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Design of Thickened Fluids Rich in Proteins Intended for Dysphagia Management

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Results of the design of food products that behave as structured fluids and that deliver attributes tailored for dysphasic people are presented in the paper. Dysphagia refers to any disruption in the swallowing processes. Dietary strategies for dysphagia management may include altering food textures and modifying liquid consistencies, while maintaining a high nutritive profile and acceptable sensory attributes. Products presented in the paper are designed by taking into account sustainability criteria. Further knowledge is needed at the product's mesoscale to improve physical behaviour of the designed structured fluids.

1. Introduction

The structure of foods plays a vital role in the way foods are perceived and processed in the body. Many foods are structured solids or soft solids and the mesostructure determines their taste, texture and usability. The research that is here presented is intended as an underpinning support to the disease-oriented soft food formulation and drives innovation forward into new areas demanded for sustainable diets. Efforts were focused on understanding and manipulating the mesostructure of soft food to engineer products that deliver tailored attributes for dysphasic people.

Swallowing disorders i.e. dysphagia are highly prevalent in Europe amongst the elderly while more than 25 % of Europeans will be over 65 in 2040. Dysphagia refers to any disruption in the swallowing processes. Diet modifications are frequently recommended. Dietary strategies for dysphagia management may include altering food textures and modifying liquid consistencies. These are known to help improve swallowing safety while promoting oral intake, which may in turn improve patients' ability to meet their nutritional needs. A range of textures of thickened fluids suitable for dysphagia diets has been standardized worldwide through the International Dysphagia Diet Standardization Initiative (IDDSI) (American Dietetic Association, 2002). The classes are those with low viscosity "thin" (1–50 mPa s), medium "nectar" (51-350 mPa s), medium-high "honey" (351-1,750 mPa s) and high "pudding" (1,751 mPa s). Same classes have been implemented in Europe (syrup/slightly thick, nectar, honey and pudding) (Cichero et al., 2017).

The mentioned values refer to viscosity obtained at a shear rate of 50 s^{-1}, which is believed to represent the average shear rate in the oral cavity (Sopade et al., 2007).

The goal of the research was to design products through the IDDSI range of textures. This paper presents a first set of rheological tests which were finalized to thickened fluids formulations.

When designing and manufacturing new food products, whatever the final use, elements of sustainability are nowadays required by the market. To this goal, plant-based ingredients are widely used. Health and sustainability credentials are recognized for hemp seeds proteins that are used in the formulation of products here presented. They are considered a complete protein source, which means that they provide all the essential amino acids, including methionine and cysteine, as well as very high levels of arginine and glutamic acid (Odani and Odani, 1998).

The digestibility of hemp protein is also very good, better than protein from many grains, nuts and legumes (House et al., 2010). High acilated gellan is utilized as tickening and gelling agent due to its compatibility with protein. Gellan gum (E 418) is authorised as a food additive in the European Union (EU) in accordance with Annex II and Annex III to Regulation (EC) No 1333/2008 on food additives. High acyl gellan gels are very

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weak because of the bulky acetyl and glyceryl groups that prevent close association between gellan polymer chains in double-helix formation and hinder compact packing of the cross-linked double helix (Mao et al., 2000). Gellan shows attractive technological functionalities since it demonstrates high viscosity at low shear rate, but low viscosity at high shear rate. Besides, gellan gum shows high yield point (YP), especially at low salinity. On the other hand, viscosity of gellan gum solution declines at high temperature.
The evaluation of the rheological behaviour is critical for soft solid foods under study, intended for dysphagia patients.

2. Materials and methods

2.1 Materials

High-acyl gellan gum (HA-GG) (Kelcogel HA-B) kindly provided by CP-Kelco (San Diego, USA) was used without further treatment. The protein powder was obtained by micellation proteins extraction from a side product of the oil extraction process from Hemp Seeds (hemp meal-HMP-), furnished by Next Farm (Bagnolo Cremasco, Italy). Sodium Chloride was provided by Sigma-Aldrich (St. Louis, MO, USA). Ultra-pure water Milli-Q (Millipore, Bedford, MA, USA) was used throughout experiments.

2.2 Preparation of protein isolates with micellation extraction

Hemp meal (HMP) was defatted by using a double hexane extraction, followed by air-drying at room temperature (23 °C). Defatted hemp meal was dispersed in NaCl solution (pH 7.0) and stirred. The suspension was centrifuged and the supernatant was dialysed against water (pH 7.0) using ultrafiltration membranes of cellulose. The precipitated proteins were recovered by centrifugation, dried at 30 °C and stored at 20 °C until further analysis.

2.3 Gellan gum hydrogels preparation

The Gellan hydrogels were prepared following three steps (dispersion, hydration and gelation) as indicated by Sworn (2009). Powdered HA-gellan gum (HA-GG) was gradually added to distilled water and dispersed at 20 °C. Hydration was achieved by keeping the solution in a water-bath at 75 °C under constant stirring. The gelation step then was left to occur at 20 °C. The concentrations tested for of high-acyl Gellan solution were 0.2, 0.5, 0.7 and 1.0 % (w/v) respectively. Samples were stored at 5 °C overnight before further analysis.

2.4 Preparation of formulated products

Biopolymer dispersions at high protein content (5 % w/w) were prepared following two steps: dissolution of protein and gelation with HA-GG. Samples were prepared with two different methods of dissolving protein: as concern gels A and C, where salt was included in the formulation, hemp protein extract powder was dissolved in an aqueous solution of NaCl 0.7 M. On the other hand, gels B and D (gels without salt) were dissolved in an acid solution (pH=3). The gelling agent HA-GG was added at concentrations lower than 1%. Finally water was added to the targeted volume. After a further stirring at room temperature, dispersions were heated at 60 °C, finally cooled to 20 °C and then stored at the temperature of 5 °C.

2.5 Rheological analysis

The rheological properties of the gellan dispersions and of the formulated products rich in proteins were studied using a CMT rheometer (DHR-2, TA Instruments, USA), equipped with a 40 mm diameter plate-plate geometry. For all tests temperature was kept constant a 20 °C and a solvent trap was used to prevent loss of solvent.
For Shear Flow Tests, samples were tested in the range of shear rate from 10 up to 200 s^{-1} in order to define the gels viscosity behavior in physiological conditions (Qazi et al., 2019).The apparent viscosity ($\eta_{a,\,50}$) at 50 s^{-1} was considered as reference viscosity for the swallowing process.
The viscoelastic behavior of the material at the mesoscale was investigated by means of dynamic measurements and mechanical spectra were obtained through the dynamic storage modulus G'(ω) [Pa] and the dynamic loss modulus G''(ω) [Pa] are presented for their dependence on frequency.
The range of linear viscoelastic response under oscillatory shear conditions was previously identified by means of a strain sweep test: the sample was subjected to an extended field of strains (0.01-100 %) at a constant frequency of 1 Hz. The mechanical spectra were then drawn by performing a frequency sweep test over the range 0.1-100 Hz frequencies, at a constant strain in the LVE region (1.5 %).
Data were elaborated through software TRIOS 3.0.2.

3. Results

The parameters to be accounted for in designing the targeted dysphagia products are:
- viscosity in the range of shear rate between about 23 and 209 s^{-1}, typical of swallowing;
- high protein content, since the product is intended for special diets as people with dysphagia often suffer of undernutrition;
- neutral pH, since one of the symptoms of dysphagia is gastroesophageal reflux, it is preferable to obtain a product with a neutral pH so as not to increase the acidity caused by reflux;
- savory to ensure good palatability;
- absence of phase separation over time of the dispersion.

The thickening agent is therefore strategic for an appropriate formulation. High acilated gellan is utilized as thickening and stabilizing agent. Gellan behaviour was first investigated in aqueous dispersion at different concentrations, 0.2, 0.5, 0.7 and 1.0 % (w/v), respectively. Flow curves of gellan gum dispersions are shown in Figure 1. All samples show a shear thinning behaviour and viscosity decay follow a power law trend. Two gellan concentrations, namely 0.5 % and 0.7 % (w/v), resulting in dispersions' viscosities, measured at 50 s^{-1} shear rate, equal to 2,030 mPa s and 2,233 mPa s respectively, were selected for formulated products devising. These values of viscosity seem to be in principle high for the specific dysphasia physiological requirement, but are expected to decrease when the gellan is used in formulation with other components of the dispersion.

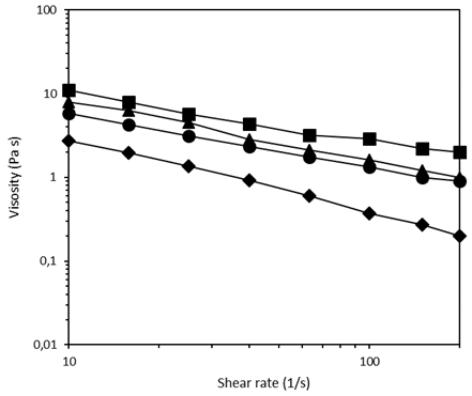

Figure 1: Viscosity as function of shear rate of gellan gum hydrogels at different concentrations: 0.2, 0.5, 0.7 and 1.0 % w/v, with rhombus, circle, triangle and square symbols, respectively

In order to get a formulated product with high added nutritional value, as it is advised for dysphasic-patients' special diets, high protein dispersions (5 % w/w) were here prepared. Since food and pharmaceutical companies look for more natural and environmentally friendly ingredients, hemp seed protein isolated from the seed meal were chosen.
Formulations of protein-rich dispersions are presented in Table 1.

Table 1: Samples formulation

Sample	Gellan gum content (% w/w)	Protein content (% w/w)	Salt content (mM)
Dispersion A	0.5	5	700
Dispersion B	0.5	5	-
Dispersion C	0.7	5	700
Dispersion D	0.7	5	-

All samples A, B, C and D appeared macroscopically stable with no macroscopic phase separation, but a difference in cohesiveness was clear with the naked eye, depending on the presence or less of NaCl (Figure 2). The goal of adding the salt was twofold: to give flavour and to modify the protein solubility and stability within the polysaccharide/protein dispersion. Preliminary tests were tentatively carried out with sodium chloride varying from 100 mM up to 700 mM and the higher salt concentration was selected for system's homogeneity reasons. In addition, since gastroesophageal reflux is one of the possible symptoms of dysphagia, it is important to underline that the final pH of the formulations was set neutral (pH = 7).

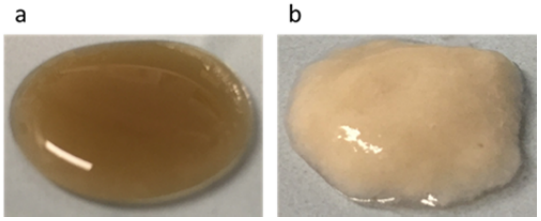

Figure 2: Images of dispersions formulated with (a) or without (b) NaCl

It is commonly accepted that food rheology is of the highest importance to prevent choking and ease swallowing. The flow behavior of the dispersion was therefore evaluated. Test was operated in the shear rate range of 10-200 s^{-1}, as similar as possible to the shear rate regime occurring during the act of swallowing. (Qazi et al., 2019).
The power law model perfectly fitted the experimental data displaying coefficients of determination, R^2, between 0.992 and 0.998. In Table 2, power law index (n) and the apparent viscosity $\eta_{a,50}$ at the swallowing shear rate condition (50 s^{-1}) are listed.

Table 2: Apparent viscosity ($\eta_{a,50}$), at the swallowing shear rate condition (50 s-1) and power law index (n)

Sample	Viscosity $\eta_{a,\,50}$ (mPa s)	Power law index n
A	85	0.103
B	600	0.05
C	510	0.09
D	1,015	0.07

At the reference swallowing shear rate condition (50 s^{-1}), viscosities were equal to 85 mPa s and 600 mPa s for the salted dispersion (A) and for the unsalted dispersion (B), respectively, both using a thickener concentration equal to 0.5 % (w/v). Viscosities were equal to 510 mPa s and 1,015 mPa s for the salted dispersion (C) for the unsalted dispersion (D) when the thickener agent was used at the concentration equal 0.7 % (w/v). The higher ionic strength, due to NaCl, prevents the formation of inter-chain bonds in high acyl gellan (Bradbeer et al., 2014).
It can be concluded that two over four IDDSI textures have been achieved: slightly thick, mildly thick. Precisely, the thickened dispersions A can be classified as medium viscosity (nectar), while gels B, C and D as medium-high viscosity (honey).
Finally, rheological evaluations were carried out in a dynamic mode to quantify the viscoelasticity of the dispersions. Mechanical spectra were obtained by means of frequency sweep tests performed at a strain values below the critical strain γ_c, in the LVE zone (1.5 % for all samples). For all samples (Figure 3), the solid-like character ($G'(\omega)$) predominates over liquid-like, viscous response ($G''(\omega)$) by around an order of magnitude, which is the typical rheological behaviour of true gels. This reflects the existence of three-dimensional networks similar to those strong gels. Thus, in the LVE region, the sample shows solid-like properties. The linear viscoelasticity zone (LVE) was obtained by means of a strain sweep (data not shown). It was found that all gels resist to strain values equal or greater than 100 % of deformations, indicating good stability.
From mechanical spectra it is also noticeable that viscoelasticity decreases when salt is added at high concentration (700 mM), regardless of gellan gum concentration (0.5 or 0.7 % w/v).
Similar results were obtained by Seo and Yoo (2013), who studied two different kind of commercial food thickeners used in dysphagia food.
The behaviour of the storage and of the loss muduli over frequency is different for weak and true gels. “Weak gels” should not be confused with conventional gels that are “weak” only in the sense of having low moduli. Conventional gels, which are often described as “self-supporting” or “demouldable”, or as “true gels”, respond to high stress by fracturing, whereas “weak gels” flow. To avoid such confusion, the term “structured liquid” might be better used (Morris et al., 2012).

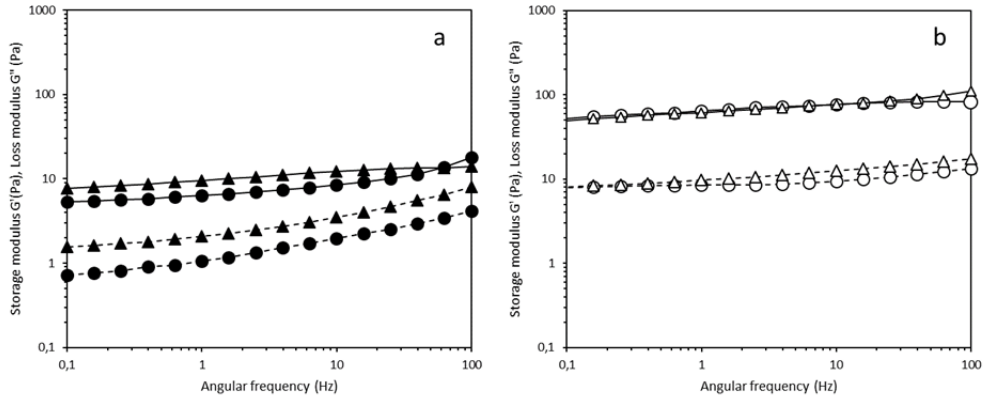

Figure 3: Storage (G', continuous line) and loss (G", dashed line) moduli vs oscillation strain (γ) of: (a) Gel A (full circle) and Gel C (full triangle) and (b) Gel B (open circle) and Gel D (open triangle)

Mechanical spectra of structured liquid normally differ from those of true gels in having greater frequency-dependence of G' and G" and smaller separation between the two moduli. Even if a macroscopic "weak gel" behaviour is observed (Figure 2), Figure 3 shows how the intrinsic rheological properties of the gellan/hemp proteins preparations does not fully reflect the behaviour expected for structured liquids, the recorded mechanical spectra being more similar to the typical true gel spectrum shown with G' about an order of magnitude greater than G", and with both moduli showing only a slight decrease with decreasing frequency.
Gellan molecules undergo a gel formation depending on the ionic strength and pH of the solution. In water, at low ionic strength and neutral pH, aggregation of the helices is impeded by the electrostatic repulsion between negatively charged carboxylic groups on the gellan. The addition of salt or reduction in pH decreases intermolecular repulsion between the helices enhancing junction zone formation and consequently, the gel strength. But viscoelastic properties the structured liquids shown in Figure 3 do not fit with the biopolymers' behaviour above described. Gelling agent behaviour in mixtures with other biopolymers might in fact result in more complex structuring phenomena. First, the high salt concentration cannot be overlooked. It has been suggested (Bradbeer et al., 2014) that no cation-mediated aggregation occurs directly with high acyl gellan, but any salt, that may be present within the initial high acyl gellan sample composition, is likely to be forced to order and aggregate within the gel matrix, whereby a considerably more aggregated, true gel form exists consisting of a larger number of elastically active network chains and junction zones.
The weaker viscoelastic intensities of salted preparations A and C (salted gels) with respect to B and C (unsalted) should therefore to be assigned to hemp proteins. The isoelectric pH of hemp proteins(pH = 4.9-6.0) is lower than the pH of structured fluids under study (pH =7) Tang et al.(2006) and Malomo & Aluko (2015) showed that the isoelectric point of these proteins was about 5, while Michaelis and Mendelssohn (1914) determined at 5.6 the pI of edestin, the main hemp protein.
At pH above their isoelectric point, proteins carry a net negative charge as indeed happens to the gellan, considering that the electrostatic repulsion between gellan carboxyl groups tended to increase approaching pH=7. Similar charge of proteins and polysaccharides are supposed to lead to electrostatic repulsion, hence to phase separation in the system, driven by volume exclusion. It can be supposed that a micro-phase separation occurred in the polysaccharide/protein mixture. (Buldo et al., 2016).
Electrostatic stabilization phenomena and phase separation on biopolymer mixtures (Turgeon & Laneuville, 2009) are in any case a complex issue, and it deserves further studies on the thermodynamic compatibility of the two polymers in the physicochemical environment that is imposed with the formulation of the structured fluids under study.
The evaluation of the viscoelastic properties was also carried out at a temperature of 37 °C (data not shown) to reproduce the physiological conditions of swallowing. Mechanical spectra can be superimposed on the corresponding one at 20 °C.

4. Conclusions

There is a good chance of designing new products for the dysphagia diet and penetrate the market. Sustainability criteria of product design can be fulfilled by using vegetable proteins. Hemp seeds are considered an eco-sustainable source and the extracted proteins are highly digestible and rich of all essential aminoacids, as it is required for dysphagia diets. Furthermore, as a structuring agent, gellan gum has proved

to be an excellent solution, as it allows to cover various viscosity levels established by the National Dysphagia Diet, varying its concentration slightly. Added salt gives a more savory taste, which can be appreciated by the patients, but also affects aesthetical appearance, since it guarantees a homogeneous macroscopic texture with respect to salt-free formulations.
Knowledge acquired through this work can be applied to further development of dysphagia-designed food products. Physico-chemical evaluation of phase separation behaviour of mixed gellan/hemp protein dispersions in different ionic environments are under work together with a monitoring of the flow behaviour of the high-nutritional-density-soft gels on mucose-models.

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