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An investigation of the influence of pH and ionic strength on the adsorption and interfacial dilatational properties at the oil–water interface of pumpkin (*Cucurbita pepo*) seed protein hydrolysate

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Abstract: Pumpkin (*Cucurbita pepo*) seed protein hydrolysate (PSPH) was obtained by enzymatic hydrolysis of pumpkin seed protein isolate using pepsin. Influence of pH (3, 5 and 8) and ionic strength, I_c (0–1 mol dm^{-3}), on the adsorption kinetics of PSPH (diffusion rate constant, k_{diff}, and adsorption rate constant, k_{ads}), interfacial pressure (π) and interfacial dilatational properties (dilatational elasticity, E', and viscosity, E'') of the oil–PSPH solution interfaces was investigated at different PSPH concentrations (c = 0.0014–14 g dm^{-3}). It was found that PSPH adsorbs to the interface at c > 0.0014 g dm^{-3}, regardless of pH and ionic strength, as evidenced by the increase in interfacial pressure. The k_{diff} and k_{ads} value were found to be the highest at pH 3 and the lowest at pH 5 at the corresponding concentrations. The dilatational properties of the interfaces, which were investigated at different oscillation frequencies, ν, 0.01–0.2 Hz, showed that the E' of the oil–PSPH solution interfaces is much higher than its E''. Moreover, E' increases with increasing PSPH concentration at pH 5 and 8, and with increasing I_c, regardless of the pH, while E'' changes only minimally.

Keywords: natural surfactants; enzymatic hydrolysis; oil–water interface; interfacial dilatational rheology; dilatational elasticity.

INTRODUCTION

Many food products are emulsion based-systems the preparation of which requires the use of surfactants to stabilize their large interfacial area.[1,2] Surfactants adsorb to the interface, thereby reducing the interfacial tension and affect

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the mechanical properties of the interface, *i.e.* they change the way the interface responds against deformation. Since these systems are in most practical cases subjected to dynamic conditions, the dynamic interfacial tension and interfacial rheology are relevant in many technical applications, such as mass transfer, foaming, emulsification, oil recovery or high-speed coating.[1,3,4]

Proteins are of particular interest in terms of their surface activity, due to their amphiphilic nature and interfacial film-forming properties.[5,6] Namely, unlike small molecular weight surfactants that diffuse rapidly to the interface and have excellent emulsion forming properties, proteins tend to be bulkier and diffuse at a much slower rate, but, once at the interface, they develop strong viscoelastic films that resist mechanical stresses, and provide steric stabilization in addition to electrostatic stabilization of the droplet.[2,5,7] The interfacial behavior of proteins (adsorption, structure, mechanical properties, *etc*.) depends on their physical, chemical, and conformational properties (size, shape, amino acid composition and sequence, charge and charge distribution, *etc.*), which are affected by environmental factors such as pH and ionic strength.[8,9] In particular, the functionality of proteins is limited at their isoelectric point (p*I*) and at increased ionic strength (I_c),[4] which is most often at pH ≈5 and I_c>0.5 mol dm^{-3}, conditions typical for food formulations.[8] One way to increase protein functionality under these particular conditions is the utilization of enzymatic hydrolysis. The enzymatic modification of protein molecular structure yields polypeptide mixtures with lower molecular weight and less secondary and tertiary structures in comparison to the native protein. Accordingly, the functionality of hydrolyzed proteins could be enhanced over a wide range of pH and other processing conditions, *i.e.*, hydrolyzates could stabilize a larger interfacial area, their diffusional transport and affinity for adsorption to the interface is accelerated and the formation of more robust adsorption films at the oil–water interface is enabled.[4,5,10,11] In addition, protein hydrolysis improves nutritional and bioactive properties, such as digestibility, antioxidant properties and reduces allergenic properties.[12,13]

Therefore, enzymatic hydrolyzates are of special interest nowadays, especially those of plant origin since they can be a good replacement for animal-based proteins, they increase food safety and sustainability, they have greater consumer/market acceptability and they can be obtained from cheap and renewable resources.[6,8–10,14] One of such hydrolyzates is pumpkin (*Cucurbita pepo*) seed protein hydrolysate (PSPH), which can be obtained from an oil cake, a by-product of the oil industry with a high protein content (up to 65 %).[15] Hitherto, PSPH were reported on their bioactivity in terms of antioxidant and antiradical activity and blood pressure-lowering (ACE inhibitory) effects,[15–17] whereas investigations on their functional properties focused mainly on their solubility. In this regard, the solubility of PSPH was found to be significantly improved in comparison to the solubility of PSPI over a wide range of pH and ionic strength

values, especially when pH = p*I* of PSPI, *i.e.*, pH 5, where an up to four-fold increase in solubility was observed.[18] Moreover, enzymatic hydrolysis was found to improve foaming and emulsifying properties of pumpkin seed proteins but proper understanding of its behavior at the interface under dynamic conditions is still lacking.[16,18] In fact, supporting data on interfacial film forming properties of protein hydrolyzates in general are scarce, while their interfacial film forming properties under different conditions of pH and ionic strength have not hitherto been investigated.

The aim of this study was to investigate influence of pH (3, 5 and 8) and ionic strength (0–1 mol dm^{-3}) on the adsorption kinetics, dynamic interfacial pressure and interfacial dilatational properties at oil–PSPH solution interfaces, where PSPH was obtained by enzymatic hydrolysis of pumpkin (*Cucurbita pepo*) seed protein isolate.

EXPERIMENTAL

Materials

Pumpkin (*Cucurbita pepo*) seed oil cake was obtained from "Agrojapra", Bosnia and Hercegovina. It was stored at the temperature of 4 °C and ground in a coffee grinder before use. Caprylic/capric triglyceride oil was obtained from "Centrohem d.o.o.", Serbia. Ultrapure MilliQ water was used as a solvent. Pepsin (0.7 FIP–U mg^{-1}) and all other chemicals were obtained from Sigma–Aldrich Co. and were of at least extra pure quality. Buffer solutions were prepared by mixing 0.2 mol dm^{-3} di-sodium hydrogenphosphate and 0.1 mol dm^{-3} citric acid in proportions defined for each pH value.

Enzymatic hydrolysis

Enzymatic hydrolysis was realized on pumpkin seed protein isolate (PSPI) using pepsin. PSPI was obtained according to a procedure described in Bučko *et al.*[18] A PSPI suspension (10 g dm^{-3}) was prepared by suspending the required amount of PSPI in an aqueous solution of pH 3, where the pH was set and controlled by the addition of 1 mol dm^{-3} HCl. The enzymatic hydrolysis was performed in a batch reactor at 37 °C and an enzyme to substrate ratio of 0.02 g g^{-1}. The reaction conditions were set so that the degree of hydrolysis was 19±1 % at the end of the reaction. The enzymatic hydrolysis was completed after 90 min. The degree of hydrolysis and the conditions of the enzymatic hydrolysis, which were selected as optimal, were based on previous experiments on functional properties of enzymatically hydrolyzed cucurbitin, the main protein fraction of PSPI.[16] The hydrolyzed suspension was then vacuum filtered and the filtrate was dried on a "Büchi 190" spray drier at an inlet temperature of 120 °C and outlet temperature of 70 °C to obtain pumpkin seed protein hydrolysate (PSPH). Molecular weight of the thus obtained hydrolysate is below 20 kDa and zeta potential are 20.5±0.5, 0.72±0.4 and –3.41±1.7 mV at pH 3, 5 and 8, respectively.[18] PSPH is composed of 92.13±1.70 % proteins, 5.78±0.00 % moisture and 2.13±0.04 % ash.[18]

Determination of the degree of hydrolysis (DH)

The degree of hydrolysis (DH) was determined according to a slightly modified method of Tsumura *et al.*[19] Namely, the same volumes of hydrolyzated suspension and trichloracetic acid (0.44 mol dm^{-3}) were mixed and incubated at 4 °C, for 30 min. Thereafter, the mixture was centrifuged (Eppendorf mini Spin Plus, 14500 rpm, 10 min). Obtained 0.22 mol dm^{-3}

trichloroacetic acid soluble protein fraction and the hydrolysate mixture without addition of trichloroacetic acid were each analyzed to determine the protein content by the Lowry *et al*. method[20] using bovine serum albumin as the standard protein. *DH* was calculated as the ratio of 0.22 mol dm^{-3} trichloroacetic acid soluble proteins to total proteins in the hydrolysate, expressed as a percentage.

PSPH dissolution

PSPH suspensions were prepared by suspending the required amount of PSPH powder in a buffer solution of different pH (3, 5 or 8) and ionic strength, I_c (0–1 mol dm^{-3}), in order to obtain a PSPH solution of 14 g dm^{-3}, according to previously determined PSPH solubility profiles by Bučko *et al.*[18] (Table I). The ionic strength was manipulated by the addition of the required amount NaCl.

TABLE I. Influence of pH and I_c on PSPH solubility[18]

pH	I_c / mol dm^{-3}	Solubility, %
3	0.0	127
	0.1	126
	0.5	121
	1.0	104
5	0.0	106
	0.1	100
	0.5	100
	1.0	73
8	0.0	91
	0.1	91
	0.5	94
	1.0	102

The suspensions were agitated at room temperature (25 °C) during 40 min, in order to allow dissolution. The soluble hydrolyzates were separated from the undissolved particles by centrifugation (Eppendorf centrifuge 5415C) at 10000 rpm for 20 min to obtain a PSPH solution. The PSPH solutions were diluted with buffer to the required concentrations, c_{sol}, in range 0.0014–14 g dm^{-3}.

Measurement of interfacial pressure and interfacial dilatational properties

Measurement of the interfacial tension of an oil–PSPH solution was realized using a drop profile analysis tensiometer PAT1 (SINTERFACE Technologies, Germany). A pendant droplet of oil was formed at the tip of a stainless steel capillary that was surrounded by a PSPH solution. After formation of a fresh droplet at the capillary tip, the interfacial tension was monitored as a function of time, during 60 min at room temperature (24±1 °C). The basic principle of the method is to fit the theoretical drop profile given by the Laplace equation of capillarity to the experimental drop profile, where the surface tension is generated as the fitting parameter. Other parameters, such as contact angle (when sessile drops are used), drop volume, surface area, and three-phase contact angle, can also be obtained.

The measured interfacial tensions of oil–PSPH solutions were used to calculate the corresponding interfacial pressures:

$$\pi = \sigma_0 - \sigma \quad (1)$$

where σ_0 and σ are the interfacial tension of the oil–buffer solution and the oil–PSPH solution, respectively, measured 60 min after drop formation.

Sixty minutes after the oil drop had formed in a protein solution, a sinusoidal perturbation was induced at the interface by injecting and extracting liquid into and out of the droplet, respectively. The oscillations were performed under the conditions of a constant amplitude of 10 % at five different frequencies, 0.01, 0.02, 0.04, 0.1 and 0.2 Hz, with a resting period of 100 s between each frequency change. The surface area perturbations lead to a respective harmonic surface tension response. Subsequently, Fourier transformation was performed on the experimental data to obtain the dilatational parameters of the interfacial layer. The surface dilatational modulus is a complex term, first derived by Gibbs, as the change in interfacial tension (dilatational stress) induced by a small change in surface area, A (dilatational strain). The Gibbs dilatational modulus exhibits two contributions: an elastic component, accounting for the recoverable energy stored in the interface (storage modulus or dilatational elasticity, E'), and the dissipative component, accounting for energy lost through relaxation processes (loss modulus or dilatational viscosity, E''). By using a complex notation, a quantity, the complex dilatational modulus E^*, can be introduced. E^* is composed of a real and an imaginary part, related to the elastic and viscous component, respectively:[9,11]

$$E^* = E' + iE'' = \frac{\Delta\sigma}{\Delta A / A_0}\cos\delta + i\frac{\Delta\sigma}{\Delta A / A_0}\sin\delta \quad (2)$$

where A_0 is the unperturbed interfacial area of the drop, $\Delta\sigma$ and ΔA are the measured changes in stress and strain amplitude, respectively, and δ is the phase angle between stress and strain. For an ideal elastic material, stress and strain are in phase, $\delta = 0°$, and the imaginary term is zero. In the case of ideal viscous material $\delta = 90°$ and the real part is zero. The measurements were performed in triplicate and average data are presented.

Adsorption kinetics data analysis

The rate of protein adsorption to the interface can be modeled *via* diffusion, penetration, and rearrangement mechanisms. During the first step, at relatively low surface pressures when diffusion is the rate-determining step, a simplified form of the Ward and Tordai equation was used to correlate the change in the surface pressure with time:[9,21]

$$\pi = 2ck_bT(Dt/3.14)^{1/2} \quad (3)$$

where D is the diffusion coefficient, k_b is the Boltzmann constant, T is the absolute temperature, and t is time. Linear dependence in the plot of π against $t^{1/2}$ indicates that the kinetics of adsorption process is controlled by the rate of protein diffusion to the interface. The linear slope in this plot was used to obtain the diffusion rate constant.[8,9,21] At longer adsorption times, the rate of adsorption decreases indicating that a protein molecule has to pay an additional energy penalty in order to adsorb to the interface containing previously adsorbed protein molecules. The adsorption kinetics is now controlled by rate of protein penetration to the interface, their unfolding, and rearrangement at the interface, which could be described by applying a first-order phenomenological equation:

$$\ln((\pi_{3600}-\pi_t)/(\pi_{3600}-\pi_{400})) = -k_i t \quad (4)$$

where π_{3600}, π_t and π_{400} are the interfacial pressures after 3600 s, at any time t, and at the initial time, respectively, and k_i is the first-order rate constant. The plot of the equation usually gives two or more linear regions. The first linear region is associated with the process of penetration and unfolding, while the second liner region is associated with molecular rearrange-

ment at the interface.[8,9,21] In this work the first linear region was used to obtain the adsorption rate constant, k_{ads}, which corresponds to the rate of protein penetration and their unfolding at the interface. However, no attempt was made to discuss the experimental data for the second rearrangements step of previously adsorbed protein molecules because protein adsorption at fluid interfaces is a very time consuming process.

RESULTS AND DISCUSSION

The influence of pH (3, 5 and 8) and ionic strength (0–1 mol dm^{-3}) on the interfacial properties of PSPH solutions at different concentration (0.0014–14 g dm^{-3}) were investigated. The influence of time on the interfacial pressure, π, of representative oil–PSPH solutions (c = 0.14 g dm^{-3}, I_c = 0 mol dm^{-3}) at three different pH is illustrated in Fig. 1.

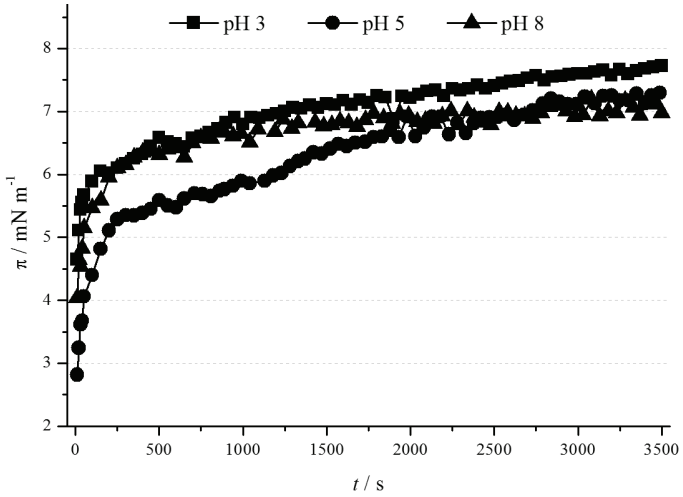

Fig. 1. The influence of time on the interfacial pressure, π, of representative oil–PSPH solutions (c = 0.14 g dm^{-3}, I_c = 0 mol dm^{-3}) at three different pH.

PSPH adsorbs at the interface at each pH, as indicated by the increase in interfacial pressure. The value of π increased throughout the measurements, regardless of the solution pH. Initially, π increased sharply due to the population of the bare interface by PSPH molecules, while as time preceded, the increase in π becomes more gradual.[9,21–23]

The influence of the concentration of the PSPH solution on the value of π for an adsorption time of 3000 s at three different pH values is shown in Fig. 2. It can be seen that π strongly depends on the PSPH solution concentration as evidenced by its continuous increase as PSPH concentration increased. At the lowest concentration, c = 0.0014 g dm^{-3}, the PSPH solutions at pH 5 and 8 had a minimal influence on the value of π, which remained ≤1 mN m^{-1}, whereas at pH 3, π increased by 5 mN m^{-1}. However, further increase in PSPH concentration

brought about more steep increase in π at pH 5 and 8 in comparison to the increase in π at pH 3. Namely, at pH 5 and 8, π increased by >10 mN m^{-1} when c was increased from 0.0014 to 14 g dm^{-3}, while at pH 3, the increase was ≈5 mN m^{-1}. Such influences of the pH of the PSPH solution on the interfacial pressure could be attributed to the differences in the solubility of PSPH and the zeta potential at different pH values. Namely, PSPH is most soluble at pH 3, which allows its molecules to diffuse readily to the interface and therefore, the interfacial pressure decreases even at low solution concentrations. However, at pH 5 and 8, the PSPH molecules are more aggregated because of their lower solubility, which could account for lower ability of PSPH to increase the surface pressure at the lowest concentration.[4,8,9,18] On increasing the concentration of the PSPH solution, the interface becomes more and more populated with PSPH molecules, and further adsorption is governed by charge effects from the adsorbed and adsorbing PSPH molecules.[4] The zeta potential of PSPH at pH 3 is 20.5 mV, while at pH 5 and 8, they are close to zero.[18] Therefore, charge repulsion between the adsorbing and adsorbed PSPH molecules at pH 3 limits further adsorption when PSPH concentration in solution is increased, which consequently results in only a modest increase in π on increasing c. As opposed to this, minimal zeta potential at pH 5 and 8 results in the absence of charge repulsion.

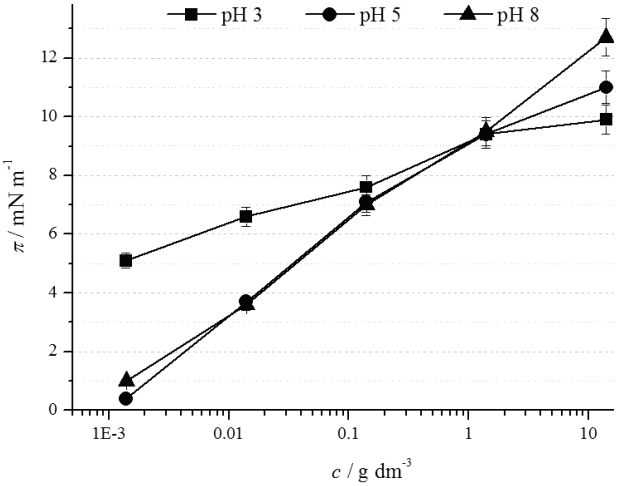

Fig. 2. Influence of the concentration of the PSPH solution on π, after 3000 s of adsorption at three different pH.

PSPH diffusion from the bulk phase to the subsurface and subsequent adsorption to the interface could be described in more detail by the diffusion rate constant, k_{diff}, and the adsorption rate constant, k_{ads}. The results of the determination of k_{diff} and k_{ads} of representative PSPH solutions at each pH value are presented in Fig. 3a and b, respectively, where k_{diff} was determined as the slope

of the linear plot of π against $t^{0.5}$ (Fig. 3a) and k_{ads} as the first linear region in the plot of $\ln[(\pi_{3600}-\pi_t)\ (\pi_{3600}-\pi_{400})^{-1}]$ against time (Fig. 3b).[8,9,21]

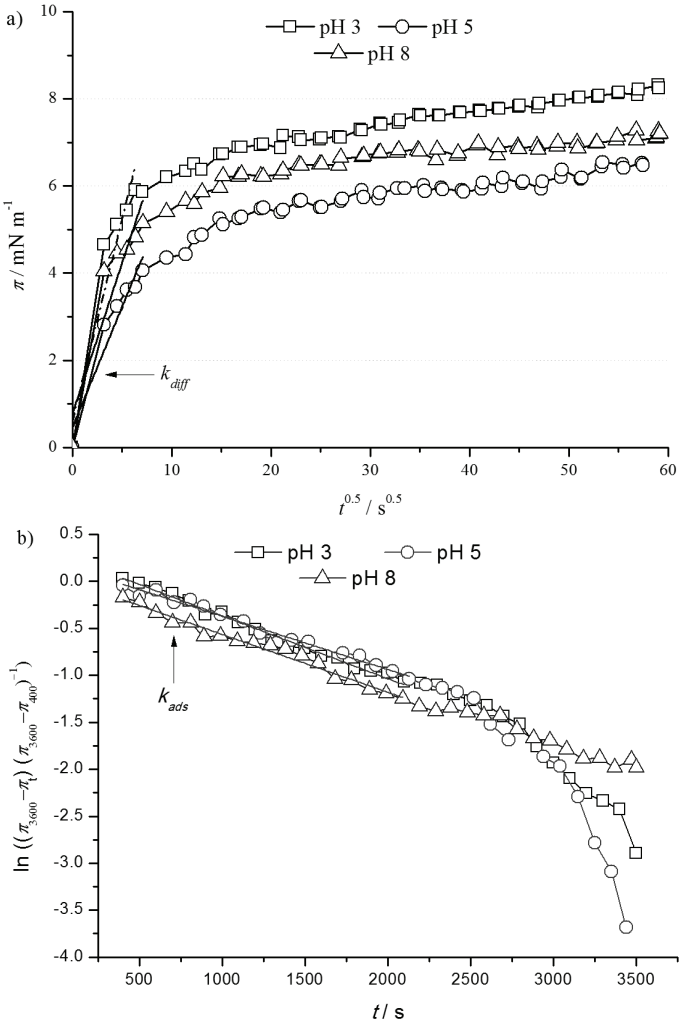

Fig. 3. Graphical determination of a) k_{diff} and b) k_{ads} of representative oil–PSPH solutions at different pH values; c=0.14 g dm^{-3}.

The diffusion rate constants, k_{diff}, and the adsorption rate constants, k_{ads}, obtained for PSPH solutions of different concentrations (0.0014–14 g dm^{-3}) at three different pH values (3, 5 and 8) are presented in Table II.

The k_{diff} increases as the concentration of the PSPH solution increases, regardless of the pH, whereby, at the two highest concentrations (1.4 and 14 g dm^{-3}), the diffusion rate was too fast to allow k_{diff} determination by the method

used in this study. The k_{diff} was also found to be affected by the pH of the solution. The highest k_{diff} was obtained for PSPH solutions at pH 3, when compared to the PSPH solutions at pH 5 and pH 8 at the corresponding concentrations, while the lowest k_{diff} of 0.01 mN m^{-1} s$^{0.5}$ was exhibited by PSPH solutions at the lowest concentration at both, pH 5 and pH 8. This is in agreement with the trends of increasing π on increasing c (Fig. 2). The diffusion phase is followed by the adsorption phase – a phase where the adsorption is limited by the rate of the protein adsorption to the interface, rather than by the rate of diffusion, which arises as a result of the energy barrier increasing as the interface becomes more populated by the molecules.[9,22] The k_{ads} decreases as PSPH concentration increases, with the exception for the lowest concentrations, at all three pH values investigated. Among the pH values studied, k_{ads} was the highest at pH 3 and the lowest at pH 5 at corresponding concentrations (Table II). These results are in agreement with the influence of pH and the hydrolysate concentration on k_{diff} and k_{ads} obtained for enzymatic hydrolyzates of soy glycinin and β-conglycinin.[8,9]

TABLE II. Diffusion rate constants, k_{diff}, and the adsorption rate constants, k_{ads}, obtained for PSPH solution of different concentrations (0.0014–14 g dm^{-3}) at three different pH values (3, 5 and 8); I_c = 0 mol dm^{-3}

pH	c / g dm^{-3}	k_{diff} / mN m^{-1} s$^{0.5}$	k_{ads} / 10^{-4} s^{-1}
3	14	–	–
	1.4	–	5.9±0.4
	0.14	0.82±0.2	7.5±1.3
	0.014	0.22±0.0	9.7±0.1
	0.0014	0.26±0.0	6.0±0.1
5	14	–	–
	1.4	–	3.8±0.4
	0.14	0.55±0.1	4.2±0.3
	0.014	0.06±0.0	5.4±0.3
	0.0014	0.01±0.0	1.5±0.3
8	14	–	–
	1.4	–	5.3±0.3
	0.14	0.69±0.1	5.5±0.5
	0.014	0.08±0.0	6.5±0.3
	0.0014	0.01±0.0	4.2±0.4

The dilatational properties of the oil–PSPH solution interfaces were characterized by measuring the response of the interface to introduced sinusoidal perturbations at different frequencies, 0.01, 0.02, 0.04, 0.1 and 0.2 Hz, and subsequent calculation of the dilatational parameters of the interface, *i.e.*, dilatational elasticity, E', and dilatational viscosity, E''. The influence of the oscillation frequency, ν, on the E' and E'' values of representative PSPH solutions (c = 0.14 g dm^{-3}; I_c = 0 mol dm^{-3}) at pH 3, 5 and 8 are presented in Fig. 4.

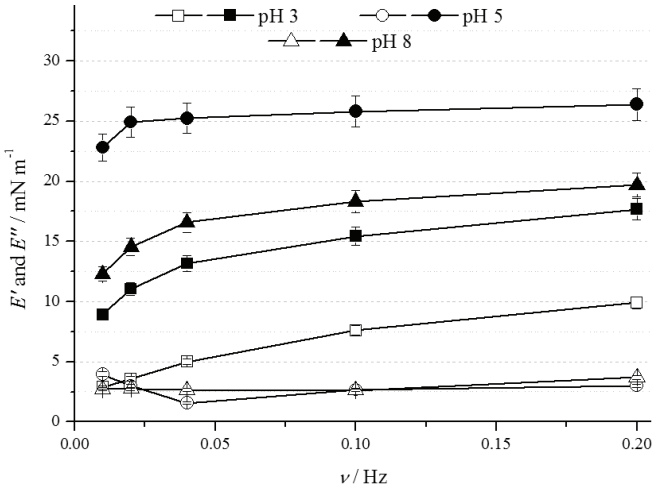

Fig. 4. The influence of the oscillation frequency on the dilatational elasticity, E', and the dilatational viscosity, E'', of representative PSPH solutions. E': closed symbols; E'': open symbols; c = 0.14 g dm^{-3}; I_c = 0 mol dm^{-3}.

It was found that E' increases with increasing oscillation frequency and tends to a plateau value at higher frequencies, regardless of the solution pH. The higher is the PSPH solution concentration, the more frequency independent E' becomes (data not shown). The influence of the oscillation frequency on E'' is more complex. Nevertheless, the obtained E'' values were a few times lower than E' at all tested frequencies, which is a characteristic that PSPH shares with protein hydrolyzates of soy proteins and β-lactoglobulin.[11,24,25]

The influence of PSPH solution concentration on the dilatational elasticity, E', and dilatational viscosity, E'', of the oil–PSPH solution interfaces at three different pH values (3, 5 and 8) and ν = 0.02 Hz is illustrated in Fig. 5.

Increasing the PSPH concentration at pH 3 affected dilatational elasticity only slightly, while at pH 5 and 8, increasing the PSPH concentration increased E' significantly, Fig. 5. At the lowest concentration, the highest E' value was obtained for the interface of the oil–PSPH solution at pH 3, while at any other concentration, the E' at pH 5 takes preponderance. The lower E' at c = 0.0014 g dm^{-3} at pH 5, as well as at pH 8, than at pH 3 could be ascribed to the lower drive of PSPH molecules towards the interface, as evidenced by the lower k_{diff} and k_{ads} at pH 5 and 8 in comparison to the k_{diff} and k_{ads} at pH 3 (Table II). The E'' values remained <4 mN m^{-1}, which are much lower than the E′ values, regardless of pH and PSPH concentration, indicating that the interfacial relaxations, which are manifest in E'', seem to be reduced due to the simple internal structure of the PSPH molecules, and/or they may be occurring more rapidly than the measurement timeframe.[24]

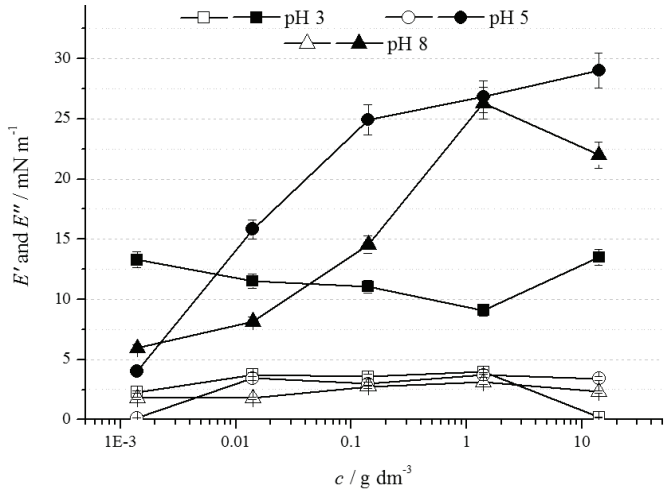

Fig. 5. The influence of PSPH solution concentration on the dilatational elasticity, E', and dilatational viscosity, E'', of oil–PSPH solution interface at three different pH (3, 5 and 8). E': closed symbols; E'': open symbols; I_c = 0 mol dm^{-3}; ν = 0.02 Hz.

Influence of ionic strength

Influence of the ionic strength, I_c (0–1.0 mol dm^{-3}), on the interfacial pressure of the oil–PSPH solution interface at different pH values is shown in Fig. 6.

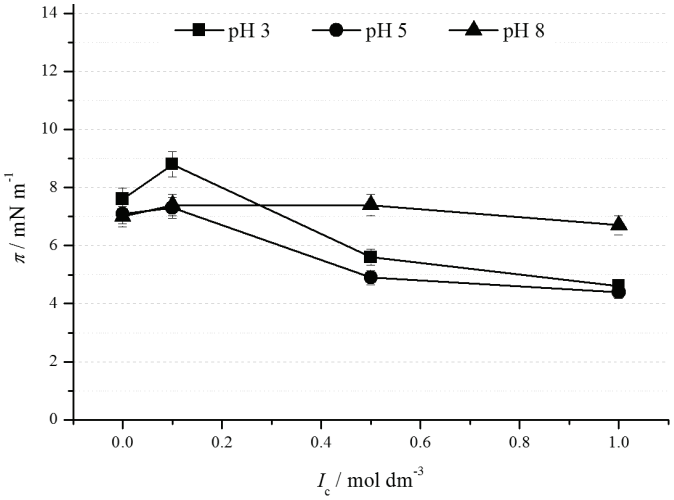

Fig. 6. The influence of ionic strength on the interfacial pressure, π, of PSPH after 3000 s at different pH values; c = 0.14 g dm^{-3}.

An increase in I_c to 0.1 mol dm^{-3} slightly increased π at all pH values, while further addition of NaCl brought about a decrease in π at pH 3 and 5. At pH 8, π

remained almost constant at all tested I_c values. The negative influence of the increased I_c is in accordance with the influence of I_c on solubility. Namely, at pH 3 and 5, the solubility decreased while at pH 8, the solubility increased upon increasing ionic strength, indicating that aggregation retards the diffusion of PSPH molecules at pH 3 and 5 and, consequently, impairs the interfacial activity PSPH.

The influence of ionic strength on k_{diff} and k_{ads} values of PSPH solutions at different pH values are presented in Table III.

TABLE III. The influence of ionic strength on k_{diff} and k_{ads} of PSPH solutions at different pH values, c = 0.14 g dm^{-3}

pH	I_c / mol dm^{-3}	k_{diff} / mN m^{-1} s$^{0.5}$	k_{ads} / 10^{-4} s^{-1}
3	0.0	0.82±0.2	7.5±1.3
	0.1	0.76±0.1	8.9±0.2
	0.5	0.34±0.0	8.7±0.2
	1.0	0.11±0.0	6.5±0.2
5	0.0	0.55±0.1	4.2±0.3
	0.1	0.48±0.1	6.9±0.3
	0.5	0.23±0.0	6.6±0.1
	1.0	0.26±0.0	6.5±0.1
8	0.0	0.69±0.1	5.5±0.5
	0.1	0.64±0.2	4.6±0.4
	0.5	0.53±0.1	6.1±0.3
	1.0	0.26±0.1	9.1±0.5

The addition of NaCl resulted in a decrease in k_{diff}, regardless of the pH. The k_{diff} decreased by more than twice its value at pH 3 and 5 as the ionic strength was increased from 0 to 0.5 mol dm^{-3}, as opposed to the much lower decrease at pH 8, which implies increased aggregation of the PSPH molecules with increasing I_c. The k_{ads} value at pH 3 and 5 first increased at I_c = 0.1 mol dm^{-3}, whereas further increases in the ionic strength resulted in its decrease. On the contrary, at pH 8 the k_{ads} was found to decrease on increasing the I_c to 0.1 mol dm^{-3}, while further addition of NaCl brought about an increase in k_{ads}.

The influence of the ionic strength on the E' and E'' of the oil–PSPH solution interfaces at different pH at ν = 0.02 Hz is illustrated in Fig. 7.

Increasing the ionic strength only slightly affected the dilatational viscosity of oil–PSPH solution interfaces. At the same time, the dilatational elasticity was found to increase upon NaCl addition, suggesting that the formation of the intermolecular interactions was enhanced by the reduction in electrostatic repulsion. The most prominent increase in E' was at pH 3, the pH at which PSPH charge was the highest (20.5 mV).[18] E'' remained much lower than the E' at all the tested pH values.

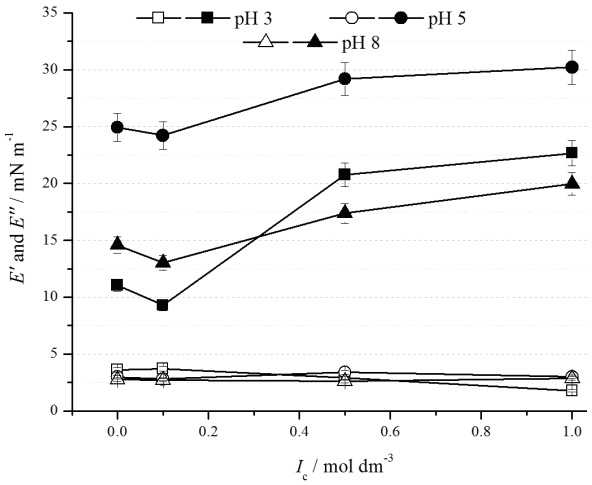

Fig. 7. The influence of the ionic strength on the dilatational elasticity, E', and dilatational viscosity, E'', of oil–PSPH solutions at different pH values. E': closed symbols; E'': open symbols; c = 0.14 g dm^{-3}; ν = 0.02 Hz.

CONCLUSIONS

The influence of pH (3, 5 and 8) and ionic strength, I_c, (0–1 mol dm^{-3}) on the adsorption and dilatational properties of caprylic/capric triglyceride oil–PSPH solution interface was investigated at different PSPH concentrations (0.0014–14 g dm^{-3}). PSPH was found to adsorb to the interface at all the tested pH values as evidenced by the increase in interfacial pressure at PSPH solution concentrations higher than 0.0014 g dm^{-3}. The diffusion rate constant, k_{diff}, and adsorption rate constant, k_{ads}, were found to be affected by the pH of the solution. The highest values of both k_{diff} and k_{ads} were obtained at pH 3, while the lowest values were obtained at pH 5. The dilatational properties of the oil–PSPH solution interfaces were described by the dilatational elasticity, E', and dilatational viscosity, E''. The value of E' was found to be dependent on the pH and PSPH solution concentration while the aforementioned conditions had little effect on the value of E''. Nevertheless, the obtained values for E' were found to be a few times higher than values of E'' at all the tested pH values and PSPH solution concentrations. Increasing the ionic strength up to 1 mol dm^{-3} resulted in a decrease in k_{diff}, regardless of pH, and a decrease in π, especially at pH 3 and 5. On the other hand, it induced an overall increase in the dilatational elasticity of the oil–PSPH solution interfaces at all three pH values.

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ИЗВОД

ИСПИТИВАЊЕ УТИЦАЈА pH И ЈОНСКЕ ЈАЧИНЕ НА АДСОРПЦИЈУ И ДИЛАТАЦИОНЕ ОСОБИНЕ ПРОТЕИНСКИХ ФИЛМОВА НА ГРАНИЦИ ФАЗА УЉЕ–РАСТВОР ПРОТЕИНСКОГ ХИДРОЛИЗАТА СЕМЕНА ТИКВЕ (*Cucurbita pepo*)

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Ензимском хидролизом протеинског изолата семена тикве (*Cucurbita pepo*) пепсином добијен је протеински хидролизат семена тикве, PSPH. Особине PSPH као што су кинетика адсорпције на границу фаза уље–раствор хидролизата (константа брзине дифузије, k_{diff}, и константа брзине адсорпције, k_{ads}), међуповршински притисак, π, као и реолошке особине протеинских адсорпционих филмова (дилатациони еластицитет, E', и дилатациони вискозитет, E'') испитиване при различитим условима средине, pH вредности (3, 5 и 8) и јонске јачине, I_c (0–1 mol dm^{-3}), и различитој концентрацији PSPH (0,0014–14 g dm^{-3}). PSPH се адсорбује на границу фаза при концентрацији >0,0014 g dm^{-3} без обзира на pH вредност средине, судећи по повећању површинског притиска. Утврђено је да k_{diff} и k_{ads} имају навише вредности на pH 3 док су најниже вредности забележене на pH 5, на одговарајућим концентрацијама PSPH. Дилатационе особине протеинских адсорпционих филмова, које су испитиване при различитим фреквенцијама осциловања, ν, 0,01–0,2 Hz, указују на то да је дилатациони еластицитет протеинских филмова на граници уље–PSPH раствор много израженији од дилатационог вискозитета. Поред тога, E' се повећава са повећањем PSPH концентрације на pH 5 и 8, као и са повећањем I_c, независно од pH, имплицирајући ефикасније формирање интермолекуларних интеракција између PSPH молекула адсорбованих на граници фаза.

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