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Research of the effect of cold atmospheric plasma on gluten proteins from gluten-free flour

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Abstract

A gluten-free diet is the only available therapy for people with celiac disease (CD) and wheat allergy (WA). Given that this type of diet is difficult to implement because there is a possibility that gluten-free products could be contaminated with gluten, the aim of this paper was to examine the effect of cold plasma treatment on gluten proteins present in gluten-free flour. Samples were treated in a solid state (4 min) and as extracts (1 min). After treatment, proteins were separated on an HPLC apparatus. Then, the samples that were treated (in the solid form and as extracts) were compared to the untreated samples. Based on the obtained results, fewer proteins were isolated from the samples that were treated, compared to the untreated ones.

Keywords: gluten, gluten-free flour, cold atmospheric plasma (CAP), RP-HPLC

1. INTRODUCTION

For people who suffer from celiac disease (CD), wheat allergies (WA) and non-celiac gluten sensitivity, consumption of food containing gluten can lead to serious health consequences (Elli et al. 2015; Serena, D'Avino, & Fasano 2020). Gluten is the main protein of wheat. It contains fractions that are toxic. These are gliadins (monomeric proteins) and glutenins (polymeric proteins) (Balakireva & Zamyatnin 2016; Shewry 2019; Wieser 2007). Gliadins are separated chromatographically into four fractions (ω5, ω1,2, α+β and γ) and glutenins into three (ωb gliadins, HMW and LMW glutenins) (Pilolli et al. 2019; Schalk, Lexhaller, Koehler, & Scherf 2017). Gluten proteins are responsible for the elasticity and stretchability of dough (Kumar 2014; Rai, Kaur, & Chopra 2018). In the United States and Northern Europe, celiac disease (CD) affects about 1% of the population (Gujral 2012; Taraghikhah et al. 2020). It is one of the most common hypersensitive conditions (Gumienna & Górna 2020). Pathogenic microorganisms, the time of eating food containing gluten, and breastfeeding are also factors influencing the development of this disease (Caminero & Verdu 2019; Lionetti & Catassi 2015; Silano 2010). The most common symptoms that occur in people who are suffering from this disease are stomach pain, diarrhea, constipation, weight loss, and inflammation of the small intestine (Caio et al. 2019). A wheat allergy is an allergy that occurs as a result of a reaction to wheat proteins. The symptoms are similar to those that occur when people are allergic to other products. These are rashes on the skin, difficulty breathing, and, in the most severe cases, anaphylaxis and possibly death (Cianferoni 2016; El-Sayed & Shousha 2020). Non-celiac gluten sensitivity occurs when symptoms similar to celiac disease appear, but it is not understood how the immune system might be involved (Holmes 2013; Sergi, Villanacci, & Carroccio 2021). A gluten-free diet is the only available therapy for people with celiac disease (CD) and wheat allergy (WA) (Itzlinger, Branchi, Elli, & Schumann 2018). According to the *Codex Alimentarius* standard, gluten-free food is food that has a natural deficiency or an acceptable level of gluten (<20 mg/kg) (Codex Alimentarius Commission

and others 2008). People who need to eat a gluten-free diet can eat foods that naturally do not contain gluten, such as meat, fish, dairy products, vegetables, nuts, fruits, and gluten-free grains. These are rice, corn, millet and pseudocereals such as amaranth and buckwheat (Saturni, Ferretti, & Bacchetti 2010; Wieser, Segura, Ruiz-Carnicer, Sousa, & Comino 2021). Gluten-containing raw materials from wheat, rye, and barley can be made gluten-free by specialized processing, such as starch washing, peptidase treatment for beverages, and the use of gluten-free strains (Khairuddin & Lasekan 2021; Walter 2014). Implementing a gluten-free diet is difficult because there is a possibility of unintentional contamination of products with gluten, improper declaration, social restrictions, and the presence of gluten proteins in food and pharmaceutical products (Wieser, Ruiz-Carnicer, Segura, Comino, & Sousa 2021). Contamination of gluten-free foods with gluten can occur at many stages of food production and also in households (Wieser, Ruiz-Carnicer, et al. 2021; Wieser, Segura, et al. 2021). Today, there are numerous methods for determining gluten proteins in food products. The following methods are the most commonly used: isoelectric focusing (IEF), sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE), reversed-phase high pressure liquid chromatography (RP-HPLC), exclusion chromatography (SE-HPLC), high-pressure zone electrophoresis (HPCE), coupled liquid chromatography-mass spectrometry (LC-MS/MS), enzyme-immunochemical method (ELISA), and polymerase chain reaction (PCR) (Gojković-Cvjetković et al. 2019; Vensel, Tanaka, & Altenbach 2014). Given that a gluten-free diet is difficult to implement because there is a possibility of accidental contamination of products with gluten during production, processing, transport, and storage, the aim of this paper was to examine the effect of cold plasma treatment on gluten proteins obtained from gluten-free flour. After plasma treatment, the proteins were separated by RP-HPLC chromatography and the treated samples (in the solid state and as extracts) were compared to the untreated samples.

2. MATERIALS AND METHODS

Gluten-free flour samples in the solid form (protein content 3.6 g/100 g) and their extracts were treated with cold atmospheric plasma. Gliadin and glutenin extracts were obtained according to the modified method of Wieser, Antes, and Seilmeier (1998) (Gojković-Cvjetković et al. 2019). Gliadin was extracted with 70% (v/v) ethanol, and glutenins were extracted with 50% (v/v) 1-propanol to which Tris-HCl was added (0.05 mol/l, pH=7.5), urea (2 mol/l) and dithioerythritol (DTT, 1%). Prior to analysis on an HPLC apparatus, the samples were filtered through a 0.45 μm filter.

2.1. Measurement procedure

Gluten-free flour samples in a solid state and their extracts were treated with cold atmospheric plasma. The treatment was done using a custom-built device composed of a dielectric barrier source (SDBD) attached to the top cover of a plastic box. The samples were placed in the box that was tightly closed with a lid. The plasma was ignited in the surrounding air. During the treatment, the input frequency was 50 Hz and the input voltage was 50 V. The samples in the solid state were treated for 4 minutes and the extracts for 1 minute.

2.2. RP-HPLC chromatography

After plasma treatment gliadin and glutenin separation was performed on an HPLC apparatus (Agilent Technologies 1260 Infinity). It was performed on a column (Zorbax 300 SB-C3 Agilent), set at 45 °C (gliadins) and 40 °C (glutenins) for 16.0 min (gliadins) and 21.0 min (glutenins). Absorbance was measured at 210 and 280 nm.Separation was performed according to the modified method of Gojković-Cvjetković et al. (2019).

2.3. Statistical analysis of results

For the statistical analysis of the results IBM SPSS Statistics 26 was used. The average amount of proteins (Xav), standard deviation (SD), std. error, min and max value were calculated. In order to examine the effect of cold plasma treatment on the average amount of proteins and their relative concentration, a one-way analysis of variance with different groups was used. The significance of differences between the average amounts at the p=0.05 level was assessed by post-hoc Tukey' HSD tests.

3. RESULTS AND DISCUSSION

Table 1 shows the total amount of gliadin proteins (TAP), the amount of protein within fractions (ω5, ω1.2, α+β and γ gliadins) obtained from gluten-free flour samples and separated on an HPLC apparatus, with absorbance measured at 210 nm.

Based on the obtained results, the highest amount of protein was obtained from T1 samples (Xav=15.17), and the lowest from T3 samples (Xav=9.33). The effect of cold plasma treatment on the total amount of protein was investigated by one-factor analysis of variance of different groups. It was found that there is a statistically significant

Table 1. TAP and amount of gliadin proteins by fractions (ω5, ω1.2, α+β and γ gliadins) from gluten-free flour observed during RP-HPLC analysis; solvent: 70% (v/v) ethanol; absorbance measurement at 210 nm.

Treatments		N	Xav	SD	Std. Error	Min	Max
Total amount of proteins (TAP)	T1	6	15.17	0.98	0.40	14	16
	T2	6	12.00	1.26	0.52	11	14
	T3	6	9.33	0.82	0.33	8	10
ω5 gliadins	T1	6	1.67	0.52	0.21	1	2
	T2	6	2.17	0.41	0.17	2	3
	T3	6	1.17	0.41	0.17	1	2
ω1,2 gliadins	T1	6	3.00	0.63	0.26	2	4
	T2	6	2.83	0.75	0.31	2	4
	T3	6	1.83	0.75	0.31	1	3
α+β gliadins	T1	6	5.17	0.41	0.17	5	6
	T2	6	4.50	0.84	0.34	4	6
	T3	6	3.83	0.98	0.40	3	5
γ gliadins	T1	6	5.33	0.52	0.21	5	6
	T2	6	2.50	0.55	0.22	2	3
	T3	6	2.50	0.55	0.22	2	3
ANOVA (TNP)	F(2.15)=47.47, Sig.=0.000, eta square=102.33/118.50=0.86						
ANOVA (ω5)	F(2.15)=7.50, Sig.=0.005<0.05, eta square=3/6=0.50						
ANOVA (ω1,2)	F(2.15)=4.67, Sig.=0.03<0.05, eta square=4.78/12.44=0.38						
ANOVA (α+β)	F(2.15)=4.36, Sig.=0.03<0.05, eta square=5.33/14.50=0.37						
ANOVA (γ)	F(2.15)=55.58, Sig.=0.000, eta square=32.11/36.44=0.88						

T1 – CONTROL, T2 – extracts treated with plasma for 4 min and T3-extracts treated for 1 min after extraction.
Xav – the average amount of isolated proteins, SD – standard deviation.

difference, F(2.15)=47.47, Sig.=0.000<0.05. A post-hoc Tukey's test found that samples T1 and T2; T1 and T3; T2 and T3 differed statistically significantly. The highest amount of protein within the fraction of ω5 gliadins was obtained in T2 samples (Xav=2.17), and the lowest in T3 samples (Xav=1.17). Within the ω1.2 gliadin fraction, the highest amount of protein was obtained in T1 samples (Xav=3.00), and the lowest in T3 samples (Xav=1.83). The highest amount of protein within the α+β gliadin fraction was obtained in T1 samples (Xav=5.17), and the lowest in T3 samples (Xav=3.83). Within the γ gliadin fraction, the highest amount of protein was obtained in T1 samples (Xav=5.33), and the lowest in T2 and T3 samples (Xav=2.50). Table 2 shows the total amount of gliadin proteins (TAP) and the amount of protein within fractions (ω5, ω1.2, α+β and γ gliadins) obtained on an HPLC apparatus, with absorbance measured at 280 nm.

The highest total amount of protein was obtained in T1 samples (Xav=15.17), and the lowest in T3 samples (Xav=8.83). One-factor analysis of the variance of different groups showed that there is a statistically significant difference, F(2.15)=43.04, Sig.=0.000<0.05. The post-hoc Tukey test showed that samples T1 and T2; T1 and T3; T2 and T3 differed statistically significantly. Within the ω5 gliadin fraction, the highest amount of protein was obtained in T1 samples (Xav=1.83), and the lowest in T3 samples (Xav=1.50). The highest amount of protein within the ω1.2 gliadin fraction was obtained in T1 samples (Xav=3.00), and the lowest in T3 samples (Xav=2.33). Within the α+β gliadin fraction, the highest amount of protein was obtained in T2 samples (Xav=5.50), and the lowest in T3 samples (Xav=3.17). Within the γ gliadin fraction the highest amount of protein was obtained in T1 samples (Xav=6.00), and the lowest in T3 samples (Xav=2.17). Table 3 shows the total amount of glutenin proteins (TAP), the amount of protein within fractions (ωb gliadins, HMW and LMW glutenins) obtained from gluten-free flour samples and separated on an HPLC apparatus, with absorbance measured at 210 nm.

Based on the obtained results, the highest total amount of protein was obtained in T1 samples (Xav=13.67), and the lowest in T3 samples (Xav=7.17). One-factor analysis of the variance of different groups showed that there was a statistically significant difference, F(2.15)=78.56, Sig.=0.000<0.05. The post-hoc Tukey test showed that samples T1 and T2; T1 and T3; T2 and T3 differed statistically significantly. Within the ωb gliadin fraction, the highest amount of protein was obtained in T1 samples (Xav=4.50), and the lowest in T3 samples (Xav=0.50). The highest amount of protein within the HMW glutenin fraction was obtained in T1 samples (Xav=4.50), and the lowest in T3 samples (Xav=2.00). Within the LMW glutenin fraction, the highest amount of protein was obtained in T2 samples (Xav=5.00), and the lowest in T1 and T3 samples (Xav=4.67). Table 4 shows the total amount of glutenin proteins (TAP), the amount of protein within fractions

Table 2. TAP and amount of gliadin proteins by fractions (ω5, ω1.2, α+β and γ gliadins) from gluten-free flour observed during RP-HPLC analysis; solvent: 70% (v/v) ethanol; absorbance measurement at 280 nm.

Treatments		N	Xav	SD	Std. error	Min	Max
Total number of proteins (TAP)	T1	6	15.17	0.98	0.40	14	17
	T2	6	13.00	1.41	0.58	11	15
	T3	6	8.83	1.17	0.48	8	11
ω5 gliadins	T1	6	1.83	0.41	0.17	1	2
	T2	6	1.67	0.52	0.21	1	2
	T3	6	1.50	0.55	0.22	1	2
ω1,2 gliadins	T1	6	3.00	0.63	0.26	2	4
	T2	6	2.67	0.82	0.33	2	4
	T3	6	2.33	0.52	0.21	2	3
α+β gliadins	T1	6	4.33	0.82	0.33	3	5
	T2	6	5.50	0.55	0.22	5	6
	T3	6	3.17	0.75	0.31	2	4
γ gliadins	T1	6	6.00	1.26	0.52	5	8
	T2	6	3.17	0.98	0.40	2	4
	T3	6	2.17	0.41	0.17	2	3
ANOVA (TNP)	F(2.15)=43.04, Sig.=0.000, eta square=124.33/146.00=0.85						
ANOVA (ω5)	F(2.15)=0.68, Sig.=0.52>0.05						
ANOVA (ω1,2)	F(2.15)=1.50, Sig.=0.25>0.05						
ANOVA (α+β)	F(2.15)=15.98, Sig.=0.000<0.05, eta square=16.33/24.00=0.68						
ANOVA (γ)	F(2.15)=26.04, Sig.=0.000<0.05, eta square=47.44/61.11=0.78						

T1 – CONTROL, T2 – extracts treated with plasma for 4 min and T3-extracts treated for 1 min after extraction.
Xav – the average amount of isolated proteins, SD – standard deviation.

Table 3. TAP and amount of glutenin proteins by fractions (ωb gliadins, HMW and LMW glutenins) from gluten-free flour observed during RP-HPLC analysis; solvent: 50% (v/v) 1-propanol to which Tris-HCl (0.05 mol/l, pH=7.5), urea (2 mol/l) and dithioerythritol (1%) were added; absorbance measurement at 210 nm.

Treatments		N	Xav	SD	Std. error	Min	Max
Total amount of proteins (TAP)	T1	6	13.67	0.82	0.33	13	15
	T2	6	10.00	0.89	0.36	9	11
	T3	6	7.17	0.98	0.40	6	9
ωb gliadins	T1	6	4.50	0.55	0.22	4	5
	T2	6	2.33	0.52	0.21	2	3
	T3	6	0.50	0.55	0.22	0	1
HMW glutenins	T1	6	4.50	0.55	0.22	4	5
	T2	6	2.67	0.82	0.33	2	4
	T3	6	2.00	0.00	0.00	2	2
LMW glutenins	T1	6	4.67	1.03	0.42	3	6
	T2	6	5.00	0.89	0.36	4	6
	T3	6	4.67	0.82	0.33	4	6
ANOVA (TNP)	F(2.15)=78.56, Sig.=0.000, eta square=127.44/139.61=0.91						
ANOVA (ωb)	F(2.15)=83.27, Sig.=0.000, eta square=48.11/52.44=0.92						
ANOVA (HMW)	F(2.15)=31.21, Sig.=0.000, eta square=20.11/24.94=0.81						
ANOVA (LMW)	F(2.15)=0.26, Sig.=0.77>0.05						

T1 – CONTROL, T2 – extracts treated with plasma for 4 min and T3 – extracts treated for 1 min after extraction.
Xav – the average amount of isolated proteins, SD – standard deviation.

(ωb gliadins, HMW and LMW glutenins) obtained from gluten-free flour samples and separated on an HPLC apparatus, with absorbance measured at 280 nm.

Based on the obtained results, the highest total amount of protein was obtained in T1 samples (Xav=13.33), and the lowest in T3 samples (Xav=7.00). One-factor analysis of variance of different groups showed that there is a statistically significant difference, F(2.15)=74.45, Sig.=0.000. The post-hoc Tukey test showed that samples T1 and T2; T1 and T3; and T2 and T3 differed statistically significantly. The highest amount of protein within the fraction of ωb gliadins was obtained in T1 samples (Xav=4.17), and the lowest in T3 samples (Xav=1.17). Within the HMW glutenin fraction, the highest amount of protein was obtained in T1 samples (Xav=3.83), and the lowest in T3 samples (Xav=1.50).

Table 4. TAP and amount of glutenin proteins by fractions (ωb gliadins, HMW and LMW glutenins) from gluten-free flour observed during RP-HPLC analysis; solvent: 50% (v/v) 1-propanol to which Tris-HCl (0.05 mol/l, pH=7.5), urea (2 mol/l) and dithioerythritol (1%) were added; absorbance measurement at 280 nm.

Treatments		N	Xav	SD	Std. error	Min	Max
Total amount of proteins (TAP)	T1	6	13.33	0.52	0.21	13	14
	T2	6	9.83	1.17	0.48	8	11
	T3	6	7.00	0.89	0.36	6	8
ωb gliadins	T1	6	4.17	0.41	0.17	4	5
	T2	6	2.33	0.52	0.21	2	3
	T3	6	1.17	0.41	0.17	1	2
HMW glutenins	T1	6	3.83	0.98	0.40	3	5
	T2	6	3.00	0.63	0.26	2	4
	T3	6	1.50	0.55	0.22	1	2
LMW glutenins	T1	6	5.33	0.82	0.33	4	6
	T2	6	4.50	0.55	0.22	4	5
	T3	6	4.33	0.52	0.21	4	5
ANOVA (TNP)	F(2.15)=74.45, Sig.=0.000, eta square=120.78/132.94=0.91						
ANOVA (ωb)	F(2.15)=68.61, Sig.=0.000, eta square=27.44/30.44=0.90						
ANOVA (HMW)	F(2.15)=15.10, Sig.=0.000, eta square=16.78/25.11=0.67						
ANOVA (LMW)	F(2.15)=4.19, Sig.=0.04<0.05, eta square=3.44/9.61=0.36						

T1 – CONTROL, T2 – extracts treated with plasma for 4 min and T3 – extracts treated for 1 min after extraction.
Xav – the average amount of isolated proteins, SD – standard deviation.

The highest amount of protein within the LMW glutenin fraction was obtained in T1 samples (Xav=5.33), and the lowest in T3 samples (Xav=4.33). Table 5 shows the total relative concentration (TRC), the relative concentration of protein within fractions ω5, ω1.2, α+β and γ gliadin) obtained from gluten-free flour samples and separated on an HPLC apparatus, with absorbance measured at 210 nm.

Based on the obtained results, the highest relative concentration within the ω5 gliadin fraction was obtained in T2 samples (RC=9.98%), and the lowest in T1 samples (RC=4.67%). The highest relative concentration within the ω1.2 gliadin fraction was obtained in T1 samples (RC=7.34%), and the lowest in T3 samples (RC=4.43%). Within the α+β gliadin fraction, the highest relative concentration was obtained in T1 samples (RC=40.58%) and the lowest in T3 samples (RC=23.63%). The highest relative concentration within the γ gliadin fraction was obtained in T3 samples (RC=60.23%), and the lowest in T2 samples (RC=47.24%). Table 6 shows the total relative concentration (TRC), the relative concentration of protein within fractions (ω5, ω1.2, α+β and γ gliadin) obtained from gluten-free flour samples and separated on an HPLC apparatus, english with absorbance measured at 280 nm.

The highest relative concentration within the ω5 gliadin fraction was obtained in T2 samples (RC=17.06%), and the lowest in T3 samples (RC=4.39%). Within the ω1.2 gliadin fraction, the highest relative concentration was obtained in T1 samples (RC=4.59%) and the lowest in T3 samples (RC=2.23%). The highest relative concentration within the α+β gliadin fraction was obtained in T1 samples (RC=28.61), and the lowest in T2 sample (RC=6.42%). Within the γ gliadin fraction, the highest relative concentration was obtained in T3 samples (RC=82.70%), and the lowest in T1 samples (RC=59.44%). Table 7 shows the total relative concentration (TRC), the relative concentration of protein within fractions (ωb gliadins, HMW and LMW glutenins) obtained from gluten-free flour samples and separated on an HPLC apparatus, with absorbance measured at 210 nm.

The highest relative concentration within the ωb gliadin fraction was obtained in T3 samples (RC=2.60%), and the lowest in T2 samples (RC=1.29%). Within the HMW glutenin fraction, the highest relative concentration was obtained in T3 samples (RC=16.63%), and the lowest in T1 samples (RC=3.04%), and within the LMW glutenin fraction, the highest relative concentration was obtained in T1 samples (RC=94.60%), and the lowest in T3 samples (RC=80.77%). Table 8 shows the total relative concentration (TRC), the relative concentration of protein within fractions (ωb gliadins, HMW and LMW glutenins) obtained from gluten-free flour samples and separated on an HPLC apparatus, with absorbance measured at 280 nm.

Based on the obtained results, the highest relative concentration within the ωb gliadin fraction was obtained in T2 samples (RC=8.51%), and the lowest in T3 samples (RC=1.54%). Within the HMW glutenin fraction, the highest relative concentration was obtained in T3 samples (RC=16.24%) and the lowest in T1 samples (RC=6.17%), while within the LMW glutenin fraction, the highest relative concentration was obtained in T1 samples (RC=89.16%), and the lowest in T2 samples

Table 5. TRC and relative concentration of gliadin by fractions (ω5, ω1.2, α+β and γ gliadin) observed during RP-HPLC analysis of gluten-free flour; solvent: 70% (v/v) ethanol; absorbance measurement at 210 nm.

Treatments		N	RC (%)	SD	Std. error	Min	Max
Total relative concentration (TRC)	T1	6	100	0.00	0.00	100	100
	T2	6	100	0.00	0.00	100	100
	T3	6	100	0.00	0.00	100	100
ω5 gliadins	T1	6	4.67	0.67	0.27	3.81	5.38
	T2	6	9.98	1.06	0.43	8.14	11.34
	T3	6	9.61	0.68	0.28	8.48	10.29
ω1.2 gliadins	T1	6	7.34	0.74	0.30	6.43	8.35
	T2	6	6.60	0.88	0.36	5.89	8.16
	T3	6	4.43	0.92	0.38	5.12	7.75
α+β gliadins	T1	6	40.58	1.57	0.64	37.53	41.66
	T2	6	36.17	2.24	0.92	33.84	39.28
	T3	6	23.63	1.87	0.76	21.11	25.82
γ gliadins	T1	6	47.40	1.31	0.54	45.89	49.20
	T2	6	47.24	3.01	1.23	42.26	50.14
	T3	6	60.23	2.26	0.92	56.14	62.68
ANOVA (ω5)	F(2.15)=78.11, Sig.=0.000, eta square=105.62/115.76=0.91						
ANOVA (ω1.2)	F(2.15)=1.92, Sig.=0.18>0.05						
ANOVA (α+β)	F(2.15)=126.81, Sig.=0.000, eta square=928.49/983.40=0.94						
ANOVA (γ)	F(2.15)=63.81, Sig.=0.000, eta square=676.08/755.54=0.89						

TT1 textendash CONTROL, T2 textendash extracts treated with plasma for 4 min and T3 textendash extracts treated for 1 min after extraction, RC – relative concentration, SD – standard deviation.

Table 6. TRC and relative concentration of gliadin by fractions observed during RP-HPLC analysis of gluten-free flour; solvent: 70% (v/v) ethanol; absorbance measurement at 280 nm.

Treatments		N	RC (%)	SD	Std. Error	Min	Max
Total relative concentration (TRC)	T1	6	100	0.00	0.00	100	100
	T2	6	100	0.00	0.00	100	100
	T3	6	100	0.00	0.00	100	100
ω5 gliadins	T1	6	7.36	0.78	0.32	6.27	8.23
	T2	6	17.06	0.49	0.20	16.42	17.70
	T3	6	4.39	0.67	0.27	3.67	5.49
ω1.2 gliadins	T1	6	4.59	0.72	0.29	3.96	5.92
	T2	6	3.74	0.30	0.12	3.32	4.18
	T3	6	2.23	0.35	0.14	1.92	2.84
α+β gliadins	T1	6	28.61	0.87	0.36	27.44	29.87
	T2	6	6.42	1.06	0.43	5.33	7.76
	T3	6	10.68	1.79	0.73	9.11	13.52
γ gliadins	T1	6	59.44	1.32	0.54	57.09	60.48
	T2	6	72.79	1.16	0.48	70.76	74.06
	T3	6	82.70	1.99	0.81	79.48	84.73
ANOVA (ω5)	F(2.15)=606.23, Sig.=0.000, eta square=526.64/533.16=0.99						
ANOVA (ω1.2)	F(2.15)=35.02, Sig.=0.000, eta square=17.08/20.73=0.82						
ANOVA (α+β)	F(2.15)=489.21, Sig.=0.000, eta square=1664.50/1690.01=0.69						
ANOVA (γ)	F(2.15)=347.22, Sig.=0.000, eta square=1634.85/1670.16=0.98						

T1 – CONTROL, T2 – extracts treated with plasma for 4 min and T3 – extracts treated for 1 min after extraction, RC – relative concentration, SD – standard deviation.

(RC=75.44%). In his research, Nooji (2011) came to the conclusion that the allergenicity of wheat decreased by 37% after treatment with DBD plasma for 5 minutes. Hajnal et al. (2019) treated wheat flour with cold atmospheric plasma and monitored the *Alternaria* toxin content. After the treatment, the *Alternaria* toxin content was reduced. Upadhyay, Thirumdas, Deshmukh, Annapure, and Misra (2019) investigated the influence of low-pressure cold plasma on the modification of the properties of chia flour. The obtained results showed a significant change in the color of the flour, with an increase in lightness. Sun et al. (2020) determined the structural properties and immunoreactivity of celiac-toxic peptides after treatment with cold jet atmospheric plasma (CJAP). The mentioned properties and immunoreactivity were determined by the enzyme-immunochemical method (ELISA).

Table 7. TRC and relative concentration of glutenins by fractions (ωb gliadins, HMW and LMW glutenins) observed during RP-HPLC analysis of gluten-free flour; solvent: 50% (v/v) 1-propanol to which Tris-HCl (0.05 mol/l, pH=7.5), urea (2 mol/l) and dithioerythritol (1%) were added; absorbance measurement at 210 nm.

Treatments		N	RC (%)	SD	Std. Error	Min	Max
Total relative concentration (TRC)	T1	6	100	0.00	0.00	100	100
	T2	6	100	0.00	0.00	100	100
	T3	6	100	0.00	0.00	100	100
ωb gliadins	T1	6	2.36	0.30	0.12	1.91	2.76
	T2	6	1.29	0.22	0.09	1.01	1.66
	T3	6	2.60	0.26	0.11	2.28	2.97
HMW glutenins	T1	6	3.04	0.38	0.16	2.49	3.50
	T2	6	10.05	0.68	0.28	8.80	10.63
	T3	6	16.63	0.98	0.40	15.28	18.16
LMW glutenins	T1	6	94.60	0.50	0.20	93.93	95.28
	T2	6	88.66	0.87	0.36	87.71	90.19
	T3	6	80.77	0.85	0.35	79.56	81.75
ANOVA (ωb)	F(2.15)=42.59, Sig.=0.000, eta square=5.86/6.89=0.85						
ANOVA (HMW)	F(2.15)=526.73, Sig.=0.000, eta square=553.98/561.87=0.98						
ANOVA (LMW)	F(2.15)=498.05, Sig.=0.000, eta square=577.60/586.29=0.98						

T1 – CONTROL, T2 – extracts treated with plasma for 4 min and T3 – extracts treated for 1 min after extraction, RC– relative concentration, SD – standard deviation.

Table 8. TRC and relative concentration of glutenins by fractions (ωb gliadins, HMW and LMW glutenins) observed during RP-HPLC analysis of gluten-free flour; solvent: 50% (v/v) 1-propanol to which Tris-HCl (0.05 mol/l, pH=7.5), urea (2 mol/l) and dithioerythritol (1%) were added; absorbance measurement at 280 nm.

Treatments		N	RC (%)	SD	Std. error	Min	Max
Total relative concentration (TRC)	T1	6	100	0.00	0.00	100	100
	T2	6	100	0.00	0.00	100	100
	T3	6	100	0.00	0.00	100	100
ωb gliadins	T1	6	4.67	0.72	0.29	3.71	5.70
	T2	6	8.51	0.35	0.14	8.07	8.96
	T3	6	1.54	0.12	0.05	1.32	1.67
HMW glutenins	T1	6	6.17	0.93	0.38	4.91	7.59
	T2	6	16.05	1.02	0.42	14.48	17.21
	T3	6	16.24	1.47	0.60	14.59	18.12
LMW glutenins	T1	6	89.16	1.33	0.54	86.71	90.29
	T2	6	75.44	1.24	0.51	73.83	77.36
	T3	6	82.22	1.56	0.64	80.21	84.09
ANOVA (ωb)	F(2.15)=337.22, Sig.=0.000, eta square=146.59/149.58=0.98						
ANOVA (HMW)	F(2.15)=146.74, Sig.=0.000, eta square=397.98/418.32=0.95						
ANOVA (LMW)	F(2.15)=146.81, Sig.=0.000, eta square=564.74/593.59=0.95						

T1 – CONTROL, T2 – extracts treated with plasma for 4 min and T3 – extracts treated for 1 min after extraction, RC – relative concentration, SD – standard deviation.

Based on the obtained results, gliadin concentration decreased after the treatment, and a significant decrease (51.95%) was observed for 60 minutes. A decrease in gliadin concentration led to a decrease in immunoreactivity. In the available literature, the effect of cold atmospheric plasma on gluten proteins extracted from gluten-free flours has not been investigated. However, when the results obtained in this paper are compared with the results of Nooji (2011) and Sun et al. (2020) who investigated the effect of cold atmospheric plasma on gluten proteins from wheat flour, it can be seen that they are in agreement. In this paper, it was shown that cold atmospheric plasma leads to a decrease in the total amount of proteins after treatment of samples when compared with control samples. Plasma treatment leads to the separation of large polymers into the monomers from which they are composed.

4. CONCLUSION

After treatment of gluten proteins obtained from gluten-free flour samples with cold atmospheric plasma (solid samples for 4 min and extracts for 1 min) and then separation by RP-HPLC chromatography, the following conclusions were reached. Plasma treatment leads to a reduction in the total amount of proteins compared to un-

treated samples. A higher reduction in the total amount of proteins was obtained when proteins were treated as extracts compared to solid samples.

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