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Effect of dietary fibre waste originating from food production on the gluten structure in common wheat dough

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Abstract. At present, the use of by-products from plant food production is gaining more interest because these products contain a large amount of valuable nutritional compounds *e.g.* dietary fibre, proteins, polyphenols, unsaturated fatty acids, vitamins. The by-products improve both the nutritional profile and the health-promoting properties of bakery products but simultaneously impair some technological properties, which is strongly related to the structure of the gluten network. FT-IR spectroscopy was used to determine changes in the gluten structure through the addition of by-products from the vegetable industry and cold oil pressing production. The supplements were added to the common wheat dough in the amounts of 3, 6, 9 and 12%. Analysis of the spectra indicates that changes in the gluten structure and the distribution of water populations are connected with the type of technological process from which the supplement originated and hence its chemical composition. Vegetable supplements cause the formation of aggregated structures such as pseudo-β-sheets, whereas gluten samples modified by oil supplements contain mainly basic secondary structures *i.e.* α-helices, β-turns and antiparallel-β-sheets. With regard to the water populations, oil supplements do not affect them or affect them slightly. Vegetable supplements lead to the formation of a weaker gluten network. This is observed in the form of a decrease in the number of strong hydrogen bonds.

Keywords: gluten structure, water populations, wheat dough, vegetable and oil supplements, FT-IR spectroscopy

INTRODUCTION

In recent times, the use of wastes and by-products from plant food production has been attracting more interest because these products contain a large amount of valuable nutritional compounds *e.g.* dietary fibre, proteins, polyphenols, unsaturated fatty acids, vitamins, minerals. The interest is connected with the EU zero-waste policy as well as an increasing consumer awareness of the effects of a healthy diet on human well-being. The by-products can be reused in food processing in the unmodified form or after treatments which include drying, grinding and micronization (Prakash *et al.*, 2018). These by-products, which may be regarded as functional ingredients, can be introduced to wheat products (*e.g.* bread, pasta) in the form of dietary fibre preparations (Collar *et al.*, 2007; Miś *et al.*, 2020), polyphenols extracts (Girard *et al.*, 2016), hydrocolloids (Ferrero, 2017; Nawrocka *et al.*, 2018a), and residues after oil extraction (Wirkijowska *et al.*, 2020; Rumińska *et al.*, 2020). The introduction of such additives leads to rheological as well as structural changes in the wheat dough and gluten network, respectively. These changes are strongly related to the chemical composition of the additives. For example, additives that are rich in polysaccharides and hydrocolloids

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cause an increase in the dough resistance to extension, this is observed as formation of new aggregated structures in the gluten network (Nawrocka *et al.*, 2016). According to the literature data (Bock and Damodaran, 2013; Nawrocka *et al.*, 2017), the formation of aggregated structures is related to the dehydration of the gluten network which occurs during the dough mixing process. Miś *et al.* (2020) has shown that dehydration is connected with the competition for water between the model dough components (wheat starch and wheat gluten) and polysaccharides. The formation of aggregated structures and dough strengthening may be observed as a result of wheat dough supplementation with polyphenols which are characterized by a large molecular size such as tannic acid. Opposite structural changes (random coils, non-hydrogen bonded β-structures), which were observed dough breakdown, were obtained after the addition of phenolic acids to wheat dough (Krekora *et al.*, 2021).

Although the by-products are regarded as a rich source of nutritional compounds, only 10% of the attempts to use them in bakery products have been successful. They improve the nutritional profile and health-promoting properties of bakery products, but at the same time they impair some of their technological properties. Among other bakery products, wheat bread as a staple food in the human diet is the food which is most frequently supplemented with by-products. The rheological parameters of wheat bread dough such as water absorption and mixing properties are crucial to the breadmaking process. Water absorption is negatively correlated with protein content, whereas it is positively correlated with the total dietary fibre (TDF) content of the by-products (Martins *et al.*, 2017). The mixing properties of the dough (*i.e.* dough stability, development time and the degree of softening) depend on the chemical composition of the by-products, particularly the TDF content (Majzoobi *et al.*, 2011). If the addition of by-products affects wheat dough quality, the quality of the bread is also affected. The incorporation of by-products often results in loaf volume decrease. This decrease may also be connected with the TDF content and the particle size of the by-products (Wu and Shiau, 2015). In the case of the wheat bread texture, an increase in hardness/firmness and chewiness was observed, whereas cohesiveness decreases as a result of dietary fibre supplementation (Bhol *et al.,* 2016).

Previous studies (Wirkijowska *et al.*, 2020; Zarzycki *et al.*, 2022) concerned the effect of by-products from cold oil pressing on the baking properties of the wheat flour and bread quality. The studies showed that the supplementation of the wheat dough increased water absorption and dough softening and decreased stability time. With regard to bread properties, the supplemented breads were characterized by a higher bread yield and lower baking loss, improved porosity, taste, and bread volume as compared with the control bread. The changes in the dough and bread quality are strongly connected with the structural changes in the gluten network. Therefore, the aim of the studies was to determine the changes in the secondary structure of the gluten network as well as the distribution of the water population in the gluten samples obtained from common wheat dough supplemented with six dietary fibre (DF) supplements. The DF supplements waste from the vegetable industry (vegetable supplements) and cold pressing oil production (oil supplements).

MATERIALS AND METHODS

Common wheat flour (type 750) was obtained from Polskie Młyny Sp. z o.o. (Warsaw, Poland). The technological parameters of the wheat flour used were published previously (Zarzycki *et al.,* 2022). A variety of dietary fibre (DF) supplements were used including vegetable supplements (paprika (PAP), pitted pepper (PITP), tomato pomace (TOM)), and oil supplements (Moldavian dragonhead flour (MD), coconut pomace (COC), and flax pomace (FLX)). All supplements were added to the flour in the powder form. Deuterium oxide (D_2O) was purchased from Sigma Aldrich (Poland). Double-distilled water was used.

The analysis of chemical composition included a determination of the moisture content (AACC 44-15A), ash (AACC 08-01), protein (AACC 46-08; conversion factor N × 5.7; KjeltecTM8400 with the ASN application, Foss Analytical AB, Sweden), fat (AACC 30-26; SoxtecTM8000 with AN 310 application, Foss Analytical AB, Sweden), and total (TDF), insoluble (IDF) and soluble (SDF) dietary fibre content (enzymatic method: AACC 32-07, AACC 32-21, AACC 32-05, AOAC 991.43, AOAC 985.29) (AACC, 2000; AOAC, 2016). The digestible carbohydrates were calculated according to Sobota *et al.* (2020).

The bread dough samples, both the control and the one supplemented with selected DF supplements, were prepared according to standard method ICC 115/1 using Farinograph E with a 50 g mixer (Brabender, Germany). Wheat flour – dietary fibre blends were made by substituting 3, 6, 9 and 12% of the flour with a single supplement (Nawrocka *et al.*, 2016). The dough mixing time was 20 min.

The gluten samples were prepared according to the procedure developed by Nawrocka *et al.* (2017). Briefly, the gluten samples were washed out from the dough samples using a Glutomatic 2200 (Perten Instruments, the USA). The samples were freeze-dried and powdered. The powdered gluten samples of a definite weight were moisturized using 10% aqueous solution of D_2O to eliminate water oscillations from the amide I band.

Fourier transform infrared (FT-IR) spectra were recorded with a Nicolet 6700 FT-IR spectrometer (Thermo Scientific, the USA) equipped with a diamond ATR attachment according to the procedure described by Nawrocka *et al.* (2017). The FT-IR spectra were also prepared for analysis in accordance with the above-mentioned reference using ORIGIN PRO (v.2021b, OriginLab Corporation, the

USA). An aqueous solution of D_2O was treated as an internal standard. All FT-IR spectra were normalized at a band characteristic of D_2O (2485 cm^{-1}), and the spectrum of the D_2O solution was subtracted from all gluten spectra to eliminate the water bands from the amide I band.

Changes in the secondary structure of the gluten network were determined through an analysis of difference spectra calculated in the amide I and amide III bands according to Nawrocka *et al.* (2017). Briefly, the spectrum of the control sample (gluten washed out from the unmodified dough sample) was subtracted from the spectrum of the by-product modified gluten sample. All spectra in the amide bands were area normalized.

A one-way analysis of variance (ANOVA) with a Tukey test ($p \leq 0.05$) were conducted using the software Statistica (v.13.3, Statsoft, the USA).

RESULTS AND DISCUSSION

The chemical composition of three supplements (paprika, pitted pepper and tomato pomace) is presented in Table 1. The chemical composition of the other dietary supplements may be found in the following references: Moldavian dragonhead flour (Zarzycki *et al.*, 2022), coconut pomace (Wirkijowska *et al.*, 2021), and flax pomace (Wirkijowska *et al.*, 2020). According to Miś *et al.* (2020), the most important factor affecting the interactions between the gluten network and selected fibre supplements in bread dough is the content of dietary fibre. The highest content of DF and its insoluble fraction was found for tomato and coconut pomace. Whereas the soluble fraction of DF was determined to be the highest one for paprika, Moldavian dragonhead flour and flax pomace.

The amide I band is used the most often for the determination of the secondary structure of proteins. The deconvoluted amide I band of the control sample (gluten washed out from wheat dough) is shown in Fig. 1. The control sample contains 25% of the β-sheets (βS), 69% of the α-helices (αH) and 6% of the β-turns (βT). Similar secondary structures with similar location of the Gaussians were determined by Nawrocka (2014) in the native gluten. However, the contents of particular structures differed significantly (βS – 36%, αH – 51%, βT – 13%). The differences may be connected with the kind of material from which the gluten was obtained. In previous studies, the gluten was washed out from the wheat wholemeal, not from the dough. Additionally, differences in the gluten structure can be assigned to the material from which the wheat flour was obtained (Both *et al.*, 2019).

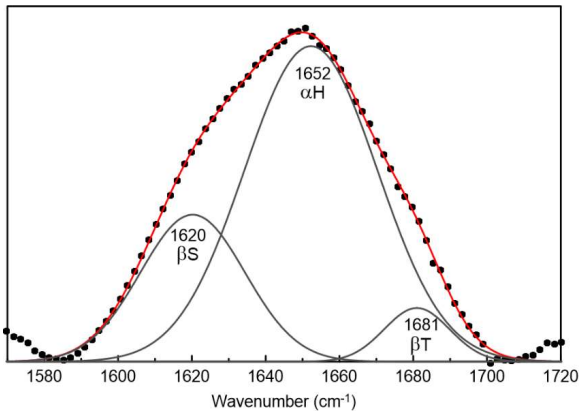

Fig. 1. Deconvoluted amide I band of the gluten network obtained from bread wheat flour. Abbreviations: βS – β-sheet, αH – α-helix, βT – β-turn.

The effects of the selected dietary fibre supplements on the gluten network in bread dough are depicted in Fig. 2 in the form of difference spectra. Each difference spectrum was calculated by subtracting the control spectrum from the spectrum of the modified samples. An analysis of the spectra indicates that the DF supplements may be divided into two groups due to the nature of the observed structural changes. The first group consists of PAP, PITP and TOM, whereas the second group contains MD, COC and FLX. The division may be connected with the type of technological process from which the waste (DF supplement) originated. PAP, PITP and TOM are wastes produced by vegetable processing, while MD, COC and FLX were obtained as a result of cold oil pressing. The previous studies of Miś *et al.* (2020) suggested that structural changes in the gluten network that were induced by DF supplements may be connected with their chemical composition. Miś *et al.* (2020) linked these changes to the content of dietary fibre. While Rumińska *et al.* (2020) assigned the structural

Table 1. Chemical composition of the selected dietary fibre (DF) supplements: paprika (PAP), pitted pepper (PITP) and tomato pomace (TOM)

DF supplement	Moisture content	Ash	Protein	Fat	TDF	IDF	SDF	CHO
		(% d.m.)						
PAP	11.84 (0.38)^{a}*	13.29 (0.13)^{a}	30.77 (0.01)^{a}	3.15 (0.04)^{c}	33.49 (0.55)^{c}	16.86 (1.56)^{b}	16.63 (1.01)^{a}	19.28 (0.47)^{a}
PITP	5.44 (0.19)^{b}	3.01 (0.12)^{c}	19.91 (0.01)^{c}	25.99 (0.03)^{a}	50.07 (0.19)^{b}	47.51 (0.38)^{a}	2.56 (0.57)^{c}	1.02 (0.06)^{c}
TOM	6.41 (0.08)^{b}	3.76 (0.01)^{b}	20.48 (0.01)^{b}	11.73 (0.14)^{b}	60.92 (0.37)^{a}	49.72 (0.36)^{a}	11.20 (0.01)^{b}	3.12 (0.21)^{b}

TDF – total dietary fibre, IDF – insoluble dietary fibre, SDF – soluble dietary fibre, CHO – digestible carbohydrates. *Means in the same column with different letters are significantly different (Tukey test, $p \leq 0.05$).

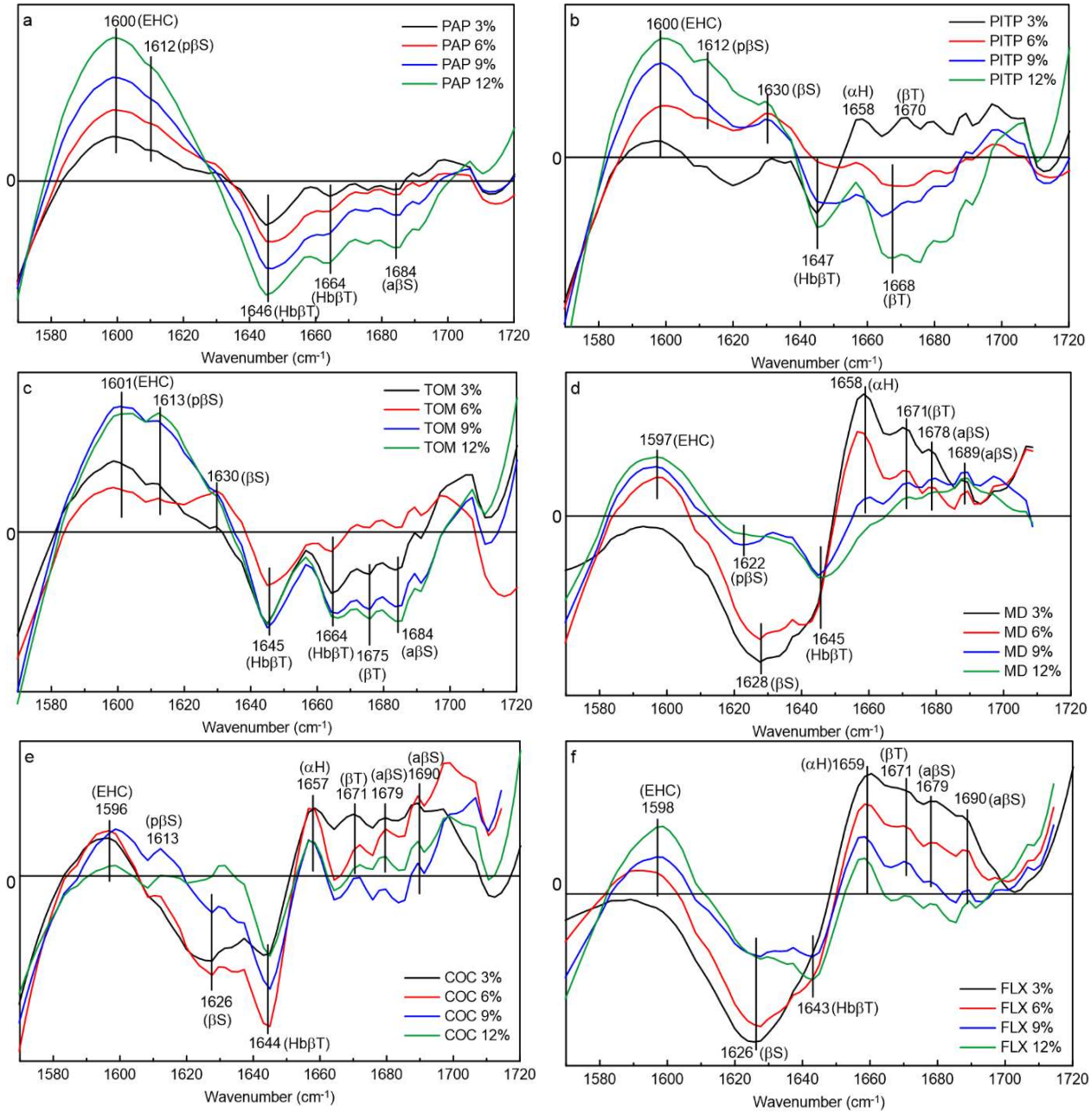

Fig. 2. FT-IR difference spectra in the amide I band for gluten samples obtained from wheat dough supplemented with paprika (PAP), pitted pepper (PITP), tomato pomace (TOM), Moldavian dragonhead flour (MD), coconut pomace (COC), and flax pomace (FLX). The following secondary structures were determined: EHC – extended hydrated chains, pβS – pseudo-β-sheets, βS – β-sheets, αH – α-helices, HbβT – hydrogen bonded β-turns, βT – β-turns, aβS – antiparallel-β-sheets.

changes to the content of fatty acids. The difference spectra of the first group may be divided into two spectral regions: positive (1 580-1 630 cm^{-1}), and negative (1 630-1 700 cm^{-1}). Whereas, the orientation of the spectral regions is opposite for the second group. According to Mangavel *et al.* (2001) and Nawrocka *et al.* (2017), the observed bands in the difference spectra may be assigned to the following secondary structures: pseudo-β-sheets (pβS, 1 612-1 625 cm^{-1}), β-sheets (βS, *ca.* 1 630 cm^{-1}), β-turns with intramolecular hydrogen bonds (HbβT, *ca.* 1 645 cm^{-1}), α-helices (αH, 1 658 cm^{-1}), β-turns with intermolecular hydrogen bonds (HbβT, *ca.* 1 664 cm^{-1}), β-turns (βT, *ca.* 1 670 cm^{-1}), and antiparallel-β-sheets (aβS, 1 678-1 690 cm^{-1}). The band at *ca.* 1 600 cm^{-1} may be connected with extended hydrated chains (EHC) (Feeney *et al.*, 2003) or the NH_2 scissoring of the glutamine side chains (Secundo and Guerrieri, 2005).

Analysis of difference spectra, which are shown in Fig. 2a-c, indicates that the addition of vegetable supplements induces the formation of hydrated extended chains and pseudo-β-sheets (aggregated structures) at the expense of hydrogen bonded β-turns and antiparallel-β-sheets. An increase in the supplement content leads to an increase in the amount of aggregated structures in the modified samples. The most intense band is observed at *ca*. 1 600 cm^{-1}. A similar band was observed by Nawrocka *et al.* (2017) as a result of the supplementation of the model wheat dough with dietary fibre preparations (fruit and vegetable). Simultaneously, there was a negative band assigned to the antiparallel-β-sheets. Nawrocka *et al.* (2017) claimed that the presence of these two bands indicated the formation of the extended hydrated chains from antiparallel-β-sheets. The presented analysis confirms this finding. However, the present difference spectra also show the positive and negative bands assigned to pseudo-β-sheets and hydrogen bonded β-turns, respectively. This suggests that pseudo-β-sheets and extended hydrated chains can be formed by both H bonded β-turns and antiparallel-β-sheets.

Gluten samples supplemented with oil supplements (MD, COC and FLX) contain mainly basic secondary structures *i.e.* α-helices (*ca*. 1 658 cm^{-1}), β-turns (*ca*. 1 670 cm^{-1}) and antiparallel-β-sheets (*ca*. 1 680 and 1 690 cm^{-1}) (Fig. 2d-f). The quantity of these structures declines with the increasing content of these supplements. Simultaneously, a slight increase in the content of the hydrated extended chains is observed. Additionally, the difference spectra show a reduction in the content of β-sheets and H-bonded β-turns (bands at *ca*. 1 626 and 1 644 cm^{-1}, respectively). Similar changes in the secondary structure of gluten were observed by Rumińska *et al.* (2020) in samples supplemented with hemp and primrose. These supplements also were waste of oil cold-pressing and contained the highest amounts of two unsaturated fatty acids: linoleic (C18:2 cis-9,12) and γ-linoleic (C18:3 cis-6,9,12) (Rumińska *et al.*, 2020). The band at *ca*. 1 658 cm^{-1} can also be assigned to unconjugated olefins (oleic and linoleic acids) (Vongsvivut *et al.*, 2014) because the FT-IR spectra of the DF supplements show wide bands with a maximum spanning the spectral region 1 620-1 660 cm^{-1}.

Generally, the difference spectra in the amide I band indicate that structural changes in the gluten network, which were observed as a result of DF supplements presence during dough mixing, depend on the chemical composition of the supplement. However, the presence of all supplements leads to the formation of extended hydrated chains (EHC) at the expense of β structures. The content of these structures is related to the process of obtaining the supplements. Vegetable supplements (PAP, PITP, TOM) cause the formation of more EHCs as compared to oil supplements (MD, COC, FLX).

The lack of water oscillations in the FT-IR amide III band allows for the analysis of the secondary structure of the proteins without any special preparation of the protein samples. Analysis of this band provides information that is complimentary to those obtained from analysis of the amide I band. Difference spectra, that show which secondary structures appear/disappear as a result of DF supplements addition to the wheat dough, are presented in Fig. 3. Analysis of the difference spectra also allows for the division of the DF supplements into two similar groups, as was the case with the amide I band. The spectra of the vegetable supplements (PAP, PITP, TOM) show a strong positive band at *ca*. 1 228 cm^{-1}. This band has the same orientation, it is weak, or it is not visible in the case of the second group of supplements. According to Nawrocka *et al.* (2018b), this positive band may be assigned to β-sheets connected with intermolecular hydrogen bonds and may be regarded as a sign of aggregation. Analysis indicates that vegetable supplements induce the aggregation of gluten polypeptide chains to a greater extent than oil supplements. A similar band was observed in the studies of Nawrocka *et al.* (2018a, b) in which the effect of apple and citrus pectin and different celluloses on gluten structure was studied, respectively. Previous studies concerning the effect of fruit, vegetable and cereal dietary fibre preparations on the gluten structure have shown an additional positive band at *ca*. 1 216 cm^{-1} that was assigned to the hydrogen bonds formed between gluten polypeptide chains and DF polysaccharides (Nawrocka *et al.*, 2020). This band indicates the incorporation of DF polysaccharides into the gluten network. The absence of this band in the present studies suggests that polysaccharides from DF supplements do not incorporate into the gluten network. As for the changes occurring in the β-sheets region, a strong negative band at *ca*. 1 245 cm^{-1} is observed in the spectra of MD and FLX. Simultaneously, a strong positive band at *ca*. 1 280 cm^{-1} is observed in the spectral region assigned to β-turns. The presence of these bands may suggest that MD and FLX supplements disrupt the formation of β-sheets. A similar orientation of the bands connected with β-sheets and β-turns are observed in the amide I band (Fig. 2d-f). Moreover, the present results show that the bands at *ca*. 1 628 and 1 245 cm^{-1} are assigned to the same secondary structure – parallel β-sheets without intermolecular hydrogen bonds. The spectral ranges assigned to random coils and β-turns show bands at *ca*. 1 260 and 1 275 cm^{-1}, respectively, in the case of vegetable supplements (Fig. 3a-c). If the band connected with the β-turns is strong, the random coils band is weak or *vice versa*. These results indicate that vegetable supplements contain some compounds which induce the formation of β-turns or random coils. The band characteristic for these two structures in the amide III band were observed for pumpkin and milk thistle pomaces (Rumińska *et al.*, 2020), and two dietary fibre preparations – chokeberry and cacao (Nawrocka *et al.*, 2017). All of these additives are characterized by high

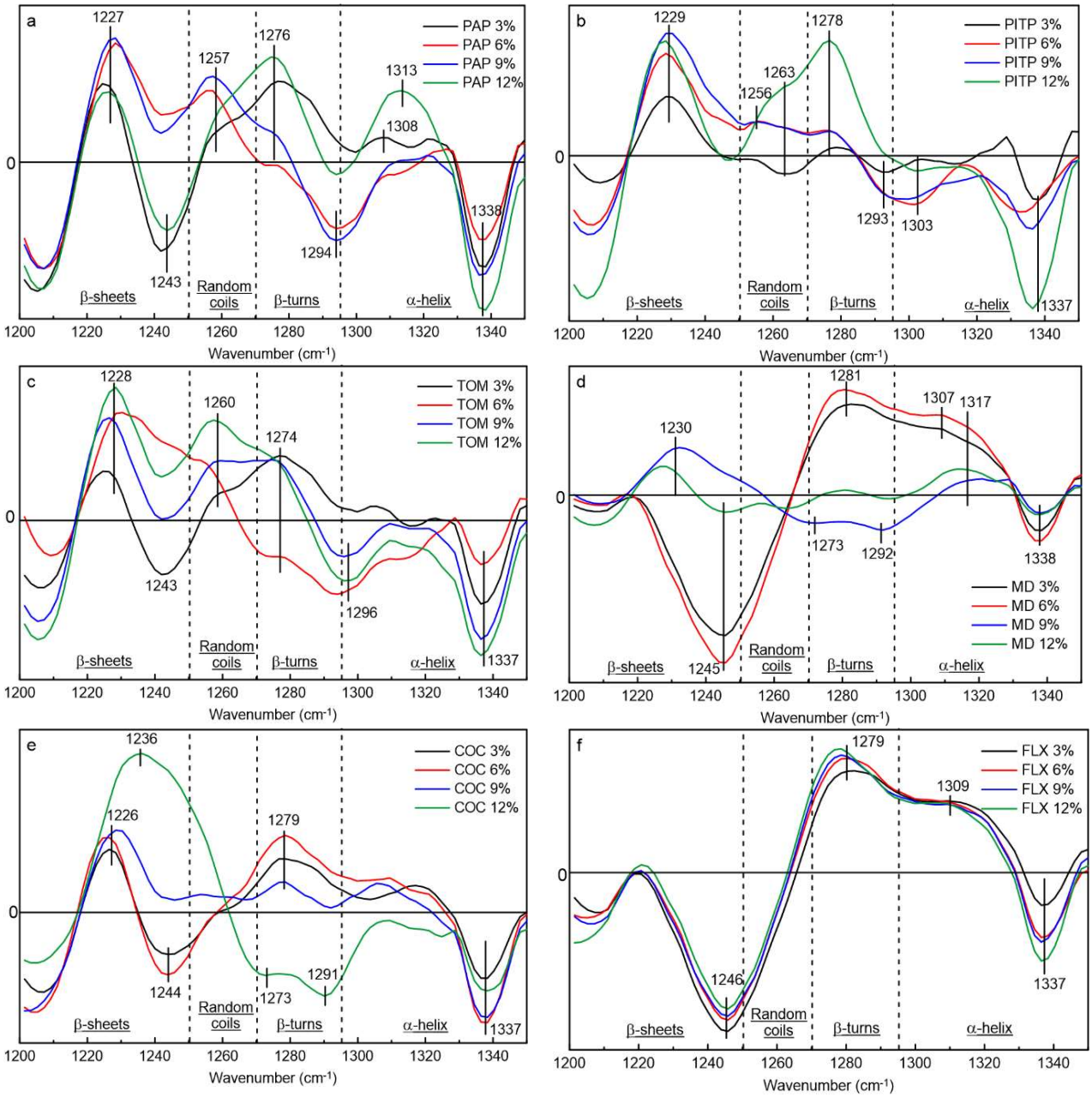

Fig. 3. FT-IR difference spectra in the amide III band for gluten samples obtained from wheat dough supplemented with paprika (PAP), pitted pepper (PITP), tomato pomace (TOM), Moldavian dragonhead flour (MD), coconut pomace (COC) and flax pomace (FLX).

total polyphenol content. Similar bands were observed by Krekora *et al.* (2021) as a result of model dough supplementation with caffeic, chlorogenic and ferulic acids. In the case of oil supplements, apart from the negative band at 1 245 cm^{-1}, the positive bands assigned to β-turns and α-helices are present. These results suggest that these supplements can protect gluten polypeptide chains from the formation of aggregated structures.

Analysis of the OH stretching region provides information about water populations present in the wheat dough. According to Bock and Damodaran (2013) and Nawrocka *et al.* (2020), the water populations can be divided into a few groups: strongly and weakly H-bonded to the gluten network (*ca.* 3 050 and 3 180 cm^{-1}, respectively), hydrogen bonded associated chains of water molecules in the bulk water or water molecules of which both hydrogen atoms

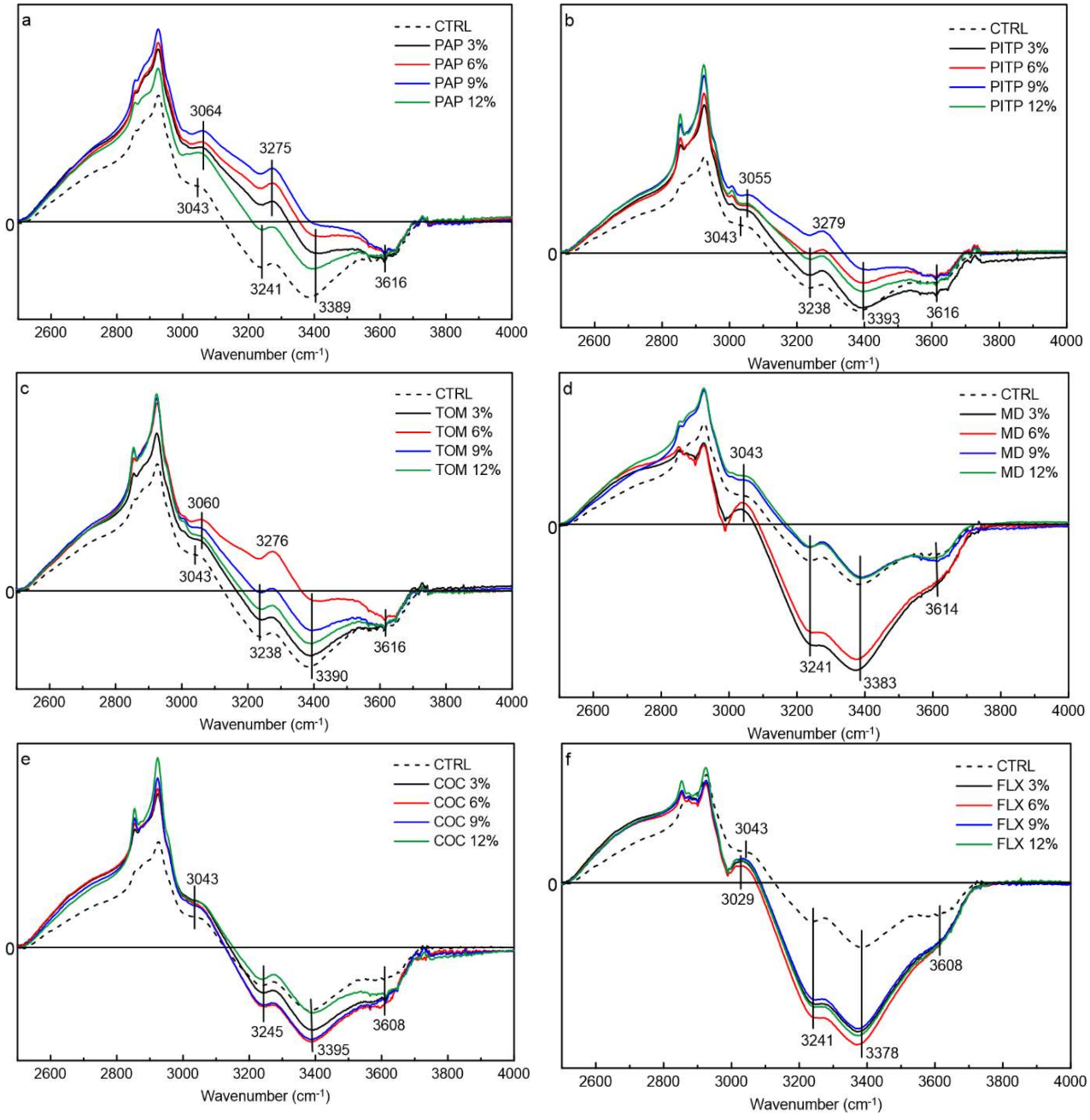

Fig. 4. Difference spectra in the OH stretching region for gluten samples obtained from wheat dough supplemented with paprika (PAP), pitted pepper (PITP), tomato pomace (TOM), Moldavian dragonhead flour (MD), coconut pomace (COC) and flax pomace (FLX).

participate in the formation of H bonds (*ca*. 3 280 cm^{-1}), small hydrogen bonded water clusters (*ca*. 3 380 cm^{-1}), water molecules involved in interactions with the carboxyl anion and the aggregation of water molecules around this anion (*ca*. 3 450 cm^{-1}), and free water (*ca*. 3 640 cm^{-1}). The distribution of the water populations in the wheat dough may vary as a result of the modification of the dough with various additives. The presence of different additives, characterized by different chemical composition, may cause competition for water between gluten proteins and particular components of the additives, especially from those characterized by a high value of the water holding capacity (WHC). As a result of the improper hydration of the wheat dough during the mixing process, wheat bread of a reduced quality is obtained.

The effects of six DF supplements on the distribution of water populations in a common wheat dough are depicted in Fig. 4. Analysis of the spectra shows that the control sample (black dotted line) only contains water molecules connected with gluten polypeptide chains through strong

hydrogen bonds (positive band at 3 043 cm^{-1}). Control samples from previous studies also contained other water populations such as water molecules weakly H-bonded to the gluten network and water molecules with both hydrogen atoms participating in the formation of H bonds (Bock and Damodaran, 2013; Nawrocka *et al.*, 2017; Rumińska *et al.*, 2020). However, other types of raw materials were used in those studies, *i.e.* wheat gluten and model flour reconstituted from wheat starch and wheat gluten.

Analysis of the spectra in the OH stretching region for modified samples indicates the division of the DF supplements into two groups. The groups content is similar to that obtained as a result of the analysis of both amide bands. In the case of the first group (PAP, PITP, TOM), the presence of vegetable supplements induces changes in the number of water molecules strongly H bonded to the gluten network observed as a shift of the band from 3 043 cm^{-1} (control) to *ca*. 3 060 cm^{-1}. This shift suggests a decrease in the number of strong H bonds formed between gluten polypeptide chains and water molecules (Liu *et al.*, 2002). Additionally, a certain number of H bonds, in which both hydrogen atoms of water participate, are formed. This is observed as appearance of a positive band at *ca*. 3 280 cm^{-1}. It suggests that one water molecule can connect gluten proteins with particular components of DF supplements as well as gluten polypeptide chains with each other. The difference spectra of the MD, COC and FLX are similar to the spectrum of the control sample. Only in the case of the FLX – gluten spectrum, a shift of the band from 3 043 cm^{-1} (control sample) to 3 029 cm^{-1} was observed. This shift is in the opposite direction compared to the first group of supplements, and indicates an increase in the number of strong H bonds formed between the gluten polypeptide chains and water molecules (Liu *et al.*, 2002). This suggests the formation of a stronger gluten network after the supplementation of the model dough with FLX supplement.

CONCLUSIONS

1. Analysis of the spectra indicates that changes in the gluten structure and in the distribution of water populations are connected with the type of technological processes from which the dietary fibre supplement originated and hence its chemical composition.

2. The supplementation of the wheat dough with dietary fibre supplements leads to the formation of extended hydrated chains. Vegetable supplements induce the formation of a larger number of these structures than oil supplements.

3. Vegetable supplements cause the formation of aggregated structures such as pseudo-β-sheets, whereas gluten samples modified with oil supplements contain mainly basic secondary structures *i.e.* α-helices, β-turns and antiparallel-β-sheets.

4. With regard to the water populations, oil supplements do not affect them or affect them slightly. Vegetable supplements lead to the formation of a weaker gluten network. This is observed in the form of a decrease in the number of strong hydrogen bonds.

Conflict of interest: The authors declare no conflict of interest.

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