INTERNATIONAL
Agrophysics
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Int. Agrophys., 2024, 38, 127-137
doi: 10.31545/intagr/184005

Effects of phenolic acid molecular structure on the structural properties of gliadins and glutenins**

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Received November 29, 2023; accepted February 12, 2024

Abstract. The aim of this research is to determine how phenolic acids affect the individual structure of gluten proteins: gliadins and glutenins, by understanding the underlying molecular interactions. Fourier transform infrared spectroscopy was used to determine changes in the secondary structure of the individual gluten network proteins: gliadins and glutenins, after addition of selected phenolic acids to the model dough. Phenolic acids were added to the model dough at the following concentrations: 0.05, 0.1 and 0.2% (w/w). The phenolic acids induce changes in the secondary structure of the gliadins and glutenins. The degree of interaction depends on the structure and concentration of the added phenolic acid. In most cases, these interactions lead to the formation of disordered structures in both gliadins and glutenins. From the results obtained, it can be concluded that the inclusion of certain phenolic acids in the dough affects the hydrogen bonding in gliadins and glutenin, and that phenolic acids interact non-covalently with these gluten proteins. The findings could potentially be applied to food chemistry and may have an impact on the allergenic properties of gluten, particularly in relation to the reduction of the β-turn content within glutenins.

Keywords: FT-IR spectroscopy, gliadin, phenolic acids, secondary structure, glutenin

1. INTRODUCTION

Common wheat (*Triticum aestivum* L.) is a significant food crop, accounting for 20% of all calories consumed by humans. It contains a substantial amount of proteins, vitamins, and minerals. The storage proteins in wheat are commonly known as gluten, although gluten is actually a composite of two primary protein types: gliadins and glutenins. Gliadins and glutenins make up approximately 30 and 50% of the total protein in wheat grain, respectively (Urade *et al.*, 2017). Gliadins, existing solely in their monomeric form, lack the ability to promote cross-linking. They contribute to the viscosity and extensibility of the dough. On the other hand, polymeric glutenins exert their primary influence on dough strength and elasticity through cross-linking and other molecular interactions (Fevzioglu *et al.*, 2020).

The growing interest in polyphenols and the number of studies on them is growing due to their properties and effects on human health and well-being. Among its many pro-health properties are anti-inflammatory, anticarcinogenic, antioxidant, antiallergenic, cardiovascular protective, and antimicrobial. Polyphenol-protein interactions have been investigated as potential therapeutic strategies for food allergies, with results showing that immunoglobulin (IgE) responses can be reduced and allergenic protein digestion can be blocked (Van Buiten and Elias, 2021). Świeca *et al.* (2014) demonstrated that the fortification of wheat bread with ingredients rich in phenolic compounds is an efficacious method for enhancing the antioxidant potential of preserved bread. Additionally, Han and Koh (2011) have successfully enriched breads with phenolic acids, providing clear evidence that the phenolics responsible for the increased antioxidant capacity are firmly attached to wheat

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**This work was supported by the National Science Centre, Poland (grant number: 2019/35/B/NZ9/02854).

flour constituents during the dough-mixing phase. The complexation between phenols and gluten proteins may influence the rheological, functional, and pro-health attributes of the resulting wheat bread. As such, elucidating the underlying mechanism of interactions between polyphenols and the specific components of wheat dough, from which bread is subsequently produced, becomes a matter of significant importance.

Interactions between proteins and phenolic compounds are influenced by properties such as size, structural flexibility and functional group type. The interactions can occur through formation of both covalent and non-covalent bonds. During process of dough development, these interactions play an important role (Ozdal *et al.*, 2013; Xu *et al.*, 2019). Phenolic acids are a class of plant phenols with simple structure that can be linked to sugar units. The compounds can be ca-tegorised according to the general formula C1-C6, and are referred to as hydroxybenzoic acid derivatives (C1-C3) and hydroxycinnamic acid derivatives (C6-C3) (Czubinski and Dwiecki, 2017).

The molecular docking analysis revealed that the chosen phenolic acids could interact with glutenin or gliadin protein via hydrogen bonds (Feng *et al.*, 2022). The addition of phenolic acids would cause a certain degree of structural change in gluten proteins, such as the exposure of amino acid groups and the extension of the peptide chain. This results in a variety of interactions between protein molecules and phenolic acids, which includes hydrophobic and electrostatic interactions (Feng *et al.*, 2022). Gliadins are an especially interesting protein target for polyphenol interactions because they are rich in proline residues and have natively unfolded structures with polyproline II helical (PPII) motifs. These characteristics have been shown to favour interactions with polyphenols (Van Buiten and Elias, 2021).

Spectroscopic methods can be used to determine a protein's secondary structure. The most commonly used method for this purpose is Fourier transform infrared spectroscopy (FT-IR) (De Meutter and Goormaghtigh, 2021). This technique serves as a significant tool for investigating protein structure, the molecular mechanisms of protein reactions, as well as protein folding, unfolding, and misfolding processes (Barth, 2007). It is well known that amide I band are one of the most useful tool for secondary structure determination (De Meutter and Goormaghtigh, 2021). Despite its low intensity, the amide III band also proves to be highly sensitive to protein secondary structure and remains unaffected by water absorption. Both amide bands provide complementary structural information (Stani *et al.*, 2020). Amide I band provide information about basic secondary structures e.g. α-helices, β-sheets, β-turns and random coils as well as aggregated structures like hydrogen bonded β-turns and pseudo-β-sheets (Nawrocka *et al.*, 2017b, 2018b). In addition, analysis of the amide I band also provides information regarding changes in the hydrogen bonds' pattern of proteins showing formation of aggregated structures. Moreover, the study of the β-sheet segment in the amide III band can unveil the formation of two types of hydrogen bonds (type I and type II) during dough mixing (Zhang *et al.*, 2008). These hydrogen bonds may be very important when studies concern interactions between protein complex and chemical compound affecting protein structure. The type I H bonds are formed between polypeptide chains of gluten network, whereas the type II can be formed between gluten polypeptide chains and different additives (Nawrocka *et al.*, 2018a).

Changes in the secondary structure of proteins may be studied using two methods. The first method is based on the deconvolution of the amide I band. Location of the used Gaussians or Lorentzians should correspond to spectral ranges assigned to adequate secondary structure. This method provides percentage content of particular secondary structures. Deconvolution of the amide I band gives quantitative information about changes in the protein structure (Gruszecki *et al.*, 2009). The second method is calculation of difference spectrum. The calculation involves subtraction of the control spectrum from the spectrum of modified sample. Both spectra are normalized using field under the band/spectrum before subtraction. The difference spectrum contains positive and negative bands. Positive bands inform about structures which appear as a result of molecular interactions, whereas negative bands provide information about structures which disappear or their amount decreases considerably. For these reasons, the difference spectrum gives qualitative rather than quantitative information (Nawrocka *et al.*, 2015).

In our study, FT-IR spectroscopy was used to identify changes in the secondary structure of individual gluten proteins: gliadins and glutenins, by supplementing model bread dough with specific phenolic acids. These acids include hydroxycinnamic derivatives (caffeic, ferulic, coumaric, sinapic) and hydroxybenzoic derivatives (4-hydroxybenzoic, protocatechuic, vanillic, syringic). Each of these compounds differs in structure and contains different functional groups on the aromatic ring. This study explores the complex interactions between these polyphenols and gluten proteins, focusing on the structural modifications in the proteins. By comprehending the underlying molecular interactions, the aim of this research is to enhance understanding of how phenolic acids influence the structure of individual gluten proteins.

2. MATERIALS AND METHODS

2.1. Materials

Wheat starch, gluten, ethanol, 4-hydroxybenzoic (4XY), ferulic (FER), caffeic (CAF), vanillic (VAN), protocatechuic (PCAT), and coumaric acids (COU), were purchased from Sigma Aldrich (Poland), whereas syringic acid (SYR)

was purchased from Alfa Aesar (Poland), and sinapic acid (SYN) was purchased from Apollo Scientific (UK). Double-distilled water was used.

2.2. Samples preparation

2.2.1. Dough preparation

The procedure for preparing the dough samples was described in previous articles (Kłosok *et al.*, 2022, 2023). Briefly, the samples were collected prior to dough breakdown, with the collection time varying depending on the type of phenolic acid used. To ensure the structural integrity of the dough and prevent its destruction, the collection time was calculated by subtracting four minutes from the average dough breakdown time. This adjustment ensured that the proper dough structure was obtained for all repetitions. Control samples were collected at three different times due to variations in the time required for dough breakdown, depending on the phenolic acid type used. Specifically, control samples were collected at 14 min for CAF, FER, and SYN, 22 min for PCAT and COU, and 36 min for 4XY, SYR, and VAN.

2.2.2. Gliadin and glutenin extraction

The gliadins were extracted from powdered gluten using the Taddei *et al*. (2013) method, with minor adjustments. Specifically, the sample was dissolved in 40 ml of 70% ethanol (120 mg), and stirred on a magnetic stirrer at room temperature (25°C) for 4 h. The resulting mixture was centrifuged at 4000 x g for 10 min. Supernatant containing gliadin proteins was obtained, collected into a flask, and lyophilized to yield powdered gliadins for further analysis. To ensure complete extraction of the gliadins, the process was repeated three times. The obtained ethanol-insoluble protein fraction represented the glutenins. They also were freeze-dried. Following lyophilisation, the samples were placed in a desiccator to prevent variations in the water content until the start of FT-IR measurements.

2.3. Fourier transform infrared spectra (FT-IR) collection and data manipulation

The FT-IR spectra were recorded using a Nicolet 6700 FT-IR spectrometer (Thermo Scientific, USA) equipped with a diamond ATR attachment. The procedure developed by (Nawrocka *et al.*, 2017a) was used for spectra collection and data manipulation. The spectra were recorded in the spectral range of 4 000-400 cm^{-1} with a resolution of 4 cm^{-1}, and the signal was averaged over 128 scans. The analysed spectra were averaged over five registered spectra. ORIGIN (v. 9.0 PRO, OriginLab Corporation, USA) was utilized to perform a structural analysis of the amide I band (1 580-1 720 cm^{-1}) and amide III band (1 200-1 320 cm^{-1}). The secondary structure of gluten proteins was determined according to Kłosok *et al*. (2023) and Yang *et al*. (2015) for the amide I band, and according to Stani *et al*. (2020) for the amide III band. To detect changes in the secondary structure, difference spectra were calculated for both bands by subtracting the spectrum of gluten washed out from gluten or model dough from the spectrum of gluten-phenolic acid (Nawrocka *et al.*, 2017a). Any peaks or shifts in the resulting difference spectrum will indicate changes in the secondary structure that are specifically induced by the interaction with the phenolic acid.

3. RESULTS AND DISCUSSION

3.1. Changes in the secondary structure of gliadins

The amide I band was used for the evaluation of changes in the secondary structure of gliadin and glutenin due to interactions with eight phenolic acids, divided into two groups - the hydroxycinnamic and hydroxybenzoic acids. The results of amide I band analysis are shown in Fig. 1.

Analysis of changes in the secondary structures of gliadins following the addition of phenolic acids has revealed a complex range of structural changes (Fig. 1). The positive band at 1 660 cm^{-1}, which is associated with the hydrogen-bonded ß-turns (HbßT), is observed (Secundo and Guerrieri, 2005) in gliadins supplemented with 4XY and COU suggesting that these phenolic acids have an influence on the hydrogen bonds in gliadin. An increase in α-helix (αH) content was observed for gliadin – CAF, FER and SYN sample, which appears to correspond to a decrease in the amount of pseudo-β-sheets (β-sheetswith intermolecular H bonds, pβS) and aggregated structures (AGR). α-helical structures present in gliadins are thought to increase the structural stability of gluten proteins. This may influence their resistance to digestion and the resulting immune response. As suggested by Lancelot *et al*. (2021), the higher content of both α-helix and antiparallel β-sheet (aβS) conformations could contribute to a more organised structure. Increase in the band at around 1 615 cm^{-1} was observed in SYN and COU modified samples at the 0.1% acid concentration. Juszczyk *et al.* (2009) suggested that these bands could be attributed to β-structures. The addition of vanillic acid (VAN) resulted in a significant increase in the intensity of bands localized at *ca*. 1 648, 1 664, 1 676 and 1 685 cm^{-1}. These bands can be connected with hydrogen-bonded β-turns and/or α-helices, β-turns and antiparallel β-sheets, respectively. As a result of the increase in these structures content, the content of pseudo-β-sheets is reduced, as can be seen as negative band present at about 1 625 cm^{-1} (Fig. 1). This suggests that certain phenolic acids, such as VAN, may influence the stability of the gluten network, as evidenced by the increased occurrence of bands assigned to β-turns and α-helices.

Further examination of the structural changes following the addition of PCAT, CAF, FER and SYN revealed a reduction in the intensity of the band assigned to the antiparallel β-sheets. This could indicate disassembly or reorganisation of these structures, which could affect the stability of the gliadin structure. The presence of a negative band attributed

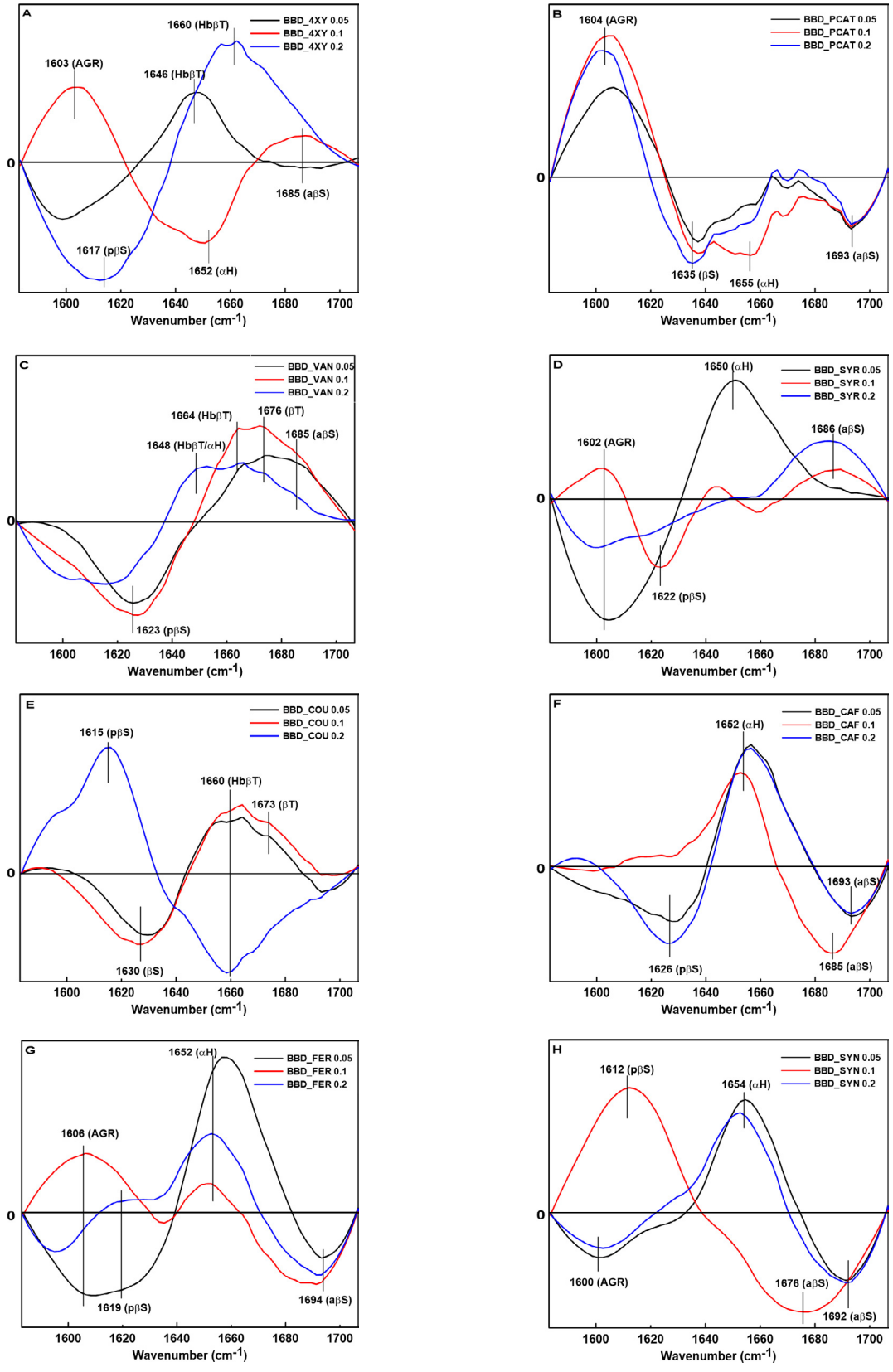

Fig. 1. Difference spectra in the FT-IR amide I band (1 580-1 720 cm^{-1}) displaying changes in the secondary structure of the gliadins modified by eight phenolic acids in concentrations of 0.05, 0.1 and 0.2%.

to antiparallel β-sheets may result in the formation of a less stable protein conformation (Chompoorat *et al.*, 2022). The increase in the intensity of these band could also indicate a disruption of hydrogen bonds within secondary structures of gliadins (Ribeiro *et al.*, 2021). In contrast to the previously mentioned compounds, the addition of 4XY and SYR to the dough resulted in the presence of a positive band (*ca*. 1 685 cm^{-1}). These results also suggest that protein aggregation occurred, as antiparallel β-sheets were commonly found in aggregated proteins (Wang *et al.*, 2017).

In the case of PCAT, a negative band is observed at *ca*. 1635 cm^{-1}, which can be attributed to the β-sheets. The research carried out by Wang *et al*. (2015) suggests that the content of β-sheets (βS) may be reduced due to the disruption of disulphide bonds. In addition, phenolic acids may have a direct effect on gluten proteins through the reduction of the hydrogen bonds and hydrophobic interactions in the intermolecular β-sheets (Wang *et al.*, 2015). Molecular docking results obtained by Feng *et al*. (2022) show that phenolic acids interact with protein molecules via hydrogen bonding. In addition, the oxygen atom from the phenolic carboxyl group was found to be more likely to interact with protein molecules. At the same time, the hydroxyl group on the benzene ring of phenolic acids appears to have a significant influence on the covalent cross-linking between the protein and phenolic acids (Feng *et al.*, 2022).

Addition of FER resulted in the appearance of the band at *ca*. 1 652 cm^{-1} which can be associated with α-helices. These result are in agreement with the research of Huang *et al*. (2018). In research of these authors, an increase in ferulic acid concentration from 1 to 4% resulted in higher α-helix and β-turn contents, while the author observed lower contents of β-sheets and random coils (RC). The authors also postulates that the reduction in β-sheets, in favour of β-turns, could affect the consistency of the gluten matrix (Huang *et al.*, 2018).

The amide III band, though less intense than the amide I band by nearly five times, remains responsive to changes in the protein's secondary structure and is not influenced by water oscillations. Specific wavenumbers for secondary structures within the amide III region have been adopted from Stani *et al*. (2020). The amide III band can be divided into four distinct spectral regions which correspond to specific secondary structures: β-sheets (1 200-1 240 cm^{-1}), random coils (1 240-1 260 cm^{-1}), β-turns (1 260-1 280 cm^{-1}), and α-helices (1 280-1 320 cm^{-1}). Amide III difference spectra are shown in Fig. 2. In general, the addition of hydroxycinnamic acid derivatives led to more disordered protein structure. The increase in β-turns at the expense of α-helices was primarily evidenced by the presence of negative bands associated with α-helices in each fortified sample. Corresponding positive bands attributed to β-turns were also detected. The β-turns can be attributed to the amorphous regions of the Gly-rich protein chains, which facilitate the formation of intramolecular antiparallel β-sheets (Rani *et al.*, 2023). The β-sheet content increased in all gliadin samples except those with lower vanillic acid concentration and those containing 0.1% 4XY. These observations were in line with the results of previous studies (Kłosok *et al*., 2022).

An increase in random coil content was observed after COU modified gliadins. As the concentration of this acid increases, the intensity of bands attributed to random coils are more apparent. Structural changes caused by SYR and VAN were observed only at the highest concentrations of these acids, with slight changes at lower concentrations. These results are similar to those obtained after analysis of samples modified by hydroxycinnamic acid derivatives. In all gliadins modified by hydroxycinnamic derivatives, negative bands in the range 1 220-1 235 cm^{-1} are observed. Hydrogen bonds of the type I have been identified in these samples. These bonds (-HN···O=C-) show a band at around 1 230 cm^{-1} and can occur between different polypeptide chains within the gluten network, favouring aggregation. Samples also contained type II hydrogen bonds (-HN···O, ether bond) with a prominent band at around 1 220 cm^{-1}. These bonds are due to the interactions between the gluten proteins and the additional compounds present in the dough, such as polysaccharides and polyphenols (Nawrocka *et al.*, 2023).

In summary, analysis of amide I band revealed that the interactions between gliadins and phenolic acids result in numerous structural modifications. The diversity of these modifications provides valuable insights into the adaptability of gliadins under the influence of different phenolic compounds. In the majority of samples analysed, changes in the α-helix conformational state of gliadins were observed following the incorporation of phenolic acids. This is supported by the fact that α-helices are the most abundant secondary structure motifs in gliadins, as suggested by Wellner *et al*. (2005). This correlation between empirical data and existing literature highlights the importance of α-helices in the molecular structure of gliadins (Wellner *et al.*, 2005). The analysis of amide III band reveals that the structural changes induced by phenolic acids are mainly connected with formation of hydrogen bonds within the gliadins. This may be confirmed by a study on proanthocyanidins, a class of polyphenols, which showed that gliadin reacts with proanthocyanidins primarily through hydrogen bonding, as shown in research performed by Girard *et al*. (2018). However, it should be noted that phenolic acids are small chemical compounds compared to proanthocyanidins. They may react differently with gliadins. The structural changes appear to be significantly influenced by the type of acid and the type of functional group on the aromatic ring. The different effect of the phenolic acids on gliadins may be related to the fact that the phenolic acids used in our study can interact with different subunits of the gliadins. In the study of Girard *et al.* (2018), specific

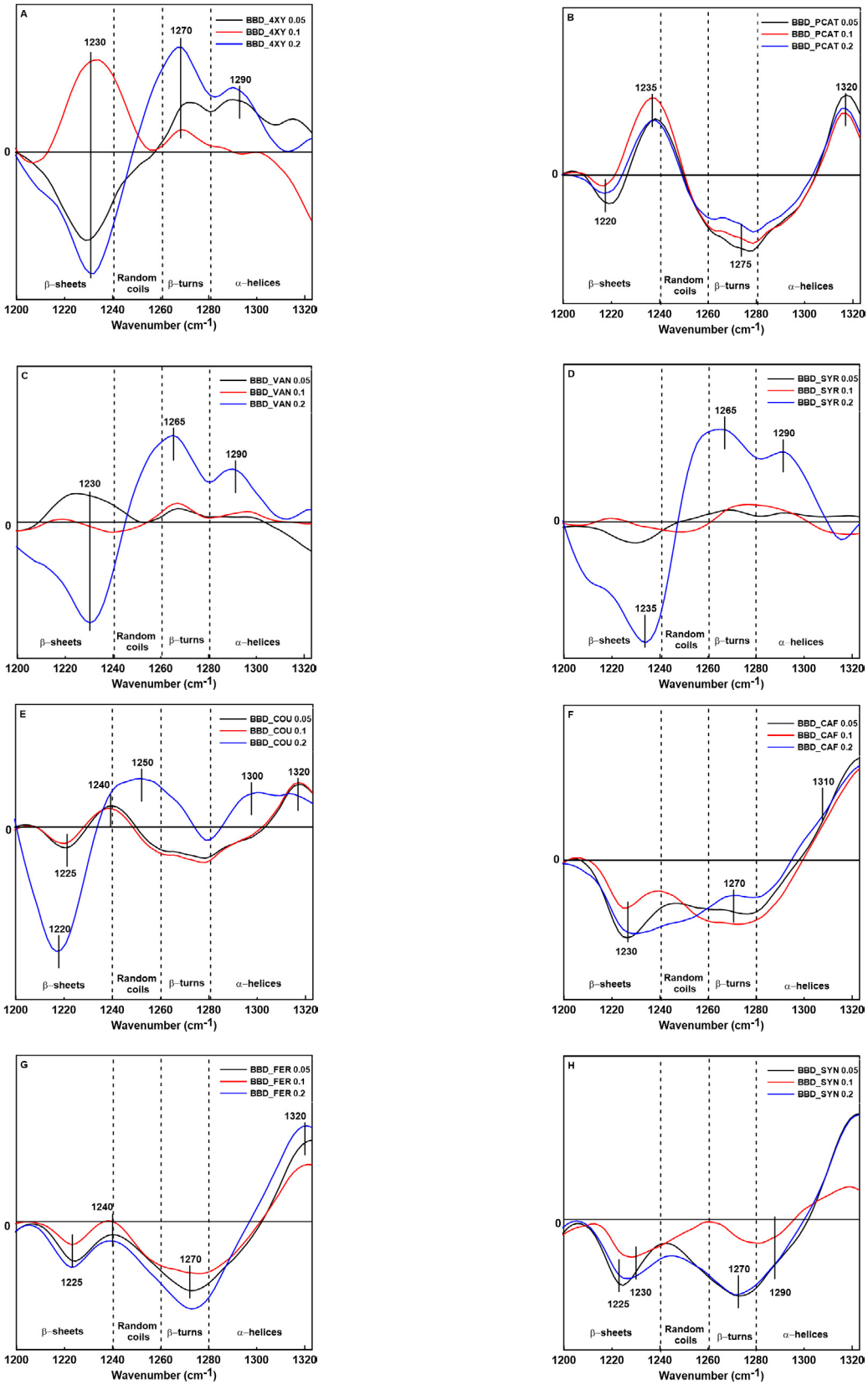

Fig. 2. Difference spectra in the FT-IR amide III band (1 200-1 320 cm^{-1}) displaying changes in the secondary structure of the gliadins modified by eight phenolic acids in concentrations of 0.05, 0.1 and 0.2%.

subfractions of gliadins also had a preferential interaction with the proanthocyanidin. The ω-gliadins preferentially interacted with the proanthocyanidins (Girard *et al.*, 2018).

3.2. Changes in secondary structure of glutenin

The amide I spectra of glutenin extracted from the model dough supplemented with different concentrations of phenolic acids are shown in Fig. 3. In all spectra, strong band at *ca.* 1 600 cm^{-1} is observed. The band at approximately 1 600 cm^{-1} could be associated with extended hydrated chains (Feeney *et al*., 2003) or NH_2-scissoring of glutamine side-chains (Secundo and Guerrieri, 2005). In addition, the spectra show that the peak attributed to the pseudo-β-sheets is present in all samples supplemented with the lowest concentration of phenolic acid (0.05%). However, there is a corresponding decrease in the content of this structure, as the concentration of phenolic acid increases. A decrease in α-helix and/or β-turn content was observed for specific acids: 4XY, COU, PCAT and CAF. The fact that these acids do not contain additional methoxyl groups attached to the aromatic ring may explain the specific interaction of glutenins with these acids. Different behaviour was observed in case of VAN, FER, SYR and SYN. Glutenins modified by these acids also showed a lower amount of hydrogen-bonded β-sheets. This structural change is probably due to the presence of a methoxyl group attached to the phenolic aromatic ring. The content of hydrogen-bonded structures can be disrupted by the additional methoxyl groups of phenolic acids It should be noted that, after the addition of CAF, a band is observed at about 1 668 cm^{-1}, which can be attributed to the β-turns. This band is also observed in the study conducted by Dhaka and Khatkar (2016). The authors found that in the high quality bread wheat, the intensity of this band was higher.

An increase of intensity in the range between 1 680 and 1 695 cm^{-1} was observed for samples modified by hydroxybenzoic acid derivatives. These bands can be assigned to strong intermolecular hydrogen bonds in the conformation of the antiparallel β-sheets. The ability of Fourier transform infrared (FT-IR) spectroscopy to distinguish between parallel and antiparallel β-sheets was demonstrated by Cerf *et al*. (2009). Authors linked the band at 1 636 cm^{-1} to both types of β-sheets. However, the existence of antiparallel β-sheets has been associated with an additional high frequency element in the range 1 685 to 1 695 cm^{-1}. The increased content of these bands suggest a possible indication of protein denaturation or a disruption of the hydrogen bonds present in certain secondary structures of glutenins. This disruption could also lead to the formation of new, stronger hydrogen bonds, such as intermolecular hydrogen bonds, which are usually associated with aggregation (Ribeiro *et al.*, 2021). A more complex pattern of interactions was observed with the glutenin modified by hydroxycinnamic acid derivatives. Only addition of sinapic acid and caffeic acid at a concentration of 0.1% showed an increased content of antiparallel β-sheets. Within this group of phenolic acids, a decrease was observed in all other samples.

Glutenins are rich in highly polar amino acids, in particular glutamine, which acts as both a donor and an acceptor of H-bonds. As such, they are involved in the formation of intramolecular hydrogen bonds with other donor and acceptor amino acids, which can serve as H bond donor or acceptor (Dhaka and Khatkar, 2016). There is also a possibility that all studied polyphenols interact with proline. Proline also causes an extended/disordered structure, which frequently includes PPII helices. This type of secondary structure increases protein backbone accessibility for interaction with polyphenols. The interactions with phenolic acids can cause structural changes in the protein, which can impact its function (Van Buiten and Elias, 2021).

Figure 4 shows the spectra of the amide III band of the phenolic acid-modified glutenins. All samples, except those containing 4XY, showed an increase in the band around 1 220 cm^{-1}, while a corresponding decrease was observed at *ca.* 1 240 cm^{-1}. The changing intensity of this region may be related to the bands attributed to the β-sheet structure and their stabilisation by intermolecular or intramolecular hydrogen bonding. These structures are typically found within a folded protein complex. β-sheet conformation allows hydrophobic amino acids to become incorporated into protein core (Wang *et al*., 2014). In addition, due to their relatively large surface area, which leads itself to the ordered development of hydrogen bonds, β-sheet structures could potentially have a significant impact on the formation of protein fibrillar aggregates. Shewry *et al*. (2000) postulated that high molecular weight glutenin subunits (HMW-GS) contribute to the viscoelastic properties of gluten by facilitating the formation of a β-sheet structure, based on secondary structure analyses using nuclear magnetic resonance (NMR) and Fourier transform infrared (FT-IR) spectroscopy (Shewry *et al.*, 2000).

After the modification of glutenin by the COU, PCAT, CAF, FER, SYN and higher concentrations of SYR, bands attributed to α-helix structures were identified in the spectral region 1 295-1 320 cm^{-1}. This observation suggests an increase in the α-helix content, which could indicate stabilisation of glutenin proteins. On the other hand, a decrease in α-helix content was observed when treated with VAN and SYR at 0.05% concentration. Since the structure of the α-helix is maintained by hydrogen bonding, any disruption of the hydrogen bond or enclosing of the polyphenols in the hydrophobic pocket of the proteins can lead to conformational changes in the α-helices (Sun *et al.*, 2022). This may explain the observed decrease in the content of these structures. In addition, an increase in β-turn content was observed at certain concentrations for 4XY (0.05%), COU (0.05, 0.1%), CAF (0.05%), VAN (0.1%), all FER concentrations, SYR (0.05, 0.1%) and SYN (0.05, 0.1%).

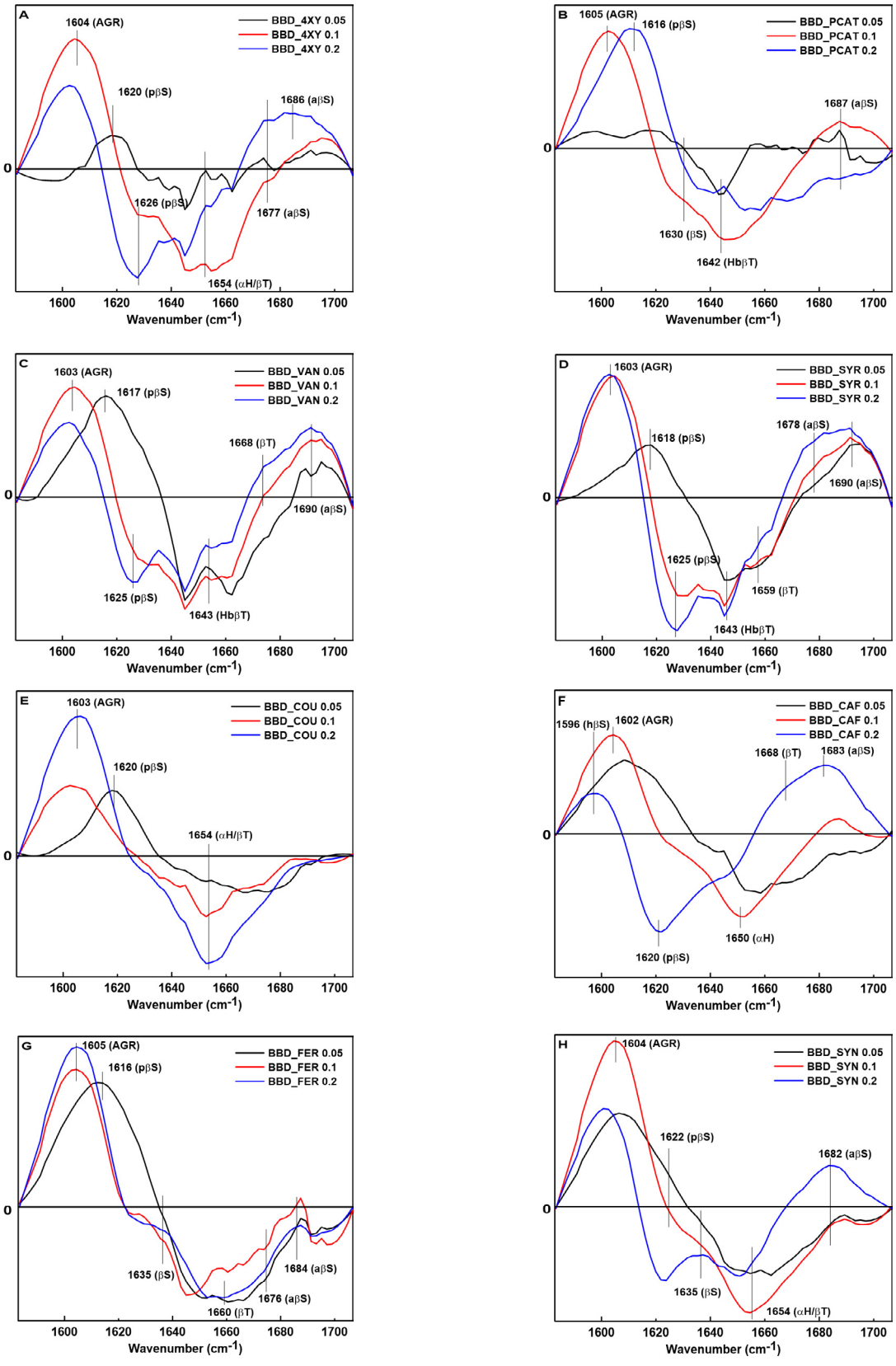

Fig. 3. Difference spectra in the FT-IR amide I band (1 580-1 720 cm^{-1}) displaying changes in the secondary structure of the glutenins modified by eight phenolic acids in concentrations of 0.05, 0.1 and 0.2%.

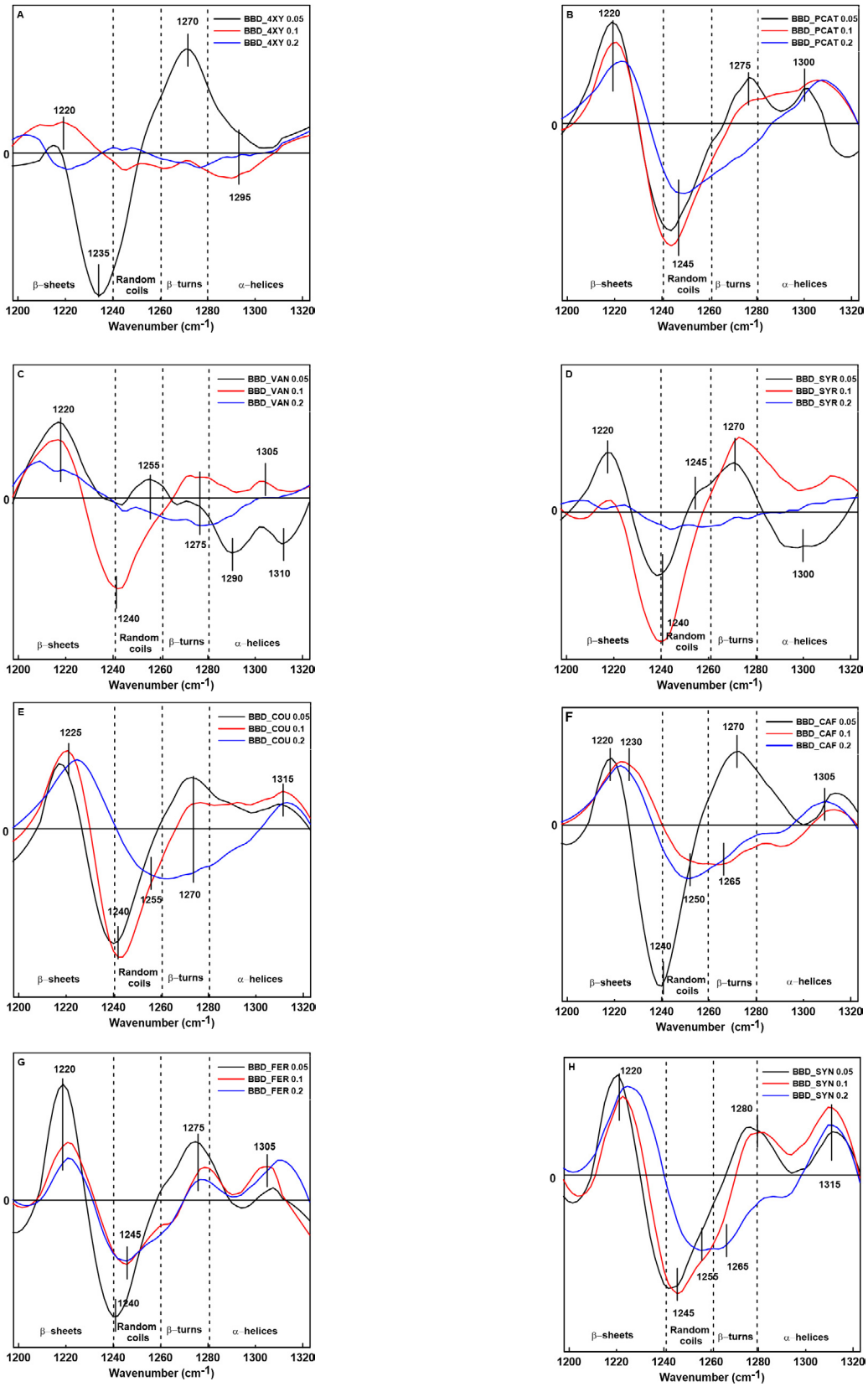

Fig. 4. Difference spectra in the FT-IR amide III band (1 200-1 320 cm^{-1}) displaying changes in the secondary structure of the glutenins modified by eight phenolic acids in concentrations of 0.05, 0.1 and 0.2%.

In contrast, COU (0.2%), PCAT (0.2%), CAF (0.1%), VAN (0.2%) and SYN (0.2%) showed a decrease in β-turn content.

The analysis of difference spectra showed changes in secondary structures occurring due to the addition of phenolic acids. Most of the samples showed changes resulting in increase in β-sheet structures and changes in the content of α-helix structures that were dependent on both the concentration and structure of the phenolic acid. Additionally, alterations in β-turn content were observed with different phenolic acids at specific concentrations, hinting at complex interactions between phenolic acids and glutenin.

4. CONCLUSIONS

The obtained results indicate that incorporation of the phenolic acids into the dough alters the structural conformation of gliadins as well as glutenins. These changes occur within the gluten network. Both gliadins and glutenins interacted with the phenolic acids during dough mixing process. As shown by difference spectra analysis, all phenolic acids induced changes in the secondary structure of gluten proteins. The degree of interactions is affected by the concentration and structure of the phenolic acid used in the research. The interaction of phenolic acid with gliadins or glutenins typically results in the formation of aggregated structures. The structural changes in phenolic acid-modified gliadins are more complex than those in phenolic acid-modified glutenins. Spectral analysis of the modified glutenins indicates that the predominant protein changes involve β-structures. This is supported by the presence of positive bands attributed to these structures and occurs at the expense of α-helices. Inclusion of phenolic acids in the dough led to disruption of the secondary structure of glutenins, but it is difficult to establish a direct correlation between changes in protein secondary structure and phenolic acid structure and concentration. Our study suggests that phenolic acids influence the formation of hydrogen bonds in both gliadins and glutenin. The changes in glutenin structure induced by phenolic acids may have an impact on the allergenicity of gluten proteins. In particular, the allergenicity of gluten proteins could be affected by a change in the β-turn structure. As the allergenicity of proteins has been linked to the β-turn, strategies aimed at reducing this secondary structure could potentially reduce the allergenic reactions to gluten proteins. This knowledge is crucial to understanding the underlying mechanisms of these interactions, with potential applications in food chemistry and related fields. Advancements in our understanding of the molecular interactions between gluten proteins and particular groups of polyphenols could lead to the development of functional foods enriched with polyphenols as well as characterized by optimized gluten structure. These foods could be tailored to meet the dietary needs of individuals with specific dietary requirements, including those with gluten sensitivity or a preference for an improved nutritional profile.

Conflicts of Interest: The authors declare no conflict of interest.

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