Surface Properties of Whey Protein Gels

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Summary: Surface properties of whey protein gels are reviewed based on traditional microscopic techniques and new methods, as optical profilometer and contact angle measurements. Optical profilometer is an instrument allowing measurement of surface roughness and contact angle measurements to determine the surface wettability behavior (hydrophobicity/hydrophilicity) of the gels. Investigation of surface properties of whey protein gels is very important, as it can transform this product to a new level of application. It could be used as a matrix for an active ingredient release, material for tissue engineering, e.g. scaffolds, i.e. temporally structures biodegraded in the human organism.

Keywords: Microscopy, Wettability, Contact angle, Roughness, Surface hydrophobicity, Spectroscopy, Rheology.

Introduction

Whey proteins are becoming very popular as functional ingredients in foods. Their application in food products will increase in the future, as consumers pay more and more attention on valuable sources of exogenous amino acids. It is connected with increasing interest of population in sport and recreation. Whey proteins are generally marketed in three forms: whey protein concentrate, whey protein isolate and whey protein hydrolysate [1]. The concentrate contains fat and lactose along with the quintessential proteins (29–89%); the isolate is made of 90% protein and the hydrolysate is the semi-digested form of the protein. Whey protein enriches foods in the most valuable amino acids and shapes their texture, mouthfeel, water and flavor holding capacity [2, 3]. Gelation is the most important functional property of whey protein and it is a key process to generate food texture. Properties of the gel depend on the ionic strength of the solution, temperature and the time of heating. Heating of whey protein solution above the denaturation temperature (greater than 65° C), causes unfolding and aggregation of proteins. After cooling down, at low ionic strength, a thick solution can be obtained. Addition of ions will result in electrical shielding of charges and formation of a gel [4, 5]. Whey protein gel microstructure has been observed to change from fine stranded to particulate with increasing salt concentration [6]. The properties of whey protein gels are greatly modified by the presence of dispersed filler particles, for example oil droplets, within the protein gel matrix, due to interfacial filler particle gel matrix interactions [7 – 10]. The gelling ability of proteins provides important textural and water-holding properties in many foods [11 – 13]. The mechanism of heat-induced gelation of whey proteins is not completely understood. Former investigations interpreted the gelation as a two-phase process consisting of unfolding of the globular structure, and subsequent aggregation of protein chains into a three-dimensional network. Today, gelation of whey proteins is basically considered as a four-phase process consisting of unfolding of the native structure, aggregation of the unfolded protein molecules, string formation of the aggregates, and linkage of the strings to a three-dimensional network [14]. Partially stable intermediates of the three-dimensional structure of whey proteins, called the "molten globule state", are of particular importance during gelation [12, 15]. The formation of heat-induced whey protein gels, mainly due to disulfide bridges and hydrophobic interactions, is irreversible [14, 16, 17]. The structure and texture of the gels depend on protein concentration, ion strength and type as well as on pH value, temperature and degree of denaturation [14, 16 – 19]. Furthermore, the origin of the whey (rennet casein cheese or acid casein) as well as the operations used for concentrating,

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isolating and/or fractionating proteins is particularly relevant for the resulting properties of the whey protein products [14, 20].

The cold gelation process itself is induced by adding salt or acid to the heat-treated protein solutions [21 – 24]. Comparative studies on the compositional, physicochemical and functional properties of whey protein concentrate (WPC) and whey protein isolate (WPI) are widely described in the literature [20, 25 – 28]. In this review different techniques of investigating surface properties of whey protein gels are presented. Due to growing interest in the study of biopolymers, this paper aims to review up-to-date publications on the surface properties of whey protein gels investigated by traditional and the recently applied technologies.

Methods/Techniques for the analysis of the surface properties of whey protein gel

Traditional Microscopic Methods

Traditionally the surface of the gels is observed using different types of microscopy. For the examination of the gel structure different types of microscopy (scanning electron microscopy (SEM) [5, 29 - 33], atomic force microscopy (AFM) [29, 34 - 38], transmission electron microscopy (TEM) [39 - 41] or confocal laser scanning microscopy (CLSM) [42 - 48] are applied. Some of them require a special sample preparation. The combination of microscopy with other techniques allows the direct study for the investigation of gel structure. The choice of the adequate method for the examination of the structure of the gels is essential for a successful research in this field. A broad variety of microscopic methods has been applied for whey protein gels and it is beyond the scope of this review to discuss them. We are focused on some interesting examples from research conducted by our research group, especially aerated gels.

Confocal laser scanning microscopy (CLSM)

Confocal laser scanning microscopy (CSLM) is very suitable for observing the structure of gels without finer details such as casein micelles or protein clusters [49 – 51]. It enables to observe gel structure without any preparation procedure, as the laser beam penetrates the sample at a desired depth, without changes in the gel properties [52]. The microstructure of aerated gels was investigated using confocal laser scanning microscopy [53]. At different pH, a very interesting structure of air bubbles was observed, larger bubbles contained smaller ones and these contained other smaller bubbles. This microstructure resembled a fractal structure. Ikeda *et al*. [54] reported a fractal nature of whey protein gels. Similarly, the fractal nature of the whey protein gels surface was investigated by Chen and Dickinson [55]. Tomczyńska-Mleko and Mleko [56] showed that different microstructure of the ion induced aerated gels was obtained at different ions. For Ca^{2+} induced gels a particulate gel was formed and for Fe^{2+} and Mg^{2+} fine-stranded microstructure was noticed. The aerated gel induced by Fe^{2+} ions was the most transparent. Air bubbles were composed of fractal structures as larger bubbles contained smaller ones in the same geometrical pattern. Kharlamova *et al*. [46] studied whey protein isolate gels formed by acidification of fractal aggregates with different sizes. The microstructure was analyzed by CSLM and it was found, that varying the protein composition, the microstructure changed drastically. The images showed more homogeneous structure when the protein content increase. Similar microstructure was observed by Ju and Kilara [57] for gels formed from whey protein isolate by adding GDL or $CaCl_2$.

Scanning electron microscopy (SEM)

Comparing this technique with light microscopy, the resolution is notably improved with electron microscopy. This technique has been used to study representative surface structure of whey protein gels and to assess their homogeneity. The microstructure of ion induced whey protein aerated gels has been investigated by Tomczyńska-Mleko [5]. It was reported that at higher calcium concentration more porous structure of particulate whey protein gel was obtained. It resulted in higher syneresis. An opposite effect of syneresis was observed for increased protein concentration. It was caused by more packed gel structure with higher number of bonds between protein molecules. Fine-stranded gel microstructure produced at pH values far from the isoelectric point is characterized by lower porosity with lower syneresis [58]. Similar results were observer by Liu *et al*. [33]. They investigated the influence of Ca^{2+} and Na^{+} ions on the properties of whey protein isolate/lotus root amylopectin composite gel system. The SEM images showed a high number of pores in the network of WPI-LRA gels without salt ions, however the addition of Ca^{2+} (0.05M-0.1M) resulted in a denser and more homogeneous microstructure, in case of Na^{+} the images showed a more compact gel microstructure than without Na^{+} (0.5M). They concluded that an appropriate amount of Ca^{2+} or Na^{+} results in a more compact and stable gel network structure with compact cavities. In SEM images, the addition of salt

ions in low concentration resulted in a denser and more homogeneous gel network, however large cavities and irregular structure in the gel network appeared increasing the salt concentration.

Aerated whey protein isolate gels were obtained at different pH using reversibility of heat-induced gels [53]. SEM micrographs indicated that at different pH (3.0, 9.0, 10.0) a smooth gel microstructure was observed. At these pH values, repulsive electrostatic force between charged protein chains is effective [22]. Tomczyńska-Mleko *et al*. [59] observed aerated whey protein gels induced by calcium ions on SEM micrographs. They showed that increased ions concentration causes higher aggregation of protein matrix and more porous microstructure is present at the interface of the gel and air. Similar changes were observed by Croguennec *et al*. [60]. This is in an agreement with results presented by Tomczyńska-Mleko *et al*. [61], which demonstrated that at higher magnesium ions concentrations, stronger ionic interactions were possible with more magnesium bridges being formed. This resulted in a more aggregated structure. Maltais *et al*. [62] observed fine-stranded microstructure for a gel at low salt concentration (10 mM of calcium chloride) and a particulate, unordered gel structure was noticed at higher calcium chloride concentration. At higher salt concentration, the surface microstructure becomes rougher. Nayebzadeh *et al*. [63] observed natural, not-dried mixed whey protein/xanthan gels using a modified, steam filled column method of scanning electron microscopy. Microscopic images were used for calculation of roughness these mixed gels surface. It was noted that xanthan has a surface smoothing effect on the heat-set whey protein gels.

Transmission electron microscopy (TEM)

Transmission electron microscopy allows observing the microstructure of the gels at very high magnitude. For a new high-resolution transmission electron microscopy, it is possible to obtain above 50 million times magnification with a resolution of 1 Ångström. The problem is with the preparation of the sample which must be cut into ultrathin section less than 100 nm thick. It is possible to observe the surface section of the gel, but usually this technique is used to observe protein gels in terms of the degree of aggregation and the shape of protein aggregates. Tomczyńska-Mleko *et al*. [61] showed denser microstructure of the gels with increased magnesium ion concentration which coincided with more elastic behavior. Increased elasticity of the gels was caused by a higher number of bonds between whey protein molecules. A higher concentration of divalent magnesium ions probably caused a stronger screening effect, which facilitated more powerful hydrophobic interactions [61]. Recently Jiang *et al*. [39] used the transmission electron microscopy to compare the microstructure characteristics of polymerized whey protein isolate and concentrate. The TEM images revealed that the network of the isolate form was more homogeneous, stable and denser than the concentrate form. Uzun el al. [41] proposed an interesting way for developing delivery system. The morphology of dried WPI gels containing lutein droplets was observed by TEM~~,~~. The images of gels confirmed that the process preserved the original size of lutein droplets.

New methods

Optical profilometer

Optical profilometer is an instrument allowing measurement of surface roughness. Microscopic methods do not allow for quantification of this property. There are very scarce research studies on whey protein gel surface using optical profilometry.

Optical profilometry is a non-contact technique which uses a light source to investigate the surface. The key component to this technique is directing the light in a way that it can detect the surface in 3D. Optical profilometry is faster than contact profilometry with sacrifices in lateral resolution. It is completely non-destructive to samples that are not sensitive to light and very soft surface can be scanned. Based on the images, the computer program calculates the parameters determining the surface properties. The gels roughness is described using three different parameters: Ra, average roughness, is the main height as calculated over the entire measured length or area. It is useful for detecting general variations in overall profile height characteristics. Rq is a quadratic mean of the surface roughness and is given by the standard deviation of the vertical values for the gel area. The root means square index RMS (Rq) is the best parameter to compare roughness of the gels, as it is insensitive to local surface topographical heterogeneity. Rt represents the distance between the highest peak and the lowest valley on the measured gel surface. Generally, gels with more aggregated microstructure have surfaces with higher values of roughness parameters. Chen *et al*. [64] noticed that whey protein gel obtained without any salt addition had a very smooth surface with Rq and Ra of 0.20 and 0.18 μm, respectively. For the gel obtained at 200 mM of sodium chloride, a much rougher surface with

large Rq and Ra values were produced. It was caused by increased protein aggregation caused by high concentrations of salt [58]. Higher magnesium ion concentration produced gels with a rougher surface structure i.e. with a higher quadratic mean of the surface roughness Rq and maximum roughness height Ra [65]. Tomczyńska-Mleko and Mleko [56] reported that the roughness of the obtained gel surface depends on the type and concentration of added salt. Higher concentration of cations resulted in gels with a higher quadratic mean of the surface roughness and maximum roughness height. Aerated calcium ion induced gels were used for hydrolysis experiments in an artificial stomach [59]. There was a linear correlation between the quadratic mean of the surface roughness (Rq) and the maximum roughness height (Rt). Similar correlation was observed for the non-aerated egg white gels [66]. Surface roughness of the gels influences different active ingredients release from the gel [67]. Tomczyńska-Mleko and Mleko [56] found, that microstructure and different surface roughness of whey protein gels influenced contact area of the gels with pepsin, which resulted in different release time of active ingredients. In recent research Terpiłowski *et al*. [68] examined whether activation of glass support can influence surface properties of ion-inducted whey protein gels deposited on this support. The gel surface was observed using an optical profilometer. Increasing of ion concentration resulted in the rougher structure of the obtained gel (Fig. 1). It is in line with our previous results on the aerated whey protein gels [61]. Whey protein gel layer is smooth on the untreated glass while for the argon treated sample it is rough with big deep holes on the surface. Gels deposited on the air treated glass plate have also a different structure which is rougher than for untreated glass support (Fig. 2).

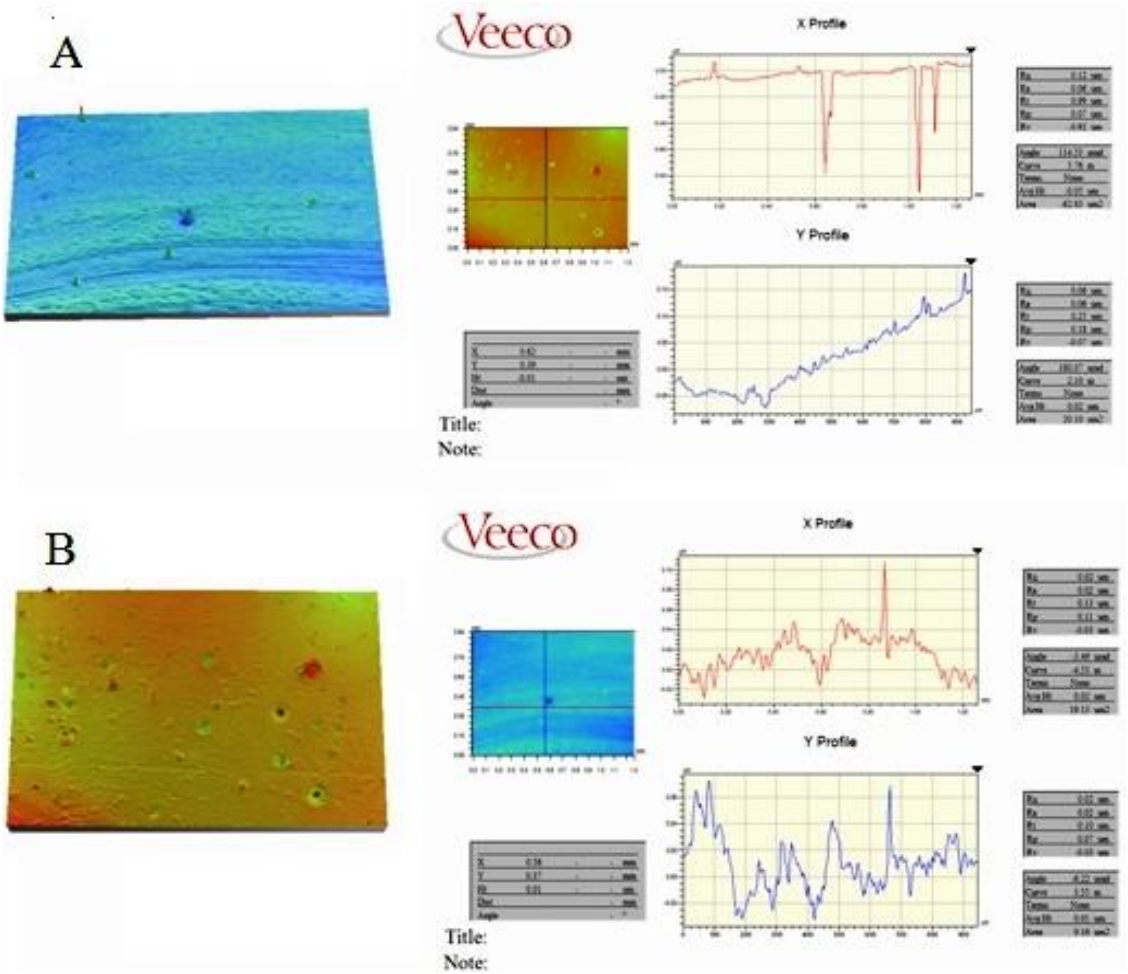
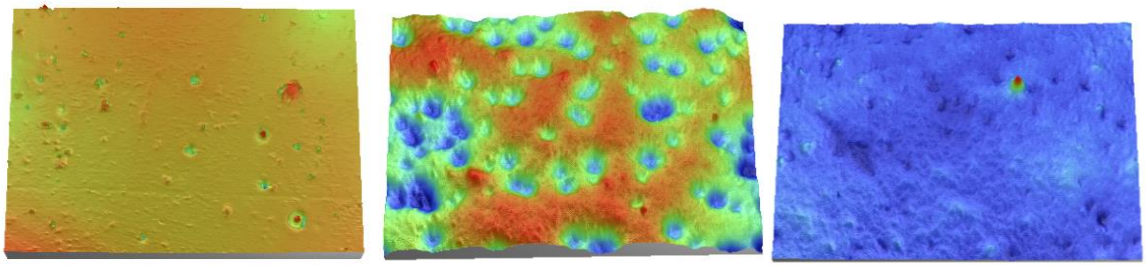


Fig. 1: Optical profilometer images (0.9 x 1.3 mm) and side profiles for whey protein gels deposited on untreated glass surface A: 20 mM Ca^{2+} , B: 30mM Ca^{2+}.



A B C

Fig. 2: Optical profilometer images (47 x 62 µm) for whey protein gels induced by 30 mM Ca^{2+} deposited on: A - untreated glass surface, B - Ar plasma activated glass and C - air plasma activated glass.

Contact angles and surface free energy (SFE)

Another important surface property is its wettability. It is a physical property relating to the ability of a liquid drop to spread on a surface of a solid or liquid material. The strength of the attractive or repellant force is closely related to the "contact angle" between the water drop and the surface (Fig. 3). When the solid has a high affinity for water, this kind of material is called hydrophilic (e.g. glass), the contact angle will be less than 90°. Water drop tends to spread out and "wet" the surface. In the opposite case of hydrophobic (e.g. Teflon) surface, the contact angle will be greater than 90°, and instead, forms at equilibrium a spherical cap resting on the substrate with a "contact angle", the water drop tends to bead up on the surface (Fig. 3). Measurements of contact angles require the solid surfaces to be rigid, smooth and homogeneous, so the Young's equation is calculated for the appropriate equilibrium condition. The solid surfaces should be as inert as possible, so that effects such as swelling and chemical reactions are minimized.

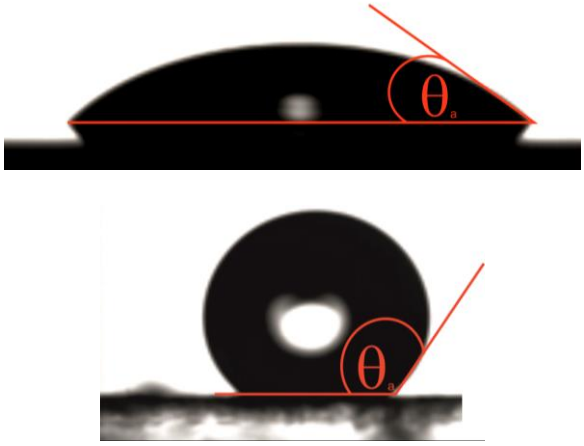


Fig. 3: Different contact angles for a hydrophilic (left) and a hydrophobic surface (right).

Two different theoretical approaches to interfacial interactions are presented for the determination of very useful parameter - surface free energy (SFE), which quantifies the characteristic of the solid surface and its wettability. Acid–base (LWAB) and hysteresis (CAH) approaches are presented bellow.

In the LWAB approach of Van Oss *et al.* [69] the surface free energy is showed as the sum of two constituents: a polar Lifshitz-Van der Waals γ_i^{LW} and Lewis acid–base γ_i^{AB} :

$$\gamma_i = \gamma_i^{LW} + \gamma_i^{AB} \quad \text{Eq. 1}$$

Besides dispersion interactions, the component γ_i^{LW} includes the dipole orientation and the induction ones which were considered to be polar earlier. The component of acid–base interactions γi^{AB} can be expressed by the geometric mean:

$$\gamma_i^{AB} = 2\,(\gamma_i^+ \gamma_i^-)^{1/2} \quad \text{Eq. 2}$$

Based on such model the adhesion work can be written by means of the constituents:

$$\gamma_l(1+\cos\theta) = 2\sqrt{\gamma_s^{LW}\gamma_l^{LW}} + 2\sqrt{\gamma_s^+\gamma_l^-} + 2\sqrt{\gamma_s^-\gamma_l^+} \quad \text{Eq. 3}$$

where: γ_l liquid surface tension, θ-contact angle (advancing), $\gamma_{s/l}^{LW}$ solid and liquid apolar Lifshitz van der Waals interactions, $\gamma_{s/l}^{+}$ solid and liquid electron acceptor parameter of surface free energy, $\gamma_{s/l}^{-}$ solid and liquid electron donor parameter of surface free energy. The quantity of the constituent γ_s^{LW} and the parameters γ_s^+, γ_s^- of surface free energy can be calculated from Eq. 3 by measuring the wetting angle of three different liquids with known

surface tension constituents: γ_l^{LW}, γ_l+ and γ_l-. A set of three equations with three unknown values (γ_s^{AB}, γ_s^+, γ_s^-) allows to calculate the energy components (γ_i^{LW}, γ_i^{AB}) and finally its total value. Very important in determination of surface free energy of solids is the selection of liquids used for measuring wetting angles [70]. The most used is a set of three liquids of which one is a polar liquid with high surface tension and the other two are polar liquids with differences in the quantities of γ_i^{LW}, γ_l^+ and γ_l^-. The most often the following liquids are used: diiodomethane (γ_l = 50, 8 mJ/m^2) or 1-bromonaphtalane (γ_l = 44.4 mJ/m^2) with formaldehyde and water as polar liquids [71].

The other approach was proposed by Chibowski [51 – 53] and is based on the contact angle hysteresis (CAH). It relates γ_S to the surface tension of the probe liquid γ_L and CAH, which is defined as the difference between the advancing θ_a and receding θ_r contact angles:

$$\gamma_S = \left(\frac{\gamma_L(1+\cos\theta_a)^2}{(2+cos\theta_a+cos\theta_r)}\right) \quad \text{Eq. 4}$$

where γ_s is apparent surface free energy, γ_L is liquid surface tension, θ_a is advancing contact angle, and θ_r is receding contact angle.

The apparent surface free energy can be calculated from Eq. 4 using the liquid surface tension and advancing and receding contact angles. The calculated free energy value depends on the physicochemical properties of the used liquid. For the equilibrium contact angles used for calculation of apparent surface free energy, Eq. 4 transforms into:

$$\gamma_s = \frac{\gamma_L}{2}\left(1+\cos\theta_{Eq}\right) \quad \text{Eq. 5}$$

where γ_L is liquid surface tension and θ_{Eq} is equilibrium contact angle.

Changes in the gel surface topography causes the changes in the wettability and the differences in the value of apparent surface free energy. For the hydrophilic surface the wettability can be described by the Wenzel model and for hydrophobic surface by the Cassie-Baxter model [72]. Nature of structure of gels can be explained by contact angle measurements. Białopiotrowicz [75] concluded that starch gel surface maintains maximal hydrophobic character with polar domains created by the functional glucose groups with the branched chain of amylopectin directed into air [76]. Tomczyńska-Mleko *et al*. [61] applied contact angles measurements to characterize surface properties of the aerated whey protein gels. It was reported that with increasing the concentration of the $MgCl_2$, the surface became more hydrophobic. Increasing the concentration of the $FeCl_2$ had a contrary effect: the surface became hydrophilic. So it was found that the obtained surfaces were influenced not only by the type of the added salt but also by their concentration. The pendant drop method was applied to determine the contact angle of whey protein gels. Moreover, the surface free energy was determined by hysteresis (CAH) and acid-base (LWAB) approaches in order to have more info about energetic changes on the surface. In CAH approach a decrease of SFE was observed with the addition of $MgCl_2$, however with the addition of $FeCl_2$ an increase of SFE was found. In the other approach (LWAB) the SFE was confined only to the dispersion components which increased after $MgCl_2$ addition, however a significant decrease of electron donor parameter was found. They conclude that the difference in SFE is caused by the change in surface topography. In a recent publication by Terpiłowski *et al*. [69] the contact angle values were assessed to determine the surface wettability behavior (hydrophobicity/hydrophilicity) of the ion-induced whey protein gels deposited on plasma activated glass support. For increased contact angles, an increase in surface roughness was observed. This relationship depended on the type of ion used for the gelation induction. Besides, wettability properties were affected by the electron donor parameter of energy obtained using (LWAB) approach and contact angles measurements, which value increased for the samples obtained on the plasma activated supports, especially the air treated ones. Kokoszka *et al.* [77] studied the wetting properties of whey protein isolate/glycerol mixed gels influenced by varying theirs proportions. It was found that the surface with higher plasticizer content showed more hydrophilic behavior. They concluded that the presence of the plasticizer decreased the protein-protein interaction and increased the chain mobility improving the water absorption. Similar results were obtained by Ramos *et al.* [78], but in this case for the whey protein concentrate/glycerol mixed gels, once again, the surface became more hydrophilic after the addition of glycerol. These results are consistent with the claim by Sobral *et al*. [79], who reported that increasing concentrations of glycerol facilitate water absorption and transport within the films.

Conclusion

Modification of surface properties of whey protein gels can transform this product to a new level of application. It could be used as a matrix for an

active ingredient release, material for tissue engineering, e.g. scaffolds, i.e. temporary structures biodegraded in the human organism. Some traditional techniques, like microscopy are used for investigation of protein gels for many years, but other methods, like optical profilometer and contact angle measurements reveal other very important properties of whey protein gels.

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