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INFLUENCE OF HIGH-VOLTAGE ELECTRICAL DISCHARGE TREATMENT ON OIL BINDING CAPACITY OF FAVA BEAN PROTEIN AND COCOA SHELL

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Introduction. Formulation of the problem

Oil binding capacity (OBC) is the ability of a matrix to retain oil within its three-dimensional network. This property is of special importance in oleogels, which are recently extensively studied as alternatives for saturated fats in foods. The entrapment of oil within the three-dimensional network of fibre, protein or starch is important from the aspect of texture, mouthfeel, and stability of food products with oleogels [1]. Often, native proteins and fibre are not sufficiently effective in entrapment of oil, and are modified to

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Abstract. Food industry generates large amounts of side-streams, from waste water to by-products, leaving large mark on the environment. Food industry by-products should not be treated as waste, as they contain numerous compounds that may be used in food, feed, pharmaceutical products and cosmetics. Plant-based by-products are source of fibre, pigments, bioactive compounds, and often good sources of protein. In addition, new European regulation regarding sustainable supply of cocoa will reduce available quantities of cocoa and raise cocoa prices in the European Union. This increases the need for better use of cocoa bean, e.g. use of cocoa shell. Therefore, the aim of this research was to examine potential of food industry by-product: cocoa shell and novel protein source: fava bean protein for binding liquid oils for the purpose of application in food industry. The potential of high voltage electrical discharge treatment in improving this ability was investigated. Different treatment conditions (time: 30 s – 20 min, frequency: 20 – 80 Hz; injection of air and N₂) were explored and binding of sunflower oil was determined after each treatment. The results showed that high-voltage electrical treatment with injection of gas improves oil binding capacity of cocoa shell and fava bean protein, with the maximum value of 3.41 g/g observed for cocoa shell treated for 10 min at 20 Hz with air injection. Some investigated treatment conditions caused the partial loss of phenolic compounds in cocoa shell, and all investigated treatments reduced foaming capacity of fava protein. The results are a good ground for further research of use of cocoa shell and fava bean protein as oleogel raw-materials for production of substitutes of saturated and hydrogenated fats in food products. Further research is needed for optimization of high-voltage electrical treatment conditions, and exploration of oleogel production.

Keywords: high voltage electrical discharge (HVED), oil binding capacity (OBC), fibre, protein, polyphenols, foaming capacity.

enhance this ability. It has been established that high voltage electrical treatment (HVED) may be used as tool for enhancing oil binding of proteins and fibre.

Analysis of recent research and publications

HVED is a process in which intense electric field is established between two electrodes in contact with food or aqueous system. This generates shock waves, UV radiation, generation of reactive species such as ozone, free radicals, NO_x, electric winds, and surface charging [2]. Application of high-voltage treatment in the form of pulsed electric field increases water-holding

and oil-holding capacity, gel strength, changes in secondary and tertiary structure of proteins, and moderate electric fields reduce free SH content and particle size of protein, changing its secondary and tertiary structure and surface hydrophobicity [2]. Thongkong et al. [3] reported increase of OBC after treatment of rice bran proteins with PEF and Agoua et al. [4] reported improved OBC of β -lactoglobulin after HVED treatments.

Fibres are also modified by the influence of HVED. Our previous research showed that ratio of soluble/insoluble fibre is changed and that tannins are formed as a result of HVED treatment of cocoa shell [5] and Brahim et al. [6] reported improved delignification, better enzymatic digestibility and heat resistance of lignins extracted from rapeseed straw by HVED. As a result, OBC may be increased, as shown in our previous research [7].

However, the effect of HVED on OBC is material- and condition-dependant, so optimizing energy input, field strength and treatment time is needed.

The purpose and objectives of the study.

Therefore, the aim of this research was to examine the influence of different conditions of HVED treatment on OBC values of cocoa shell and fava bean protein. Cocoa shell was selected as a by-product from chocolate industry with great potential for use in food production due to high contents of fibre and phenolic compounds, while fava bean protein is a novel protein ingredient at the market, offered as plant-based alternative for animal proteins. In addition to OBC examination, samples of treated matrices were chosen to monitor changes in nutritional (cocoa shell) and functional (fava protein) properties that might influence their applicability in food.

Research materials and methods

Fava bean protein was kindly provided by Nutris, Novi Senkovac, Croatia. Cocoa shell was obtained from the local chocolate producer, after deshelling roasted cocoa beans.

Treatment of cocoa shell and fava bean protein was conducted in custom-made equipment for high-voltage electrical treatment designed by Faculty of Food Technology Osijek and Ingeniare, Osijek. The impulse generator had a frequency range of 20 – 100 Hz, medicinal needle was used as discharge electrode, and ground electrode was 45 mm plate. For each treatment, new needle was used.

For the purpose of HVED treatment, cocoa shell was milled in IKA MF 10 mill for 1 min and 300 mL of 10% suspension in distilled water was taken for treatment. Fava bean protein was treated as a 20% suspension.

Treatments were conducted at 20, 40, 60 and 80 Hz, for 30 s, 1 min, 5 min, 10 min or 20 min, without gas injection, with nitrogen and with air, in four replicates.

After the treatment, samples were centrifuged at 3000 rpm for 20 min (CentraIEC) to remove surplus water and dried in convection oven at 50 °C. Afterwards, samples were milled in IKA mill with 1 mm sieve.

Oil binding capacity was determined by preparing a suspension of 2.5 g of the sample in 30 mL of sunflower oil (Tena, Čepin, Croatia). Suspension was vortexed every 5 min, and after 30 min samples were centrifuged at 3000 rpm for 15 min. Oil binding capacity was calculated according to Eq. 1. Three replicates were done for each sample.

$$OBC (g/g) = \frac{\text{mass of the residue after centrifugation}}{\text{mass of the sample}} \quad (1)$$

The influence of HVED treatment on *phenolic compounds in cocoa shell* was screened on selected samples from treatments without injection of gas to obtain comparable results to our previous research. Samples with the highest OBC values from each set of samples were taken (10 min/20 Hz, 1 min/60 Hz, 5 min/60 Hz, 10 min 80 Hz, 20 min/80 Hz). Samples were defatted prior to analysis by triple extraction with n-hexan, and phenolic compounds were extracted twice with 70% methanol, assisted by ultrasound. Total phenolics were determined according to method of Singleton et al. [8].

Foaming capacity and emulsifying activity indices of fava bean protein were screened on samples treated 5 min at 20 Hz, 5 min and 20 min at 40 Hz, 10 min and 20 min at 80 Hz. For *foaming capacity* (FC), distilled water was added to 5 g of sample until 100 mL volume was reached. Suspension was mixed with hand mixer at maximum speed for 5 min and afterwards, the volume of the formed foam was determined. Foaming capacity (FC) was calculated from the Eq. 2:

$$FC (\%) = (\text{foam volume}/\text{initial volume}) \times 100 \quad (2)$$

For *emulsifying activity index* (EAI), 0.96 g of protein was suspended in 96 mL of distilled water and 24 mL of sunflower oil was added. The mixture was sheared at 12000 rpm during 3 min. 0.01 of emulsion was taken to 5 mL of water containing 0.1 g SDS/100 g water and vortexed. The absorbance of the suspension was measured at 500 nm and emulsifying activity index was calculated according to Eq. 3:

$$EAI (m^2/g) = (2 \times 2.303 \times A_0 \times N) / (c \times \psi \times 10000) \quad (3)$$

Where: A_0 , absorbance of the emulsion immediately after preparation,

N , dilution factor (500)

C , mass concentration of protein in suspension prior emulsification,

Ψ , volume fraction of oil in emulsion.

To test the difference between treatments, results for OBC were analysed using Wilcoxon Rank-Sum test in which statistic value is converted into a p-value. A lower p-value reflects a value that is more significantly

different across samples. Statistical difference for total phenolic content, foaming capacity and emulsifying activity index was determined using one-way ANOVA at $p < 0.05$.

Results of the research and their discussion

Oil binding capacity values of the cocoa shell and fava bean protein treated with HVED are shown in Table 1. For cocoa shell, it is evident that gas injection increases oil binding capacity regardless of time and frequency of treatment, with more pronounced effect of air. For fava bean protein, injection of nitrogen and air had similar effect, with very close values of OBC obtained after both treatments. On the other hand, when comparing different treatment times for the same frequency and different frequencies for the same treatment times, no visible trend is revealed. For instance, for HVED treatment without gas injection at 40 Hz, the parabolic curve is formed with the minimum at 5 min duration, and sinusoid curve is formed at the treatment 60 Hz. In most cases, different treatment times of fava bean protein by HVED with N₂ did not have marked effect on OBC: at 20 Hz, virtually the same values were obtained from 1 min to 20 min treatment (2.15 – 2.17 g/g), at 40 Hz 2.20 g/g was observed for treatments from 30 s to 5 min etc. Evidently, it is hard to predict and control the extent of physical and chemical changes occurring during HVED treatment. When a high-voltage electric fields is applied between two electrodes in water suspension, reactive species are generated, along with cavitation, UV emission and

shock-waves [9, 10]. The performance of the process is the combined result of the energy input, number of pulses, electrode distance, liquid-to-solid ratio, type of the treated matrix, type of solvent, duration of the process [10, 11].

The plasma channel between the electrodes may be formed through developing gaseous phase and/or by ionization of the solvent [12]. This phenomenon is revealed in the present research as well: generally, the treatment without gas injection resulted with lower OBC values at all treatment times (Fig. 1), and all frequencies (Fig. 2) for both investigated matrices (cocoa shell and fava bean protein), indicating more severe changes during treatment when gases were applied. Among reactive species formed during high voltage electrical treatment in presence of air and/or N₂ are nitric oxide compounds. [13] and reactive oxygen species [14]. Larger number of these oxidative compounds will form in presence of air/N₂, giving more pronounced effect on matrix change.

The statistical difference (Wilcoxon rank sum test p -value of order 10^{-16}) between HVED treatment without and with injection of gas is visible from the median and mean values shown in Table 2 as well, showing positive effect of gas injection during treatment on OBC values.

The air was more effective (statistical difference at Wilcoxon rank sum test p -value of order 10^{-16}) when cocoa shell was treated, while for fava bean protein difference between N₂ and air was not statistically significant (Wilcoxon rank sum test p -value 0.2263) (Table 3).

Table 1 – Oil binding capacity of cocoa shell and fava bean protein (*Vicia faba* L.) treated with HVED in different conditions

Treatment	Oil binding capacity (g/g)					
	Cocoa shell			<i>Vicia faba</i> L.		
	without gas	with N ₂	with air	without gas	with N ₂	with air
30 s 20 Hz	2.66±0.09	3.25±0.02	3.31±0.02	1.71±0.05	2.25±0.04	2.19±0.06
1 min 20 Hz	2.50±0.06	3.20±0.05	3.32±0.05	1.80±0.00	2.17±0.08	2.17±0.03
5 min 20 Hz	2.58±0.03	3.18±0.01	3.31±0.01	1.86±0.00	2.16±0.09	2.26±0.03
10 min 20 Hz	2.71±0.04	3.22±0.02	3.41±0.02	1.77±0.04	2.15±0.02	2.16±0.03
20 min 20 Hz	2.66±0.05	3.29±0.06	3.38±0.02	1.77±0.00	2.16±0.05	2.22±0.03
30 s 40 Hz	2.68±0.06	3.22±0.06	3.29±0.02	1.82±0.02	2.20±0.06	2.16±0.02
1 min 40 Hz	2.61±0.03	3.16±0.04	3.31±0.02	1.78±0.01	2.20±0.12	2.15±0.02
5 min 40 Hz	2.57±0.01	3.22±0.04	3.35±0.03	1.93±0.06	2.20±0.06	2.21±0.02
10 min 40 Hz	2.61±0.02	3.17±0.07	3.30±0.02	1.83±0.00	2.12±0.04	2.20±0.01
20 min 40 Hz	2.72±0.04	3.18±0.02	3.33±0.02	1.86±0.01	2.12±0.09	2.16±0.05
30 s 60 Hz	2.69±0.06	3.18±0.02	3.34±0.04	1.75±0.01	2.13±0.02	2.17±0.02
1 min 60 Hz	2.74±0.02	3.15±0.08	3.28±0.03	1.72±0.02	2.10±0.05	2.17±0.02
5 min 60 Hz	2.72±0.01	3.10±0.03	3.30±0.03	1.77±0.02	2.25±0.18	2.14±0.05
10 min 60 Hz	2.66±0.06	3.18±0.02	3.28±0.03	1.80±0.00	2.11±0.08	2.16±0.01
20 min 60 Hz	2.72±0.05	3.25±0.02	3.25±0.07	1.77±0.03	2.14±0.02	2.17±0.03
30 s 80 Hz	2.67±0.06	3.19±0.04	3.26±0.04	1.71±0.00	2.15±0.07	2.14±0.03
1 min 80 Hz	2.68±0.00	3.18±0.02	3.31±0.02	1.73±0.01	2.21±0.12	2.24±0.02
5 min 80 Hz	2.67±0.04	3.24±0.02	3.18±0.02	1.73±0.02	2.24±0.05	2.19±0.03
10 min 80 Hz	2.89±0.07	3.27±0.01	3.26±0.02	1.85±0.01	2.25±0.06	2.25±0.02
20 min 80 Hz	2.74±0.01	3.30±0.03	3.25±0.03	1.90±0.03	2.24±0.10	2.23±0.04

Untreated cocoa shell: OBC 2.34(±0.04) g/g, untreated *Vicia faba* L. OBC 1.96(±0.01) g/g

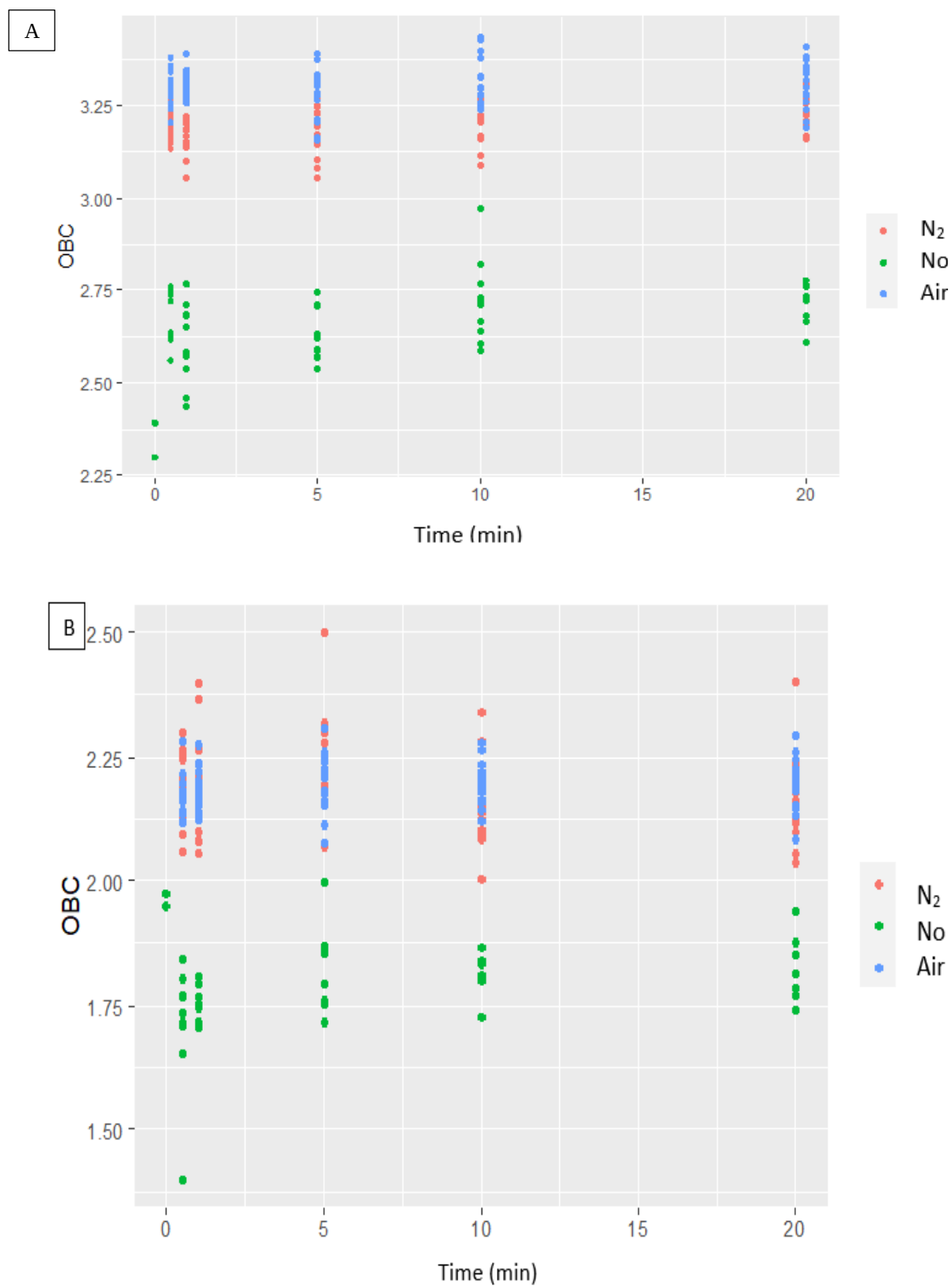


Fig. 1. Scatter diagram of oil binding capacity (OBC) of cocoa shell (A) and fava bean protein (B) based on time of treatment

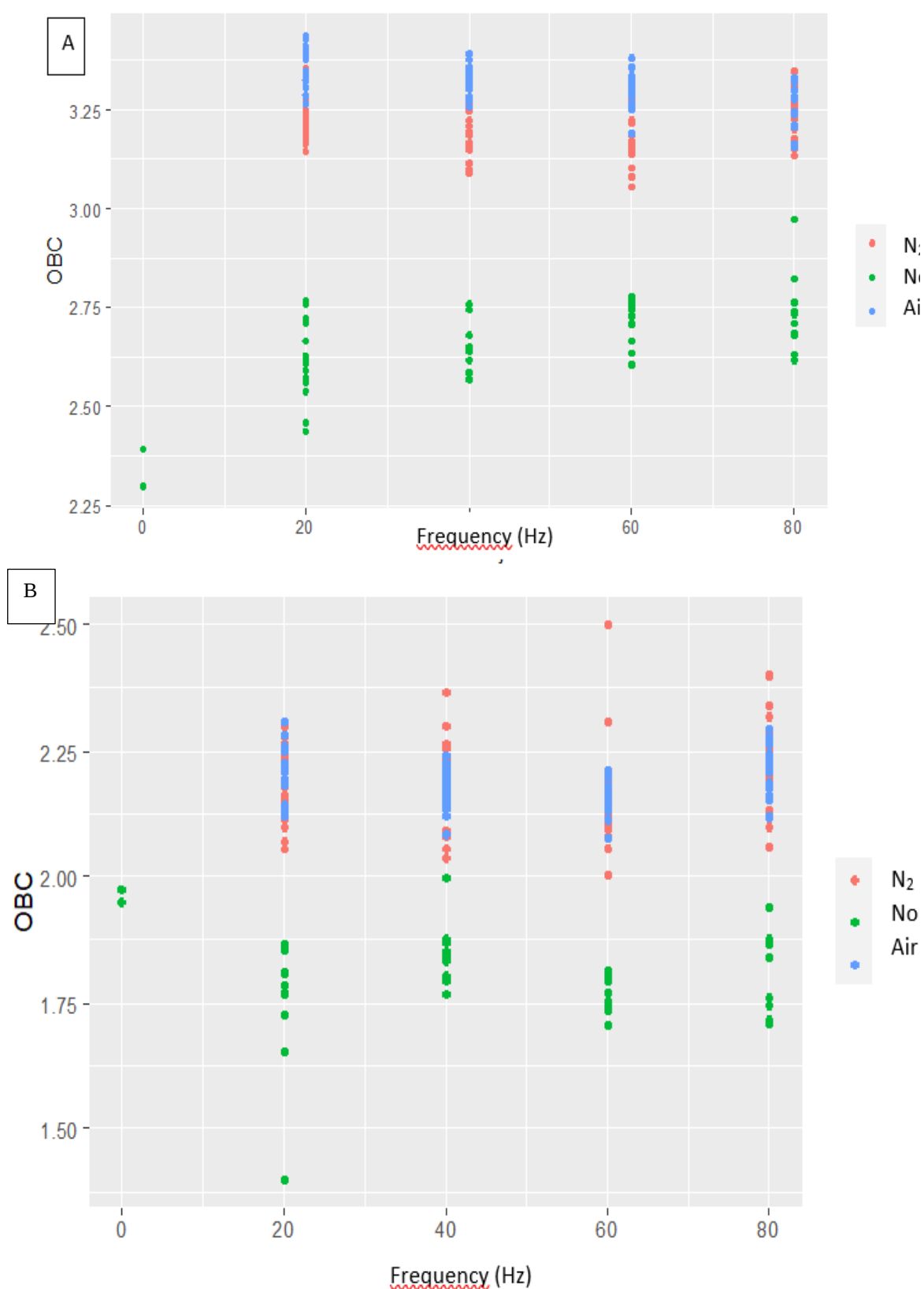


Fig. 2. Scatter diagram of oil binding capacity (OBC) of cocoa shell (A) and fava bean protein (B) based on frequency

Table 2. Influence of gas injection on OBC of HVED-treated cocoa shell and fava bean protein (*Vicia faba* L.)

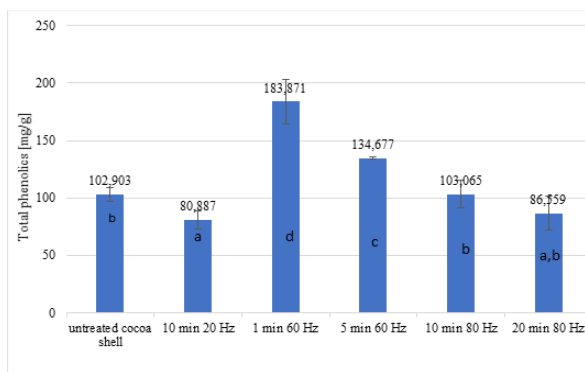
OBC	Cocoa shell		<i>Vicia faba</i> L.	
	without gas	with gas	without gas	with gas
N	50	160	43	160
Median (IQR)	2.66 (2.59, 2.73)	3.26 (3.20, 3.30)	1.80 (1.75, 1.85)	2.18 (2.14, 2.22)
Mean (SD)	2.66 (0.11)	3.25 (0.08)	1.80 (0.10)	2.18 (0.07)
Range	2.30, 2.97	3.06, 3.44	1.40, 2.00	2.00, 2.50

Table 3. Influence of type of gas injected during treatment on OBC of HVED-treated cocoa shell and fava bean protein (*Vicia faba* L.)

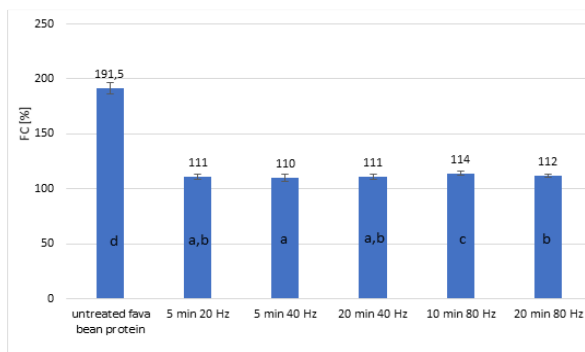
OBC	Cocoa shell		<i>Vicia faba</i> L.	
	N ₂	Air	N ₂	Air
N	80	80	80	80
Median (IQR)	3.21 (3.17, 3.25)	3.30 (3.27, 3.34)	2.17 (2.12, 2.22)	2.19 (2.15, 2.22)
Mean (SD)	3.21 (0.06)	3.30 (0.06)	2.18 (0.09)	2.19 (0.05)
Range	3.06, 3.35	3.16, 3.44	2.00, 2.50	2.08, 2.31

In addition to fibre, nutritional value potential of cocoa shell lies in phenolic compounds that are highly represented in it. The present research revealed 102.9 mg/g of phenolic compounds in the untreated sample. Martinez-Inda et al. [15] reported 9.15 mg/g of phenolics in the ethanol extract of cocoa bean shell, and Disca et al. [16] reported 2.89 – 6.37 mg phenolics/g of cocoa bean shell, depending on the extraction method. Fakhlai et al. [17] reported values similar to ours for roasted cocoa shell. The 10 min-treatment at 20 Hz and 20 min-treatment at 80 Hz reduced the content of phenolics in cocoa shell, while other screened treatments resulted in the increase of their contents. HVED has been reported as an effective method of extraction of polyphenols from *Satureja montana* L. [18], grape pomace, pomegranate peel etc. [12], but the effectiveness of the process depends on many, afore mentioned, factors. Abbaspour et al. [12] emphasize that stability of polyphenols is a challenge for their extraction via HVED. They may undergo oxidation, form pyrocatechol, hydroquinone, resorcinol, oxidize to quinones or condensate into more complex compounds. In addition, the method of determination of phenolics is based on oxidation reaction with Folin-Ciocalteu reagent and certain substances that are either not phenols or seldom thought of as phenols also contribute to the result [8]. Therefore, higher contents of phenolics determined after some HVED treatments in the present research may be a result of interfering compounds formed during the treatment (such as free radicals or oxydising compounds), although our previous research showed that part of polyphenolic compounds, such as (-)-epicatechin gallate remain retained in the matrix [19].

When proteins are considered, along with their obvious nutritional values as source of amino acids, they have an important functional role in food, such as foaming [20] and emulsifying [21, 22].

**Fig. 3. Total phenolics in untreated and cocoa shell treated by high-voltage electrical discharge without gas injection (bars with different letters are statistically different, $p < 0.05$)**

Foaming capacity of fava bean protein in the present research reduced after all screened HVED treatments, without significant difference between the treatments (Fig. 4).

**Fig. 4. Foaming capacity (FC) of untreated and fava bean protein treated by high-voltage electrical discharge without gas injection**

Foaming properties of protein depend on high solubility in the aqueous phase [20, 23] and their adsorbing at air-water interfaces, forming viscoelastic interfacial layers through molecular rearrangements [24]. Apparently, solubility of fava protein decreased after HVED treatment, probably due to partial unfolding and exposure of hydrophobic groups [25]. However, this should be further investigated by a more detailed characterization of protein after the treatments.

Structural changes of fava bean protein caused by HVED treatment are further supported by reduction of emulsifying activity indices, shown in Fig. 5.

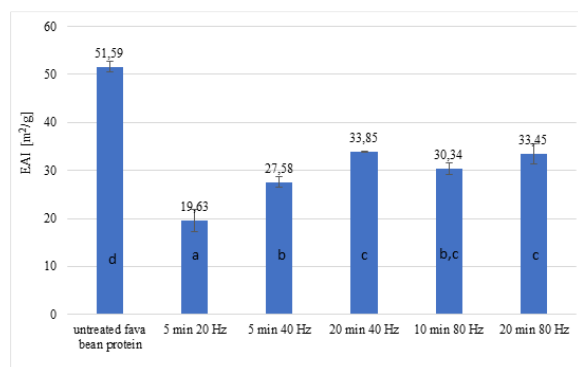


Figure 5. Emulsifying activity index (EAI) of untreated and fava bean protein treated by high-voltage electrical discharge without gas injection

The most pronounced effect was observed after the 5 min-treatment at 20 Hz, whereas, differences between two treatments at 80 Hz and the 20 min-treatment at 40

Hz was less pronounced. The emulsifying activity of protein shows its ability to reduce surface tension at the oil-water interface and stabilise the system [20], and the values obtained in this research show better emulsifying properties of fava bean protein compared to rapeseed proteins in research of Chen et al. [20].

Conclusion

The present research has shown that HVED conditions impact oil binding capacity of cocoa shell and fava bean protein. Generally, injection of gas improves oil binding capacity of both fibrous and protein matrices, where fibres are influenced by the type of gas – better properties are achieved with air injection compared to N₂, and protein is not. The largest values of OBC were observed for cocoa shell treated for 10 min at 20 Hz with air injection (3.41 g/g).

HVED reduced total phenolic contents of cocoa shell, and foaming capacity and emulsifying activity of fava bean protein, showing negative impact on nutritional and functional properties. However, further optimization, as well as more detailed characterization of modified substrates might reveal more positive effects of HVED treatment

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