D [10].

Physical treatments are currently used to modify the immunological properties of wheat gluten. It was shown that pulsed light and microwave irradiation decrease significantly the immunoreactivity of wheat gluten [11, 12]. Instant controlled pressure drop technology (French: *Détente Instantanée Contrôlée*-DIC), as the name suggests, is a thermo-mechanical treatment method that combines both mechanical and heat impacts of saturated steam pressure over a short period of time.

The DIC process is carried out into two main steps. The first step is based on heating through the use of dry saturated steam pressure from 100 to 700 kPa. The steam condensing on the surface of product results in a quick increase in the product temperature up to a level usually defined in the range of 100–165 °C and thermal treatment time between 5 and 60 s. The objective is to ensure homogeneous heat temperature and water repartition within the product. The second step is based on an abrupt pressure drop toward a vacuum (4–5 kPa) with a very high decompression rate (higher than 500 kPa s⁻¹) which is generally maintained for 5–20 s [13, 14].

Very few studies have addressed the effects of DIC on food proteins immunoreactivity. The overall findings appear to suggest that DIC has different effects on protein immunoreactivity depending on the nature of the protein and the treatment conditions [15–18]. Lupin and soybean proteins treated by DIC at 159 °C for 3 min have been shown to completely lose their binding ability of IgE [15–17]. In chickpea and lentil treated at 132.5 °C and 159 °C for 3 min and whey protein treated at 144 °C and 159 °C for 25 s, DIC induced partial loss of IgE-binding ability, whereas, in roasted peanut proteins treated at 132.5 °C and 159 °C for 1 and 3 min and

milk caseins treated at 144 °C for 25 s, the immunoreactivity was shown to have increased [17, 18].

In the present study, the possibility of using DIC to reduce or abolish the immunoreactivity of wheat gluten as an alternative to a gluten-free diet was investigated. As far as we know, there are no such studies examining the impact of DIC on chemical, functional and immunological properties of wheat gluten.

2 Materials and Methods

2.1 Extraction of Wheat Gluten

For extraction of wheat gluten, 300 g of flour was first mixed with 160 ml of NaCl 0.4 M for 5 min in a kneading machine. After 8-min resting time, the dough was washed with a NaCl solution (0.4 M, 3 L) until viscoelastic gluten was formed. Then, the crude gluten was again washed with deionized water to remove NaCl [19]. Finally, the wet gluten was dried, ground and sieved to obtain a homogeneous powder.

2.2 DIC Treatment

DIC treatment was carried out by a laboratory-scale DIC unit (provided by ABCAR-DIC Process, La Rochelle, France). Briefly, the gluten powder was placed in a processing chamber and exposed to a dry saturated steam pressure (up to 100 kPa) at high temperature (up to 100 °C) over a relatively short time (few seconds to some minutes). This high temperature–short time was followed by an instant pressure drop toward a vacuum at about 5 kPa. This abrupt pressure drop, at a rate $\Delta P/\Delta t$ higher than 500 kPa/s, simultaneously provokes an autovaporization of a part of the water in the product and instantaneously cools the products, which stops thermal degradation [20].

2.3 Experimental Design

In this study, response surface methodology (RSM) with central composite design of experiments (CC-DoE), the most popular of all second-order designs, was applied to reduce experimental runs and to determine the significance of the factors. This DoE is composed of three portions: $2k$ factorial portion ($-1, +1$), $2k$ axial portion ($-\alpha, +\alpha$) and n_0 center points replicates (0, 0). In the actual case, two factors of temperature (X_1) and processing time (X_2) were the independent variables ($k=2$). Thus, the DoE total number was $n=2^2+2*2+5=13$ runs. Therefore, the five levels for each factor were $-\alpha, -1, 0, +1$ and $+\alpha$ corresponded, respectively, to 100, 109.5, 132.5, 155.5 and 165 °C for temperature and 20, 25.9, 40, 54.1 and 60 s for time treatment. The CC-DoE matrix was made with Minitab software (version 13.31, Minitab Inc., USA). The



responses were expressed in terms of the factors according to the following equation:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{12}X_1X_2$$

where Y is response variable, and X_1 , X_2 are independent variables, processing temperature and time, respectively. b_0 is constant coefficient of intercept, b_1 and b_2 are regression linear coefficients, b_{11} and b_{22} are regression quadratic coefficients, and b_{12} is interaction regression coefficient.

2.4 Human Sera

Individual serum samples from three diagnosed celiac disease patients (CD) were collected from Ibn Sina Clinic (Constantine, Algeria). Untreated CD patients were numbered P1 (female aged 24), P2 (female aged 7) and P3 (male aged 17). They showed digestive and extra-digestive symptoms and positive IgA antibodies toward gliadin (> 25 RU/ml), tissular transglutaminase tTG (> 20 RU/ml) and endomysium ($> 1/10$). These patients were selected on the basis of their high IgA antigliadin antibodies concentration (> 2000 RU/ml). The sera were stored at -20°C until use.

2.5 Determination of Changes in Secondary Structure

Fourier transform infrared spectroscopy (FTIR) was used to study the impact of DIC on wheat gluten secondary structure. All samples, gluten control and DIC-treated gluten samples, were analyzed by FTIR. FTIR spectra were recorded between 4000 and 400 cm^{-1} with 32 scans and 8 cm^{-1} resolution against the background using an IR Affinity-1S Fourier transform infrared spectrophotometer (SHIMADZU, IZASA Scientific, France) equipped with a single-reflection diamond attenuated total reflection (ATR) crystal and a mercury–cadmium–telluride (MCT) detector. Each sample was placed on the surface of the ATR crystal at room temperature. The measurement was repeated five times to get the average value.

The spectra were manually corrected with a linear baseline in the LabSolutions IR software. Structural analysis of the amide I (1600 – 1700 cm^{-1}) was conducted using Origin software (version 8.0724 PRO, Origin Lab Corporation, USA) and peak-fit software (version 4.6, USA). The baseline correction and derivation were performed with Origin software. The spectra were deconvoluted and curve fitted to obtain percentages of secondary structure components using peak-fit software.

2.5.1 Determination of Free Thiol Content

Colorimetric measurements of free thiol content were taken using DTNB (5,5'-dithiobis-2-nitrobenzoic acid)

according to the reported method of Jansens et al. [21] slightly modified. A dispersion of the samples (2 mg/ml) was prepared in sample buffer [0.05 mol L^{-1} phosphate buffer (pH 6.5) containing 2% (w/v) SDS, 3.0 mol L^{-1} urea and 1.0 mmol L^{-1} EDTA (ethylenediaminetetraacetic acid)]. The dispersion was left for 1 h with intermittent vortexing every 15 min. Then, $400\text{ }\mu\text{l}$ of DTNB reagent [0.1% (w/v) in sample buffer] was added, incubated for 45 min with intermittent vortexing and centrifuged at $3000\times g$ at room temperature for 10 min. The supernatant was collected, filtered ($0.45\text{-}\mu\text{m}$ RC, Millipore), and the absorbance of the filtrate was taken at 412 nm using UV spectrometer (Secomam, Prim, France). The absorbance value was divided by the molar extinction coefficient of 13,600 to obtain the thiol content, expressed as $\mu\text{mol/g}$ of protein.

2.5.2 Electrophoresis Separation

2.5.2.1 Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS-PAGE) SDS-PAGE was performed according to Laemmli protocol following the Instruction Manual of Mini-PROTEAN 3Cell (BioRad) using 6%/12% stacking/resolving gel. Three milligrams of each sample, gluten control and DIC-treated gluten, was extracted by $300\text{ }\mu\text{l}$ $2\times$ Laemmli sample buffer (BioRad)-contained β -mercaptoethanol and then was heated in boiling water for 3 min, and the same amount (4 – $4\text{ }\mu\text{l}$) of samples was loaded onto the gel. The separation was achieved at 200 V for approximately 65 min at room temperature. After electrophoresis, the separated proteins were fixed on a gel with 20% TCA (trichloroacetic acid) for 20 min, and then, the gel was soaked in acetic acid/ethanol washing liquid 3 times for 10 min. Staining was performed with Coomassie Brilliant Blue R-250 (Reanal, Hungary). The background staining was removed by 10% acetic acid. Imaging of the gels was carried out with BioRad Gel Doc 2000 system.

2.5.3 Two-Dimensional Gel Electrophoresis (2D-PAGE)

Two-dimensional gel electrophoresis (2D-PAGE) was performed according to Laemmli [22] and Hajós et al. [23]. Only gluten control and gluten samples DIC treated at the same time (40 s) and different processing temperatures (100 , 132.5 and 165°C) were analyzed by 2D electrophoresis. The first dimension, the isoelectric focusing, was run in the immobilized pH gradient (IPG strip, 7 cm) strip with pH range of 3–10 using an isoelectric focusing chamber (PROTEAN IEF Cell, BioRad). A quantity of 0.6 mg dry matter of each sample was used for each strip. It was dissolved (rehydrated) in 250 ml rehydration buffer (8 mol L^{-1} urea, 1% CHAPS, 0.02 mol L^{-1} DTT) with $600\text{ }\mu\text{l}$ mineral oil (BioRad) surface top put into a i12 rehydration/equilibration tray (BioRad).



Then, the isoelectric focusing (IEF) was carried out by linearly increasing voltage from 250 to 24,000 Vh. After reaching the equilibrium, each strip was incubated in 1.5 ml equilibration solution [first for 15 min at room temperature in I. Buffer: 6 mol L⁻¹ urea, 1.5 mol L⁻¹ Tris-HCl (pH 8.8), 2% w/v DTT, 2% SDS, 20% glycerol and secondly for 15 min at room temperature in II. Buffer: 6 mol L⁻¹ urea, 1.5 mol L⁻¹ Tris-HCl (pH 8.8), 2.5% w/v iodoacetamide, 2% SDS, 20% glycerol] with gentle shaking before being transferred to the second dimension.

In the second dimension, the separation of proteins was carried out by SDS-PAGE in thin layer gels (75 mm_83 mm_1.5 mm) by using a Mini-PROTEAN II cell (BioRad). The total acrylamide content of the running gel was 12%. Imaging of the gels was carried out with BioRad Gel Doc 2000 system.

2.6 Determination of Functional Properties

2.6.1 Determination of Protein Solubility

Protein solubility (*S*%) of wheat gluten control and DIC-treated gluten samples was investigated according to the method of Casella and Whitaker [24] with some modifications. Solubility was performed at pH 3 at 1% (w/v) concentration in distilled water. The gluten protein and distilled water were first stirred up in glass beaker (50 ml) for 5 min to obtain a pre-mixed blend. Then, the pH values of suspensions were adjusted to 3 with 0.5 mol l⁻¹ HCl and stirred at room temperature for 1 h on a magnetic stirrer. They were centrifuged at 1640×g for 15 min at room temperature, and the supernatant was filtered through a Whatman No. 1 filter paper to obtain a clear filtrate. The amount of soluble protein in the filtrate was determined by the method of Lowry et al. [25] with bovine serum albumin as standard.

The solubility *S*% was calculated with the following equation:

$$S\% = \frac{C}{C_0} \times 100$$

where *C* and *C*₀ are the protein concentrations of DIC-treated gluten and untreated gluten, respectively.

2.6.2 Determination of Emulsifying Capacity

Emulsifying capacity (EC) of wheat gluten control and DIC-treated gluten samples was determined according to the method of Yasumatsu et al. [26]. EC of the DIC-treated glutes and gluten control at 1% (w/v) concentration in distilled water was examined at pH 3. Protein solutions were prepared as described in the determination of solubility properties. In total, 5 ml of the protein solution was homogenized with

5 ml of corn oil by ultraturax (IKA model T18D digital, USA) at 23,500 rpm for 90 s to form an emulsion. The emulsion formed was then centrifuged at 3630×g for 25 min.

EC was calculated by measuring the height of the emulsified layer in the centrifuge tube (*H*₁) and the total height of the liquid (*H*_T) and reported as:

$$EC(\%) = \frac{H_1}{H_T} \times 100$$

2.6.3 Determination of Foaming Ability

Foaming ability (FA) was evaluated by Caessens et al. [27] method. Firstly, 10 ml of gluten proteins solution at 1% (w/v)/pH 3, prepared as previously described, was placed in a beaker. After, the solution was homogenized with Ultraturax (IKA model T18D digital, USA) at 1000 rpm for 2 min. Foaming ability (FA) of samples was calculated by the following equation:

$$FA(\%) = \frac{V - V_0}{V_0} \times 100$$

*V*₀ is the total volume of the initial solution and *V* is the volume of the solution no foaming (ml).

2.6.4 Determination of IgA Antigliadin Antibodies Immunoreactivity

A non-competitive indirect enzyme-linked immunosorbent assay (ELISA) was performed to measure the immunoreactivity of IgA antibodies from celiac patients against gliadins from DIC-treated gluten samples. For gliadin extraction, 50 mg of gluten powder was dispersed in 4 ml of 70% ethanol, mixed for 1 h and centrifuged at 3000×g for 10 min at room temperature, and supernatant was freeze-dried. Briefly, microplates were coated with 5 µg/mL gliadins in coating buffer (0.1 mol l⁻¹ NaHCO₃, pH 9.6) overnight. After washing (3 times) with phosphate-buffered saline Tween (PBST) (15 mmol l⁻¹ KH₂PO₄, 0.15 mol l⁻¹ NaCl, pH 7.4, containing 0.2% Tween 20), the plates were blocked with 1% BSA in PBST for 2 h at room temperature followed by 3 washes and incubated with a human serum samples (3 CD-diagnosed patients) diluted 1: 201 in PBST containing 0.1% gelatin (PBSTG).

After incubation for 30 min at room temperature, plates were washed 3 times and incubated with HRP-conjugated antihuman IgA antibodies in PBSTG (1: 2000 dilution). After three washes, activity was developed with chromogen/substrate in the dark. After 15 min, the reaction was stopped with 1 mol l⁻¹ H₂SO₄ and the absorbance was measured at 450 nm (microplate reader, TECAN, Infinite F50, Switzerland). Percentage of immunoreactivity was



calculated as the ratio of the absorbance at 450 nm of gliadins from DIC-treated gluten with respect to gliadins from the control reaction. Immunoreactivity was duplicated for each sample [28].

2.7 Statistical Analysis

The functional properties and free thiol content data were studied through the analyses of variance (ANOVA) and multiple regressions using Minitab software (version 13.31, Minitab Inc., USA). Similarly, the secondary structure data and the immunoreactivity data of each patient were studied by analysis of variance (ANOVA) and Tukey's test using GraphPad Prism software (version 7, Inc., USA). Statistical significance was accepted at a level of $p < 0.05$. The response surfaces were plotted with

STATISTICA software (version 7.0, StatSoft, Inc., USA) (Table 1).

3 Results and Discussion

3.1 Model Fitting

The results of multiple regression analysis of DIC wheat gluten properties are presented in Table 2 through the coefficients of the linear and quadratic effects of each of the factors and the interaction between them. The adequacy of the model is indicated through the model significance ($p < 0.05$) and satisfactory of determination coefficient ($R^2 > 70\%$) [29, 30]. The multiple regression analysis of solubility indicated that the model was significant ($p = 0.015$) and it could be explained by 82.1% of the variance in data. The significant p values of emulsifying capacity, foaming ability and free thiol content were 0.000, 0.001 and 0.000, respectively. Their models got data variance R^2 of 96%, 92.8% and 95.5%, respectively. The statistical results allowed the validation of the experimental results.

3.2 Impact of DIC on Chemical Properties

Fourier transform infrared spectroscopy (FTIR) analysis was conducted to investigate the changes in the secondary structure of wheat gluten treated by DIC. The composition of all samples, untreated and DIC-treated gluten, is shown in Table 2. The gluten control contained 7.77% β -sheets, 12.11% β -turns, 23.88% α -helixes, 30.17% random coil, 22.35% antiparallel β -sheets and 3.73% intermolecular β -sheets.

The results obtained in this study were different than those reported by Nawrocka et al. [31]. They observed that wheat gluten extracted from dough contained 11% β -sheets, 13% β -turns, 60% α -helixes, 8% antiparallel β -sheets and 8%

Table 1 Design of experiments of DIC treatment

Sample	Codes (X_1 , X_2)	Temperature ($^{\circ}\text{C}$) (X_1)	Time (s) (X_2)
G_1	(0, 0)	132.5	40
G_2	($+\alpha$, 0)	165.0	40
G_3	(0, $+\alpha$)	132.5	60
G_4	(0, 0)	132.5	40
G_5	($+1$, $+1$)	155.5	54.1
G_6	($+1$, -1)	155.5	25.9
G_7	(0, 0)	132.5	40
G_8	(-1 , -1)	109.5	25.9
G_9	(-1 , $+1$)	109.5	54.1
G_{10}	(0, 0)	132.5	40
G_{11}	($-\alpha$, 0)	100.0	40
G_{12}	(0, $-\alpha$)	132.5	20
G_{13}	(0, 0)	132.5	40

Table 2 Regression models for the response variables

	Functional properties			
	Solubility (%)	Emulsifying capacity (%)	Foaming ability (%)	Free thiol content ($\mu\text{mol/g}$ of protein)
Temperature (X_1)	−4.33NS	−5.818*	−0.01145*	0.2030*
Time (X_2)	−7 NS	−1.134*	−0.0666*	−0.0106NS
Temperature/time (X_1X_2)	−28.19*	−0.333NS	−0.0375NS	−0.0412NS
Temperature ² (X_1^2)	−15.93*	−1.417*	−0.1006*	−0.679*
Time ² (X_2^2)	2.64NS	0.250NS	−0.0006 NS	−0.5596*
R^2 (%)	82.1	96	92.8	95.5
p (Model)	0.015*	0.000*	0.001*	0.000*

NS not significant

*Significant at $p < 0.5$



aggregates. This discrepancy can be attributed to numerous factors such as washing out, freeze-drying and pulverizing [31]. This difference is probably also related to wheat varieties used to produce the flour.

As shown in Table 2, we note major changes in the secondary structure of wheat gluten after DIC treatment. All components of the secondary structure were significantly different than those of wheat gluten control. Table 2 also shows that β -sheet structure of DIC-treated gluten ranging from 9.62 to 43.91% has increased in relation to the one of control (7.77%). Our result is in agreement with those of Georget and Belton [32] who found that hydration and heating implied changes when the β -sheet structure occurs. The low hydration due to the steam condensing on the surface of gluten and high temperature during DIC treatment causes this change.

Moreover, the β -turns structure included between 8.51 and 24.75% showed an increase except for β -turns for wheat gluten treated at 109.5 °C for 54 s. As reported in the literature [19], the increase in structures β resulted in a more hydrophobic and rigid structure which explains the rigidity and hardness of wheat gluten powder after DIC treatment as mentioned in Fig. 1.

According to Yong et al. [33], the β -turn structure is composed mainly of Glu and Asp and β -sheet is formed from Gln residues in gluten. Therefore, β -turns have increased due to the release of Glu and Asp. A significant loss was observed for α -helixes structure, ranging from 6.16 to 16.43%. As for random coil, it decreased except for gluten samples treated at 132.5 °C for 40 s (31.20%) and 132.5 °C for 20 s (31.55%). Similarly, antiparallel β -sheets decreased compared to that for wheat gluten control (22.35%) except of that for DIC-treated gluten at 100 °C during 40 s (24.31%) (Table 3).

However, intermolecular β -sheet due to protein aggregation, including between 5.49 and 10.93%, was increased. These results showed clearly that DIC treatment involved the unfolding of gluten proteins. Guerrieri et al. [34] argued that the denaturation of wheat gluten can be detected above 80 °C. As reported by Mounir and Allaf [14], the protein denaturation is among the irreversible changes in microorganism cells treated by DIC.

Further information about chemical modifications induced by DIC treatment was obtained by free thiol content measurement. Figure 2d illustrates the effect of DIC treatment on free thiol content of gluten. The thiol content of gluten control was 6.43 ± 0.072 $\mu\text{mol/g}$ of protein, whereas its concentrations after DIC treatment, ranging from 3 to 5 $\mu\text{mol/g}$ of protein, decreased. Figure 2d also indicates that both factors had a negative quadratic effect on thiol content of DIC-treated gluten. The concentration of free thiol was correlated with the increasing factors (temperature, time treatment) until limits were reached, where the response decreased beyond the central zone of the experimental

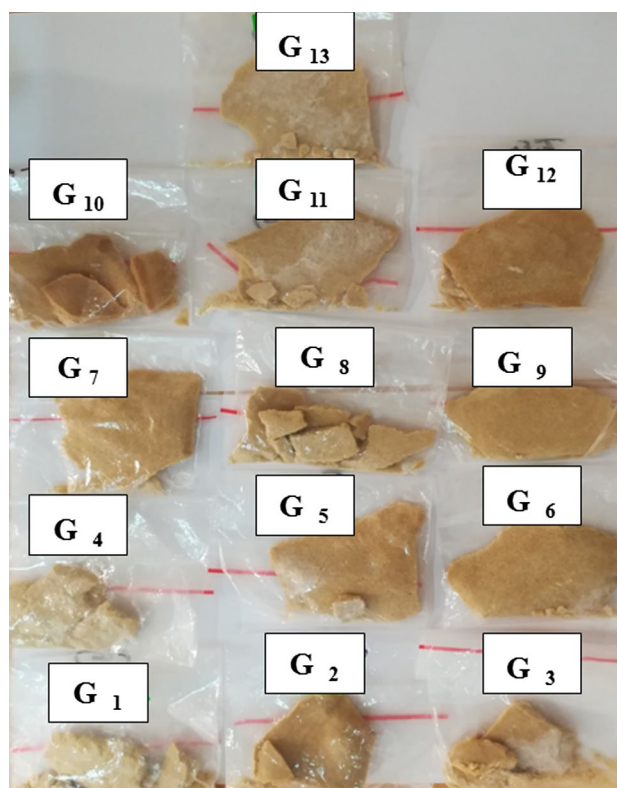


Fig. 1 Aspect of wheat gluten powder after DIC treatment: rigidity of wheat gluten powder GC: gluten control, G1–G13: DIC-treated glutes

domain. It increased from 3 $\mu\text{mol/g}$ at 5 $\mu\text{mol/g}$ of protein in the treatment ranges of 100–132.5 °C of temperature and 20–40 s of time. However, it decreased from 5 to 3 $\mu\text{mol/g}$ of protein in the treatment intervals of 132.5–165 °C of temperature and 40–60 s of time.

Disulfide bonds of gluten proteins have a key role in their functionality in wheat flour. The α - and γ -gliadins contain six cysteine and eight cysteine residues, respectively, leading to intra-chain disulfide bonds contrary to ω -gliadins that lack cysteine, whereas the low molecular weight glutenin subunits (LMW-GS) contain both intra- and inter-chain disulfide bonds among themselves and with high molecular weight glutenin subunits (HMW-GS) forming glutenin polymers [35]. The quantity of free thiol content depended on numerous factors such as temperature, pH and hydrostatic pressure. These results showed that DIC treatment probably induces oxidation of free thiol; in other words, the formation of a new disulfide bridge indicates aggregate formation.

Guerrieri et al. [34] reported that changes in disulfide bridging are involved at temperatures above 100 °C. The rearrangements and the formation of these bonds can increase by unfolding the native structure under high pressure/high temperature causing denaturation and polymerization of wheat gluten proteins [36].



Table 3 Secondary structure components of untreated gluten and DIC-treated gluten samples determined by FTIR spectroscopy

Band position (cm ⁻¹)	[1687, 1690]	[1665, 1670]	[1650, 1660]	[1640, 1645]	[1629, 1632]	[1612, 1614]
Secondary structure	β -Sheets (%)	β -Turns (%)	α -Helixes (%)	Random coil (%)	Antiparallel β -sheets (%)	Intermolecular β -sheets due to protein aggregation (%)
Gluten control	7.77 $\pm$ 0.01 ^a	12.11 $\pm$ 0.01 ^b	23.88 $\pm$ 0.03 ^h	30.17 $\pm$ 0.04 ^h	22.35 $\pm$ 0.02 ⁱ	3.73 $\pm$ 0.05 ^a
132.5 °C, 40 s	16.15 $\pm$ 0.12 ^e	13.27 $\pm$ 0.05 ^c	16.23 $\pm$ 0.06 ^f	31.20 $\pm$ 0.12 ⁱ	15.38 $\pm$ 0.05 ^h	7.77 $\pm$ 0.16 ^c
165 °C, 40 s	15.43 $\pm$ 0.16 ^d	15.59 $\pm$ 0.15 ^e	16.25 $\pm$ 0.15 ^f	25.43 $\pm$ 0.24 ^d	18.16 $\pm$ 0.15 ^g	9.13 $\pm$ 0.52 ^f
132.5, 60 s	9.62 $\pm$ 0.06 ^b	20.08 $\pm$ 0.03 ⁱ	16.43 $\pm$ 0.03 ^g	28.03 $\pm$ 0.05 ^g	17.36 $\pm$ 0.02 ^f	8.48 $\pm$ 0.06 ^e
155.5 °C, 54.1 s	43.19 $\pm$ 0.12 ^h	24.75 $\pm$ 0.14 ^j	12.01 $\pm$ 0.07 ^c	9.10 $\pm$ 0.05 ^a	5.46 $\pm$ 0.03 ^a	5.49 $\pm$ 0.16 ^b
155.5 °C, 25.9 s	13.16 $\pm$ 0.21 ^c	17.22 $\pm$ 0.13 ^g	15.82 $\pm$ 0.12 ^e	27.16 $\pm$ 0.21 ^e	15.72 $\pm$ 0.12 ^d	10.93 $\pm$ 0.37 ^g
109.5 °C, 25.9 s	16.86 $\pm$ 0.12 ^f	18.22 $\pm$ 0.08 ^h	14.10 $\pm$ 0.06 ^c	27.63 $\pm$ 0.13 ^f	14.33 $\pm$ 0.05 ^c	8.85 $\pm$ 0.2 ^{ef}
109.5 °C, 54.1 s	43.91 $\pm$ 0.16 ⁱ	8.51 $\pm$ 0.06 ^a	6.16 $\pm$ 0.04 ^a	23.22 $\pm$ 0.16 ^c	8.93 $\pm$ 0.0 ^b	9.27 $\pm$ 0.16 ^f
100 °C, 40 s	17.80 $\pm$ 0.11 ^g	16.03 $\pm$ 0.07 ^f	12.18 $\pm$ 0.05 ^b	22.44 $\pm$ 0.09 ^b	24.31 $\pm$ 0.05 ^j	7.24 $\pm$ 0.15 ^d
132.5 °C, 20 s	16.62 $\pm$ 0.14 ^f	14.28 $\pm$ 0.06 ^d	14.88 $\pm$ 0.06 ^d	31.55 $\pm$ 0.13 ^j	16.78 $\pm$ 0.03 ^e	5.89 $\pm$ 0.13 ^b

Different letters (a–j) indicate significant difference ($p < 0.05$)

DIC-treated gluten proteins and control gluten proteins were separated according to their molecular weights by SDS-PAGE under reducing conditions. The SDS-PAGE patterns are illustrated in Fig. 3a. The different fractions of gluten protein were determined by referring to molecular weight markers and literature data [37]. The protein profiles of thirteen DIC-treated gluten and untreated gluten did not show a significant difference.

Furthermore, we observe that the number and the intensity of the bands of all samples did not decrease or increase. The bands ranged between 70–90 kDa and 30–45 kDa corresponding to HMW-GS and LMW-GS, respectively, whereas the bands had 28–45 kDa corresponding to ω -gliadins, α -, β - and γ -gliadins. These results suggest that DIC treatment did not induce any modification of protein fractions gluten.

The same results were found when the glutes treated at different temperatures (132.5, 165 and 100 °C) and during the same period of time (40 s) were separated according to their isoelectric points and molecular weights as shown in Fig. 3b. There are no significant differences between untreated gluten and the different DIC-treated gluten patterns. These results indicate that the wheat gluten proteins had the same molecular weight and the same charge after DIC treatment. Our results are in good agreement with Boughallout et al. [18], which showed that the SDS-PAGE patterns of DIC-treated milk proteins, bovine caseins and whey protein, at 144 °C for 25 s, are identical with one of the untreated milk proteins.

However, this observation is contrary to the study of Guillamón et al. [15], which suggested that SDS-PAGE patterns of proteins extracted from lupin seeds treated by DIC at 132.5 °C (300 kPa) and 159 °C (600 kPa) for 1 and 3 min showed a disappearance of conglutin β and a decrease in bands intensity of conglutin α as treatment time was

increased. Therefore, the effect of DIC on proteins depends on treatment duration and food matrix.

3.3 Impact of DIC on Wheat Gluten Functional Properties

Gluten may be used as a functional protein additive in various applications other than baking due to its desirable structure-enhancing properties [38]. Figure 2a presents the response of the surface of the effect of DIC treatment on gluten solubility. As shown in this figure, DIC treatment reduced significantly the solubility, changing from 20 to 100%. A negative quadratic effect of temperature and a negative interactive effect on gluten protein solubility were observed. The minimum values of solubility were obtained at 100 °C for 20 s and at 165 °C for 60 s, whereas the maximum values were observed at 100 °C for 60 s and 165 °C for 20 s.

In general, the emulsifying capacity (EC) is the first functional property determinate during the development and testing of new protein ingredients [39, 40]. Figure 2b illustrates the effect of DIC treatment on EC. The values of EC for DIC-treated gluten, varied from 65 to 80%, were lower than EC of untreated gluten 88.33 $\pm$ 5.55%. The response surface showed that both temperature and time treatment had a linear negative effect on EC. In addition, the temperature had a negative quadratic effect. The EC maximum values (80%) corresponded to the lowest temperature of 100 °C and the shortest time of 20 s and vice versa.

Concerning foaming ability (FA), the same effect of DIC treatment was observed. All the FA values of DIC-treated gluten were lower than the one of gluten control, which was 130 $\pm$ 31%, as shown in Fig. 2c. It is varied from 50 to 110%. Both temperature and time treatment had a negative linear effect on foaming ability. Negative quadratic effect of



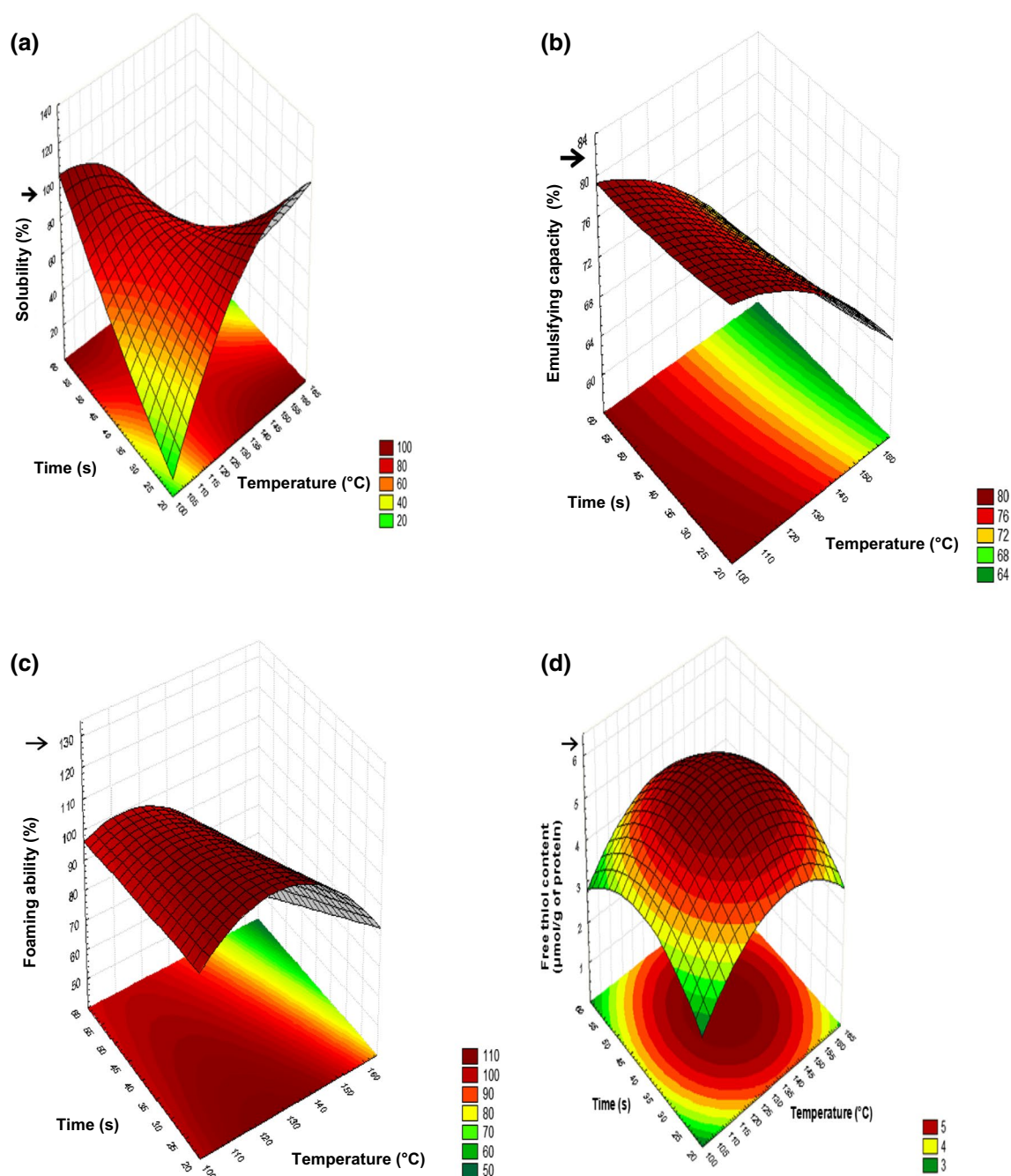


Fig. 2 Response surfaces of impact of DIC treatment on protein solubility (a), emulsifying capacity (b), foaming ability (d) and free thiol content (d), →: untreated gluten value

temperature was also observed. The lowest foaming ability of DIC-treated wheat gluten was noted at the highest DIC treatment (165 °C for 60 s), whereas the maximum values were recorded in a large zone ([100, 150] °C for [20, 55] s).

The functional properties of proteins are affected by numerous factors such as source, pH, concentration, solubility and temperature. The protein solubility is tightly related to the content of hydrogen bonds and hydrophobic and

hydrophilic residues on the protein surface. It is the result of the active properties of proteins such as foaming, emulsification, fat and flavor-binding properties. This surface active depends principally on composition and distribution of amino acid, molecular flexibility and to a small degree on the shape and the size [26].

The solubility is affected by the degree of thermal and non-thermal processing such as the DIC procedure that



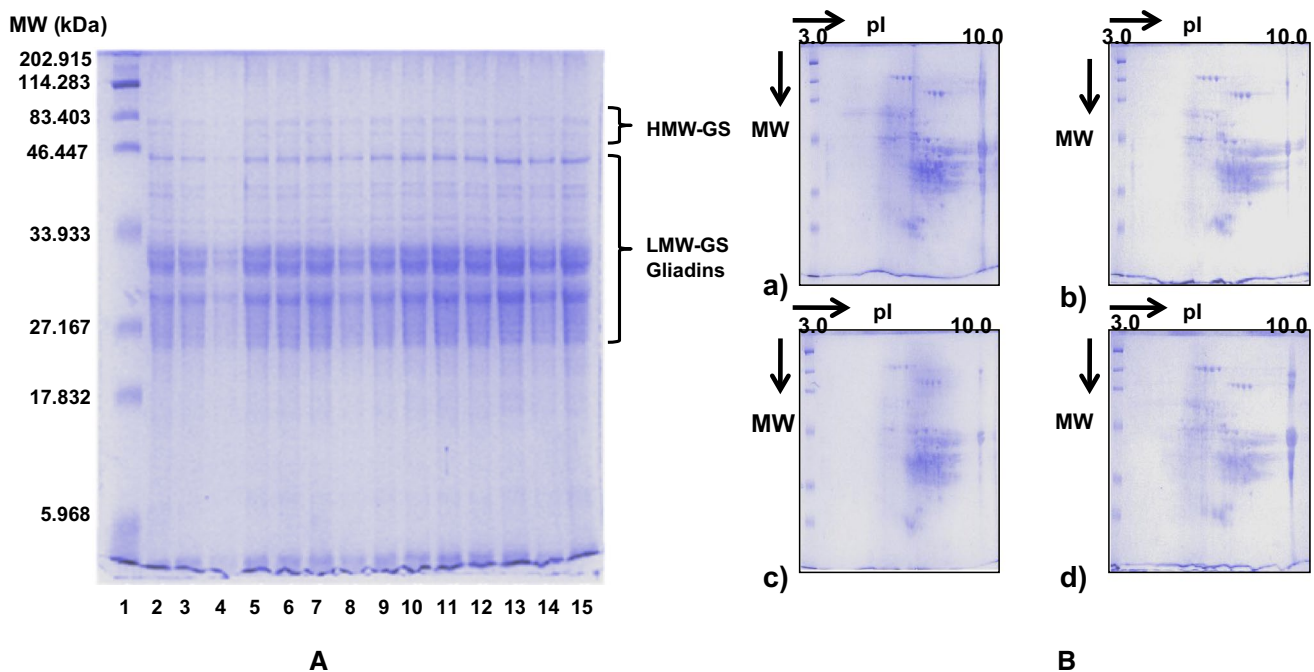


Fig. 3 Electrophoresis patterns of wheat gluten untreated and treated by DIC. **A**/SDS-PAGE pattern of gluten in reducing conditions (1: molecular mass marker kDa. 2: lane untreated gluten proteins. 3–15: lanes DIC-treated gluten proteins G₁ to G₁₃. HMW-GS: high molecular weight glutenin subunits, LMW-GS: low molecular

weight glutenin subunits. **B**/Two-dimensional electrophoresis pattern (IEF×SDS-PAGE) of untreated and DIC-treated gluten [**a**: untreated gluten, **b**: DIC-treated gluten G₁ (132.5 °C, 40 s), **c**: DIC-treated gluten G₂ (165 °C, 40 s), **d**: DIC-treated gluten G₁₁ (100 °C, 40 s)]

combines heat and steam pressure in a short time following by an instant pressure drop to vacuum. These processing methods lead to the formation of oligomers, changes in surface hydrophobicity and denaturation of proteins that responsible for solubility reduction [34].

Han et al. [41] reported that the functional properties are closely related to the secondary structure. The changes of protein structure can alter the functional properties including solubility, apparent viscosity, emulsifying, foaming, immunoreactivity and gelling properties. These results are in good agreement with the previous results of FTIR. The increase in β -structures (β -turns, β -sheets and intermolecular β -sheets) was probably responsible for protein solubility reduction. Despite the short time of DIC treatment (20–60 s), the high temperature applied in this treatment involved the diminution of solubility. As observed by Morel et al. [42], heat treatment of wheat gluten induced solubility loss. The solubility is strongly correlated with other properties such as emulsification and foaming characteristics. The diminution of foaming ability and emulsifying capacity could be explained by the diminution of the solubility.

3.4 Impact of DIC on Celiac IgA Antigliadins Antibodies Immunoreactivity

Celiac IgA antibodies immunoreactivity against gliadin extracted from untreated and DIC-treated gluten samples was determined by indirect ELISAs using three celiac disease patients' sera. The effect of DIC on immunoreactivity is presented in Fig. 4. For patient 1, the immunoreactivity of DIC-treated gluten varied between $107.22 \pm 4.69\%$ and $226.54 \pm 12.29\%$ corresponded, respectively, to the immunoreactivity of gluten samples DIC treated at 155.5 °C for 25.9 s and 165 °C for 40 s. There is a significant difference between gluten control immunoreactivity (100%) and those of DIC-treated gluten sample ($p < 0.0001$) except the gluten treated at (155.5 °C, 25.9 s) which did not indicate any difference. The IgA-binding ability of DIC-treated gluten significantly enhanced.

For patient 2, the immunoreactivity changed from $86.24 \pm 1.58\%$ to $144.37 \pm 12.55\%$ successively corresponding to immunoreactivity of gluten DIC treated at 100 °C for 40 s and 132.5 °C for 60 s. The results of ANOVA showed a significant difference between the immunoreactivity of all samples ($p < 0.0001$). The



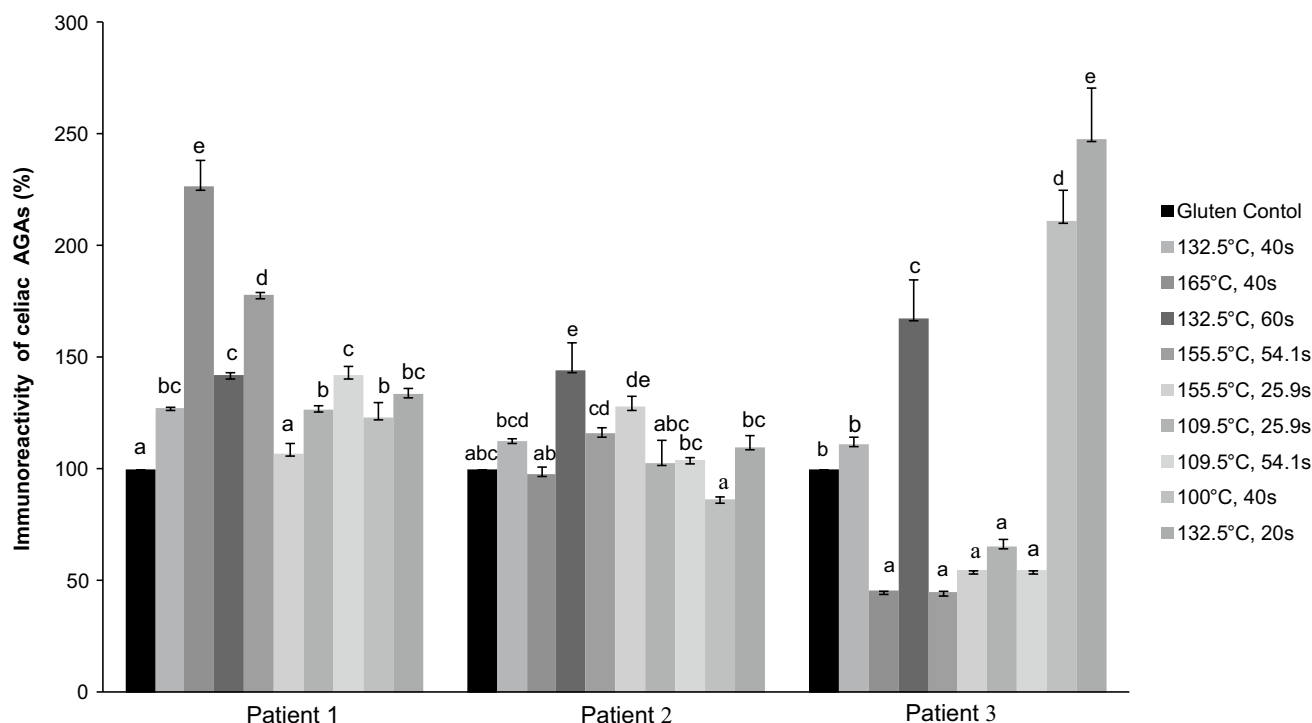


Fig. 4 Immunoreactivity of celiac IgA anti-gliadin antibodies (AGAs) determined by indirect ELISA. Different letters (a–e) indicate significant difference ($p < 0.05$)

multiple comparisons showed that only the immunoreactivity values of gluten samples DIC treated at 155.5 °C for 26 s and 132.5 °C for 60 s, were significantly different than the immunoreactivity of gluten control.

For patient 3, the immunoreactivity varied between $44.79 \pm 1.04\%$ and $247.91 \pm 22.91\%$ for gluten DIC treated at 155.5 °C for 54.1 s and 132.5 °C for 20 s, respectively. The ANOVA results showed that there is a significant difference between immunoreactivity samples ($p < 0.0001$). Compared to gluten control immunoreactivity 100%, only the immunoreactivity of gluten treated at 132.5 °C for 60 s, 100 °C for 40 s and 132.5 °C for 20 s, significantly increased, whereas the immunoreactivity values of DIC gluten differently treated at 155.5 °C for 54.1 s, 165 °C for 40 s, 109.5 °C for 54.1 s, 155.5 °C for 25.9 s and 109.5 °C for 25.9 s, were lower than the one of untreated gluten.

These results demonstrated that the same DIC treatment had different effects on the binding of IgA from different patients to gliadins. These differences can be related to the age of patients and immunogenic epitopes [28, 43]. Immunodominant epitopes were recognized by nearly all CD patients; however, simply immunogenic epitopes were recognized by only some CD patients. Gluten toxicity in celiac disease is due to an immunoresponse that involves the innate and adaptive systems, and the immunoresponse varies considerably between patients with CD [44].

Globally, the DIC treatment increased the celiac IgA anti-gliadin antibodies' immunoreactivity. α -Gliadin was indicated as the most immunogenic wheat proteins with regard to celiac disease following to some extent γ -gliadins and glutenins [45]. The conformational changes in food proteins induced by severe thermal processing are known to affect the immune recognition properties [46]. The structural changes of DIC-treated gluten detected by FTIR could lead to a higher exposure of immunogenic epitopes of gliadins, and their unmasking may explain the marked increase in gluten immunoreactivity.

Numerous toxic epitopes are found within areas characterized by conformations stabilized by disulfide bonds [47, 48]. Most of all, the increase in the β -turn structure resulted from rearrangement and included the 33-mer peptide (57–89 residues of α -gliadin) could also explain this increase [37]. Color changes in DIC-treated gluten (Fig. 1) associated with non-enzymatic browning products, a reaction between proteins and gluten sugars, may also affect the allergenicity of proteins [49, 50].

4 Conclusion

This study aimed to determine the impact of DIC treatment on the structural, functional and immunological properties of wheat gluten. The results of this research indicated that



the DIC treatment gives a rigid aspect to wheat gluten due to the enhancement of β -structures.

In addition, the loss of secondary structure and formation of disulfide bonds led to the denaturation and the aggregation that reduced the solubility, emulsifying capacity and foaming ability. Although the DIC treatment of wheat gluten can be perfectly controlled to perform such structural and functional properties, it is important to identify its specific and effective way of reducing gluten immunoreactivity, which normally should depend on the patients, whereas the immunoreactivity values of differently DIC-treated gluten samples were lower than the immunoreactivity of the untreated gluten. Thus, depending on the patient, the DIC treatment of gluten would then be optimized to reach a specific immunoreactivity.

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Compliance with Ethical Standards

Conflict of interest The authors report no conflicts of interest.

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