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Novel approaches in healthy cream development: oleogels and a natural sweetener

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Abstract

Physical and sensory performance of creams are strictly related to the presence of a large amount of saturated fat and sugar, whose consumption should be reduced since increases the incidence of cardiovascular diseases, obesity, diabetes and other health implications. With regards to hardstock fat reduction, oleogelation is a promising and healthy method to transform liquid oil into a 'gel-like' structure with viscoelastic properties called oleogels. On the other hand, an innovative approach to replace sugar could consist in the application of natural sweeteners plant-based with a low glycemic index, such as lucuma (*Pouteria obovata*) containing beneficial bioactive compounds. To date, very few studies exist regarding using wax oleogels in nut creams and there are no studies that have investigated the effect of using oleogels, as well as Lucuma, on refining in a ball mill. Based on this background, the study aimed to investigate novel approaches for healthy spreadable creams production in line with consumer needs. Firstly, a market online survey was carried out to define the concept of healthy cream to develop, taking into account the opinion of consumers with different healthy choice attitudes. Results showed that a healthy spread is in line with the current trend market. To develop a new healthy cream, the percentage of cocoa and dried fruit could be increased, the amount of sugar and fat could be reduced using alternative natural sweeteners and, the use of vegetable oils with beneficial properties could be considered as well. The second and third project sections focused on the development and characterization of novel oleogels prepared with uncommon vegetable oils naturally rich in vitamins and with an interesting nutritional profile, such as pumpkin seed oil. The effect of the oil type on the gelling capacity of oleogels was reported in the literature but not completely elucidated. Therefore, the crystallization and gelation kinetics of two oleogelators, beeswax and carnauba wax, in six different oils (pumpkin, hemp, almond, rice, sesame and grape seed), were studied and modelled by a modified Avrami model. Subsequently, the characterization of pumpkin seed oil was optimized by analyzing the effect of the beeswax and carnauba wax concentration (4%, 5%, 6% and 8%) on the formation mechanisms and physical properties of oleogels. To obtain information on the microstructure of oleogels, the fractal dimension model was used by exploiting their rheological properties. All developed oleogels were characterized in terms of physical properties. The oil type did not affect the crystal growth mechanism (Avrami index ranged from 1.00 to 1.43) since both, beeswax and carnauba wax, showed a 1D-2D mixed crystallization in all the oils analysed. On the other hand, the fatty acid composition of oils affected the self-assembling of oleogelators during the gelation, which occurs significantly later than the onset of nucleation. Hempseed, rice and pumpkin seed oils, the richest in saturated fatty acids among the oils analysed, formed a more interconnected structure when gelled with carnauba wax, following a 3-D network formation mechanism. The strength and yield stress of carnauba wax oleogels increased with increasing saturated fatty acid amount, while in beeswax-based oleogels a more interconnected structure was associated with the length of the saturated fatty acid chain. Considering the oleogels based on pumpkin seed oil, wax concentration affected both, thermal parameters and gelation kinetics. At the end of the cooling stage, all the oleogels has not completely formed and needed a setting

stage at 25°C to totally gel. The higher the concentration of oleogelator, the greater the floc number and their interactions, leading to the formation of a stronger crystalline network, hence a harder oleogel, with higher solid fat content, more resistant to melting. Carnauba wax proved to be a more efficient oleogelator in pumpkin seed oil than beeswax since a lower concentration is required to obtain an oleogel with excellent physical properties. Based on fractal dimension theory, all the oleogels followed a strong-link regime and the fractal dimension of the network (D) was comparable to fats widely used in food production, such as cocoa butter and palm oil. It was therefore highlighted how very small differences in the chemical composition of the oil can affect the formation mechanisms of oleogels, providing the criteria for choosing the most suitable oil and oleogelator to design oleogels with the desired physical properties. Finally, the impact of both, a novel oleogel and uncommon sweetener on the structure of healthy creams was studied. Three different creams were prepared in a stirred ball-mill: a reference cream R with cocoa butter and sugar; a cream A with a pumpkin seed oil and carnauba wax oleogel and sugar; a cream B with both oleogel and Lucuma powder. The effect of replacements on the structure of creams at different refining times was investigated analysing their granulometric and rheological behaviour and physical properties including apparent viscosity, oil binding capacity, water activity, colour and Turbiscan stability. Similar creams were obtained by changing only the fat phase, in terms of particle size distributions and rheological properties. Lucuma seemed to slow down the refining while the oleogel accelerated the refining process, which could also be stopped at 120 minutes since a particle size of 30 μm, do not perceive by papillae, was obtained. All the samples exhibited pseudo-plastic behaviour, showing a good oil binding capacity, and they were microbiologically and physically stable since showed water activity and Turbiscan stability index values close to 0.5 and 1, respectively. The oleogel based on pumpkin seed oil and carnauba wax could be totally replaced the cocoa butter, and Lucuma could partially decrease the sugar intake. Starting from the consumer's needs, it was possible to functionalize polyunsaturated and monounsaturated oils, designing novel structures, the oleogel, successfully applied in a complex food matrix such as cream, improving the nutritional profile, not only reducing the saturated fatty acids, but also by introducing a natural sweetener, providing viable alternatives for reformulating foods with healthier nutritional profiles.

Riassunto

Le performance fisiche e sensoriali delle creme sono strettamente correlate alla presenza di grandi quantità di grassi saturi e zuccheri, il cui consumo dovrebbe essere ridotto poiché aumenta l'incidenza di malattie cardiovascolari, obesità, diabete e problematiche. Per quanto riguarda la riduzione dei grassi solidi, l'oleogelazione è una strategia promettente e innovativa per trasformare un olio liquido in una struttura "simile a un gel" con proprietà viscoelastiche, ottenendo prodotti chiamati oleogel. Per quanto riguarda la sostituzione dello zucchero, un approccio innovativo consiste nell'applicazione di dolcificanti naturali a basso indice glicemico e contenenti composti bioattivi benefici come la Lucuma (*Pouteria obovata*). Ad oggi, esistono pochissimi studi sull'introduzione di oleogel a base di cere naturali nelle creme spalmabili, e l'effetto dell'oleogel, così come della Lucuma, sul processo di raffinazione in un mulino a sfere non è mai stato studiato. Partendo da queste ipotesi, il presente progetto si è focalizzato sulla messa a punto di nuove strategie per lo sviluppo di creme spalmabili salutistiche che fossero in linea con le esigenze dei consumatori. In primo luogo, è stata condotta un'indagine di mercato online per definire il concetto di crema da sviluppare, considerando l'opinione di consumatori con diverse abitudini alimentari. I risultati hanno mostrato che una crema salutistica è in linea con l'attuale trend di mercato, e che nello sviluppo di una crema salutistica sicuramente andrebbe considerato l'aumento di frutta secca, una riduzione di zuccheri e grassi saturi, l'utilizzo di dolcificanti naturali e di oli vegetali. La seconda e la terza fase si sono focalizzate sullo sviluppo e la caratterizzazione di nuovi oleogel a partire da oli poco o mai studiati in letteratura (in termini di oleogelazione), che fossero naturalmente ricchi di vitamine e con un interessante profilo nutrizionale, come l'olio di semi di zucca. Pertanto, sono state studiate le cinetiche di cinetica di cristallizzazione e gelificazione di due oleogelatori, la cera d'api e la cera carnauba, in sei oli diversi (zucca, canapa, mandorla, riso, sesamo e vinaccioli), modellate utilizzando un modello di Avrami modificato. Nella terza fase è stata ottimizzata la caratterizzazione di uno degli oli investigati nella seconda, ovvero l'olio di semi di zucca, analizzando l'effetto della concentrazione di cera d'api e carnauba (4%, 5%, 6% e 8%) sui meccanismi di formazione e sulle proprietà fisiche degli oleogel. Per ottenere informazioni sulla microstruttura degli oleogel è stato utilizzato il modello della dimensione frattale sfruttando le proprietà reologiche. Tutti gli oleogel sviluppati sono stati caratterizzati in termini di proprietà fisiche. Da un lato, il tipo di olio non ha influenzato il meccanismo di crescita dei cristalli (indice di Avrami che variava da 1,00 a 1,43) che era dovuto ad una cristallizzazione mista 1D-2D delle cere in tutti gli oli analizzati. Dall'altro, la composizione chimica degli oli ha influenzato l'autoassemblaggio degli oleogelatori durante la gelificazione, influenzando il meccanismo di formazione del network tridimensionale. In particolare, gli oli più ricchi di acidi grassi saturi come l'olio di semi di canapa, riso e semi di zucca, gelificati con cera di carnauba, seguivano un meccanismo di formazione 3-D, indice di una struttura più interconnessa. La formazione degli oleogel a base di cera d'api era associata, invece, alla lunghezza della catena di acidi grassi saturi piuttosto che alla loro quantità. Analizzando nel dettaglio gli oleogel a base di olio di semi di zucca, è emerso che la concentrazione di cera ha avuto un effetto sia sui parametri termici, che sulle

cinetiche di gelificazione. Al termine della fase di raffreddamento, tutti gli oleogel non si sono completamente formati ed è stata necessaria una fase di *setting* a 25°C per ottenere una gelificazione completa. Maggiore è la concentrazione di oleogelatore, maggiore è il numero di flocculi e le loro interazioni, portando alla formazione di una rete cristallina più forte, quindi un oleogel più duro, con un *solid fat content* maggiore, più resistente alla fusione. La cera carnauba si è rivelata un oleogelatore più efficiente nell'olio di semi di zucca rispetto alla cera d'api, poiché era necessaria una minore concentrazione per ottenere un oleogel con eccellenti proprietà fisiche. In base alla teoria della dimensione frattale, tutti gli oleogel seguivano un regime reologico *strong-link* e la dimensione frattale stimata (D) degli oleogel era paragonabile a quella dei grassi generalmente utilizzati nella produzione di creme spalmabili, come il burro di cacao e l'olio di palma. È stato quindi evidenziato come piccolissime differenze nella composizione chimica dell'olio possano influenzare i meccanismi di formazione degli oleogel, fornendo i criteri per la scelta dell'olio e dell'oleogelatore più adatti per progettare oleogel con le proprietà fisiche desiderate. Infine, è stato studiato l'impatto dei nuovi ingredienti sulla struttura di nuove creme salutistiche considerando il reale processo produttivo. Sono state preparate tre diverse creme raffinate in un mulino a sfere: una crema di riferimento R con burro di cacao e zucchero; una crema A con oleogel, a base di olio di semi di zucca e cera carnauba, e zucchero; una crema B sia con oleogel che con polvere di Lucuma. È stato studiato l'effetto delle sostituzioni sulla struttura delle creme a diversi tempi di raffinazione analizzandone il comportamento granulometrico e reologico e le proprietà fisiche tra cui la viscosità, la capacità di legare l'olio, l'attività dell'acqua, il colore e la stabilità fisica. Modificando solo la fase grassa, sono state ottenute creme simili sia in termini di distribuzione granulometrica che di proprietà reologiche. In presenza di Lucuma la raffinazione procedeva più lentamente, mentre l'oleogel sembrava accelerare la raffinazione che, per questa crema, si sarebbe potuta stoppare a 120 minuti, poiché si era ottenuta una granulometria ideale intorno ai 30 μm, non percepibile dalle papille gustative. Tutti i campioni presentavano un comportamento pseudo-plastico, avevano buona abilità nel trattenere olio ed erano microbiologicamente e fisicamente stabili mostrando valori di attività dell'acqua e di indice di stabilità Turbiscan prossimi a 0,5 e 1, rispettivamente. L'oleogel a base di olio di semi di zucca e cera di carnauba si è rivelato un buon sostituito dal burro di cacao, e la Lucuma potrebbe parzialmente diminuire l'assunzione di zucchero. Partendo dalle esigenze del consumatore, è stato possibile funzionalizzare oli vegetali polinsaturi e monoinsaturi, creando nuove strutture ovvero oleogel, applicate con successo in un prodotto alimentare complesso come la crema, riducendo gli acidi grassi saturi e il saccarosio, fornendo valide alternative per riformulare alimenti con profili nutrizionali più sani.

Summary and aim of the PhD project

Creams represent an important ingredient in different confectionery foods but are generally characterized by a medium-high caloric power due to the presence of a large amount of saturated fat and sugar providing taste, texture, mouthfeel and flavour to the product. Creams are to be considered an unhealthy product that is often consumed because perceived as very palatable and easy to consume. The confectionery market aimed to develop new products based on consumers' motivations and behaviours. In recent years the demand for healthy products is increasing, the spreadable cream market is in constant development and a new healthy cream could be used as attractive food to enhance healthy eating behaviours.

The physical and sensory performance of creams is improved by the presence of fat, especially hard fats rich in saturated fatty acids, whose consumption can increase the incidence of cardiovascular diseases, obesity, diabetes and other health implications. Vegetable oils play an important role in food nutritional quality due to the recognized beneficial effects of increased consumption of unsaturated fatty acids. However, directly replacing hard fats with liquid oil can lead to technological problems such as texture weakness and oil leakage in several food products due to their low viscosity (Kim, Lim, Lee, Hwang & Lee, 2017). Against this background, oleogelation is a promising and healthy method to solidify oil and replace the traditional solid fat in a foodstuff, obtaining new gelled oils called oleogels. The formation of oleogel requires two components, a liquid oil and a gelling agent (oleogelator) able to transform liquid oil into a 'gel-like' structure with viscoelastic properties. A large number of oleogelators and oils type can be used to prepare oleogels with different physical properties (Kamali, Sahari, Barzegar, Gavlighi, 2019). Natural waxes, such as beeswax and carnauba wax, are efficient low-cost oleogelators and are food grade as they are commonly used in the food industry as glazing and coating agents, conferring a glossy appearance (e.g. cheese, apples or candies).

Several studies have shown that a diet high in sucrose is associated with several diseases; replacing sugars seem to reduce the energy intake decreasing body weight. Current focus on reducing sucrose intake concerns its replacement with artificial sweeteners which, even if they are calorie-free, can impart off-flavour to the product. The combined use of alternative sweeteners and bulking agents is still one of the most used approaches to reduce sugar content in food, e.g. intense sweeteners together with fibres (Di Monaco, Miele, Cabisidan & Cavella, 2018). An innovative approach could consist in the application of a natural ingredient with a low glycemic index, such as lucuma (*Pouteria obovata*), naturally rich in potentially beneficial bioactive compounds (Ak-Cvitanovìc et al., 2015). Through this strategy, the caloric content will be reduced and the nutritional profile improved.

Starting from this hypothesis, these specific goals have been addressed along with the PhD project:

- collecting information on consumer expectations and defining the ingredient of healthy cream to develop through an online market survey;
- understanding the effect of the use of different uncommon oils, with an interesting healthy profile, on gelling process and properties of new oleogels;
- developing and characterizing novel oleogels based on pumpkin seed oil and natural wax as an innovative strategy to provide additional options for tailor-made formulations;
- exploring the physical properties of healthy spreadable cream with oleogels and lucuma powder as fat and sugar replacer at different refined degree.

Overview of PhD research activities and publications

Borriello A., Miele N. A., Masi P. Aiello A. & Cavella S. (2022). Effect of fatty acid composition of vegetable oils on crystallization and gelation kinetics of oleogels based on natural wax. *Food Chemistry*, 375, 131805.

Borriello A. Novel approaches in healthy cream development. In Proceedings of First Virtual (XXV) Workshop on the "Developments in the Italian Phd Research on Food Science Technology and Biotechnology", Palermo, Italy, 2021.

Borriello A., Masi P. & Cavella S. (2021). Novel pumpkin seed oil-based oleogels: Development and physical characterization. *LWT-Food Science and Technology*, 152, 112165.

Miele N.A., **Borriello A**., Fidaleo M., Masi P. & Cavella S. (2020). Modelling grinding kinetics of fat based anhydrous pastes. *Journal of Food Engineering*, 268, 109732.

Cavella S., Miele N.A., Fidaleo M., **Borriello A**. & Masi P. (2020). Evolution of particle size distribution, flow behaviour and stability during mill ball refining of a white chocolate flavouring paste. *LWT-Food Science and Technology*, 132, 109910.

Miele N.A., Armini V., **Borriello A**. *, Torrieri E., Sacchi R., Cavella S. Oxidation kinetics of ready to use therapeutic food formulations. In Proceedings The 9th Shelf-Life International Meeting, Naples, Italy, 2019. *Corresponding author: angela.borriello@unina.it

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CHAPTER 1

1.1 Spreadable creams

Spreadable creams are widely consumed products used both as filling cream, representing an important ingredient in different confectionary foods, and alone as spreads. Among them, creamy and smooth nut spreads are preferred, especially by children (Shakerardekani, Karim, Ghazali & Chin, 2013). Spreadable creams are generally anhydrous sweet products, with a water content of less than 1% which guarantees the microbiological stability of the product and a shelf-life of 24 months. They are sugar and fat mixtures, even if also other ingredients are included in the recipe and they influence many characteristics of the final product. They are required not to solidify during storage at room temperature. This peculiar property is guaranteed by a considerable amount of fats mixed with different dry ingredients. They could be defined as a concentrated suspension of different solid particles (sugar, cocoa powder, milk whey, milk powder, dehydrated cream, nut solids, etc.) in a continuous fat-phase represented by oil and solid fat (Miele, Borriello, Fidaleo, Masi & Cavella, 2020). The quality, quantity, and type for each ingredient used in a formula, particularly fats, affect the cream rheological and textural properties (Glicerina, Balestra, Pinnavaia, Dalla Rosa & Romani, 2013).

Sugar is generally the main ingredient, and highly refined white sugar is 99.9% sucrose, a non-reducing disaccharide (Birkett, 2009). The greater the quantity of sugar in the cream recipe the harder and 'drier' will be the cream, the larger the sugar crystal size the grittier will be the cream in the mouth. Sugar performs a dual function, acting as a sweetener and as a bulking agent, increasing the cream volume and also affecting the palatability and the pleasant sensation perceived in the mouth during the tasting. Particle size of sugars less than 30 μm is required since those particles should not be perceived in the palate (Puleo, Miele, Cavella, Masi & Di Monaco, 2020).

The fat is the second 'bulk' component of creams after sugar. The total amount of oils/fats in a spreadable cream ranged between 28–60%, depending on the desired cream cost and quality level. To have a good structure it is necessary to use fats that are solid at ambient temperatures and above, but melt at mouth temperature. This often means using fats that are rich in saturated and trans-fatty acids which unfortunately gives the product undesirable nutritional characteristics (Pipoyan et al., 2021). A combination of different types of fat can be used: polymorphic non-lauric fats, such as cocoa butter, cocoa butter equivalents or fats based upon palm fractions; non-polymorphic and non-lauric fats, based upon hydrogenated rapeseed, soybean and palm fractions; non-polymorphic lauric fats, based upon coconut and palm kernel

oil (Birkett, 2009). The directives 2000/36/EC of the European Parliament and of the council of 23 June 2000 relating to cocoa and chocolate products intended for human consumption define cocoa butter as the fat obtained from cocoa beans or parts of them with a free fatty acid content (expressed as oleic acid) less than 1.75 % and an unsaponifiable matter (determined using petroleum ether) less than 0.5 %, except in the case of press cocoa butter (< 0.35 %).

Spreadable cream with different consistency could be obtained by changing the liquid oil/solid fat ratio. The more liquid the fat phase, the less saturated it is, but with a high probability of fat phase separation and unacceptably soft product. Generally, a 60/40 ratio is required to develop a fat crystal network that ensures spreadability, at room temperature, and stability. The fat crystal network is the product of an aggregation process of molecules into crystals and of crystals into larger clusters, until a space-filling three-dimensional network is formed (Awad & Marangoni 2006). Both, physical and sensory performances of spreads are strictly affected by the mechanical strength of the fat crystal network providing texture, mouthfeel and flavour to the product and acting as a binder between cream and product to be filled (Fayaz et al., 2017).

Dry ingredients are mainly sugar and cocoa powder but also nuts and flavours are usually used in a spreadable cream recipe. Most spreadable creams are nut and cocoa-based and the type of nut essentially depend on the flavour required. Dry roasted hazelnut, almond, and peanuts are the most common ones, but also cashew, macadamia nut, pecan, pistachio and walnut are available. Similar spreads can also be made from other seeds such as sesame seed, pumpkin seed, soybean and sunflower seeds. The roasting condition of kernels should be properly controlled because they affected the moisture content of nuts, the development of flavour, aroma and also the colour of the final product (Shakerardekani et al., 2013). Their amount in a spreadable cream could range between 13-45%.

The directives 2000/36/EC of the European Parliament and of the council of 23 June 2000 relating to cocoa and chocolate products intended for human consumption define cocoa powder as the product obtained by converting into powder cocoa beans previously cleaned, shelled and roasted, and which contains not less than 20 % cocoa butter, calculated according to the weight of the dry matter, and not more than 9% water. The cocoa powder confers a characteristic flavour and aroma to the spreadable cream and it is classified according to the quantity of cocoa butter that remains after pressing. The most widely used is a degreased cocoa powder, which is characterized by the commercial-grade 10/12, with a cocoa butter content lower than 20%. The amount of cocoa in a spreadable cream generally ranges between 4-10%.

Lecithin is the most frequently used emulsifier in fat-based fillings because it gives the filling an acceptable viscosity, reducing the fat content, and a water-binding ability if water is present.

Lecithin can be sourced from soy, rape, and sunflower (Birkett, 2009). This emulsifier speeds the mixing of the cream but tends to give softer creams after cooling. It has the technological function of stabilizing the crystallization of fats during cooling, modifying the consistency of the product acting at the interface between the solid and liquid phases since it has amphipathic characteristics. The recommended amounts of use are between 0.1 and 0.3% since excessive quantities could alter the flavour of the final product, and lead to an excessively fluid cream at high temperatures (Loncarevic et al., 2016).

Finally, aromas also can be added to the formulation playing an important role in the characterization of the product. Aromas can be in different forms: essential oils, oil-soluble flavours or powder flavours. It is preferred to avoid those containing water as they could compromise the consistency and stability of the cream. One of the most used aromas is the vanilla aroma, in concentrations higher than 0.05% (Birkett, 2009). In all cases, the optimum effect is achieved if colour is added to suggest the flavour.

1.1.1 Refining process

Refining, also called grinding, is a fundamental unit operation of spreadable creams processing aimed to reduce the solid particle size. This is a critical step in the production of spreadable creams because the solid particle size and particle distribution are important parameters that influence the overall quality of the final product. Refining was mostly carried out with roll refiners and generally consist of two steps: a pre-refining in three-roll refiners followed by five-roll milling (Beckett, 1999; Loncarevic et al. 2016). During this process of mixing, grinding, and in some cases recirculation, the size of the solid particles is reduced and their surface gets wrapped by the fat phase (Petkovic, Pajin, & Tomic, 2013), conferring to the cream its rheological and textural properties. For small-scale production, stirred ball mills represent a valid alternative for creams production (Fidaleo, Mainardi & Nardi, 2017a; Fidaleo, Miele, Mainardi, Armini, Nardi, & Cavella, 2017b; Konar & Bingol, 2019; Pajin et al., 2011; Toker, Zorlucan, Konar, Daglıoglu, Sagdic, & Sener, 2017). Stirred ball mills were introduced at the beginning of the 20th century in the refining of paintings and were later applied to the powder and confectionery industries (Alamprese, Datei & Semeraro, 2007). About 60–80% of the void volume is filled with the grinding media, typically balls made up of stainless steel, steel, ceramic or other materials. In such mills, the product and spheres are stirred in the mill tank by the arms of a rotating shaft that result in impact and shearing actions reducing the solid particles as well as determining their homogeneous dispersion (Cavella, Miele, Fidaleo, Borriello & Masi, 2020). During milling, part of the mechanical energy is absorbed by the tailings of large

particles, determining an increase in surface energy and a decrease in particle size. To obtain the right palatability, which means that papillae do not perceive the graininess of the particles, the particle size should be smaller than 30 μm, even if among consumers a different graininess sensitivity has been observed (Puleo et al., 2020). Moreover, Afoakwa, Paterson, Fowler, & Vieira (2009) reported that 90% of the particle size smaller than 30 μm is necessary to inhibit the separation of the lipid fraction phases obtaining a homogeneous cream. Low acceptability of creams can occur if they have particles larger than 35 μm as they become grainy or coarse in the mouth. These machines are generally equipped with a thermostat control with a water circuit to provide accurate temperature control during creams processing. The solid fats, previously melted, are generally added in a second step after mixing of nut paste and powders, keeping the processing temperature around 35 °C. Moreover, since cocoa butter is a polymorphic fat and it can exist in six different crystalline forms, creams based on this fat require a tempering operation after refining. This means that the product must crystallize at a temperature below 32 ° C through a cooling/ heating cycle vigorously mixing to promote the formation of crystals in the V form, which is the most stable one having the capacity to trap liquid oil within its crystal network. However, very few studies have dealt so far with food refining employing stirred ball mills (Fidaleo et al., 2017b).

1.1.2 Physical properties

The physical and sensory properties of spreadable creams are strictly affected by processing techniques as well as their formulation. Therefore, it is important to investigate the relationship between particle size distribution, rheological behaviour and colloidal stability because it is strongly related to the overall quality of the final product (Cavella et al., 2020).

From a physical point of view, spreadable creams could resemble a concentrated suspension where the matrix is fully packed by the particles that fill the space available. Coussot & Ancey (1999) described concentrated suspensions as “complex systems within which particles interact strongly, giving rise to viscosities much higher than the viscosity of the suspending media”. In these systems, the interactions between particles dominate over the hydrodynamic forces (Brownian forces) exhibiting a complex flow behaviour.

From a rheological point of view, spreadable creams are pseudo-plastic fluids, showing a shear-thinning behaviour. Their viscosity decreases with increasing shear rate due to the loss of structure in the material. In detail, these materials can exhibit three different regions during flow (Figure 1.1): a first region where the limiting viscosity at zero shear rate (η_0) is constant with changing shear rates; a second region characterized by a decreasing of apparent viscosity

(η) with shear rate; a third region where the material has reached a steady-state where the intermolecular forces are in equilibrium and no further decrease of viscosity is observed (the limiting viscosity at infinite shear rate η_∞).

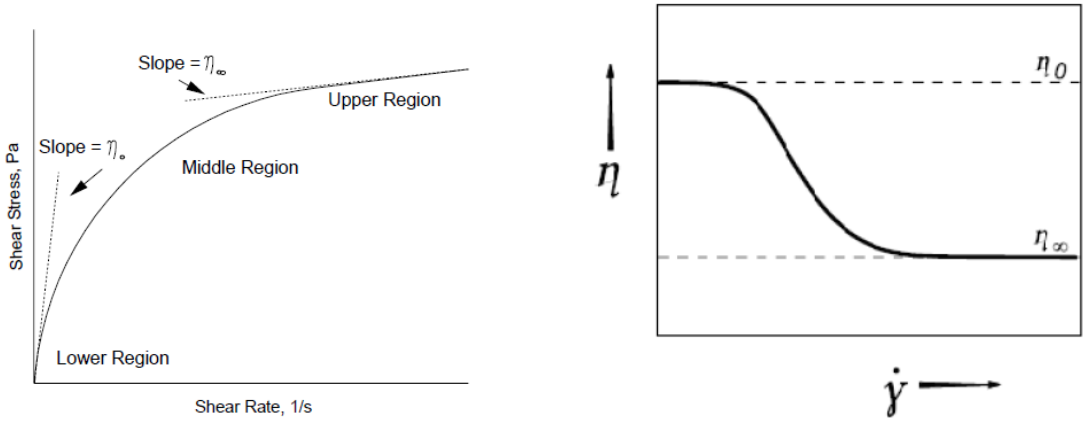

Figure 1.1 Pseudo-plastic behaviour (Adapted from Steffe, 1996 and Spetch et al., 2007)

Several rheological models could be used to describe flow behaviour of these multiphase matrices, and the most suitable ones are the Ostwald model, also called Power Law model, and Casson model. The Power Law model is expressed by the following equation (1):

$$\eta = k(\dot{\gamma})^{n-1} \quad (1)$$

where η is the viscosity (Pa·s), K is the consistency index (Pa·s^n), $\dot{\gamma}$ is the shear rate (1/s) and n is the dimensionless flow behaviour index. The Casson equation has been adopted by the International Office of Cocoa and Chocolate for interpreting chocolate-based product behaviour. The rheological model of Casson is represented in the following equation (2):

$$(\eta)^{1/2} = (\frac{\sigma_0}{\dot{\gamma}})^{1/2} + (\eta_\infty)^{1/2} \quad (2)$$

where σ_0 is the Casson yield stress and η_∞ is the infinite-shear viscosity, also called the Casson plastic viscosity. The yield stress can be used to calculate whether a sample is likely to settle in situ, or whether it will be difficult to start pumping or stirring (Steffe, 1996; Shakerardekani et al., 2013).

Moreover, stain and frequency sweep tests could be performed to investigate other structural properties of creams. Those are dynamic tests independent of the strain applied but are only related to the structure of the product (Baldino, Gabriele & Migliori, 2010). Strain sweep test,

performed by increasing the amplitude of strain and keeping constant the frequency of oscillation, is carried out to identify the linear viscoelastic range (LVR).

Frequency sweep tests are applied to evaluate the storage G′ and the loss modulus G″, which are indices of the elastic and viscous behaviour, respectively. Rheological parameters of spreadable creams are strictly affected by the type, quality and quantity of each ingredient used in a recipe, particularly fats (Fang and Zhang 1997). Larsson and Quinn (1994) and Ribeiro, Grimaldi, Gioielli & Gonçalves (2009) reported that a high amount of saturated fatty acids strongly increases the consistency of the product. In the work of Glicerina, Balestra, Dalla Rosa, Bergenhstål, Tornberg, & Romani (2014) is also reported that nut creams obtained with a high level of hydrogenated fats are characterized by a stronger fat network and higher viscosity values than the cream with only palm oil. Aydemir (2019) studied the use of different oils and fats on cocoa hazelnut cream production, showing that the apparent viscosity, the consistency index, and both, the storage and loss modulus increased as the amount of solid fat increased.

For chocolate-based products, it is well known that the size of the solid particles, as well as their distribution, directly influences the rheological properties. During refining the surface of the solid particles is wrapped by the fat phase, conferring to the paste its rheological properties. Particle size distribution becomes homogeneous, particle size and flow index decrease, viscosity and consistency of creams increase, as refining proceeds. However, too viscous creams could be obtained by excessive refining, making the product difficult to spread and swallow. Therefore, the control of solid particle size is useful to monitor the grinding process and avoid an over-grinding and a sub-sequent use of fats and emulsifier to restore the right viscosity. The particle size of solid particles also affects the colloidal stability of creams. Especially during creams storage, destabilization phenomena occur, mainly due to solid particle sedimentation and consequent oil separation. Many investigators showed that the fat phase separation is enhanced by the presence of larger particles, while in products containing small particles this phenomenon is reduced (McCarthy & McCarthy, 2008; Dahlenborg, Millqvist-Fureby, Bergenståhl & Kalnin, 2015; Cavella et al., 2020).

1.2 The Oleogelation: a novel strategy to structure vegetable oils

In recent years, food choices are mainly driven by attention to health and the consumers are increasingly informed on the damage that negative eating behaviour could have on their health. The demand to reduce saturated and/or trans-fats from the consumers' diet is constantly increasing because it is well known that the consumption of excessive amounts of saturated fats increases the incidence of cardiovascular and other related diseases. On the other hand, vegetable oils play an important role in food nutritional quality due to recognized beneficial effects of increased consumption of unsaturated fatty acids (Pehlivanoglu, Demirci, Toker, Konar, Karasu, & Sagdic, 2018).

However, fats and oils are differently distributed in the food matrix, playing different technological functions. Generally, oil globules are evenly distributed and are micrometric in size, while solid fats exist as larger globules of various sizes, at least 100 μm (Youssef & Barbut, 2009). These hard fats are generally used in confectionery products where some degree of hardness and structure is required, which consequentially contain a significant level of saturated fatty acids. Therefore, directly replacing solid fats with liquid oil could change both physical and sensory properties of products, such as structural weakness and oil separation. Hydrogenization, interesterification, and fractionation are widely used methods to structuring vegetable oils, producing hydrogenated fat rich in *trans* fatty acids which also have negative health implications, such as increasing low-density lipoprotein (LDL) and increasing triglyceridemic (Principato & Spigno, 2019).

Moreover, The World Health Organization (WHO) indicated that the amount of saturated and trans-fat must be less than 10% and 1% of the total daily intake, respectively. Finding replacements to hard fats has proven to be difficult and the challenge consists of developing valid alternatives to saturated and hydrogenated fats that can perform their technological function, with a better nutritional profile. Within this context, oleogelation is a promising and healthy method to solidify oil obtaining new gelled oils called oleogels and replacing the traditional solid fat in foodstuffs. Using the oleogels, trans-fat free products, low in saturated fats and high in unsaturated fats could be obtained. Structured oil systems formed through oleogelation are also known as organogels, lipid gels or simply, oleogels (Patel & Dewettinck, 2016).

1.2.1 Oleogels: definition and formation mechanism

According to Lloyd's gel definition, gels are three-dimensional structures consisting of two phases, a liquid fraction and a gelling agent, also called structuring agent or gelator. According to the polarity of the liquid phase, it is possible to differentiate the hydrogels, in the case of a polar liquid phase, such as water, from the organogels, where the phase is a non-polar liquid, like organic solvents. An organogel with vegetable oil as a liquid phase could be also called oleogel. After Lloyd (1926), Ferry and Hermans expanded the definition of gel adding that gel was a system showing unstable flow, with mechanical properties of a solid-like. Oleogels are generally described as a complex microstructured system with a gel-like structure that has rheological properties, viscoelasticity, spreadability, firmness and other typical properties of a solid fat with a low saturated fat content (Co and Marangoni, 2012). Gelator molecules assemble in supramolecular aggregates (building blocks) forming a 3-D network able to trap a large amount of oil (≥ 90 %wt) (Patel & Dewettinck, 2016). Different ways to categorize oleogels are commonly used, e.g. based on the type of interaction that occurs during formation, on the chemical nature of oleogelators, or on the type of building blocks and structuring principles involved in gelation.

According to the type of interaction responsible for the formation of the 3D network, the organogels can be defined as chemical gels, which are structured by primarily covalent bonds, or as physical gels formed by physical secondary forces such as hydrogen bonding, hydrophobic interactions and van der Waals forces. Another subdivision can be done according to the chemical aspect of the gelling agents, which can be polymeric or low molecular weight. Polymeric oleogelators can form both, chemical and physical gel, when their chains are crosslinked or entangled macromolecular chains, respectively (Ract, da Cruz & Pereira, 2019). However, most of the oleogels are formed from non-polymeric species forming supramolecular structures via noncovalent interactions. Low molecular weight oleogelators (<3000 Da) can self-assemble in long aggregates, arranged by physical interactions, that entrap the oil phase (Co & Marangoni, 2012). Oleogels based on low molecular weight oleogelators are self-standing, thermo-reversible, anhydrous, viscoelastic materials and highly sensitive to temperature (Rogers, Wright, & Marangoni, 2009).

Depending on the class of oleogelators used, oleogels can show a different structure. In detail, the three-dimensional structure of the oleogels is formed by building blocks that can belong to one of the categories described below and shown in **figure 1.2** (Marangoni & Garti, 2011).

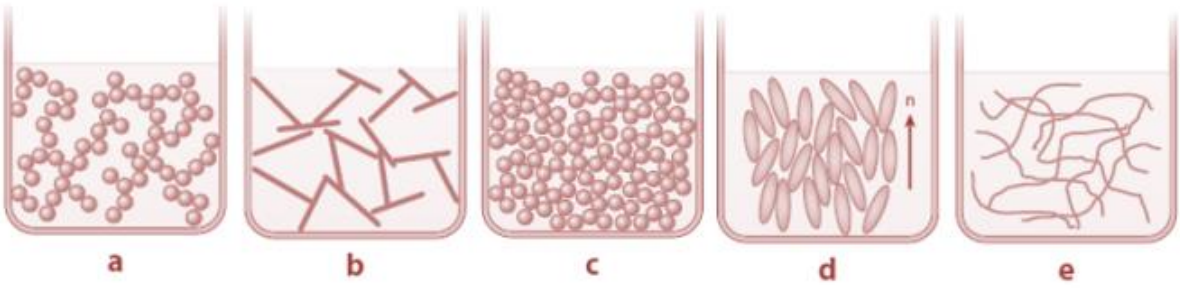

Figure 1.2 Schematic illustration of type of building blocks and structuring principles involved in gelation: crystalline particles (a), fibril network (b), particle fillers (c), liquid crystalline esophase (d), polymer network (e). (Schematic illustration reported form Organogelation: It's Food Application Kaushik et al., 2017).

Crystalline particles self-assemble forming crystalline three-dimensional networks similar to the traditional triacylglycerides crystalline structures. A large variety of oleogelators falls in this group, such as n-alkanes, waxes, fatty acids, mono, di, and triacylglycerol and ceramides. *Crystalline fibres* also self-assemble developing fibrillary networks, such as 12-hydroxstearic acid and sterol/phytosterol mixtures. *Polymeric strands*, such as ethylcellulose, are derived from cellulose where hydroxyl groups have been ethylated and it can be soluble in water or organic solvents depending on the degree of substitution.

Particle-filled networks consist of closed-packed networks where the oil phase is immobilized by a high concentration of adjacent solid particles. This is the case of oleogels formed from silica particles, although the most cited example to clarify this mechanism is the peanut butter one, where peanut particles act as a colloidal system able to restrain the liquid oil phase.

Lastly, *liquid crystalline mesophases* show characteristic properties between the solid (crystalline) and liquid (isotropic) phases and are characterized by an intermediate mesophase called liquid crystals. This system type contains ordered solid lattice structures despite showing a liquid-like flow behaviour. Examples include a system of phospholipids, and monoglycerides, with oil as the continuous phase and cylindrical structures filled with water.

The most used method to prepare an oleogel is the direct dispersion of oleogelators in the solvent. Oil is warmed up until the melting temperature of the oleogelators has been reached, and the gelling agent is added under shear until a clear solution is obtained. The system is then cooled at environmental temperature either under shear or static conditions. Oleogel based on lipid oleogelators, such as waxes, fatty acids, fatty alcohols and monoglycerides, are usually prepared in this way. During the oleogelation different steps occur, starting from nucleation, crystal growth and aggregation, until forming a networked structure.

It is well known that the oleogel formation, as well as a gel, requires two phases, an oil liquid phase and an oleogelators. It is possible to obtain oleogel with different physical properties by

changing both, the dispersion and the dispersed phase. The network structure is affected by several factors, such as the nature of the solvent, the type and concentration of the oleogelator.

1.2.2 Natural waxes as oleogelators

The oleogelator role consists in the immobilization of the liquid oil allowing the consequential oleogel formation. The essential requirements that an oleogelator should have are the presence of lipophilic parts, surface activity, thermoreversible properties, natural origin, as well as generally recognized as safe (GRAS). A wide number of oleogelators can be used to prepare oleogels. However, waxes are a promising strategy in food applications because they are natural, already used in the food industry, inexpensive and available, easy to use and efficient, which means that their threshold concentrations are lower than 10% (Hwang, Kim, Singh, Winkler-Moser, Liu, 2012; Patel, Schatteman, De Vos, Lesaffer & Dewettinck, 2013; Pehlivanoğlu et al., 2018). Moreover, the most suitable options for spreads applications are wax-based oleogels. Natural waxes are crystalline oleogelator that can have both, animal origin such as beeswax, and vegetable origin, such as rice bran wax, candelilla wax, sunflower wax and carnauba wax. From a chemical point of view, waxes are complex heterogenous materials of long-chain esters, derived from fatty acids and fatty alcohols, and hydrocarbons. They also contain minor compounds such as n-alkanes, fatty acids, fatty alcohols, mono-, di- and triacylglycerols, ketones and sterol esters, which are present in different amounts according to the type of wax. The polarity, long chain length, and high melting point of their main components confer to natural waxes excellent crystallization and gelation properties (Liu, Ramirez, Yang & Ciftci, 2020; Doan, Tavernier, Okuro & Dewettinck, 2018). The chemical composition of the most used waxes in oleogelation is shown in the **table 1.1**.

Table 1.1 Chemical composition and thermal parameters of natural waxes reported by Blake, Toro-Vazquez, & Hwang (2018) with some modification.

Chemical composition and thermal parameters	BW	CL	CR	RBX	SFX
Esters (%)	71	27-35	84-85	92-97	97-100
Free fatty acid (%)	12	7-10	3-3,5	0-2	0-1
Free fatty alcohol (%)	-	10-15	2-3	-	-
Hydrocarbons (%)	14	50-65	2,5-3	-	-
Resins (%)	6	-	6,5-10	3-8	0-3
T_m (°C)	63.15±0.17	60-73	80-85	78-82	74-77
ΔH_m (J/g)	174.44±6.17	156±10.5	195±14.7	211±7.6	195±29.8

BW: beeswax; CL: candelilla wax; CR: carnauba wax; RBX: rice bran wax; SFX: sunflower wax; T_m: Melting temperature; ΔH_m: melting enthalpy.

Several studies (Doan, Patel, Tavernier, De Clercq & Van Raemdonck, 2016; Doan et al., 2017a; Doan et al., 2017b) investigated the crystallization behaviour of natural waxes showing that their component, such as n-alkanes, fatty acids and fatty alcohols, precipitate during cooling forming solid nuclei. Then the crystal growth, mainly due to strong crystalline interactions, and formation of supramolecular structure occurs. The network formation is mainly driven by strong primary sintered connections and weak secondary Van der Waals bonds. Wax molecules and triglyceride molecules show different configurations, linear and non-linear, respectively. This difference in molecular asymmetries allows the crystallization and assembly of solid wax particles from the liquid oils, which leads to the oleogel formation (Doan et al., 2018). Moreover, the gelation mechanism is mainly due to the arrangement of n-alkanes or wax esters into microcrystalline platelets, which aggregate to form a strong three-dimensional lattice able to immobilize the oil phase (Blake, Co & Marangoni, 2014).

Several studies report the application of natural waxes for the formation of edible oleogels, such as beeswax (Martins, Cerqueira, Fasolin, Cunha, & Vicente, 2016; Öğütcü, Arifoğlu, & Yilmaz, 2015), carnauba wax (Lim, Jeong, Oh, & Lee, 2017), rice bran wax (Wijarnprecha, Aryusuk, Santiwattana, Sonwai, & Rousseau, 2018), candelilla wax (Dassanayake, Kodali & Ueno, 2011) and sunflower wax (Hwang, Singh, Winkler-Moser, Bakota, & Liu, 2014). Beeswax (BW) and carnauba wax (CW) have been extensively studied in the literature (Doan et al., 2018).

Beeswax, secreted from bees of the genus *Apis* (*A. dorsata, A. indica, A. florea* and *A. mellifera*), is a food-grade and a purified by-product of honey processing. It is commonly used in food products as a glazing, coating agent, and also applied to improve the chewing gum texture or as an additive carrier. Their chemical compounds are predominantly based on straight-chain monohydric alcohol compounds (carbon chains from C24 to C36) and straight-chain acids (carbon up to C36), including esters, diesters and triesters of C18 hydroxyl acids (Yilmaz & Dagdemir, 2012). The main components of BW are wax esters and n-alkanes, but it also contains a considerable number of hydrocarbons and free fatty acids. Beeswax was applied to gel pomegranate oil (Fayaz et al., 2017), virgin olive oil, hazelnut oil (Yılmaz & Öğütcu, 2015) sesame oil (Moghtadaei, Soltanizadeh & Goli, 2018), fish oil (Zhang, Zhang, Hu, Xu & Xu, 2020), rapeseed oil (Gao, Li, Zhang, Wang, Yu & Han, 2021), camellia, sunflower, corn and linseed oils (Han, Chai, Liu, Xu & Tan, 2021).

Carnauba wax is extracted from the leaves of *Copernicia prunifera*, a palm native of the north-eastern Brazilian states. It has a wide range of applications such as polishing, in the pharmaceutical industry as coatings to make tablets easier to swallow, thickener in cosmetics, and it is generally used in the food industry as a glazing and bulking agent, acidity regulator,

carrier, and anticaking (Blake et al., 2014). Over 80% of its chemical composition is represented by esters, with a predominance of aliphatic esters and diesters of cinnamic acid (Silva de Freitas et al., 2019). Many studies showed the gelling ability of carnauba wax in canola oil (Blake et al., 2014) and soybean oil (Lim et al., 2017; Yang, Yang, Chen, Chen & Liu, 2020; Bultimea-Cantuà et al., 2021).

Müller, Lindner, Briesen, Sommer & Forest (2018) observed that beeswax and carnauba wax showed a similar physico-chemical composition, but this composition differs in proportions, determining also different thermal behaviour. Among plant-based wax, CW showed the highest melting point (80-90 °C), higher than BW (63-83 °C). Moreover, CW has lower viscous and higher elastic properties (Doan et al., 2018) because it contains more branched methyl groups and a higher percentage of double- and triple-bonded carbon atoms than BW (Basson & Reynhardt, 1988).

The critical gelling concentration (C*) of natural waxes in different types of oil was reported in several studies (Dassanayake, Kodali, Ueno & Sato, 2009; Morales-Rueda, Dibildox-Alvarado, Charó-Alonso, Weiss & Toro-Vazquez, 2009; Hwang et al., 2012; Blake et al., 2014). The C* corresponds to the concentration of oleogelator at which no flow can be observed in the container (Doan et al., 2018). The critical concentration, indicated as C*, is defined as the minimum percentage of oleogelator required to gel liquid oil under particular time-temperature conditions. The inverted test-tube is the easiest method to evaluate C* and thus the oleogel formation. It is determined by preparing in different containers mixtures of oil/oleogelator with increasing wax concentration. After applying the chosen time/temperature parameters, and then cooling the samples, the containers are turned upside down. If a collapse of the structure is observed, it means that the oleogel is not structured, while if the structure is self-standing, a structured oleogel is formed. Hwang et al. (2012) reported that the chemical composition of the same wax species could affects the C*, highlighting those waxes with high ester contents are more efficient oleogelators.

1.2.3 Effect of oil type on oleogelation

The oil type plays a fundamental role in oleogelation, influencing rheological, textural and thermal properties of the oleogels. Their structure seems to be affected by different characteristics of the oil, such as fatty acids composition and solvent polarity, oil viscosity and dielectric constant, length of fatty acid chain, length of triglycerides, minor components and impurity. A high level of high melting triacylglycerides, saturated fatty acids, tends to strengthen the oleogel structure (Patel, 2015). Lupi, Gabriele, Facciolo, Baldino, Seta & de

Cindio (2012) also reported that crystallization and gelling temperature, as well as the storage modulus values of oleogels, increased with the saturation degree level. Moreover, the pore diameter of oleogel structure decreased with increasing unsaturation of the fatty acids present in the solvent phase (Zetzl, Marangoni & Barbut, 2012). Preparing oleogel based on vegetable oils with high oleic content such as olive oil, rice bran oil required a lower amount of oleogelators obtaining also economic advantages (Pehlivanoglu et al., 2018). The solvent polarity can affect the structure and mechanical strength of the oleogel. The oil polarity depends on the ratio of saturated/unsaturated fatty acids, the chain length of fatty acids, the conformation of the triglyceride chain, and the presence of polar/non-polar functional groups. The higher the polarity of the oil, the harder the oleogel (Doan et al., 2018). Long Chain Triglycerides-based oleogels showed different spacing and placement between crystals, in a lamellar conformation compared to medium-chain triglycerides (Martins et al., 2016). Texture parameters of oleogels, such as firmness, seems to be correlated with the viscosity and the dielectric constant of the oils containing long-chain fatty acids. Decreasing oil dielectric constant or increasing oil viscosity, firmness and rheological parameters linearly increased (Valoppi et al., 2017). Hwang et al., 2014 also investigated the effect of 12 different oils on the physical properties of sunflower wax-based oleogels, demonstrating how the gelling ability can be easily monitored by changing the composition of the liquid oil fraction. Another factor that influences the oleogel formation is the presence of impurities and minor components, such as free fatty acids, phospholipids and sterols, which can affect the crystallization, in terms of the start of nucleation (Co & Marangoni, 2012; Smith, Bhaggan, Talbot & Van Malssen, 2011).

1.2.4 Phase transition and oleogels properties

The sol-gel transition point of the oleogels can be rigorously determined by rheological methods, measuring the viscoelastic properties of the oleogel as in terms of both storage modulus G′ and the loss modulus G″, related to the elastic and the viscous behaviour, respectively. In detail, gelling behaviour is investigated performing temperature sweep tests and time sweep tests, where dynamic moduli are determined at a constant frequency as a function of temperature and as a function of time, respectively. Before the oleogel formation, mixture oil/oleogelator is in a molten state and shows a liquid-like behaviour with loss modulus G″ higher than storage modulus G′. Cooling the system, the solubility of the wax in liquid oil decreases and the wax begins to crystallize forming clusters and then aggregates (Marangoni et al., 2018). A sudden change in moduli values occurs at a critical temperature at which the sol-gel transition begins (T_{gel} G′=G″). G′ modulus becomes higher than G″, depending on the three-

dimensional network formation as a result of strong interactions between flocs (Feng & Cavicchi, 2012). High G′ and G″ moduli are usually associated with the presence of smaller crystals forming a well-structured homogeneous network, with excellent oil-binding properties (Blake et al., 2018). Physical characterization of oleogels can be obtained by carrying out small oscillation rheological tests. Strain sweep tests are usually performed to determine the linear viscoelastic regime (LVR) of the oleogels, which can change mainly depending on the type and concentration of wax, and also to differentiate ductile or brittle oleogels, depending on the linkage strength in the crystalline network (Doan et al., 2017). The oleogels generally exhibit a gel-like behaviour, namely that the elastic modulus (G′) dominates the viscous modulus (G″). LVR usually shortens as the wax concentration increases, indicating that wax-rich oleogels are more rigid and brittle than the low-wax oleogels (Wijarnprecha, et al., 2018; Doan et al., 2018; Patel, Babaahmadi, Lesaffer, & Dewettinck, 2015). To investigate the time-dependent deformation frequency sweep tests are performed, where G′ and G″ are measured as a function of frequency at a constant temperature, also allowing classification of the oleogels as weak, strong gels or viscous sols. Generally, an increase of rheological moduli occurs with an increase of oleogelator concentration, indicating a more strengthened structure able to withstand higher stress values before irreversible deformation occurs (Blake et al., 2014). To investigate the oleogels structuring degree the following model is proposed (Steffe, 1967):

$$G' = a(\omega)^b \tag{3}$$

where G' is the storage modulus (Pa), a (Pa·s^b) and b (-) are parameters used to describe rheological behaviour, ω is the frequency (rad/s) and b is the dimensionless flow behaviour index. The moduli of a completely structured oleogel do not depend on frequency (b→0); if the oleogelator concentration is not enough to create a well-structured crystal network an increase of the moduli, as the frequency increases, is observed (Martins et al., 2016). The parameter a is related to the oleogelator concentration and increases as it increases.

The crystallization behaviour, as well as the melting, of the wax oleogels, are studied using the differential scanning calorimetry (DSC). The methodology is based on the detection of enthalpic changes due to the self-assemblies and provides an insight into the thermodynamics of the gelator–gelator interactions. As reported by Martini Tan & Jana (2015) and Doan et al. (2017a), waxes crystallization in an oil (triglyceride system) is probably induced once a supersaturated state is achieved. The initial crystallization temperature is generally reported as the onset temperature of the first crystallization peak, while the melting temperature is indicated

as the melting peak temperature (Toro-Vazquez, Morales-Rueda, Dibildox-Alvarado, Charó-Alonso, Alonzo-Macia & González-Chávez, 2007). To study the oleogel melting and crystallization transitions is necessary to consider the chemical nature of the oleogelator and its interaction with the oil phase. The thermal behaviour of oleogel depends on the wax type and concentration (Blake et al., 2018; Winkler-Moser, Anderson, Felker, & Hwang, 2019), but also on the oil type (Patel, 2015; Hwang et al., 2014). The presence of more than a single exothermic peak is the result of the heterogeneous chemical composition of the waxes (Martins et al., 2016). For example, Doan et al. (2017) reported that BW based oleogel showed a crystallization profile with a maximum and a minimum peak, the first related to wax esters (the prevailing chemical classes of BW), while the second could be attributed to hydrocarbons (the second major chemical class in BW). From the solvent point of view, vegetable oils with a higher amount of saturated fatty acid (high melting TAGs) could contribute to strengthening the structure (Patel, 2015). Many investigators have determined that crystallization and melting temperature (T_c and T_m), as well as the crystallization and melting enthalpy (ΔH_c and ΔH_m), increase linearly with the wax concentration (Toro-Vazquez et al., 2007; Blake et al., 2014; Martins et al., 2016), consequently leading to the formation of a strong crystal network. Similar thermal behaviour was reported for oleogels prepared with BW and hazelnut oil (Yılmaz & Öğütcü, 2014a). The higher melting enthalpy at higher wax concentrations was explained by the formation of more crystals due to a higher supersaturation (Doan et al. 2017a). Moreover, T_c, T_m, ΔH_c and ΔH_m values of the wax-based oleogels are typically lower than those of crude wax. It is mainly due to the colligative properties and solubility of the wax components in the oil phase, and it could be explained by the greater dissolution of some of the wax components (such as esters and hydrocarbons) in the solvent as the ratio of oil/wax increases (Blake et al., 2018).

Physical properties as firmness, oil binding capacity (OBC), solid fat content (SFC) are evaluated to macroscopically describe oleogels.

The firmness of the oleogels is a texture property defined as the maximum force, expressed in Newton (N), measured during a penetration test. As reported by Toro-Vazquez et al. (2007), firmness increases as a function of the wax concentration, and is strongly related to the cooling temperature; since the oleogels formed at 5°C were firmer than those prepared at 25°C. Many studies demonstrate that firmer oleogels were related to networks containing a higher number of small crystals able to form more junction zones and to develop a stronger network compared to a system of larger and dispersed crystals (Kerr, Tombokan, Ghosh & Martini, 2011; Hwang et al., 2012; Dassanayake et al., 2009). The crystals number and size are strictly affected by the cooling rate. The presence of a large number of small crystals is observed in quickly cooled

gels, while slower cooling produced a lower number of larger crystals. Rapid cooling increased oleogels firmness because contain a high population of small crystals able to form deformation-resistant networks (Hwang et al. 2012).

Oleogels contain a large amount of oil therefore they should have a high oil-binding capacity (OBC). The oil that is not trapped in the three-dimensional network will separate and penetrate throughout the food matrix containing the oleogel. Early studies suggested that crystal morphology affected oil binding capacity, showing how waxes with needle-like morphologies, such as rice bran wax, displayed lower critical concentrations to achieve gelation than waxes with platelet-like morphologies (e.g. candelilla wax). Blake et al. (2014) were the first to investigate the OBC of oleogels based on plant waxes and canola oil, showing that the oil loss occurred in two stages, a fast rate and a slow rate stage. They also reported that candelilla wax had a higher OBC than rice bran wax, despite the morphological difference of the crystals described above. Those results prompted the researchers to investigate the relationship between the OBC and the microstructure, in particular with the box-counting fractal dimension $D_{b,}$ which is an indicator of the homogeneity of spatial mass distribution in a colloidal system (Tang & Marangoni, 2007). Oleogels with high D_b showed high OBC due to the homogeneously distributed solid mass in the system (Blake & Marangoni, 2015). Yılmaz and Öğütcü (2014b) investigated the OBC of beeswax and monoglycerides oleogels with hazelnut oil, forcing the oil separation with a centrifugation method. They showed that even if two oleogelators developed different crystalline morphology (needle-like crystals by BW and spherulite crystals by MG), at higher wax concentrations those differences did not affect the oil binding capacity since all the oleogels were able to retain oil (OBC>99%). The total amount of solid surface area increases as the crystal size decreases, consequentially more oil can be adsorbed, suggesting that the surface area of oleogelator crystals greatly affects the OBC of oleogels.

An important parameter for fat characterization, and therefore also for oleogels, is the solid fat content (SFC), which is the ratio between the solid and liquid fraction of fats at different temperatures. SFC is an important fat technological property; spreadability, plasticity and sensory profile are related both to it and the thermal conditions during processing or consumption. Solid fat content usually decreases as temperatures increase mainly due to the greater gelator mobility as the temperature increased (Winkler-Moser et al. 2019). The SFC is usually determined with a low-resolution NMR (Nuclear magnetic resonance) spectrometer, by conditioning the oleogels at different temperatures. Higher and lower SFC values are generally related to higher texture and rheological properties, and a high solubility of the wax in the oil, respectively (Toro - Vazquez et al., 2007). Higher SFC values enhances textural and rheological

properties of the oleogels. Oleogelator with too higher solubility in the oil could determine low SFC values. In order to obtain a good balance of gelator/gelator interactions and gelator/solvent interactions, an oleogelator should be neither too soluble nor insoluble in a solvent.

1.2.5 Microstructure modelling

1.2.5.1 Fractal dimension model

The macroscopic behaviour of fat-based food, including the spreadability of margarine, butter and spreads, as well as the snap of chocolate, depends on the microstructure of the fat-crystal network. Also, plastic fats show viscoelastic behaviour and present yield stress as a result of their crystal network (Narine & Marangoni, 1999). Some authors state that the network that forms inside the oleogels, potentially consisting of wax crystals, is an aggregation, first of clusters, then of fractal flocs resulting in a three-dimensional network like the lattice of fat crystals in hard fats (Tang & Marangoni, 2007). The microstructure of the fat crystal network and other colloidal system networks (such as oleogels) can be quantified through the fractal theory. Assuming that the oleogel solid network is a collection of fractal flocs, the fractal dimension model can be applied by exploiting their rheological properties. Oleogel rheological properties depend on both the volume fraction of solids and the microstructural characteristics of the network such as size, shape and spatial distribution pattern of crystals. The elastic modulus G' obtained from small oscillation rheological measurements seems to be sensitive to the microstructure of the system and give also a measure of the hardness of the material. The yield stress σ* is also related to fractal particle network, depending on the volume fraction of solids, to the primary particle size and intermolecular forces solid-liquid interfacial tension (Marangoni & Rogers, 2003). Shih, Shih, Kim, Liu & Aksay (1990) proposed to distinguish between two different rheological regimes, namely strong-link and weak-link, based on the relative strength of the inter-floc and intra-floc links. The strong-link regime occurs at low volume fractions of solids ($\varphi < 0.1$) when the intra-floc is lower than the inter-floc interaction and γ_0 (the strain at the limit of linearity) decreases as a function of φ. The weak-link regime occurs when inter-floc links predominate at a higher volume fraction of solids ($\varphi > 0.1$), and γ_0 increases with φ. For materials following a strong-link regime G′ and σ* depend on the fractal dimension of the flocs (D), as in the following equations:

$$G' \sim \phi^{\frac{d+x}{d-D}} \tag{4}$$

(5)

$$\sigma * \sim \frac{6\delta}{a}\phi^{\frac{d+x}{d-D}}$$

where d is the Euclidean dimension of the network (usually 3); δ is the solid-liquid interfacial tension; a is a constant which is dependent on the size of the primary particles and the interactions between them; ϕ is the particle volume fraction of solid fat; x is the backbone fractal dimension that describes the tortuosity of the stress transduction chain within a cluster of particles under an externally applied stress and that is estimated between 1 and 1.3 (Shih et al., 1990). The fractal dimension is a parameter able to capture the complexity of the combined effects of morphology and spatial distribution of mass within the network in a single parameter.

1.2.5.2 Crystallisation kinetics

Crystalline network formation can be investigated by exploiting the thermal and rheological behaviour of the oleogels. The physical properties of crystalline materials such as fats as well as oleogels are related to the mode of nucleation and the dimensionality of crystal growth. Depending on the cooling profile, the nucleation mode could be defined as instantaneous (i.e., all nuclei form at once) or sporadic (i.e., nuclei form as a function of time). Following nucleation, crystal growth that indicates the number of axes in which growth of crystals takes place can occur in one two or three axes, forming 1-dimensional (fibres), 2-dimensional (plane) and 3-dimensional (sphere) crystals, respectively (Lam & Rogers, 2011). The most extensively used model to study crystallization kinetics is the Avrami model. However, especially in industrial applications, crystallization occurs under non-isothermal cooling conditions, and an Avrami modified model could be applied (Lam & Rogers, 2011). Moreover, the crystalline network formation should be monitored following the evolution of both, thermal and rheological parameters. Therefore, for non-isothermal conditions, the crystallization kinetics can be modelled using a modified Avrami model as follow:

$$\frac{Y_s}{Y_{max}} = 1 - e^{-k_{app}(t-t_0)^n} \quad (6)$$

where Y_s/Y_{max} ratio is the relative degree of crystallinity (Y_s and Y_{max} are the crystalline solid volume formed until a given time and the maximal crystal phase volume, respectively), k_{app} is the apparent crystallization rate constant, n is the Avrami index and finally, t and t_0 are the time and the induction time, respectively (Lam & Rogers, 2011; Palla, de Vicentec, Carrína & Gálvez Ruizc, 2019). The Y_s/Y_{max} ratio could be obtained by integrating the area underlying the crystallization peak according to the following equation:

$$\frac{Y_s}{Y_{max}} = \frac{\int_0^t (t) \frac{dH(t)}{dt} dt}{\int_0^\infty (t) \frac{dH(t)}{dt} dt} \quad (7)$$

where *dH(t)* is the enthalpy of crystallization during an infinitesimal time interval *dt*.
The Y_s/Y_{max} ratio could be also estimated through rheological measurements and reported as (Liu & Sawant, 2001):

$$\frac{Y_s}{Y_{max}} = \frac{\eta^*(t) - \eta_{solv}}{\eta^*_{max} - \eta_{solv}} \quad (8)$$

where $\eta^*(t)$ corresponds to the complex viscosity at time t, η^*_{max} indicates the maximum sample complex viscosity, and η_{solv} is the complex viscosity of the solvent. The complex viscosities should be obtained from temperature sweep tests.
The Avrami index allows to determine the type of nucleation and dimensionality of the crystal growth process and it should be ranged from 2 to 4, assuming integer number for homogeneous nucleation. An Avrami exponent equal to 2 indicates a 1-D crystal growth mechanism, a value of n = 3 corresponds to a 2-D mechanism and an n = 4 follows a 3-D mechanism. The Avrami index that assumes a non-integer value indicates heterogeneous and secondary nucleation.

1.2.6 Food application

Oleogels can provide a wide range of applications in the cosmetic, pharmaceutical and food industries. They could be used for different purposes, not only to replace saturated fatty acids, but also to prevent oil migration and to promote the controlled release of nutraceutical bioactive or aromatic compounds. Many variables are to be considered to best incorporate an oleogel in a food matrix, such as the selection of food-grade oleogelator and the process parameters to produce both the oleogel and food product. Moreover, a fundamental understanding of the formation mechanism and physical properties of the fat phase, especially novel oleogels, is necessary to better control their food application. Wax-based oleogels prepared with different types of vegetable oils have been used in several foodstuffs, as reported in **table 1.2**.
Numerous studies investigated the use of oleogels as a spreadable fat alternative to promote healthy eating behaviour (Hwang et al., 2013; Hwang et al., 2014; Patel et al. 2014; Yilmaz & Öğütcü 2015). Soybean oleogels based on sunflower wax resulted suitable to develop a healthy margarine since the firmness of the novel product and the reference were comparable (Hwang

et al., 2013). Yilmaz and Ögütcü (2015) tested the sensory properties and consumer acceptance of virgin olive and hazelnut oils in oleogel form prepared with beeswax and sunflower wax, revealing that both types of oleogels are physically and sensorially suitable as alternative breakfast spreads.

Zulim Botega, Marangoni, Smith & Goff (2013) used oleogels based on high oleic sunflower oil and rice bran wax to replace the solid fat in ice cream. They reported that the ice cream sample with oleogels showed similar meltdown and granulometric distribution of air cells compared to the milk fat ice cream.

Table 1.2 Summary of application of wax based oleogel in food system

Wax type	Oil type	Food system	Function	References
Carnauba Beeswax Sunflower wax rice bran wax candelilla wax	Virgin olive oil Hazelnut oil Soybean oil	Spreadable fat alternatives	Saturated fat reduction	Hwang et al., (2013); Hwang et al., (2014); Patel et al. (2014); Yilmaz and Ögütcü (2015)
Rice bran wax Candelilla wax Carnauba wax	High-oleic sunflower oil	Ice cream	Saturated fat reduction	Zulim Botega et al. (2013)
Sunflower wax Rice bran wax Beeswax Candelilla wax	Olive oil linseed oil soybean oil canola oil hazelnut oil	Cookies	Saturated fat reduction	Hwang et al. (2016); Jang et al. (2015); Mert & Demirkesen (2016); Yilmaz and Ögütcü (2015)
Candelilla wax Carnauba wax	Grapeseed oil Sunflower oil	Bakery product	Shortening replacer	Lim et al. (2017b); Kim et al. (2017); Oh et al. (2017); Pehlivanoğlu et al. (2018)
Rice bran wax Sunflower wax	soybean oil High-oleic soybean oil	Cheese products	Saturated fat reduction	Bemer et al. (2016); Park et al. (2018); Huang et al. (2018); Moon et al. (2021)
Beeswax Propolis wax	Rice bran oil Pomegranate oil	Confectionery fillings	Saturated fat reduction	Doan et al. (2016); Fayaz et al. (2017)
Beeswax Rice bran wax	linseed oil olive oil linseed oil fish oil Sesame oil Soybean oil	Meat-based products	Saturated fat reduction	Moghtadaei et al. (2018); Wolfer et al. (2018) Franco et al. (2019); Gómez-Estaca et al. (2020);
Carnauba wax	Soybean oil	Deep frying	Decreased oil absorption; saturated fat reduction	Lim et al. (2017a); Adrah et al. (2021)

Several studies have been done to replace saturated fat with oleogels in cookies. The texture, sensory properties and stability of cookies prepared with sunflower and beeswax hazelnut

oleogels were investigated by Yilmaz and Öğütcü (2015) using a commercial shortening as reference. The authors reported that cookies prepared with oleogels showed a more aerated structure; also they were softer and preferred compared to shortening cookies, as assessed by the sensory evaluation panel. Jang, Bae, Hwang, Lee & Lee (2015) tried to completely replace the shortenings of cookie doughs using oleogels based on canola oil and candelilla wax. They reported that a complete replacement of shortening with oleogels increased the level of unsaturated fatty acids from 47% to 92%, but reduced viscoelastic properties of the cookie compromising the structure. Later, a similar study was conducted by Mert and Demirkesen (2016) who demonstrated that the same type of oleogels can be used to partially replace shortening in short dough cookies. Samples prepared with oleogel-shortening mix were slightly softer and similar in texture and extensibility of dough compared to cookies containing only shortening.

The most difficult challenge using oleogels as a solid fat replacer in bakery products is preventing the volume loss, because the lower the viscosity batters, the lower the final product volume. Oleogels should be also used as shortening replacers in bakery products, including muffins and cakes. Lim et al (2017) successfully introduce grapeseed oil and candelilla wax oleogels in muffin formulation, obtaining muffin batters with lower viscosity and less elasticity when the oleogels/shortenings ratio was 1:3. Kim et al. (2017) reported that a 25% of shortening replacement with oleogels prepared with canola oil and carnauba wax should maintain the cake quality, in terms of volume, porosity and fragmentation index of cake crumbs. In the study of Oh, Amoah, Lim, Jeong & Lee (2017) on the potential application of natural wax-based oleogels in baked cakes, it was demonstrated that only beeswax oleogels were allowed to produce cakes with physical properties comparable to the control sample. Cakes prepared with carnauba wax oleogel based on high oleic acid sunflower oil and cottonseed oil were considered acceptable from a sensory point of view (Pehlivanoğlu, Ozulku, Yildirim, Demirci, Toker & Sagdic, 2018).

In a cream cheese product, vegetable fat is often preferred to milk fat for both economical and nutritional reasons, but it is important that those alternatives exhibit physical and hedonic properties like the conventional cheese products. The potential use of ethylcellulose and rice bran wax oleogels with soybean oil and high oleic soybean oil in cheese dairy products have been investigated by Bemer, Limbaugh, Cramer, Harper & Maleky (2016). The authors compared physicochemical properties of three different samples, oleogel-based cream cheese, full-fat and fat-free commercial cheeses, demonstrating an effective reduction in saturated fat and an increase in unsaturated oil using oleogels. Oleogel samples showed similar lipid globule

size and consequentially similar texture to commercial control groups (Bemer et al., 2016). Park, Bemer & Maleky (2018) and Huang, Hallinan & Maleky (2018) also used rice bran wax to gel high oleic sunflower oil and soybean oil respectively, introducing the formed oleogels in cream cheese products. In detail, the first work investigated the oxidative stability and tocopherol content of oleogels based cream cheese, using an ungelled based cheese (prepared with only oil) and commercial samples as controls. Commercial samples showed the lowest tocopherol content, while there were no significant differences in its amount for ungelled cream cheese and oleogel-based cream cheese (Park et al., 2018). On the other hand, Huang et al (2018) demonstrated the potential applications of oleogels in the replacement of milk fat producing cheese products with desirable rheological and thermomechanical properties. An imitation cheese low in saturated fat was successfully produced by Moon, Choi, Jeong, Kim & Lee (2021) using carnauba wax and canola oil-based oleogels as palm oil substitutes.

Chocolate spreads, as well as spreadable or filling creams, are generally high caloric foods, fat and sugar-based. The fat phase is commonly represented by cocoa butter, palm oil, coconut oil and other fats rich in saturated fatty acid. Therefore, two main studies have been focused on the reduction of saturated fat in confectionary fillings using wax oleogels, Doan et al. (2016) and Fayaz et al. (2017). Doan et al (2016) used rice bran oil oleogel at different beeswax concentrations to partially replace the palm oil in hazelnut fillings. They first characterized oleogels/palm oil mix, at different replacement levels (17, 33, and 50%wt), in terms of thermal and rheological behaviour, analyzing also their microstructure, solid fat content and oil binding capacity; then the effect of continuous wax-based fat phases on hazelnut fillings structure was investigated. A dilution effect occurred when palm oil was partially replaced with oleogels, consequentially a shift to lower temperatures of both crystallization and melting peaks was observed. The systems containing 17% of oleogel and 83% of palm oil was found to be the most efficient fat phase since it showed an higher oil binding capacity and crystalline density and the same strength compared to the reference sample containing 100% palm oil. In the work of Fayaz et al. (2017) oleogels-palm oil mixtures (1:1 ratio) have been used to produce functional chocolate spread. Beeswax, propolis wax and monoglycerides have been used to solidify pomegranate seeds oil, and the physical and mechanical properties of both oleogels-palm oil systems and chocolate spreads have been investigated. Although wax oleogel showed similar morphological structures, rheological parameters (storage and loss modulus) and oil binding capacity of propolis-based oleogels were found to be lower than those of beeswax wax. The authors explained that in the samples with a weak gel strength, the formation of the

crystalline network was negatively affected by phenolic compounds and the low chemical compatibility between propolis wax and pomegranate oil.

The potential application of oleogel as an animal fat replacer to improve the nutritional characteristics of meat products have been also investigated. Moghtadaei et al. (2018) evaluated the physicochemical properties of beef burgers with different levels of animal fat substitution with oleogel prepared with sesame oil at different beeswax concentrations. The introduction of oleogels into beef burger formulation positively reduced the cooking loss and the fat absorption but diminished the hardness gumminess and chewiness of the raw burgers, which were negative aspects of animal fat replacement. From a sensory point of view, no difference in texture and colour between oleogels-burgers and control samples were found (Moghtadaei et al., 2018). Oleogels prepared with soybean oil and rice bran wax have been used to replace pork back fat in frankfurter-type sausages Wolfer, Acevedo, Prusa, Sebranek & Tarté (2018). The replacement did not significantly affect the firmness, chewiness and springiness of sausages, but a reduction of the flavour occurred, even if it was not perceived by sensory judges. The pork back fat reduction using oleogels was also explored by Gomez-Estaca et al. (2019) to reduce the trans and saturated fats content and to improve the nutritional profiles of pork liver pâtés. A mix of olive, linseed, and fish oil have been structured by using different types of oleogelators, including beeswax. It was reported that sensory parameters, cooking loss and water loss values were not significantly affected by the replacement of pork back fat with beeswax oleogels (Gomez-Estaca et al., 2019).

It is well known that the consumption of fried foods is strictly related to coronary, obesity, diabetes and other diseases, especially due to the retention of the high amount of oil inside the fried product, depending also on the frying medium. Therefore, the potential use of oleogels as frying medium was investigated by Lim et al. (2017) and Adrah, Adegoke, Nowlin & Tahergorabi (2021) using different foods, instant noodles and chicken products, respectively. The first work compared semisolid medium, made up of carnauba wax and soybean oil oleogels, with conventional soybean and palm liquid oil for frying noodles. A great reduction in saturated fat content and the lowest oil uptake was obtained using oleogels. The oxidation stability of the soybean oil was retarded when it was in gel form, however the highest oxidation stability belonged to palm oil. In the second investigation chicken samples were deep-fat fried in canola oil and carnauba wax oleogels, using canola oil as reference. Also, in this case, a lower fat uptake was observed using oleogels. Samples fried in different frying mediums differed in colours properties in the crust structure and moisture values but showed similar protein and ash contents.

1.3 Natural sweeteners as sucrose substitutes

Sugars daily intake should be less than 10% as stated by the WHO guidelines, because its consumption negatively affects health increasing several chronic diseases risk, such as obesity and diabetes (Patel, Moghadam, Freedman, Hazari, Fang & Allen, 2018). On the other hand, sugar plays an important role in many foodstuffs, conferring sweet taste, influencing flavour mouthfeel and texture profile to the products. A spreadable cream contains generally more than 30%-40% of sugar dispersed in the fat phase. The functional properties of solid sugar particles include sweetness, stability, particle size distribution, mouthfeel (texture), and strictly influence rheological properties of the product which are fundamental for chocolate-based products (Jeffery, 1993). The most used strategy to reduce sugar in the food system is product reformulation, but also other methods could be used including the application of multisensory interaction. Product reformulation includes partially or totally sugar replacement or directly a sugar amount reduction with no substitution (Di Monaco et al., 2018). For example, in Oliveira et al. (2016) the sugar reduction approach was shown as an easy method to produce low sugar chocolate-flavoured milk without affecting consumers' perception (Oliveira et al., 2016). However, the latter method could be harder to be applied in foods where sugar plays an important structural role.

To produce healthy products, the use of high-intensity sweeteners (HIS), which are calorie-free, allows to reduce the sugar content, but they may confer undesirable flavours that can limit their applications. Many of the previous studies have focused on artificial non-nutritive sweeteners (NNS) such as neotame, sucralose, or aspartame (Morais, Morais, Cruz, & Bolini, 2014). However, the demand for natural ingredients is increasing, thus the availability of products with natural sweeteners should be expanded to satisfy consumer needs. Products with natural sweeteners are often preferred over artificial sweeteners by parents for the nutrition of their children, as reported by Li, Lopetcharat & Drake (2014) with regard to chocolate flavoured milk. Moreover, alternative natural sweeteners contain beneficial bioactive compounds, such as polyphenolic compounds with antioxidant properties. Natural sugars can be obtained from plant leaves such as maple syrup, stevia and agave nectar, grains such as molasses, barley malt and brown rice syrup, or vegetables and fruits like carrots. Lucuma, explained later in detail, is low glycemic natural sweetener that also could be used to reduce the sucrose intake and improve the nutritional profile due to the high content of potentially beneficial bioactive compounds (Ak-Cvitanovìc et al. 2015).

1.3.1 Lucuma

Lucuma (*Pouteria Obovata*) is a subtropical fruit belonging to the *Sapotaceae* family, with a dark green peel and a yellow pulp with a mealy texture. Most of the world production (88%) is in Peru, especially in Lima, but it is also cultivated in Chile and Ecuador, where it also is known as "Inca Gold" due to its high nutritional qualities. Its consumption seems to help children's physical development and regulate the metabolism of adult people because of fibre and iron amount. It is also a source of niacin and beta-carotene, which are useful to prevent depression problems and heart attack, respectively, and increase the immunologic system efficiency, as well as reducing cholesterol and triglycerides in the blood. Moreover, it shows a high content of phenol and flavonoid components that make it a superfood (Taiti, Colzi, Azzarello & Mancuso, 2017). The Peruvian fruit shows a sweet, pleasant taste that is like caramel thus it should be used as natural food sweetener (Banasiak, 2003; Dini, 2011; Rojo et al., 2010). Different products have been produced using lucuma as an ingredient, such as ice cream, juices, cakes, biscuits, yogurt, chocolate, baby food and pies (Durakowa et al., 2019). Flour or pulp are the best ways to export Lucuma without compromising the quality parameters such as colour, structure and texture. Fresh lucuma fruits are generally processed at the maturation point to retain taste and colour in the final flour. Lucuma powder is obtained from the dried and pulverized pulp; from 4 kilograms of fresh fruit 1 kilogram of lucuma powder was obtained.

1.4 Project outline

The PhD thesis focuses on the development of a new spreadable cream by using oleogels as solid fat replacers and natural sweeteners to reduce sucrose intake. Firstly, participants were asked to fill in an online questionnaire specially designed to explore the consumers' variables that may drive the food healthy choices and to define the possible ingredients to use for the development of a new healthy spreadable cream (**chapter 2**). Secondly, the core of the project was focused on the development of oleogels able to reduce the solid fat, and consequentially the saturated fatty acid amount, usually used in cream production. Studying the formation mechanism and physical properties of novel oleogels that have not yet been explored is necessary to better control their food application. Therefore, the effect of fatty acid composition and viscosity of 12 uncommon vegetable oils on network formation mechanism and physical properties of oleogels was studied (**chapter 3**). In the **chapter 4** a novel pumpkin seeds oil-based oleogel was developed and characterized, analysing the effect of wax type and concentration on microstructural, rheological and thermal behaviour. The substitution of solid hard fat with food grade oleogels could affect the cream performances and thus the product

quality. It is therefore of utmost importance to study the physical properties of both the mimicking fat and the oleogels based creams. Moreover, it should be considered that powder type could also affect refining process as well as the structure of a cream. Therefore, the last step aimed to assess how the physical properties of the creams, with different fat and/or sugar phase, can be modified during refining (**chapter 5**).

Finally, **chapter 6**, provides final remarks and reflects on potentials and limits of the project. Main conclusions and a brief consideration on future perspective are also given.

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CHAPTER 2

Submitted on Applied Food Research

WHAT CONSUMERS WITH OPPOSITE EATING BEHAVIORS WANT TOWARDS A NEW HEALTHY CREAM

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Abstract

Consumers are increasingly aware of health and nutrition. Individual food choices are influenced by many interacting factors, and in turn, play a pivotal role in health. Nowadays, the demand for healthy sweet products is growing, and a new healthy sweet spreadable cream is in line with the market trend as long as it respects the needs of consumers. Indeed, the intervention strategies to reduce the number of calories need to be consumer-driven to be successful. The first aim of this study was to investigate how individual variables, such as gender, age, BMI and health interest (HTAS) may drive healthy food choices. The second aim was to select some ingredients to develop a new healthy spreadable cream, taking into account the opinion of subjects with different healthy choice attitudes.

To these objectives, 400 consumers filled in a market online survey about cream consumption, individual variables, psycho-attitudinal factors, and food preferences and choices. A forced choice questionnaire was developed to evaluate preferences within a pair of food items similar in flavour but different in calories content. Subjects were then classified into two groups "healthy lovers" and "unhealthy lovers" based on the calculated healthy choice index (HCI) and the effect of HCI on individual variables and food preferences were studied. The unhealthy lover group was more represented by young, which were regular consumers of sweet products, including sweet creams, and showed more positive attitudes in using food as reward and in craving for sweet food. They were also more familiar with products perceived as fatter, compared to healthy lovers. A healthy sweet cream would be consumed by both categories of consumers, which considered an increment of cocoa, dried fruit and natural sweeteners as a valid alternative to reduce the calories content of the product.

Keywords: healthy choice index; spreadable cream; consumer expectations; HTAS; food choice; consumption frequency.

1. Introduction

It is well known that the consumption of foods recognized as junk foods negatively affects health. To prevent a multitude of diseases, such as obesity, cardiovascular disease and cancer, it is necessary to follow healthy dietary guidelines. Consumers have been urged to consume foods with low sugar, salt, and fat content owing to health concerns (Bagheri, Radi & Amiri, 2014).

Intervention strategies that reduce the number of calories consumers choose should potentially achieve both economic benefits and improvements of personal and public health (Brownell et al., 2009). A wide range of intervention strategies, including public campaigns targeted at promoting increased consumption of healthy foods, have been implemented to reduce obesity prevalence (Capacci et al., 2012). The intervention strategies on public health issues can be applied at the education-, marketing- and/or law level (Rothschild, 1999). However, systematic reviews focused on the effectiveness of intervention strategies shows mixed and inconclusive results (Brambila-Macias et al., 2011; Capacci et al., 2012; Kesten, Griffiths & Cameron, 2011). Andreasen (2002) contends that strategies should be consumer-driven to be successful.

Indeed, intervention strategies should be designed to introduce new healthy foods on the market, through the understanding of consumers' experiences, beliefs, and desires, all of which contribute to consumer preference and choice. Food choice is a highly complex process with variation both within and between individuals, and choices are often very reflective or habitual and automatic (Sobal & Bisogni, 2009). According to Wądołowska, Babicz-Zielińska, & Czarnocińska (2008) food preferences interact with many individual variables (such as BMI, gender and age (Hearty, McCarthy, Kearney & Gibney, 2007; Carrillo, Varela, Salvador & Fiszman, 2011)) and different food choice factors, in particular health (Ares & Gámbaro, 2007; Marsola, Cunha, Carvalho-Ferreira & da Cunha, 2020), which in turn interacts with the frequency of food intake. The healthiness concept is mainly influenced by the perception of the fat and calorie content (Carels, Harper & Konrad, 2006; Oakes & Slotterback, 2001; Carels, Konrad & Harper, 2007). On the other hand, unhealthy food products, especially those with high-fat content, are often preferred because perceived as more palatable (Fazzino, Rohde & Sullivan, 2019). Also, the sensory performance of many foodstuffs is improved by the presence of fat, especially hard-stock fats rich in saturated fatty acids.

Among the vastness of unhealthy products, spreadable creams cover an important market share (IRI research institute). Spreadable creams can be defined as complex food systems consisting of several solid particles (sugar, nut solids, cocoa powder, milk whey, milk powder, dehydrated cream, etc.) dispersed in a continuous liquid (oil) or semi-solid phase (cocoa butter, palm or

coconut oils) (Miele, Borriello, Fidaleo, Masi & Cavella, 2020). They represent an important ingredient in different confectionery foods but are generally characterized by a medium-high calorific power due to the presence of a large amount of saturated fat and sugar providing taste, texture, mouthfeel and flavour to the product. From the data recorded by the IRI research institute on the Italian total of hyper-, super- and mini-markets, spreadable creams were among the stationary market segments in 2017-2018. In the following two years, there was an increase in their sales, ranking among the product categories that generated the most additional turnover in large-scale distribution: 67 million euros more, between January and mid-May 2020. The lockdown related to the Covid-19 emergency was characterized by a "provisions effect" and by a (forced) rediscovery of breakfast at home which prompted Italians to buy more sweet spreads (https://www.ilsole24ore.com/art/il-consumo-creme-spalmabili-cresce-anche-il-lockdown-maggio-vendite-188percento-ADSN0yb (accessed on 29/10/2021)). The confectionery products that were previously consumed as "solid-snacks" are now re-proposed in a spreadable form. Moreover, the market for new spreadable products that follow the current trends, such as organic, vegan and "free-from", is also growing.

Therefore, according to this background, a new healthy sweet spreadable cream development would be in line with the needs of the current market and could be used as an attractive strategy to encourage healthy eating behaviours.

This work aimed to explore the consumers' variables that may drive the food healthy choices and to define the possible ingredients to use for the development of a new healthy spreadable cream. To take into account the opinion of people with different eating habits and preferences, subjects were classified into two groups "healthy lovers" and "unhealthy lovers" based on the healthy choice index (HCI). Therefore, firstly, the effect of HCI on gender, age, BMI, consumption frequency, Health and Taste Attitude Scale (HTAS), food familiarity and liking was studied. Secondly, the effect of HCI on a list of ingredients was investigated to identify those that could be used to develop a new healthy spreadable cream and, simultaneously, move the unhealthy lovers towards healthy food choices.

Methods

2.1 Overview of data collection

Participants were asked to fill in an online questionnaire at home. A platform called "Survio" (Survio® 2012-2021) was used to create the questionnaire. The questionnaire was divided into different sections (**Table 1**) explained in detail in the following paragraphs. First, socio-demographic (self-reported gender, age, education) and socio-economics information were

collected. Next, participants were asked to fill in a set of questionnaires to measure attitudes towards choice, food, liking, and familiarities. To conclude, they indicated their preferences about spreadable creams, such as taste and flavours, to understand the consumer expectations towards a product that is not born to be healthy.

Considering the high number of questions, trap questions were used to ensure the data quality. Therefore, among the questions, there was a block of text that asked the respondents to ignore the question and their real answer, and to instead select certain response options as instructed. This technique was used to identify non-attentive respondents who did not pay attention to the instruction. Moreover, subjects were explained that they could interrupt the questionnaire anytime and resume whenever they wanted from the stopping point.

Table 1 Questionnaire sections, their relative options, items, categories and rating scale.

Questionnaires	Variables	Options
Socio-demographics & socioeconomics	Gender	M/F
	Age	Years old at the moment of the test (18-100)
	Place of birth/residence	Place of birth/residence Province and Council
	Educational level	None/Primary/Lower secondary/Upper secondary/Degree/Post-degree
	Marital status	Married or living with a partner/Divorced or separated/widowed/Celibate or maiden
	Employment status	Employed/student/freelancers/housemaker/retired/un employed
	Number of persons in the house	1-7
	Monthly food spending (euro)	Up to 200/From 201 to 400/From 401 to 600/More than 600
	Monthly confectionary food spending (euro)	Up to 100/From 101 to 200/From 201 to 300/More than 300
Anthropometric	Weight	(self-reported)
	Height	(self-reported)
	Diet	11 items (adapted from De Backer & Hudders, 2015).
Physical health	Practice of restrictive diets	No/Yes, low calorie diet Yes, for medical reasons (if yes, which one:*) Yes, but not for medical reason
	Food allergies	Yes/No; if yes, which one
	Food intolerances	Yes/No; if yes, which one
	Use of medicines	Use for: Flow of bood/Blood pressure/Arthritis pain/To sleep/Headaches/Digestion/Diabetes/To facilitate the movement/Memory/Arrhythmias/ Antidepressants/Hormonal therapies/None
	Use of supplements	Use for: sport/health and well-being/diabetes/against aging/beauty/none
forced choice questionnaire	17 pairs	Forced-choice between two options
Frequency of consumption	- Confectionary food - Sweet creams - Healthy food	7-point category scale (everyday; 3 to 4 times a week; 2 times a week; once a week; 2 to 3 times a month; at least once a month; never)

	- Functional food	
Health and Taste Attitudes Scale (HTAS)	38 items – 6 domains: - general health interest (GHI) - light product interest (LPI) - natural product interest (NPI) - craving for sweet foods (CSF) - food as a reward (FR) - pleasure (P)	7 point Likert scale (1 = disagree strongly; 7 = agree strongly) Roininen et al. (1999)
Familiarity	30 items – 2 categories: - Ingredients (14) - Confectionary products (16)	5-point labeled scale (1 = I do not recognize it; 2 = I recognize it, but I have never tasted it; 3 = I have tasted it, but I don't eat it; 4=I occasionally eat it; 5 = I regularly eat it); (Tuorila et al., 2001)
Liking	30 items – 2 categories: - Ingredients (14) - Confectionary food (16)	9-point hedonic scale (1 = extremely disliked; 5 = neither liked nor disliked; 9 = extremely liked); (Peryam & Pilgrim, 1957) + option: ''I have never tasted it"
Specific questions about creams	If you had the opportunity to create a sweet cream, what would you add to make it healthy?	Open answer
	If you had the opportunity to create a sweet cream, what would you remove or reduce to make it healthy?	Open answer
	If you had the opportunity to create a sweet cream, which ingredients would you choose?	Wot, hazelnut, pistachio, almond, chestnut, coconut, coffee, macadamia nut, cocoa, cashew nuts, peanuts, white chocolate

2.1.1 Forced choice questionnaire

A binary forced-choice questionnaire was carried out to obtain information on the importance given by the consumer to the healthy food category. In particular, a binary answer was chosen instead of the 7-point scale generally used (Dinnella, Recchia, Tuorila & Monteleone, 2011), to optimize the discriminating capacity of the questionnaire. The forced-choice questionnaire was developed to evaluate preferences within a pair (similar in flavour but especially different for the caloric count) of 17 items developed based on calories dichotomies. The presentation order of the food items, within and between each pair, was randomised across participants.

2.1.2 Consumption frequency

Information about frequency of consumption was collected for confectionery food, sweet creams, healthy and functional food. A 7 point labelled scale (1 = everyday; 2 = 3 to 4 times a week; 3 = 2 times a week; 4 = once a week; 5 = 2 to 3 times a month; 6 = at least once a month; 7 = never) was used. Frequency of consumption of those foods was expressed as yearly frequency (e.g. 1 time/month =12, evryday =365, etc.) and log-transformed to reduce skew (Puleo, Castillo, Di Monaco & Stieger, 2021).

2.1.3 Health and Taste Attitudes Scale

The individual orientation towards the hedonic and health characteristics of food was measured with the Health and Taste Attitudes Scale (HTAS) questionnaire (Roininen, Lähteenmäki &Tuorila, 1999; validated in Italian by Saba et al., 2019), organized into 6 different domains: three domains were health-related (the general health interest (GHI), i.e., interest in eating healthily; light product interest (LPI), i.e., interest in eating reduced-fat foods; and natural product interest (NPI), i.e., the interest in eating foods that do not contain additives and are unprocessed), and three domains were taste-related (craving for sweet foods (CSF), food as a reward (FR), and pleasure (P)). The HTAS items were scored on a seven-point category scale with the scales labeled from ‘‘disagree strongly” to ‘‘agree strongly”. For each participant and each subscale, after recodification of negatively worded items, a mean score was computed from the individual scores.

2.1.4 Food familiarity and Liking

The Food Liking and Food Familiarity questionnaires were developed to measure, respectively, liking for and familiarity with a selection of 30 items divided into two categories: ingredients that could be used in a new cream formulation and confectionary food that generally contains filling creams. Stated liking was assessed using a nine-point hedonic labelled scale with the addition of the option “never tasted it”. The concept of liking is often linked to familiarity, which plays a fundamental role in the consumer's food choices, as demonstrated by several studies (Arvola, Lähteenmäki & Tuorila, 1999; Tuorila, Meiselman, Bell, Cardello & Johnson, 1994). The same items were scored on a five-point labelled scale to measure familiarity (Tuorila, Lähteenmäki, Pohjalainen & Lotti, 2001).

2.2 Participants

A total of 410 respondents took part in the survey. However, 10 individuals did not complete the questionnaire. Thus, data of 400 Italian consumers were collected through a web-based survey Participants were recruited by sharing the survey online via social network sites.

2.3 Data Analysis

Socio-demographic characteristics were descriptively analysed. The internal consistency of each domain of the HTAS was measured by Cronbach’s alpha. From the forced choice questionnaire, a choice index was calculated as a sum of the choice of the healthy options, assigning to that one a value of 1, with higher scores reflecting higher choice for the healthy

options. Based on the calculated index, subjects were classified into two sub-groups representing low (unhealthy lovers) and high (healthy lovers) scores, using the median values as cut-off (Puleo, Masi, Cavella & Di Monaco, 2021). Participants with the median score were excluded from the dataset.

The effect of HCI was calculated on gender and age, using the chi-squared test, and on BMI using one way ANOVA. One way ANOVA models were applied to test the effect of HCI on the other variables considered in the study (consumption frequency, HTAS, food familiarity and liking). The significance level was set at 0.05. All data were analyzed using XLSTAT (Version 2016.02, Addinsoft).

2. Results and Discussion

3.1 Data description

3.1.1 Consumer's socio-demographic characteristics

Characteristics of the n=400 participants are reported in **Table 2**.

Table 2 Socio-demographic characteristics of survey respondents.

Characteristics of the Participants	***%***
Gender	%
Women	69
Man	31
Age	%
< 30	55
> 30	45
BMI	%
Slight underweight	25
Normal weight	49
overweight	26
Education level	***%***
middle school	4
high school	31
degree	52
post degree	13
Marital status	*%*
celibate/maiden	69
married	29
divorced	1
widowed	1
Family members (n)	4 (0.52 SD)
Profession	%
employed	37
student	34
busy	15
freelancers	5

housewife	5
retired	2
unemployed	2
Expense for food (monthly, €)	%
up to 200	17
from 201 to 400	47
from 400 to 600	23
more than 600	13
Expense for confectionary foods (monthly, €)	%
up to 100	86
from 101 to200	12
from 201 to 300	2
more than 300	0
Diet	*%*
none	70
hypocaloric diet	11
specific diet for health reasons	3
specific diet not for health reasons	16
Food allergies/intolerances	*%*
none	79
food allergies	10
food intolerances	12
Food supplements use	23 %
Medicines use	29 %

3.1.2 Healthy choice index

The healthy choice index was calculated, for all the participants, as a sum of the choices of the healthy option assigning to that one a value of 1 with higher scores reflecting higher choice of the healthy options (range from 0 to 17). From the distribution of the calculated healthy choice index, the median value was extrapolated to classify subjects into two groups of preference. Subjects with a healthy choice index less than the median value (median=9) were classified as subjects who preferred the unhealthy version of the proposed foods (n=184); subjects with a healthy choice index higher than the median value, on the other hand, were classified as subjects who preferred the healthy version of the proposed foods (n=172).

3.1.3 HTAS data

Concerning the internal consistency of each Health and Taste domain, only Natural Product Interest and Pleasure revealed a low internal validity (Cronbach's α=0.6 and 0.5, respectively). These two domains were excluded from the dataset and not considered for the next data

analysis. The Cronbach's α values for General Health Interest, Light Product Interest, Craving for Sweet Foods and Using Food as Reward were higher than 0.7.

3.2 Relationships between healthy choice index and individual variables

No significant relationships were found between healthy choice index and gender (χ^2=0.81, p=0.367), and BMI (p=0.751). A significant relationship was found between healthy choice index and age (χ^2=9.36, p=0.002). In particular, the healthy lover group was more represented by adult (60%) than young subjects (40%), while the unhealthy lover group was more represented by young (72%) than adult subjects (28%). Gender and BMI are generally associated with food choice (Hearty et al., 2007; Gough & Conner, 2006; Sobal, 2005; Roininen et al., 2001).

However, in our research, there was a higher proportion of female participants, and BMI were calculated from self-reported height and weight, which could explain the absence of correlation. Carrillo et al. (2011) reported that women and adults were linked with healthier lifestyles if compared with men adults. However, the same work also reported that men and women between 17-29 years presented an intermediate behaviour toward controlling weight. This suggests that age is one of the major factors determining healthy food choices.

Probably, the fact that they are more aware of preventing chronic diseases may lead adults to prefer lower-calorie foods and adopt a healthy eating behaviour. On the other hand, people under 30 were not yet fully aware of this risk. Moreover, Perez-Rodrigo et al. (2017) showed that a higher proportion of people aged 18–30 years was classified into the "Poor diet-low physical activity-sedentary lifestyle pattern". Our results were in line with those of Wongprawmas et al (2021), who stated that the food choices of adults were mainly driven by the health-related aspect, compared to the choices of young consumers, which were highly affected by their mood or emotional state.

Moreover, a significant relationship was found between the healthy choice index and the frequency of consumption of different foods, as shown in **table 3**.

Differences in frequency of consumption between the two groups were mostly found in subjects who consumed regularly sweet products, sweet creams, healthy and functional foods, suggesting different attitudes of the population toward these kinds of food products.

Table 3. Relationship between frequency of consumption of different types of foods and healthy- and unhealthy-lovers' groups - χ^2 analysis on frequencies (showed as percentages).

Frequency of consumption	**Healthy lovers (%)**	**Unhealthy lovers (%)**	**Sign. (p)**
Sweet products			
Regularly	24	76	0.03
Weekly	57	43	-
Monthly	43	57	-
Sweet creams			
Regularly	20	80	0.01
Weekly	39	61	-
Monthly	70	30	0.01
Healthy foods			
Regularly	68	32	0.002
Weekly	27	73	0.002
Monthly	45	55	-
Healthy sweet foods			
Regularly	43	57	-
Weekly	44	56	-
Monthly	51	49	-
Functional foods			
Regularly	78	22	0.02
Weekly	58	42	-
Monthly	35	65	0.02

As it can be observed in **table 3**, regular consumers of sweet products, in general, were significantly more represented by unhealthy lover- (76%) than healthy lover (24%) subjects. Similarly, regular consumers of sweet creams were significantly more represented by unhealthy lover- (68%) than healthy lover (32%) subjects; on the other hand, monthly consumers were significantly more represented by healthy lover- (70%) than unhealthy (30%) lover subjects. On the contrary, regular consumers of healthy foods, in general, were significantly more represented by healthy lover- (68%) than unhealthy (32%) lover subjects; instead, weekly consumers were significantly more represented by unhealthy lover- (73%) than healthy lover (27%) subjects. Considering the healthy sweet foods, the healthy and unhealthy lover subjects were equally distributed among the consumption frequency groups. Finally, the regular consumers of functional foods were significantly more represented by healthy lover- (78%) than unhealthy (22%) lover subjects; instead, the monthly consumers were significantly more represented by unhealthy lover- (65%) than healthy lover (35%) subjects. The current finding confirmed that individuals who paid more attention to healthful food choices daily consumed

healthy and functional products. This could be due to the fact that such individuals, paying more attention to their diet, are more aware of what should be limited (e.g., fat and sugar) (Wongprawmas et al., 2021). Interestingly, despite the unhealthy lovers being habitual consumers of sweet products, and in particular of sweet creams, the frequency of eating healthy sweet foods did not differ according to the healthy choice index. This result suggests not only that the intake of this product category was similar for the two clusters, but also that consumers with healthier attitudes would consume sweet products provided that make them physically healthier.

The Health and Taste Attitude Scales (HTAS) were developed to evaluate the importance that consumers assign to perceived health and hedonic characteristics of foods in relation to their food choices (Roininen et al., 1999). Consumers who belonged to different HCI groups responded differently to the HTAS questionnaire domains. As shown in **Figure 1**, significant relationships were found between the healthy choice index and *General health interest* (GHI) (p=0.000), *Craving for sweet foods* (CSF) (p=0.010) and *Food as a reward* (FR) (p=0.000). The healthy lover group rated higher GHI (5.4) and the lower CSF and FR (4.5 and 3.6, respectively) compared to unhealthy lovers. The two clusters did not significantly differ for the *Light product interest* (LPI) domain (p=0.904).

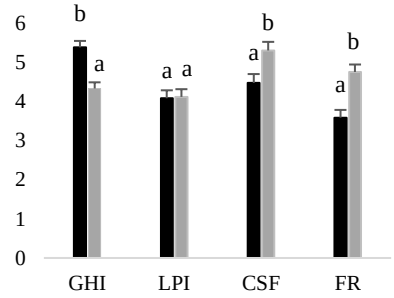

Figure 2 Mean scores and standard error of Health and Taste Attitudes Scale (HTAS) questionnaire, for subjects classified in two groups according to the healthy choice index: healthy lovers in black, unhealthy lovers in grey. Different letters indicate significant differences by Duncan's test ($p<0.05$). GHI=General health interest; LPI=Light product interest; CSF=Craving for sweet foods; FR=Food as a reward.

Our results were in line with those of Zandstra, de Graaf & Van Staveren (2001), who showed that people who had lower fat intakes rated high on general health interest. The same author found that respondents scoring high in general health interest consumed both, high-fat and low-fat sweet spreads. Whereas, respondents scoring high in craving for sweet foods consumed more high-fat sweet snacks. The medium-low values for light product interest indicated that respondents were poorly interested in those products, as reported by Saba et al (2019), who

characterised Italian consumers food-related attitudes with weak and strong connotations of health and taste. The unhealthy lovers showed more positive attitudes in using food as reward, meaning that people less interested in following a healthy diet could be more inclined to search for immediate rewards. Unhealthy diets may attract subjects who look for immediate gratification from foods (Barlow, Reeves, McKee, Gale, & Stuckler, 2016; MacKillop, Amlung, Few, Lara, Lawrence & Munafò, 2011). Thus, healthy as well as taste-related attitudes were associated with the type of dietary behaviour. The results of this study are according to the results of the study of Roininen & Tuorila (1999) who concluded that general health interest and craving for sweet foods were good predictors of food choices.

3.3 Effect of healthy choice index and familiarity on liking for ingredients and sweet foods

Figures 2 and **3** showed the mean scores for familiarity and liking, respectively, of healthy and unhealthy lovers' groups for ingredients and sweet foods. The healthy lover group rated familiarity with higher scores for dark chocolate ($p<0.0001$), hemp fluor ($p=0.009$), hempseed oil ($p=0.011$), raspberries ($p=0.011$), blueberries ($p=0.018$), pomegranate ($p=0.016$) and almond ($p=0.001$) than unhealthy lovers. On the other hand, the unhealthy lover group had the highest mean scores of familiarities for *panna cotta* ($p=0.033$), filled croissant ($p=0.000$), chocolate snack ($p=0.000$), milk ($p=0.000$) and white chocolate ($p=0.000$), nut cream ($p=0.001$) and wafer ($p=0.002$).

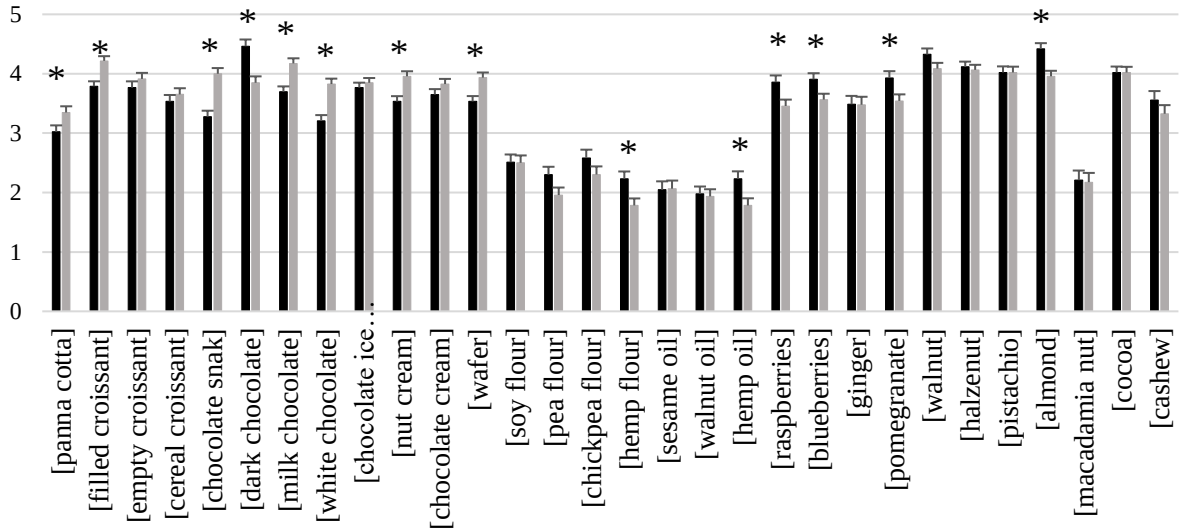

Figure 2 Mean scores and standard error of familiarity for ingredient and sweet foods, for subjects classified in two groups according to the healthy choice index: healthy lovers in black, unhealthy lovers in grey. Asterisk indicates a significant effect ($p<0.05$) of the healthy choice index and familiarity.

Significant differences between the two HCI groups in liking for several ingredients and sweet foods were also found. In particular, the unhealthy lover group rated liking with higher scores the filled croissant (p=0.017), chocolate snack (p<0.000*1*), milk (p=0.000) and white chocolate (p=0.000), chocolate ice cream (p=0.039) and nut creams (p=0.003), compared to healthy lover group. On the contrary, healthy lovers rated liking higher for hemp oil (p=0.035), raspberries (p=0.017), blueberries (p=0.013), ginger (p=0.044), pomegranate (p=0.000), almond (p=0.012) and cashew (p=0.008).

Interestingly, walnut, hazelnut, pistachio and cocoa had the highest liking scores from both, healthy and unhealthy lover groups, and no significant differences due to the healthy choice index were observed.

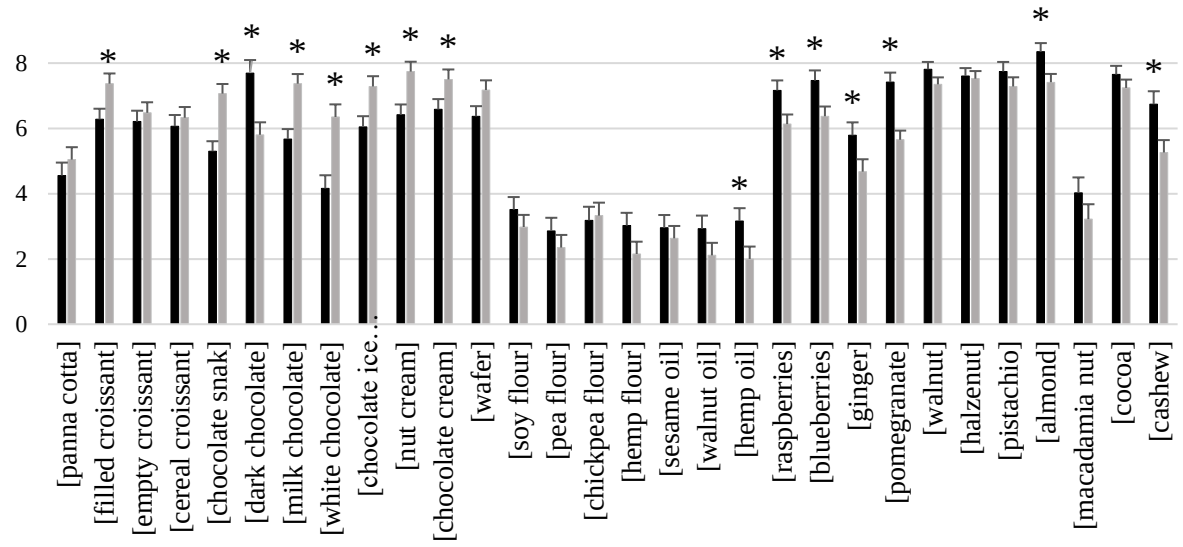

Figure 3 Mean scores and standard error of liking for ingredient and sweet foods, for subjects classified in two groups according to the healthy choice index: healthy lovers in black, unhealthy lovers in grey. Asterisk indicates a significant effect (p<0.05) of the healthy choice index and familiarity.

People with stronger positive attitudes towards following a healthy diet were more familiar with products perceived as healthier, compared to people with weaker positive attitudes, who were more familiar with products perceived as fatter. Moreover, there was a group of products that both, unhealthy and healthy lovers, poorly liked and were unfamiliar with, such as soy, pea, chickpea and hemp flour, sesame walnut and hemp seeds oil.

However, differences between the two consumers groups were also observed for some of these products. Hemp-based products were preferred by the healthy lover group, probably because they are more predisposed to experiment with new ingredients and more informed on the beneficial effects of hemp.

Metcalf, Wiener & Saliba (2021) found that consumers of hemp food placed greater importance on health, mood, natural content, familiarity, and ethical concern. They also highlight the role of familiarity in the acceptance of novel food, having implications for the way that hemp foods might be produced and marketed (Metcalf et al., 2021).

The current findings suggest that unhealthy lovers are more driven by motivations related to familiarity with the product, rather than by the perception of the healthiness of the food. Furthermore, our findings confirmed that there is a positive relationship between familiarity with and preference for foods: foods that had been less tasted often tended to be less liked (Cooke, 2007).

3.4 Ingredients to add or to reduce to develop a healthy sweet cream

With a view to develop a new product and investigate consumer perception of healthy ingredients, consumers were asked which ingredients they wanted to add or reduce to transform a sweet cream into a health product (make a cream healthy), as shown in **table 4**.

Table 4 Classified and total consumers' sample based on percentage frequencies of response to the following question: "If you had the opportunity to create a sweet cream, what would you add to make it healthy? And what would you reduce?"

To Add	Total subjects (%)	Healthy lovers (%)	Unhealthy lovers (%)
Cocoa and dried fruits	19	8	11
Fiber and protein	19	11	8
Vitamins and antioxidants	13	3	10
Fruit	14	8	6
New flavour	9	4	5
Natural sweeters	21	11	10
I do not know	5	2	3
To Reduce			
Sugar	40	22	18
Fats	29	13	16
Sugar and fats	31	14	17

The major part of the respondents wanted to add natural sweeteners (21%) cocoa and dried fruits and fibre and protein (19%). Some subjects wanted a cream enriched with vitamins and antioxidants (13%) and a fruit-based cream (14%), and only a small percentage of them wanted to add new flavours such as spices or goji berries.

The major part of the respondents wanted to cut down on sugar (40%), fats 20% or both (31%), indicating that they were consumers aware of the negative health impact of these ingredients.

These results could suggest that consumers considered an increment of cocoa, dried fruit and natural sweeteners as a valid alternative to reduce the percentage of fats and sugars, while directly associating a single component (as "fiber and protein" and/or "vitamins and antioxidant") with health. The use of natural sweeteners could potentially be employed to drive attention and to promote healthy decision-making.

3.5 Tastes for a new sweet cream

The subjects were asked which ingredients they would use if they had the opportunity to create a sweet cream, and the results are shown in **Table 5**. Hazelnut and cocoa flavours were the most frequently chosen by all subjects, followed by pistachio and almond. A significant relationship was found between the healthy choice index and walnut (χ^2=10.62, p=0.001) and white chocolate (χ^2=3.81, p=0.050) as possible tastes for a new sweet cream. In particular, consumers who chose the walnut were significantly more represented by healthy lovers (15%) than unhealthy (2%). On the contrary, consumers who have chosen white chocolate were significantly more represented by unhealthy lovers (15%) than healthy lovers (6%). No significant relationships for the others tastes were found.

Table 5 Classified and total consumers' sample based on percentage frequencies of response to the fol-lowing question: "If you had the opportunity to create a sweet cream, which taste would you use?" Results of χ^2 testing.

To Add	**Total subjects (%)**	**Healthy lovers (%)**	**Unhealthy lovers (%)**	**Sign p**
Walnut	17	15	2	0.001
Hazelnut	49	19	30	-
Pistachio	37	22	15	-
Almond	35	21	14	-
Coconut	22	10	12	-
Coffee	11	4	7	-
Cocoa	41	21	20	-
White chocolate	21	6	15	0.050

Strengths and Limitations

The strength of this study lies with the presentation and exploration of individual variables, including the healthy choice index, on a discrete group of consumers (400 subjects). Also, the present study explored a list of food ingredients which allowed us to define the future research and development of new healthy spreadable creams. However, our results should be viewed under the limitations of this research. The study, indeed, was somewhat limited by the small number of males, and also, the BMI was calculated from self-reported height and weight. Since this limit might especially affect the relationship between the health choice index and gender and BMI, those specific results should not be generalized towards the global population.

Conclusions

This study had a twofold objective. First, the authors wanted to study how individual variables, such as gender, age, BMI and health interest (HTAS) may drive healthy food choices. The second aim was to select some ingredients to develop a new healthy spreadable cream, taking into account the opinion of subjects with different healthy choice attitudes.

The healthy choice index, measured on each subject, was useful for classifying consumers with different levels of interest in health and a good predictor for healthy vs unhealthy food choices. Among the individual variables, age was the factor that most determined healthy food choices. Unhealthy lovers, more represented by young people, were daily consumers of sweet spreadable creams. Nevertheless, a healthy sweet product would be consumed by both categories of consumers. Health and taste attitudes were associated with the type of dietary behaviour. Healthy lovers were more familiar with and preferred products perceived as healthier such as dark chocolate, berries and hemp-based ingredients, compared to people with weaker positive attitudes towards eating a healthy diet. Moreover, to develop a new healthy cream, the percentage of cocoa and dried fruit could be increased, the amount of sugar and fat could be reduced using alternative natural sweeteners and, the use of vegetable oils with beneficial properties, such as hemp seeds oil, could be considered as well.

The current results can be used to develop a new healthy spreadable cream and move the unhealthy lovers towards healthy food choices.

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CHAPTER 3

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EFFECT OF FATTY ACID COMPOSITION OF VEGETABLE OILS ON CRYSTALLIZATION AND GELATION KINETICS OF OLEOGELS BASED ON NATURAL WAX

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Abstract: This study aimed to understand the effect of fatty acid composition and viscosity of vegetable oils on network formation mechanism and physical properties of oleogels. To this purpose, 12 oleogels were prepared, by choosing 6 seed oils and two waxes, at a fixed oleogelator concentration (6%). The modified Avrami model correctly describes the crystallization profile (R^2>0.98) and the oil type did not affect the Avrami index that ranged from 1.00 to 1.43. Independently from oleogelator, rice and hemp seed oils followed a 3-D network formation mechanism, while almond oil a 2-D mechanism. The strength and yield stress of carnauba wax oleogels increased with increasing saturated fatty acid amount, while in beeswax-based oleogels a more interconnected structure was associated with the length of the saturated fatty acid chain. Thus, the oleogels formation mechanism was closely related to the chemical composition of the solvent, even in highly monounsaturated or polyunsaturated oils.

Keywords: fatty acids; oil structuration; rheology; thermal behaviour; modified Avrami model; oil binding capacity.

1. Introduction

Oleogelation is a healthy method to solidify oil to replace the traditional solid fat in a foodstuff. This technique allows production of oleogels, i.e. three-dimensional supramolecular networks of self-assembled molecules (oleogelators) which trap oil, characterized by a self-standing, thermoreversible, anhydrous, and viscoelastic structure (Co & Marangoni, 2012). As recently summarized by Magri, Petriccione, Cerqueira & Gutiérrez (2020), but widely described by Blake, Toro-Vazquez & Hwang (2018), different types of 3D supramolecular networks could be formed: crystalline particles, crystalline fibres, polymer chains, liquid crystals. The final structure, as well as the structuring process, depends on both the gelator agent and the oil phase. Oleogelators are classified based on molecular weight, in low-molecular weight olegelators, polymeric olegelators and inorganic compounds (Pakseresht & Mazaheri Tehrani, 2020). They could be used both as single components, such as monoacylglycerols (MAGs) and diacylglycerols (DAGs), forming self-assembled structures in the shape of cubic, hexagonal or lamellar crystallines in hydrophobic and hydrophilic systems, and as a mixture of components, such as waxes, usually composed by aldehydes, esters, fatty alcohols, FFAs, glycerol esters or di-esters, ketones and n-alkanes in different ratios, depending on the natural sources and the extraction methodology. However, the effective gelling and physicochemical properties of self-assembled wax structure depend on the cooling kinetics and the nature of the wax-wax and wax-solvent interactions (Gravelle, Davidovich-Pinhas, Zetzl, Barbut & Marangoni, 2016).

The effect of the oil type on the gelling capacity of oleogels was reported in the literature but not completely elucidated. From the oil point of view, several oil characteristics have been reported to affect oleogel structure. Polarity and chemical structure, in terms of fatty acid composition of the oil phase, play an important role in the aggregate formation by olegelator molecules in oleogel systems (Sawalha et al., 2013). At high degree of instaurations of the oil phase seemed to correspond hard oleogels (Yu et al., 2020), the same authors also reported a relationship between oil composition and crystallization kinetics. For oils containing long-chain fatty acids, structural parameters of oleogels correlated well with the dielectric constant and/or viscosity of oils. In fact, increasing oil viscosity or decreasing oil dielectric constant, rheological parameters linearly increased (Valoppi, Calligaris, Barba, Šegatin, Poklar Ulrih & Nicoli, 2017). In oleogels structured by oryzanol and sitosterol mixture, the gelling behaviour, in terms of straight and resistance to the applied stress, varied depending on the dielectric constants of the oil phase, even if the mode of assembly was the same for all (tubule) (Sawalha, Venema,

Bot, Flöter, Lan & van der Linden, 2021). Oleogelation seems to differently take place in long-chain triglycerides-based oleogels compared to medium-chain triglycerides-based oleogels (Fayaz, Goli & Kadivar, 2017; Martins, Cerqueira, Fasolin, Cunha & Vicente, 2016), in terms of different spacing and placement between crystals. In particular, Martins et al. (2016) reported that long-chain triglycerides-based oleogels, at high oleogelator concentration, proved to be stronger than medium-chain triglycerides-based ones, since a lower space between lamellas was observed, due to larger crystal aggregation. Some authors (Co & Marangoni, 2012; Smith, Bhaggan, Talbot & Van Malssen, 2011) also reported that impurities and minor components of the oil could affect oleogelation, e.g in terms of nucleation starting.

In literature different vegetable oils (Fayaz et al., 2017) have been proposed for food application, among them, sunflower and soybean (Winkler-Moser, Anderson, Felker & Hwang, 2019; Zhao, Lan, Cui, Monono, Rao & Chen, 2020) oils were the most used ones. However, also other oils, e.g. rice oil, (Doan et al., 2017; Wijarnprecha, Aryusuk, Santiwattana, Sonwai & Rousseau, 2018), grapeseed oil (Choi, Hwang, Jeong, Kim & Lee, 2020), sesame oil (Moghtadaei, Soltanizadeh, Goli & Sharifimehr, 2021), pumpkin oil (Borriello, Masi & Cavella, 2021), seem to be suitable for oleogel production. No studies were found on the application of hemp seed oil or almond oil in food-grade oleogels. Hemp seed oil is a rich source of nutrients that provide nutritional and functional support for humans (Aiello, Pizzolongo, Scognamiglio, Romano A., Masi & Romano R., 2020; Marzocchi & Caboni, 2020). However, vegetable oil composition is variable and depends on the raw material nature, the production technology and storage, so to assess the impact of oil composition on both gelation and oleogels' properties, it is of utmost importance to characterize the oils, in terms of fatty acid composition, viscosity and thermal properties, to gain a deeper understanding on the effect of solvent quality on oleogelation, as also underlined by Scharfe, Ahmane, Seilert, Keim & Flöter (2019).

Based on those considerations, the present study aimed to understand the effect of the use of different oils, with an interesting healthy profile, on gelling process and properties of new oleogels. To reach this aim, 12 different oleogels were prepared, by choosing 6 seed oils, pumpkin, hemp, almond, rice, sesame and grapeseed, and two waxes, beeswax and carnauba wax, separately, at a fixed oleogelator concentration. First, different oils have been characterized in terms of fatty acid composition, viscosity and crystallization profile. Then, the structuring process of the different oils has been investigated through thermal and rheological analysis. Finally, the developed oleogels have been characterized in terms of physical

properties, such as rheological and thermal behaviour, solid fat content, colour and oil binding capacity.

2. Materials and methods

2.1 Materials

Beeswax (BW) was purchased online from a beekeeper (Piedmont, Italy) and micronized carnauba wax (CW) was kindly provided by a local candy company. Vegetable oils were purchased from Baule volante e Fior di Loto (Bologna, Italy).

2.2 Oleogel preparation

Pumpkin (P), hemp (H), almond (A), rice (R), sesame (S) and grapeseed (G) oil-based oleogels were prepared by using beeswax (BW) and carnauba wax (CW), separately, as oleogelator, at the same wax concentration (6%). Oleogels were prepared according to the procedure already reported in our previous work (Borriello et al., 2021).

2.3 Fatty acids profile

Fatty acid profile was determined by analysing the fatty acid methyl esters (FAMEs) obtained after trans-esterification (EEC,1991). Details on methodologies and peak identification were reported in Romano, Aiello, Meca, De Luca, Pizzolongo & Masi (2021), with some differences regarding the oven temperatures (70 °C for 1 min, 20 °C min -1, ramp to 140 °C for 5 min, and then 7 °C min -1 ramp to 240 °C for 10 min).

2.4 Thermal behaviour

Thermal properties of both, vegetable oils and oleogels, were investigated by means of differential scanning calorimeter (DSC Q200, TA Instruments, USA). Thermal behaviour of the vegetable oils was examined in a temperature range of -80 ÷-40 °C (10 °C min^{-1} cooling/heating rate). The oleogel samples were characterized as already reported in Borriello et al. (2021). Three measurements were performed for each sample.

2.5 Rheological behaviour

A Modular Advanced Rheometer System (HAAKE MARS, ThermoScientific, Waltham, USA) was used to investigate the rheological behaviour of both, vegetable oils and oleogels. To measure the oil viscosity at different temperatures, a viscosity ramp temperature test was performed at a constant shear rate of 10 s^{-1} from 25 to 80 °C. The gelation kinetics and

rheological properties of formed oleogels were carried out according to the procedure reported in Borriello et al. (2021). Results were reported as complex viscosity (η*), and storage modulus (G'). The measurements were conducted in triplicate.

2.6 Solid fat content

Solid fat content (SFC) measurement was performed at 25±1 °C °C by using a low resolution (20 MHz) NMR spectrometer (Minispec mq-20, Bruker, Milan, Italy). Previously, NMR tubes (10 mm e.d), filled with oil/oleogelator mixtures up to the height of 60 mm, were stored 12h at 80 or 90 °C, and then at 25 °C overnight. The measurements were performed in triplicate.

2.7 Oil binding capacity

A centrifuge method was used to assess the oil binding capacity (OBC) of the oleogels (Manzocco, Calligaris, Camerin, Pizzale & Nicoli, 2014; Borriello et al., 2021). The OBC was determined according to the following equation:

$$\mathrm{OBC}(\%) = \left[1 - \frac{(m_i - m_f)}{m_i}\right] \times 100 \quad (1)$$

where m_i and m_f are the mass of the initial and after centrifugation oleogel sample, respectively. Three replicates were performed for each sample.

2.8 Colour

The colour of the oleogels was determined using a colourimeter (Minolta Chroma Meter, CR 300, Japan). The Hunter parameters L* (from 0=black to 100=white), a* (- a*= greenness to + a*= redness), and b* (-b*=blueness to +b*= yellowness) were measured and averaged from three randomly positions for each sample the total colour difference (ΔE) was calculated according to the following equation:

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \quad (2)$$

where ΔL, Δa*, Δb* are the differentials between an oleogel colour parameter and the colour parameter of the respective oil.

2.9 Data analysis

The crystalline network formation has been monitored following the evolution of thermal and rheological parameters. For non-isothermal conditions, the crystallization kinetics can be modelled using a modified Avrami model:

$$\frac{Y_s}{Y_{max}} = 1 - e^{-k_{app}(t-t_0)^n} \quad (3)$$

where Y_s/Y_{max} ratio is the relative degree of crystallinity, k_{app} is the apparent crystallization rate constant, n is the Avrami index and finally, t and t_0 are the time and the induction time, respectively (Ho Lam & Rogers, 2011; Palla, de Vicentec, Carrína & Gálvez Ruizc, 2019). The Y_s/Y_{max} ratio could be obtained by integrating the area underlying the crystallization peak according to the following equation:

$$\frac{Y_s}{Y_{max}} = \frac{\int_0^t (t) \frac{dH(t)}{dt} dt}{\int_0^\infty (t) \frac{dH(t)}{dt} dt} \quad (4)$$

The Y_s/Y_{max} ratio could be also estimated through rheological measurements and reported as (Liu & Sawant, 2001):

$$\frac{Y_s}{Y_{max}} = \frac{\eta^*(t) - \eta_{solv}}{\eta^*_{max} - \eta_{solv}} \quad (5)$$

where $\eta^*(t)$ corresponds to the complex viscosity at time t, η^*_{max} indicates the maximum sample complex viscosity, and η_{solv} is the complex viscosity of the solvent. The complex viscosities were obtained from temperature sweep tests. The frequency curves were fitted according to the rheological Power Law model which is represented in the following as (Steffe, 1967):

$$G' = a(\omega)^b \quad (6)$$

where G' is the storage modulus (Pa), a ($Pa \cdot s^b$) and b (-) are parameters used to describe rheological behaviour, ω is the frequency (rad/s) and b is the dimensionless flow behaviour index.

Melting profile parameters were reported as the mean and standard error. One-way analysis of variance (ANOVA) and multiple comparisons of means (Duncan's test, $p \leq 0.05$) were performed to evaluate whether differences among the samples, due to the different oil type, were statistically significant ($p \leq 0.05$) by using SPSS for Windows version 17.0 (SPSS Inc., Chicago, IL, USA). Independent sample T-test (Levene test $p \leq 0.05$) was performed to evaluate the influence of crystallization cooling rate on the parameters of the modified Avrami model estimated from crystallization kinetics.

3. Result and Discussions

3.1 Oil characterization

The fatty acid composition, the fatty acid total amount and their ratios, the viscosity and crystallization parameters of oils used in this work are presented in **table 1**. The predominant component of almond and rice oil was oleic acid (C18:1n9c), reaching 41.81 and 67.57%, respectively, whilst hempseed, grapeseed and pumpkin oil were rich in linoleic acid (C18:2 Z 9, 12). In particular, grapeseed oil showed the highest linoleic acid content (67.40%) in line with Pardo, Fernández, Rubio, Alvarruiz & Alonso (2009). Unlike other vegetable oils, the percentages of oleic acid (40.73%) and linoleic acid (40.23%) in the total fatty acids of sesame oil were close, as reported by Hwang (2005). Therefore, the oleic/linoleic acid ratio for this oil is almost equal to 1. Hempseed oil, instead, in addition to being a good source of ω-6 fatty acid, was also the richest one in ω-3 fatty acid with 19.72% of α- linolenic acid in agreement with (Parker, Adams, Zhou, Harris & Yu, 2003). All samples presented total saturated fatty acid content less than one-fourth of the total fatty acid content (saturated and unsaturated fatty acids). The percentage of SFA (saturated fatty acid) content changed from 9.64% (almond oil) to 23.06% (rice oil). Palmitic acid (C16:0) was the major saturated fatty acid for all oils, followed by stearic acid (C18:0). Only traces of myristic (C14:0), behenic (C22:0) and lignoceric (C24:0) fatty acids were found. Hemp seed oil showed the highest content of arachidic acid (C20: 0) equal to 5.45%. Hempseed oil showed the lowest MUFA/SFA ratio (0.56), while almond oil had the highest one (7.08). However, it should be considered that hempseed oil, despite having a lower percentage of monounsaturated than saturated ones, contained more than 70% of PUFA, as also reported by Aiello et al. (2020). MUFA/SFA ratio is characteristic of each oil and is important from a nutritional point of view because it has been associated with a reduced risk of all-cause mortality (Schwingshackl & Hoffmann, 2014). Pumpkin seed oil was distinguished from others by the presence of arachidonic acid (0.27%), which is essential for the development

and optimal performance of the nervous system, especially the brain and cognitive functions, the skeletal muscle and immune systems (Tallima & El Ridi, 2018).

Table 1 Fatty acid composition (%), fatty acid total amounts and their ratios (%), viscosity (Pa·s) and crystallization parameters (mean ± standard error) of vegetable oils used.

		Hempseed oil	Almond oil	Rice oil	Sesame oil	Grapeseed oil	Pumpkin seed oil
Fatty acid composition (%)	**C14**	0.04±0.00^{a}	0.06±0.00^{b}	0.31±0.03^{d}	0.05±0.00^{b}	0.06±0.01^{b}	0.11±0.00^{c}
	C16	5.83±0.00^{b}	5.30±0.01^{a}	19.35±0.04^{f}	9.17±0.04^{d}	6.46±0.02^{c}	11.92±0.05^{e}
	C16:1	0.10±0.00^{a}	0.42±0.00^{e}	0.20±0.00^{d}	0.13±0.00^{c}	0.11±0.00^{ab}	0.11±0.00^{b}
	C18	2.18±0.00^{b}	4.25±0.01^{d}	1.99±0.00^{a}	5.95±0.01^{f}	3.66±0.00^{c}	5.70±0.04^{e}
	C18:1n9c	7.97±0.01^{a}	67.57±0.07^{f}	41.81±0.09^{e}	40.73±0.21^{d}	18.25±0.04^{b}	26.97±0.08^{c}
	C18:2 E 9, 12	0.94±0.00^{a}	1.54±0.06^{c}	1.32±0.05^{b}	1.30±0.01^{b}	1.02±0.01^{a}	0.96±0.02^{a}
	C18:2 E 9, Z 12	0.13±0.00^{b}	0.00^{a}	0.89±0.00^{d}	0.00^{a}	0.58±0.00^{c}	0.00^{a}
	C18:2 Z 9, E 12	0.13±0.00^{b}	0.00^{a}	0.82±0.00^{d}	0.00^{a}	0.53±0.00^{c}	0.00^{a}
	C18:2 Z 9, 12	54.45±0.02^{e}	20.51±0.01^{a}	29.92±0.14^{b}	40.28±0.23^{c}	67.40±0.01^{f}	52.33±0.02^{e}
	C20	5.45±0.00^{e}	0.00^{a}	0.84±0.01^{d}	0.51±0.03^{c}	0.81±0.01^{d}	0.40±0.01^{b}
	C18:3n6	0.11±0.00^{b}	0.00^{a}	0.26±0.01^{d}	0.00^{a}	0.00^{a}	0.00^{a}
	C18:3n3	19.72±0.01^{f}	0.00^{a}	0.60±0.00^{d}	0.43±0.01^{c}	0.27±0.01^{b}	0.72±0.00^{e}
	C20:1	0.55±0.00^{e}	0.00^{a}	0.43±0.01^{d}	0.18±0.01^{c}	0.42±0.00^{d}	0.06±0.00^{b}
	C21	1.57±0.00^{c}	0.00^{a}	0.14±0.01^{a}	0.80±0.46^{b}	0.00^{a}	0.00^{a}
	C22	0.35±0.00^{e}	0.00^{a}	0.25±0.00^{d}	0.11±0.00^{c}	0.10±0.00^{b}	0.11±0.00^{c}
	C20:4n6	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.27±0.00^{b}
	C24	0.15±0.00^{c}	0.00^{a}	0.00^{a}	0.07±0.00^{b}	0.22±0.05^{d}	0.00^{a}
	C24:1	0.06±0.00^{b}	0.14±0.00^{d}	0.10±0.00^{c}	0.14±0.02^{d}	0.00^{a}	0.14±0.00^{d}
Fatty acid total amount and ratio (%)	**SFA**	15.62±0.04^{c}	9.64±0.01^{a}	23.06±0.09^{f}	16.68±0.19^{d}	11.32±0.11^{b}	18.28±0.06^{e}
	MUFA	8.71±0.03^{a}	68.26±0.18^{f}	42.87±0.22^{e}	41.24±0.57^{d}	18.80±0.07^{b}	27.34±0.21^{c}
	PUFA	75.67±0.08^{f}	22.10±0.18^{a}	34.07±0.18^{b}	42.08±0.55^{c}	69.88±0.04^{e}	54.39±0.12^{d}
	MUFA/PUFA	0.11±0.00^{a}	3.09±0.03^{f}	1.26±0.01^{e}	0.98±0.00^{d}	0.27±0.00^{b}	0.50±0.00^{c}
	MUFA/SFA	0.56±0.00^{a}	7.08±0.01^{f}	1.86±0.02^{d}	2.47±0.19^{e}	1.66±0.02^{c}	1.49±0.02^{b}
	C18:1n9c/C18:2n6	0.15±0.00^{a}	3.29±0.01^{f}	1.40±0.64^{e}	1.01±0.00^{d}	0.27±0.00^{b}	0.51±0.00^{c}
Viscosity (Pa·s)	**25°C**	0.15±0.00^{a}	0.21±0.00^{c}	0.25±0.00^{d}	0.20±0.00^{c}	0.18±0.00^{b}	0.21±0.00^{c}
	80°C	0.05±0.00^{a}	0.06±0.00^{a}	0.06±0.00^{a}	0.05±0.00^{a}	0.05±0.00^{a}	0.05±0.00^{a}
Crystallization parameters	**T_{In} (°C)**	-15.31±0.32^{b}	-19.74±0.05^{a}	-4.43±0.05^{e}	-6.97±0.30^{d}	-14.32±0.50^{c}	-3.76±0.05^{e}
	T_P (°C)	-46.54±0.14^{b}	-49.82±0.26^{a}	-9.67±0.36^{f}	-37.90±0.49^{e}	-43.85±0.13^{c}	-38.88±0.11^{d}
	ΔH (J/g)	32.06±2.11^{a}	63.32±2.67^{d}	57.64±1.33^{c}	46.56±1.46^{b}	29.68±0.66^{a}	57.34±1.37^{c}

Means (in the same row) with different letters are significantly different ($p \leq 0.05$). Fatty acids percentage <0.05 have not been shown.
SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
T_{In} Crystallization onset temperature; T_P Crystallization peak temperature; ΔH Crystallization enthalpy.

All vegetable oils analyzed showed a Newtonian flow behaviour according to Santos et al. (2004). However, their viscosity depends on fatty acid composition and the unsaturation degree. However, the viscosity values varied as a function of the oil type. The lowest and highest viscosity was observed in hempseed and rice oils, respectively. The viscosity values of the other vegetable oils were similar and close to 0.20 Pa·s. The oil viscosity seemed to grow up with

increasing oleic acid amount (C18:1n9c) and a decreasing portion of linoleic acid (C18:2 Z 9, 12). A mild correlation (R^2=0.63) between the oil viscosity at 25°C and PUFA content was found. Especially, a decrease in the oil viscosity was observed with an increase of PUFA, suggesting that fatty acids with more double bonds do not have a rigid and fixed structure, being loosely packed and more fluid-like. The flow behaviours of vegetable oils were investigated as a function of the temperature ranging from 25 to 80 °C. All of the oil samples exhibited a non-linear decrease in viscosity with increasing temperature. This viscosity pattern over temperature has been attributed to decreased intermolecular interactions by great thermal molecular movement (Santos, Santos & Souza, 2005).

The crystallization profile also depended on the fatty acid composition of the oils. The crystallization onset (T_{In}) and peak (T_P) temperatures linearly increased with increasing SFA content (R^2=0.75 for both parameters), and they were the lowest for almond oil and the highest for rice oil. Moreover, the crystallization enthalpy (ΔH) proportionally decreased (R^2=0.77) with increasing PUFA amounts, from 63.3 J/g to 29.7 J/g, for almond and grapeseed oils, respectively.

3.2 Thermal behaviour

The Avrami index (n), the induction time (t_0) and the apparent rate constants (k_{app}), were determined by fitting the crystallization data to the modified Avrami model (**table 2**). The applied model was able to correctly describe the experimental points (R^2>0.98). All the oleogels showed a not integer Avrami index ranging from 1.00 to 1.43. An Avrami index *n* between 1 and 2 indicates that the crystal growth was due to 1D and 2D mixed crystallization. Generally, n of 1 indicates rod-like growth from instantaneous nuclei, while an n of 2 represents a high nucleation rate and plate-like growth, where growth is primarily along two dimensions (Meng, Yang, Geng, Yao, Wang & Liu, 2014). It is not possible to appreciate any influence of the oil type on crystallization Avrami index n. However, the results showed that both the apparent rate constant and the induction time were affected by the cooling rate and the oil type ($p<0.005$). Among the BW based oleogels crystallized at 1°C min^{-1}, the OP and OS showed different k_{app} values, close to 0.019 and 0.046 min^{-n}, respectively. For CW based oleogels OG and OR showed the lowest k_{app} values (0.010 and 0.026), while OA the greatest one (0.06). There were not many differences using the other oil or wax type. k_{app} increased by an order of magnitude as the cooling rate increased from 1 to 10 °C min^{-1}, as expected. At this cooling rate, OG and OS showed the lowest k_{app} values, OP and OR the higher ones, with both waxes used for gelation. In all vegetable oils, BW began to crystallize later than CW. This phenomenon was more

evident for slow cooling, where the greatest differences ($p<<0.001$) due to the oil type were observed between ORCW and OGCW, which showed t_0 values close to 31.7 and 36.8, respectively. Looking at oil composition (**table 1**), the effect of oil type on thermal behaviour of oleogels was not correlated to the fatty acid content and/or viscosity, but probably could be due to minor components and/or impurity of the oils, as already reported by Co & Marangoni (2012) that could affect crystallization process.

Table 2 Parameters of modified Avrami Model of BW and CW based oleogels as a function of the cooling rate used for their crystallization.

	1 °C min^{-1}			10 °C min^{-1}		
	n	k (min^{-n})	t_0 (min)	n	k (min^{-n})	t_0 (min)
OP BW	1.384±0.050	0.019±0.004^{a}	51.377±0.430	1.251±0.040	0.603±0.037^{c}	5.653±0.019
OH BW	1.206±0.009	0.033±0.001^{ab}	51.287±0.078	1.308±0.005	0.518±0.003^{ab}	5.773±0.035
OA BW	1.294±0.035	0.025±0.003^{ab}	52.275±0.442	1.350±0.053	0.491±0.038^{ab}	5.575±0.014
OR BW	1.181±0.093	0.040±0.011^{bc}	52.423±0.340	1.307±0.013	0.556±0.004^{bc}	5.560±0.064
OG BW	1.253±0.016	0.030±0.002^{abc}	51.760±0.120	1.342±0.010	0.464±0.004^{a}	5.530±0.058
OS BW	1.142±0.019	0.046±0.004^{c}	52.887±0.494	1.342±0.006	0.517±0.005^{ab}	5.397±0.090
OP CW	1.139±0.073	0.036±1.012^{ab}	34.733±0.299^{bc}	1.095±0.042	0.678±0.045^{b}	3.660±0.098
OH CW	1.128±0.056	0.033±0.007^{ab}	33.565±0.927^{b}	1.059±0.013	0.708±0.019^{b}	3.805±0.014
OA CW	1.009±0.038	0.059±0.010^{b}	35.285±0.072^{cd}	1.083±1.103	0.691±0.037^{b}	3.786±0.040
OR CW	1.207±0.030	0.026±0.004^{a}	31.730±0.098^{a}	1.088±0.006	0.637±0.011^{b}	3.705±0.078
OG CW	1.430±0.068	0.010±0.003^{a}	36.765±0.240^{d}	1.357±0.045	0.397±0.035^{a}	3.345±0.032
OS CW	1.167±0.131	0.036±0.013^{ab}	35.400±0.716^{cd}	1.420±0.184	0.443±0.104^{a}	3.440±0.127

The thermal parameters of the onset temperature (T_{In}), the endset temperature (T_{Off}), the peak temperature (T_P), and the enthalpy value (ΔH) of the melting of oleogels are shown in **table S1** (Supplementary files). The oil type and cooling rate influenced the melting behaviour of the oleogels. Among BW oleogels crystallized at 1°C min^{-1}, the oil type affected the T_{In} ($p<<0.001$) and T_P ($p=0.002$), and OPBW showed the highest values of both parameters. At slow cooling rate, all melting parameters of the CW based oleogels were significantly different ($p<<0.001$), and ΔH ranged from 4.05 (OG) to 13.66 (OR) J g^{-1}. The OA and OG based oleogels showed the widest melting peaks when were cooled faster, with both waxes. Moreover, at this cooling rate, the oil type significantly affected the T_P and ΔH values only of BW based oleogels, which were the smallest for OH and the largest for OG.

Generally, increasing the cooling rate resulted in a decrease in both the crystallization enthalpy ΔH and the onset of crystallization temperature because the molecular packing was less ordered and the wax molecules had less time to organize into a crystalline phase at higher cooling rates

(Co & Marangoni, 2012). Our results showed that this trend was mainly observed for oleogels made with BW.

3.3 Rheological gelation process

Figure 1 a and **b** reports the evolution over time of the complex viscosity (η*) for BW oleogel and CW oleogel, respectively. All these curves show similar sigmoidal shapes, which means the presence of an initial period corresponding to the completely molten sample, followed by a period of rapid increase, until reaching a constant trend corresponding to complete gelation.

All oleogels, in their molten state, show similar values of complex viscosity in agreement with literature results (Lupi, Gabriele, Facciolo, Baldino, Seta & de Cindio, 2012) which stated that the viscosity of the solvent did not affect the rheological properties of the derived oleogels in the molten state. At the end of the cooling stage, all the oleogels has not completely formed and needed a setting stage at 25°C to completely gellify.

Although BW oleogels reached greater η* values, 75 % of their gelation occurred during the setting stage, compared to 20 % for CW oleogels. According to our previous work (Borriello et al., 2021), CW oleogels showed characteristic two steps rheological curves, the first stage associated with the self-assembly of the high melting components of CW, the second could be explained by the crystallization of the low melting components. The presence of two phases was confirmed by the crystallization profiles.

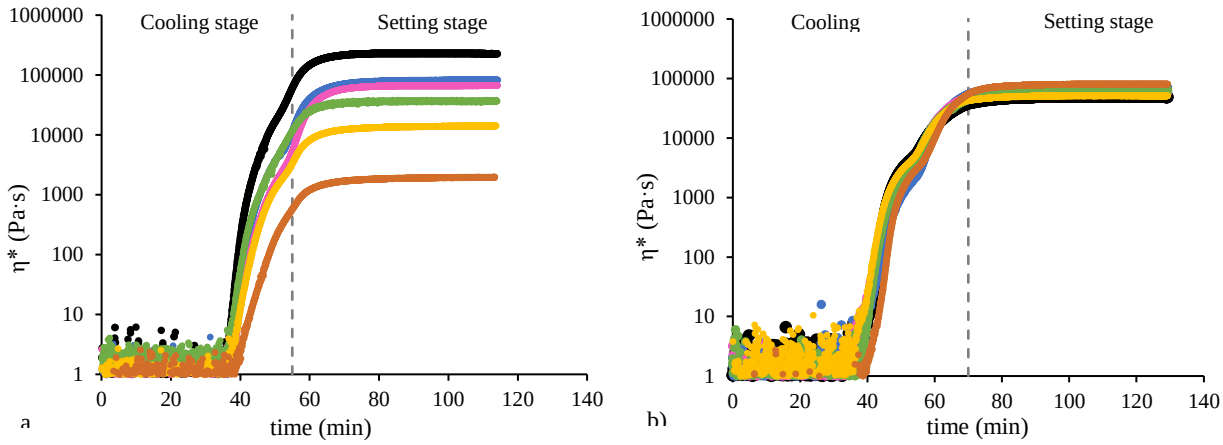

Figure 3 Evolution of complex viscosity η* (Pa·s) during global gelation process (cooling stage and setting stage) for BW oleogel (a) and CW oleogel (b) based on different oils (grapeseed black, rice blue, sesame pink, hemp green, almond yellow, pumpkin orange).

The modified Avrami model applied to the cooling stage only, well described the experimental points (R^2>0.95). However, it is important to mention that the Avrami model used to describe the rheological gelation considers both crystallization and self-assembling of crystals, thus values obtained were related to the entire process of oleogel formation. In fact, an increase in elastic properties occurs only when a three-dimensional network is formed, which occurs significantly later than the onset of nucleation.

The fitting parameters, n, t_0 and k_{app}, were affected by the oil type (**table 3**). By considering the cooling stage only, the Avrami indexes ranged from 2.75 and 4.27 and from 2.89 to 3.71, for BW and CW oleogel, respectively. Among BW oleogels, OP and OA showed n values close to 3, indicating a 2-D network formation mechanism; n values for OG OR OS and OH could be approximated to 4 and seemed to follow a 3-D network formation mechanism. Against, t_0 values exactly followed the reverse order. OP and OA showed higher t_0 values (~ 40 min) than the other samples. Therefore, a more interconnected structure was associated with high n values, which corresponded to low t_0 values, indicating the network formation started earlier. The same oils exhibited different behaviours when gelled with CW. In this case, OS OG and OA followed a 2-D network formation mechanism, while OP OR and OH a 3-D mechanism. No significant differences in terms of induction time were observed. Even if the t_0 values of the CW oleogels seem larger than those of the BW oleogels, it must consider that the experiments started from different temperatures, and therefore had a different duration. I

n effect, CW oleogels generally began to gel at higher temperatures than BW oleogels, but the oil type did not affect t_0 values of CW oleogels. All samples showed very low k_{app} values, probably due to the slow cooling rate (1°C min^{-1}). However, the oil type also affected k_{app} values of BW and CW oleogels (p=0.000). In the first case, OA and OP faster reached their final value respect with the others, while in the second, OA OG and OS showed the greatest k_{app}.

Although the model was applied only in the cooling stage, the estimated n values seemed to predict what happened during the setting stage at 25 °C. For example, among CW based oleogels, during the cooling stage OG η* values increased faster than OR; accordingly, OG k_{app} values were higher than OR and were close to 0.0001 and 0.00001, respectively. However, after the setting stage, OR were stronger than OG oleogels, and n estimated values also followed the same trend.

Table 3 Parameters of modified Avrami Model of BW and CW oleogels

	Cooling stage			
	n	k_{app} (min^{-n})	t_0 (min)	R^2
OP BW	2.75 ± 0.00^{a}	0.00093 ± 0.00^{b}	40.41 ± 0.47^{d}	0.97
OH BW	3.56 ± 0.01^{b}	0.00010 ± 0.00^{a}	38.42 ± 0.42^{ab}	0.98
OA BW	2.79 ± 0.00^{a}	0.00092 ± 0.00^{b}	39.56 ± 0.23^{d}	0.97
OR BW	3.63 ± 0.04^{b}	0.00010 ± 0.00^{a}	38.20 ± 0.22^{ab}	0.99
OG BW	4.27 ± 0.00^{c}	0.00001 ± 0.00^{a}	37.62 ± 0.26^{a}	0.97
OS BW	3.61 ± 0.08^{b}	0.00010 ± 0.00^{a}	39.03 ± 0.53^{b}	0.99
OP CW	3.67 ± 0.03^{d}	0.00001 ± 0.00^{a}	43.11 ± 0.48	0.98
OH CW	3.43 ± 0.04^{c}	0.00002 ± 0.00^{b}	41.20 ± 0.10	0.96
OA CW	2.96 ± 0.04^{a}	0.00010 ± 0.00^{d}	39.99 ± 1.35	0.97
OR CW	3.71 ± 0.02^{d}	0.00001 ± 0.00^{a}	42.43 ± 0.29	0.95
OG CW	2.89 ± 0.00^{a}	0.00011 ± 0.00^{e}	39.89 ± 0.36	0.96
OS CW	3.26 ± 0.04^{b}	0.00003 ± 0.00^{c}	40.13 ± 1.32	0.96

Comparing fatty acid content of oils (**table 1**) and rheological gelation parameters (**table 3**) of different oleogels, some relationships were observed. Only almond oil and hemp oil, with the highest and the lowest MUFA content, showed a similar network formation mechanism, despite the wax chosen. For the other oleogels, for each type of wax, the effect of the oil varied. A positive linear correlation (R^2=0.85) between Avrami index n and the total amount of saturated fatty acids (SFA) was found for CW based oleogels. In the same way, their k_{app} values linearly decreased increasing the SFA amount. These results suggest that very small differences in terms of saturated fatty acids amounts can influence the network formation mechanism, even in highly polyunsaturated oils. On the other hand, it is not completely true for BW-based oleogels and some clarifications are needed. The mechanism of formation of BW-based oleogels seems to depend more on the length of the saturated fatty acid chain than on their total quantity. Hemp seed, sesame and grapeseed oils contained higher quantities of long-chain saturated fatty acids (LCFA sat.) (C20, C21, C22 and C24) than almond and pumpkin seed oils, which had traces of them (0-0.1%). Instead, rice oil is the one that contains the greatest amount (21%) of medium-chain saturated fatty acids (C14, C16 and C18). Their presence, together with beeswax, seems to contribute to forming a more interconnected network, with high values of n and η*. Moreover, grapeseed oil is the only one that contains 0.22% lignoceric acid (C24), which is a very long-chain fatty acid (VLCFA). Quite the opposite, pumpkinseed oil showed the highest amount (0.4%) of long-chain unsaturated fatty acids (LCFA unsat.) (C20:3n3 C20:4n6 C20:5n3 C22:6n3). This is another factor that could explain the differences, in terms of the network

formation mechanism, between OGBW and OPBW oleogels. However, those results highlighted that the oleogel formation mechanisms took place differently and were closely related to the chemical composition of the solvent and the oleogelator.

3.4 Oleogels physical properties

The strain sweep (**figure 2 a, b**) and frequency sweep (**figure 2 c, d**) test were conducted to investigate the rheological properties of oleogel samples. In **table 4** are listed the strain at the limit of linearity (γ_0) and the yield stress (σ*) determined by strain sweep test; the parameters *a* and *b* estimated fitting frequency curves with the power-law model; the oil binding capacity (OBC %), solid fat content (SFC %) and colour parameters (ΔE) of BW and CW oleogels based on different vegetable oils. The elastic component (G′) was higher than the viscous component (G″ not shown) in the linear viscoelastic region (LVR) of all oleogels, indicating the solid-like behaviour of samples. Among BW oleogels, OG showed the highest strain at the limit of linearity value (γ_0=0.09), while for the other samples there were no significant differences. The strain amplitudes of OACW and OSCW were higher than the other carnauba wax oleogels, indicating that oleogels with different strengths can be equally ductile. There was great variability in terms of yield stress, corresponding to the point at which the oleogels start to behave like a liquid (Dinkgreve, Paredes, Denn & Bonn, 2016). OP and OG BW oleogels showed the lowest and the greatest σ* values, close to 18.8 Pa and 254.6 Pa, respectively. There was also an effect of the oil on the σ* values of the CW oleogels, which ranged from 176.25 to 287.77 Pa for OACW and OPCW, respectively. Those results suggested that oleogels with a more structured network required higher shear stress to flow, except for OGCW oleogel, which offered high flow resistance despite showing low η* values. For all oleogels, σ* increased with increasing PUFA amount.

The power-law model (equation 6) well-fitted storage modulus curves (R^2>0.9). For BW and CW oleogel, the parameter *b* ranged from 0.08 and 0.2 and from 0.06 to 0.13, respectively. If gelled with BW, OG and OP showed the lowest and the highest *b* value, while the opposite behaviour occurs when the same oils are gelled with CW. The parameter *a* followed the reverse order compared to *b*. Therefore, among BW oleogels, OG showed the highest *a* value (=1000000 $Pa \cdot s^b$), followed in descending order by OR, OS OH OA and OP. Among CW oleogels, OG, OA, OS and OH showed a similar and lower *a* value than OR and OP. A less frequency dependence and gel-like *a* and *b* values (Steffe, 1967) were observed for all the oleogels. Thus both, mechanical spectra and fitting parameters confirmed that a strong network was obtained for all the oleogels. However, OPBW oleogel was the only one that exhibited a

slight frequency dependence and a high *b* value, revealing the weakest gel structure. However, in all the cases self-standing oleogels were obtained, regardless of the wax type, even if the combination wax-vegetable oil strongly affected the rheological proprieties of the oleogels.

There were significant differences in the oil binding capacity (OBC) and colour parameters of oleogels made with different oils; the fatty acid composition of the oils did not affect the solid fat content (SFC) of the oleogels (**table 4**). Among the BW-based oleogels, OG OH and OS showed the highest oil binding capacity (97-98%), while OPBW retained only 68.22% oil. Among the CW-based oleogels, OH and OP showed the lowest and highest OBC, 92% and 97% respectively. No significant differences were observed for the other samples, showing OBC values close to 95%. Unfortunately, no positive correlation was observed between OBC and total saturated (or unsaturated) fatty acids (R^2=0.13).

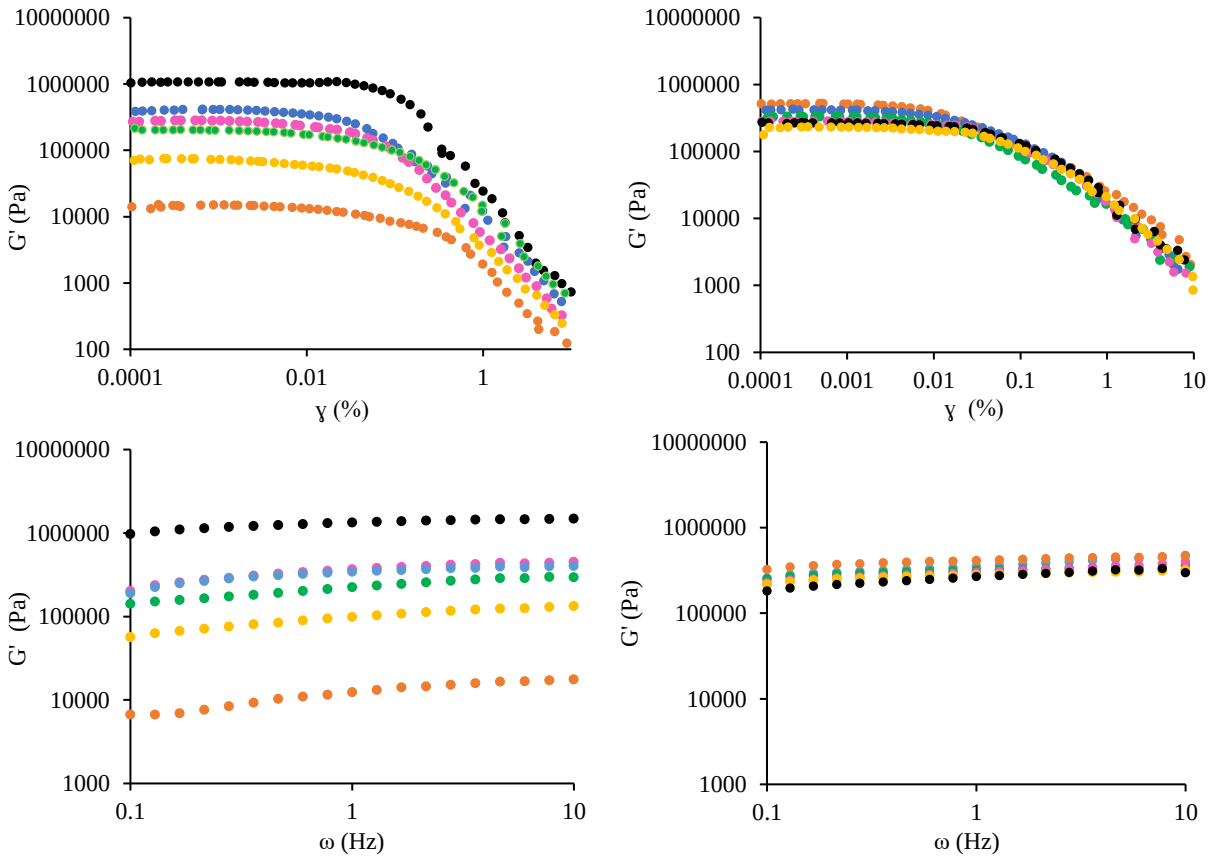

Figure 2 Strain sweeps of BW (a) and CW (c) oleogels, and frequency sweeps of BW (b) and CW (d) oleogels based on different vegetable oils (grapeseed black, rice blue, sesame pink, hemp green, almond yellow, pumpkin orange).

Table 4 Oleogels physical characterization. The strain at the limit of linearity (γ_0) and the yield stress (σ*) determined by strain sweep test values of the storage modulus ($G' = a\,\omega^b$); oil binding capacity (OBC %), solid fat content (SFC %) and colour parameters (ΔE) of BW and CW oleogels based on different vegetable oils.

	σ*	γ_0	*b*	*a* (Pa·s^b)	OBC (%)	SFC (%)	ΔE
OP BW	18.85 ± 0.98^{a}	0.04 ± 0.00^{a}	0.233 ± 0.00^{e}	11581 ± 395^{a}	68.22±0.58^{a}	3.81±0.00	4.95±0.47^{a}
OH BW	150.82 ± 8.64^{c}	0.03 ± 0.00^{a}	0.171 ± 0.01^{cd}	191224 ± 933^{c}	97.21±0.30^{d}	3.89±0.00	28.01±6.72^{c}
OA BW	30.63 ± 1.70^{a}	0.01 ± 0.00^{a}	0.180 ± 0.00^{d}	94611 ± 6623^{b}	95.35±0.39^{c}	3.84±0.00	5.34±0.62^{a}
OR BW	102.66 ± 14.74^{b}	0.03 ± 0.01^{a}	0.138 ± 0.00^{b}	324013 ± 23512^{d}	92.85±0.46^{b}	4.05±0.00	13.62±1.97^{ab}
OG BW	254.63 ± 23.67^{d}	0.09 ± 0.01^{b}	0.084 ± 0.00^{a}	1000000 ± 0.00^{e}	98.76±0.39^{e}	4.16±0.01	4.49±0.84^{a}
OS BW	73.76 ± 8.11^{b}	0.03 ± 0.01^{a}	0.163 ± 0.00^{c}	330915 ± 24737^{d}	97.06±1.22^{d}	3.79±0.03	18.25±2.59^{b}
OP CW	287.77 ± 0.15^{e}	0.01 ± 0.00^{a}	0.065 ± 0.00^{a}	489962 ± 22357^{c}	97.78±0.66^{c}	4.56±0.02	7.30±0.42^{a}
OH CW	230.09 ± 0.74^{c}	0.01 ± 0.00^{a}	0.066 ± 0.01^{a}	311846 ± 10613^{ab}	92.14±0.42^{a}	4.76±0.05	43.65±4.01^{c}
OA CW	176.25 ± 7.07^{a}	0.04 ± 0.00^{b}	0.073 ± 0.00^{a}	274801 ± 23750^{a}	94.18±1.90^{ab}	3.85±0.00	18.22±0.83^{b}
OR CW	220.91 ± 10.33^{bc}	0.02 ± 0.00^{a}	0.121 ± 0.00^{c}	354020 ± 19902^{b}	95.24±1.88^{b}	4.44±0.01	9.61±1.93^{a}
OG CW	258.81 ± 5.59^{d}	0.02 ± 0.00^{a}	0.130 ± 0.01^{d}	260862 ± 22216^{a}	95.21±0.72^{b}	4.44±0.01	10.16±1.09^{a}
OS CW	207.03 ± 3.48^{b}	0.04 ± 0.00^{b}	0.110 ± 0.00^{b}	301336 ± 31395^{ab}	95.56±0.21^{b}	4.05±0.01	53.22±1.64^{d}

The colour properties of oleogels could affect their potential food application. **Table S2** (Supplementary file) presents a summary of the results of colour parameters (L∗, a∗, and b∗) measurement of the vegetable oils and oleogels, while the total colour differences (ΔE) against the oil are listed in **table 4**. The L* value of oleogel samples was significantly affected by the oil type and exhibited the same L* value differences that existed among the oil. The lightness decreased with the oleogelation, because the refractive index of the crystalline network was lower than that of the oil. The BW enhanced the yellowness of the oleogels more than the CW, which instead seemed to influence the greenness. In Moghtadaei, Soltanizadeh & Goli (2018) was reported that less refraction of light through more compact structures produced a lighter colour. Also in our work, OPBW and OGCW, which showed a 2-D network formation mechanism, showed lower L* values, compared to OPCW and OGBW, which instead followed a 3-D mechanism. In all cases, the differences between the oil as it is and the oleogel were visible to the human eye, and the colour of hemp oil and sesame oil was most affected by the addition of wax.

Conclusions

The major components of vegetable oils were oleic acid and linoleic acid, which also seemed to make the greatest contribution to their flow behaviours, together with PUFA amounts. The oil type did not affect the crystal growth mechanism, since both beeswax and carnauba wax showed a 1D-2D mixed crystallization in all the oils analysed. On the other hand, the fatty acid composition of oils affected the self-assembling of oleogelators during the gelation, which occurs significantly later than the onset of nucleation, as highlighted by the complex viscosity. Hempseed, rice and pumpkin seed oils, the richest in saturated fatty acids among the oils analysed, formed a more interconnected structure when gelled with carnauba wax, following a 3-D network formation mechanism. Gelation kinetics of BW based oleogels seemed to depend mostly on the length of the saturated fatty acid chain. The rheological proprieties, the oil binding capacity and colour parameters of the oleogels were strongly affected by vegetable oil, whereas there were no differences in terms of solid fat content. In conclusion, the present work contributed to expanding the knowledge on the oleogelation of uncommon oils using complex oleogelators such as waxes, explaining how very small differences in terms of fatty acid amounts can influence the network formation mechanism even in highly monounsaturated or polyunsaturated oils. Finally, these findings could have a practical application as they provide criteria for choosing the most suitable oil for designing oleogels with desired physical properties and gelation process to reduce saturated fatty acids in tailor-made formulations.

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Supplementary materials

Table S1 Melting parameters (mean ± standard error) of BW and CW based oleogels as a function of the cooling rate (1 and 10 °C min^{-1}) used for their crystallization.

	1 °C min^{-1}				10 °C min^{-1}			
	T_{In} (°C)	T_{Off} (°C)	T_P (°C)	ΔH (J/g)	T_{In} (°C)	T_{Off} (°C)	T_P (°C)	ΔH (J/g)
OP BW	14.59±0.27^{c}	67.42±0.53	55.34±0.08^{d}	9.44±0.04	14.14±0.38^{c}	65.40±0.58^{bc}	52.36±0.31^{c}	8.38±0.04^{cd}
OH BW	12.01±1.12^{b}	67.59±1.11	54.86±0.17^{bc}	9.59±0.15	14.12±0.06^{c}	64.01±0.00^{a}	50.93±0.01^{a}	5.39±0.08^{a}
OA BW	7.80±0.26^{a}	68.33±0.38	55.29±0.22^{d}	8.36±0.05	11.22±0.31^{b}	65.35±0.03^{bc}	51.64±0.24^{b}	8.25±0.33^{cd}
OR BW	10.33±0.30^{b}	66.30±0.24	54.14±0.29^{a}	9.30±0.59	19.01±0.06^{e}	64.76±0.09^{b}	51.70±0.14^{b}	6.56±0.02^{b}
OG BW	10.86±0.63^{b}	66.74±0.36	54.44±0.03^{ab}	9.70±0.14	9.89±0.07^{a}	66.43±0.01^{d}	52.58±0.07^{c}	8.63±0.04^{d}
OS BW	11.18±0.06^{b}	68.09±0.18	54.75±0.02^{b}	9.40±0.39	15.59±0.03^{d}	65.73±0.03^{cd}	52.46±0.08^{c}	8.02±0.02^{c}
OP CW	19.81±0.38^{b}	91.45±0.36^{d}	77.79±0.15^{b}	10.63±0.64^{c}	19.81±0.22^{d}	91.39±0.27^{c}	75.34±0.23	10.98±0.59
OH CW	22.01±0.12^{d}	90.69±0.03^{bc}	79.91±0.10^{d}	9.47±0.26^{b}	19.94±0.25^{d}	88.69±0.37^{b}	75.93±0.00	10.31±0.19
OA CW	18.20±0.23^{a}	90.39±0.13^{b}	78.37±0.03^{c}	11.09±0.25^{c}	16.60±0.12^{a}	90.47±0.25^{c}	75.96±0.17	11.17±0.04
OR CW	21.30±0.18^{c}	91.10±0.34^{cd}	78.17±0.04^{bc}	13.66±0.31^{d}	18.79±0.03^{c}	88.71±0.28^{b}	75.03±0.06	10.16±0.52
OG CW	22.15±0.20^{d}	89.21±0.07^{a}	76.95±0.23^{a}	4.05±0.32^{a}	17.81±0.25^{b}	90.54±0.29^{c}	75.54±0.49	9.64±1.09
OS CW	19.25±0.10^{b}	89.45±0.20^{a}	77.27±0.16^{a}	11.23±0.01^{c}	19.38±0.09^{d}	87.41±0.29^{a}	75.36±0.25	10.06±0.14

Table S2. Colour parameters (mean ± error st.) of vegetable oils, BW and CW based oleogels.

	L*	a	b
OP	17.13±0.90^{a}	19.67±0.24^{e}	7.30±0.22^{a}
OH	53.72±4.02^{b}	-9.95±1.46^{a}	61.17±1.81^{d}
OA	70.24±0.81^{c}	-0.91±0.12^{d}	8.42±0.32^{a}
OR	66.92±1.39^{c}	-3.66±0.13^{bc}	20.76±1.13^{c}
OG	69.63±0.84^{c}	-2.70±0.19^{cd}	14.33±0.35^{b}
OS	68.36±1.12^{c}	-4.76±0.10^{b}	20.74±0.35^{c}
OP BW	16.11±0.02^{a}	16.44±0.24^{d}	3.90±0.10^{a}
OH BW	37.46±1.55^{b}	-4.99±0.54^{a}	39.14±2.20^{e}
OA BW	65.25±0.52^{d}	-2.36±0.24^{c}	9.61±0.26^{b}
OR BW	53.42±1.84^{c}	-3.38±0.42^{b}	21.11±0.58^{d}
OG BW	65.44±0.68^{d}	-3.49±0.18^{b}	13.53±0.37^{c}
OS BW	50.13±1.68^{c}	-4.14±0.14^{ab}	21.16±0.18^{d}
OP CW	18.25±0.39^{a}	14.18±0.17^{d}	2.68±0.11^{a}
OH CW	32.48±0.76^{b}	-5.05±0.42^{bc}	23.58±1.70^{d}
OA CW	52.80±0.13^{c}	-4.37±0.03^{c}	4.53±0.17^{a}
OR CW	57.72±1.62^{d}	-4.63±0.25^{c}	19.30±0.62^{c}
OG CW	59.95±1.61^{d}	-5.51±0.09^{b}	14.51±0.38^{b}
OS CW	118.66±0.35^{e}	-9.97±0.11^{a}	37.32±0.68^{e}

CHAPTER 4

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NOVEL PUMPKIN SEED OIL-BASED OLEOGELS: DEVELOPMENT AND PHYSICAL CHARACTERIZATION

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Abstract

The objective of this research was to develop novel oleogels based on pumpkin seed oil and natural waxes. Crystallization and gelation of 4, 5, 6 and 8% of beeswax and carnauba wax in pumpkin seed oil were investigated, and their physical properties were evaluated using an oleogel prepared with sunflower oil and beeswax as reference. In order to obtain a complete three-dimensional network, after a cooling stage a setting stage was necessary at 25°C for no longer than 60 min. Oleogels produced with pumpkin seed oil and beeswax were weaker than those made with sunflower seeds; pumpkin seed oil-based oleogels structured by carnauba wax presented higher viscoelastic properties, retained more oil and were firmer than oleogels based on beeswax. Based on scaling theory, all the oleogels followed a strong-link regime and the fractal dimension of the network (D) was comparable to fats widely used in food production. Therefore, pumpkin seed oil can be used to create novel oleogels.

Keywords: natural waxes; crystallization; gelation; fractal dimension; pumpkin seed oil.

1. Introduction

The physical and sensory performance of many foodstuffs is closely related to the presence of hard fats rich in saturated fatty acids, some of which may increase the incidence of cardiovascular diseases, diabetes and other health implications (Blake & Marangoni, 2014). Moreover, dietary guidelines recommend to consume high polyunsaturated vegetable oils as a reference for healthy living. However, directly replacing hard fats with liquid oil can lead to technological problems such as texture weakness and oil leakage in several food products due to their low viscosity.

Against this background, the oleogels represent a promising strategy to impart solid-fat functionality to liquid oils, resulting in a solid state, albeit consisting of unsaturated fats. Oleogel can be described as a complex system where an organic liquid is entrapped within a thermo-reversible three-dimensional gel network with solid-like properties (Blake, Toro-Vazquez, & Hwang, 2018; Mattice & Marangoni, 2018).

Among oleogelators that are available to gel oils, natural waxes are a promising strategy in food applications: they are inexpensive, commonly used in the food industry as glazing and coating agents, and their threshold concentrations are lower than 10 g/100 g (Pehlivanoğlu et al., 2018). Waxes may be defined as heterogeneous materials with a high ester content also containing minor components such as n-alkanes, fatty acids and fatty alcohols, which determine their thermal and rheological behavior (Doan, Tavernier, Okuro, & Dewettinck, 2018). The effect of carnauba wax quality on microstructure, textural, and rheological properties of soybean oil-based oleogels was recently investigated by Buitimea-Cantúa et al. (2021), confirming that different types of the same wax could allow the fabrication of oleogels differing in color, thermal stability, hardness and microstructure. Therefore, purity degree, chemical composition, and melting and crystallization temperatures of wax have a significant impact on the microstructure of the crystalline network that directly influences the thermal and mechanical properties of the oleogel obtained (Doan et al., 2018). Marangoni's group assumed that the oleogel solid-network, potentially consisting of wax crystals, is a collection of clusters and hence of fractal flocs, in analogy to the network of fat crystals in hardstock fats (Tang & Marangoni, 2007).

Therefore, based on the above theory, it is possible to obtain information on the microstructure of oleogels by exploiting their rheological properties. Shih H., Shih Y., Kim, Liu, & Aksay (1990) proposed the existence of two rheological regimes based on the relative strength of the inter-floc and intra-floc links. The strong-link regime occurs at low volume fractions of solids ($\phi < 0.1$) when the intra-floc is lower than the inter-floc interaction and γ_0 (the strain at the limit

of linearity) decreases as a function of ϕ. The weak-link regime occurs when inter-floc links predominate at a higher volume fraction of solids (ϕ > 0.1), and γ_0 increases with ϕ. For materials following a strong-link regime G' and σ* depends on the fractal dimension of the flocs (D), as in the following equations:

$$G' \sim \phi^{\frac{d+x}{d-D}} \quad (1)$$

$$\sigma * \sim \frac{6\delta}{a}\phi^{\frac{d+x}{d-D}} \quad (2)$$

where d is the Euclidean dimension of the network (usually 3); δ is the solid-liquid interfacial tension; *a* is a constant which is dependent on the size of the primary particles and the interactions between them; ϕ is the particle volume fraction of solid fat; x is the backbone fractal dimension that describes the tortuosity of the stress transduction chain within a cluster of particles under an externally applied stress and that is estimated between 1 and 1.3 (Shih et al., 1990).

Several types of vegetable oils can be used to prepare oleogels. In effect, BW- and CW-based oleogel was successfully produced with different oils such as virgin olive oil, hazelnut and sesame oil (Moghtadaei, Soltanizadeh, & Goli, 2018; Yılmaz, & Öğütcü, 2014b). The effect of different oil types on the rheological, textural, and thermal properties of BW oleogels was reported by Patel (2015), who found that minimum gelling concentration of BW was significantly lower in highly saturated level oils when compared to highly unsaturated level oils. Moreover, Lim, Jeong, Oh, & Lee (2017) selected carnauba wax to structure soybean-based oleogels because it allows the production of oleogels which are more responsive to temperatures changes. However, to the best of our knowledge, a pumpkin seed oil-based oleogel has not yet been explored. Pumpkin seed oil, obtained by mechanical pressing of pumpkin seeds, is dark green with a complex flavor that combines nutty, woody and roasted notes. In recent years pumpkin seed oil has gained attention because of its high nutritional value. It has been widely used as cooking oil in some countries in West Africa and Middle East, as salad oil and for margarine production (Aktaş, Uzlaşır, & Tunçil, 2018). Oral intake of pumpkin seed oil has been shown to produce beneficial effects such as prevention of prostate gland growth and alleviation of diabetes. It also appears to prevent cardiovascular disease and related complications in postmenopausal women. These positive health effects could be attributed not

only to the large amounts of polyunsaturated fatty acids, especially oleic and linoleic acids, but also to the presence of soluble substances such as pigments, phytosterols, vitamins, minerals and phenolic compounds with functional properties (Wong et al., 2019).

Thus, a pumpkin seed oil-based oleogel, naturally rich in vitamins and bioactive compounds, could be an innovative strategy to provide additional options for tailor-made formulations. Moreover, it is important to understand the formation mechanism and physical properties of a novel oleogel to better control its future inclusion in healthy products. Based on these considerations, this research aimed to develop and characterize a novel oleogel produced from pumpkin seed oil and natural wax. To this end, the crystallization and gelation of beeswax and carnauba wax in pumpkin seed oil were investigated, and the developed oleogels were characterized in terms of physical properties. An oleogel based on sunflower oil and beeswax was used as reference since it is widely studied in the literature as a solid fat replacer.

2 Materials and methods

2.1 Materials

Beeswax was purchased online from a beekeeper (Piedmont, Italy) and micronized carnauba wax was kindly provided by a local candy company. Sunflower oil (saturated/unsaturated fatty acid ratio = 8.77) was purchased from Me.pa alimentari s.r.l. (Pozzuoli, Italy) and pumpkin seed oil (saturated/unsaturated fatty acid ratio = 4.46) from Baule volante e Fior di Loto (Bologna, Italy).

2.2 Oleogel preparation

Sunflower (OS BW) and pumpkin (OP BW) seed oil-based oleogels were prepared at different beeswax concentrations (4, 5, 6 and 8 % (w/w)). Carnauba wax, at the same concentrations, was used to obtain pumpkin seed oil-based oleogels (OP CW). The oleogelator was added to the oil previously heated at 80 °C, for BW, and 90 °C, for CW; the mixture was stirred at 200 rpm, using a magnetic stirrer, until a clear solution was obtained. The mixture was then cooled to 25 °C in order to form the gels, and finally stored at room temperature for 12 h.

2.3 Thermal behavior

A differential scanning calorimeter (DSC Q200, TA Instruments, USA) was used to determine crystallization and melting profiles of the oleogels and pure waxes. Each sample (8–10 mg) was placed in a Tzero aluminum pan and hermetically sealed. The oleogel samples were heated to 100 °C, cooled (1 °C min^{-1}) from 100 °C to-20 °C, and equilibrated for 1 min. After

equilibration they were scanned (10 °C min^{-1}) from -20 °C to 100 °C. Melting and crystallization profiles of the crude waxes were also examined in a temperature range of –15-140 °C, at the same cooling/heating rate. Data were analyzed using TA Universal Analysis software. Measurements were performed in triplicate.

2.4 Rheological behavior

All rheological measurements were carried out with a Modular Advanced Rheometer System (HAAKE MARS, ThermoScientific, Waltham, USA), in a strain-controlled mode, equipped with a vane tool geometry (gap = 1 mm, ≈ 28 ml of the sample). In order to study the gelation kinetics of the oil/oleogelator mixtures, a temperature sweep (cooling stage) was performed from 80/90 °C to 25 °C at a cooling rate of 1 °C min^{-1}. The samples were then held at 25 °C for 60 min (time sweep/setting stage). All tests were carried out in the linear viscoelasticity region (LVR), imposing a strain of 0.0005 %; the oscillation frequency was 1 Hz. Strain sweep tests (strain ranging from 0.0001 to 10 %, frequency = 1 Hz) were carried out to define the LVR, and to determine the yield stress. Frequency sweep tests (frequency ranging from 0.1 to 10 Hz, γ=0.0005 %) were performed to investigate the time-dependent deformation behavior of the oleogels. Both strain and frequency sweep tests were carried out at 25 °C after controlled cooling (1 °C min^{-1}) followed by a setting of 60 min. Results were reported as storage (G') and loss (G") moduli. The measurements were conducted in triplicate.

2.5 Solid fat content

Solid fat content (SFC) was measured with a low resolution (20 MHz) NMR spectrometer (Minispec mq-20, Bruker, Milan, Italy) operating at 25 °C±1 °C. Crude waxes and gel-forming solutions were aliquoted into 10 mm external diameter NMR tubes up to the height of 60 mm, and kept at 80 or 95 °C for 12 h. The sample was then conditioned at the measurement temperature (10-100 °C) and held for at least 12 h. At each temperature SFC was acquired at different times until a constant value was reached. The measurements were performed in triplicate.

2.6 Texture analysis

The firmness of the oleogels was measured by a penetration test using TMS-Pro (Food Technology Corporation) texturometer equipped with a 50 N load cell. Gel-forming solutions (≈ 35 g) were poured into plastic containers (internal diameter ≈ 5 cm, height sample ≈ 2 cm), cooled to 25 °C, and stored at the same temperature for 24h. A PMMA (Plexiglass®) cone

(diameter 2.5 cm, 90 degrees) was used as a penetration probe. The cone penetrated the sample at a crosshead speed of 120 mm/min up to a depth of 10 mm, corresponding to about 50% of the initial height of the sample. Firmness was defined as the maximum force measured during penetration. Finally, five independent tests were performed for each sample.

2.7 Oil binding capacity

The oil binding capacity of the oleogels was determined using a centrifuge method according to Manzocco, Calligaris, Camerin, Pizzale, & Nicoli, (2014). Oleogel-forming solution (1 g) was weighed in a tube and cooled to 25 °C, and held for 12 h. Samples were centrifuged at 11,510 rcf for 15 min at 20 °C using a centrifuge (HERMLE Z 326 K). The excess oil was then decanted and the weight of the remaining oleogel was determined. Oil binding capacity was calculated as:

$$\mathrm{OBC}(\%) = \left[1 - \frac{(m_1 - m_2)}{m_1}\right] \times 100$$

where m_1 is the mass of the initial sample and m_2 is the mass of the oleogel after centrifugation. Three replicates were performed for each sample.

2.8 Data analysis

Thermal and physical analysis results were reported as the mean and standard error. One-way analysis of variance (ANOVA) and multiple comparisons of means (Tukey's test, $p \leq 0.05$) were performed to evaluate the effect of wax concentration on thermal and physical parameters on oleogels of the same type. Precisely it was done separately first for the 4 OPBW samples, then for OSBW and finally for OPCW. Furthermore, in order to evaluate the influence of the oil type on the thermal and physical parameters of OPBW and OSBW oleogels, independent sample T-test (Levene test $p \leq 0.05$) were performed at each level of wax concentration. Statistical analysis was performed using SPSS for Windows version 17.0 (SPSS Inc., Chicago, IL, USA). The fractal dimension of the oleogel crystal network (D) was estimated by using equations 1 and 2, and data were fitted by Excel with a linear regression of $\log G'$ and $\log \sigma^*$ as a function of $\log \phi$. The solid volume fraction (ϕ) was calculated as SFC at 25 °C/100.

3. Result and Discussion

3.1 Thermal behavior

Crystallization and melting profiles for OP BW, OS BW and OP CW oleogels, at different wax concentrations, are shown in **figure 1**. Thermal properties of crude BW, CW and the oleogels

are listed in **Table 1**. Thermal behavior of oleogel depended on wax concentration, as was widely found elsewhere (Blake et al., 2018; Winkler-Moser, Anderson, Felker, & Hwang, 2019), and the presence of more than a single exothermic peak is the result of the heterogeneous chemical composition of the waxes (Martins, Cerqueira, Fasolin, Cunha, & Vicente, 2016).

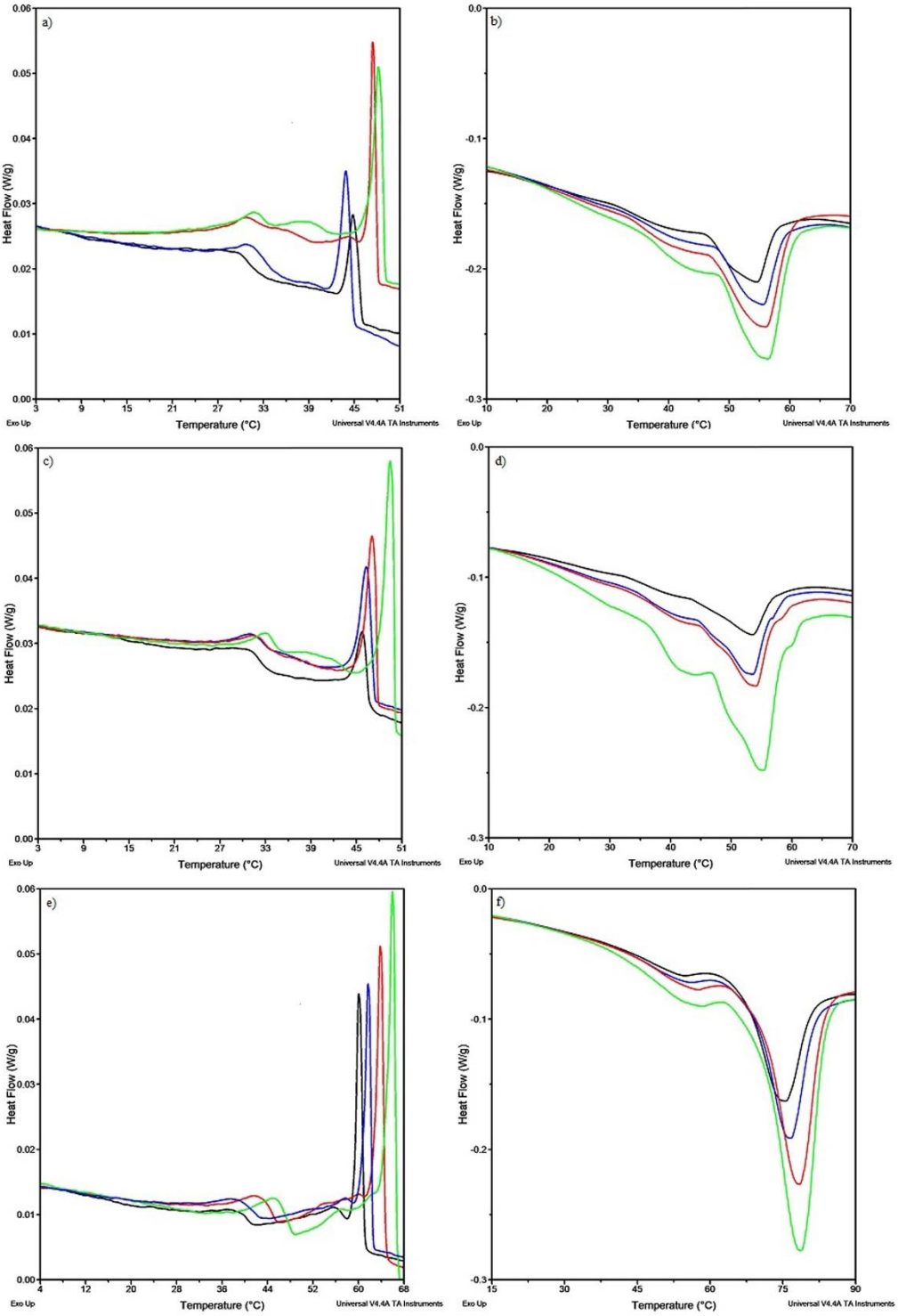

Figure 2 Crystallization (a, c, e) and melting profiles (b, d, f) for OP BW, OS BW and OP CW oleogels, at different wax concentration (4% black, 5% blue, 6% red, 8% green).

For both OP BW and OS BW oleogels, the crystallization temperatures ranged from 47 to -1 °C, with a maximum peak around 44-49 °C related to wax esters that are the prevailing chemical classes of BW, and a relative minimum peak around 30 °C (±2), that could be attributed to hydrocarbons (the second major chemical class in BW) according to Doan et al. (2017b). The crystallization peak (fig. 1a, c) became narrower, more pointed and moved toward higher temperatures with increasing wax concentration. The crystallization onset (T_{in}) and peak (T_p) temperatures slightly increased (3 and 4 °C, respectively) with the wax concentration, while there was no effect on the endset temperature (T_{off}). The crystallization enthalpy (ΔH) proportionally increased with wax concentration from 8.24±0.16 to 16.57±1.06 J/g and from 8.72±2.76 to 16.27±1.72 J/g, for OP BW and OS BW oleogels, respectively. Higher wax concentrations would lead to the formation of a strong crystalline network (Zhao et al., 2020).
OP CW oleogels showed different crystallization profiles, according to the different chemical compositions of CW compared with BW. For these oleogels, crystallization started around 69.18±0.38 and ended at about 4.51±0.89 °C. The main peak was observed around 69 °C (±4 °C), originating from wax esters (60% of CW), followed by two minor peaks, the first around 57 °C and the second around 40 °C. The presence of two minor peaks, the first related to fatty acids and the second to free fatty alcohols, seems to indicate that the CW used is of Brazilian origin, based on results reported by Doan et al. (2017a). Crystallization T_{in} and T_p, as well as the ΔH values, of the oleogels were typically lower than those of crude wax due to the colligative properties and solubility of the wax components in the oil. This could be explained by the greater dissolution of some of the wax components (such as esters and hydrocarbons) in the solvent as the ratio of oil/wax increases (Blake et al., 2018). Moreover, parameters T_{Off} and T_P of the most concentrated samples were affected by the oil type, and were lower for pumpkin seed oil than for sunflower oil-based oleogels, confirming the formation of a higher crystalline mass of BW in OS compared to OP. The BW in the two oils examined may well have a different solubility due to the different fatty acid compositions that influenced the thermal parameters of the oleogels. Indeed, the saturated/unsaturated fatty acid ratio of OS was about double that of OP. In Patel (2015) it was reported that the higher amount of saturated fatty acid content (high melting TAGs) could contribute to strengthening the structure, while a consequently lower proportion of unsaturated fatty acid (low melting TAGs) could reduce the solvency effect of liquid oils, leading to the formation of a higher crystalline mass of wax.

Table 2 Crystallization and melting parameters (mean ± standard error) of OP BW, OS BW and OP CW oleogels, at different wax concentrations (4-8 %)

	Crystallization				Melting			
	T_{In} (°C)	T_{Off} (°C)	T_P (°C)	ΔH (J/g)	T_{In} (°C)	T_{Off} (°C)	T_P (°C)	ΔH (J/g)
BW	65.67±1.97	-0.30±0.14	63.85±0.31	54.93±3.11	5.04±0.12	74.57±0.09	66.43±0.22	185.03±0.18
OPBW4	47.14±0.27 ^{b}	0.00±0.29 ^{a}	44.86±0.03 ^{b}	8.24±0.16 ^{c}	11.62±0.12 ^{c}	65.01±0.09 ^{b}	54.38±0.01 ^{b}	5.55±0.03 ^{d}
OPBW5	47.30±0.79 ^{b}	-0.64±0.33 ^{a}	44.80±0.48 ^{b}	10.52±0.34 ^{b}	13.9±0.08 ^{b}	66.91±0.43 ^{a}	54.87±0.57 ^{b}	7.77±0.66 ^{c}
OPBW6	49.94±0.43 ^{a}	-0.82±0.08 ^{a}	47.36±0.09 ^{a}	11.58±0.70 ^{b}	14.6±0.48 ^{a}	67.42±0.52 ^{a}	55.34±0.29 ^{ab}	9.44±0.56 ^{b*}
OPBW8	50.78±0.65 ^{a}	-0.96±0.17 ^{a*}	48.09±0.16 ^{a*}	16.57±1.06 ^{a}	14.8±0.25 ^{a}	68.07±0.50 ^{a}	56.08±0.08 ^{a*}	11.17±0.11 ^{a}
OSBW4	48.88±0.26 ^{b}	-1.63±0.86 ^{a}	46.04±0.58 ^{c}	8.72±1.59 ^{d}	11.83±0.81^{b}	64.13±0.68 ^{b}	53.36±0.22 ^{c*}	4.99±0.39 ^{c}
OSBW5	47.96±0.31 ^{b}	-0.45±0.30 ^{a}	46.15±0.16 ^{bc}	9.53±0.50 ^{cd}	13.90±0.33^{ab}	64.92±0.29 ^{b*}	53.29±0.06 ^{c}	6.51±0.15 ^{b}
OSBW6	48.71±0.32 ^{b}	-0.01±0.28 ^{a}	47.17±0.08 ^{b}	13.06±1.51 ^{b}	13.22±1.24^{ab}	65.50±0.59 ^{b*}	54.18±0.31 ^{b}	7.39±0.09 ^{b*}
OSBW8	51.45±0.34 ^{a}	0.46±0.29 ^{a*}	49.62±0.25 ^{ca*}	16.27±1.00 ^{a}	15.12±0.17^{a}	67.38±0.27 ^{a*}	55.23±0.17 ^{a*}	11.54±0.76 ^{a}
CR	84.35±0.02	2.31±0.12	81.60±0.02	318.70±1.31	10.27±0.23	91.95±0.14	83.30±0.08	200.2±0.51
OPCW4	63.20±0.23 ^{d}	4.51±0.89 ^{c}	59.99±0.09 ^{d}	10.03±0.91 ^{c}	17.62±0.14 ^{d}	90.37±0.61 ^{a}	74.98±0.22 ^{d}	7.03±0.00 ^{b}
OPCW5	65.55±0.05 ^{c}	5.79±0.04 ^{b}	61.72±0.24 ^{c}	10.76±0.40 ^{c}	19.18±0.21 ^{c}	91.27±0.57 ^{a}	76.52±0.22 ^{c}	8.38±0.25 ^{b}
OPCW6	67.59±0.48 ^{b}	5.86±0.08 ^{b}	62.89±0.54 ^{b}	15.81±0.89 ^{b}	19.81±0.04 ^{b}	91.45±0.28 ^{a}	77.79±0.16 ^{b}	10.63±0.72 ^{b}
OPCW8	69.18±0.38 ^{a}	6.29±0.16 ^{a}	66.10±0.09 ^{a}	22.83±0.20 ^{a}	20.35±0.07 ^{a}	91.41±0.98 ^{a}	78.60±0.07 ^{a}	14.78±1.98 ^{a}

Different letters correspond to significant differences among the samples due to the wax concentration ($p \leq 0.05$).

* corresponds to significant differences among samples due to oil type ($p \leq 0.05$).

OP BW = pumpkin seed oil and beeswax based oleogels

OS BW = sunflowers seed oil and beeswax based oleogels

OP CW = pumpkin seed oil and carnauba wax based oleogels

The wax type and concentration also influenced the melting behavior of the oleogels (Fig.1 b, d, f). As the wax concentration increased, the main melting peak became more clear-cut. Likewise, the relative minimum peak became more evident, and the melting temperatures of the high and low melting components differed much more in CW than in BW oleogels, suggesting that the large amount of wax resulted in a high influence of the low-melting components in OP CW oleogels.

For OP BW oleogels, the melting ranged from 11.62±0.12 to 68.07±0.5 °C and occurred at slightly higher temperatures than OS BW oleogels (8.72±1.6 – 67.38±0.27). However, both T_p and ΔH values were very similar. For OP CW oleogels, higher temperatures and energy amounts were needed to melt the oleogel samples. The wax concentration significantly affected all melting parameters, except T_{off} of OP CW oleogels, for which no significant differences were observed. On doubling the wax concentration (from 4 to 8 %), T_{in} increased by 3 °C for OP BW and OP CW oleogels, and by 8 °C for OS BW oleogels. However, the main effect of wax concentration was observed for melting enthalpies, which ranged from ∼5 to 11 J/g for BW-based oleogels, and from ∼7 to 15 J/g for OP CW oleogels.

3.2 Rheological behavior

3.2.1 Gelation

Figures 2, **3** and **4** show temperature sweeps (cooling stage) followed by time sweeps (setting stage) obtained for OP BW, OS BW and OP CW oleogels, respectively. The sol-gel transition was reported as storage (G') and loss (G") moduli as a function of time.

The curves can be divided into three different regions. The first is a liquid-like region (high temperature region), where the wax is still in the liquid phase and G″>G' with approximately constant moduli values. The second is a transition region where the wax begins to crystallize and clusters are forming, but a three-dimensional network has not yet formed, until a sudden change in moduli values occurs at a critical temperature at which the sol-gel transition begins (T_{gel} G′=G″). The last is the solid-like region (low temperature region). It is associated with strong interactions among flocs resulting in the gradual formation of a three-dimensional structure, where G′ values are greater than G″, as well as for gel state. Gelation kinetics increased with wax concentration, but despite the slow cooling rate (1 °C min^{-1}) the full formation of the oleogel structure needed a setting stage. The higher the concentration of oleogelator, the greater the floc number and their interactions, leading to the formation of a stronger crystalline network, hence a harder oleogel.

OP BW oleogels (**Fig. 2**) showed a liquid-like region that ended at temperatures between 42 and 48 °C, increasing oleogelator concentration.

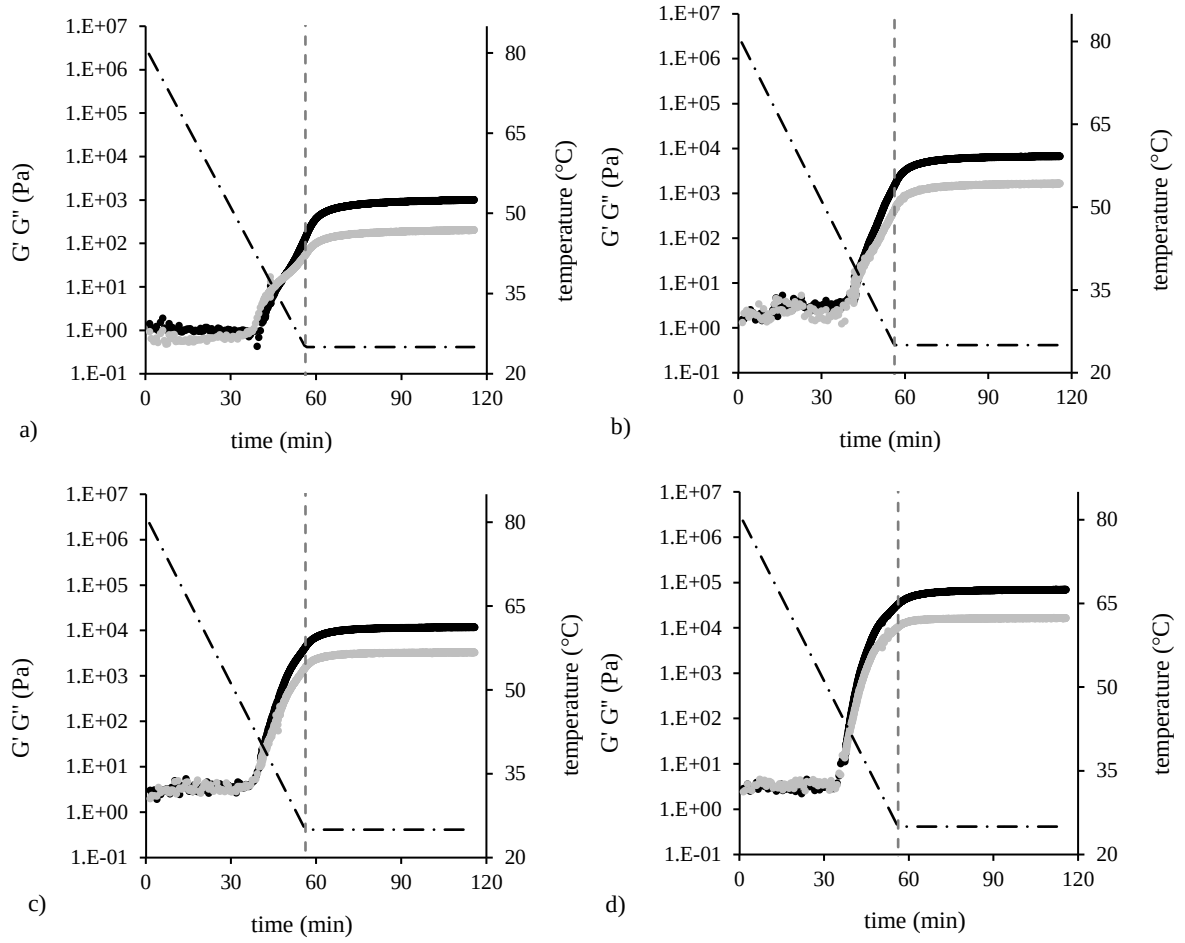

Figure 2 Sol-gel transition, during a cooling stage followed by a setting stage at 25°C, of pumpkin seed oil based oleogels at different percentage of beeswax; a) 4%, b) 5%, c) 6% and d) 8%. G′ in black and G″ in grey.

During the transition the crystallization rate increased as a function of the wax concentration, and consequentially the gelation point (T_{gel}) increased from ≈ 32.2 °C to ≈ 43.5 °C. The three-dimensional network formation was strongly affected by wax concentration; in particular, G' reached 13.5 % and 48.2 % of its final value for OP BW4 and OP BW8, respectively, before the setting stage, and then a plateau region, indicating complete oleogel formation, was reached. About 50 minutes of setting at 25°C were enough to reach the maximum values of G' for all samples ($6.7 \cdot 10^3$ Pa OP5, $1.19 \cdot 10^4$ Pa OP6, $7.0 \cdot 10^4$ Pa OP8) except for OP4 ($1.0 \cdot 10^3$ Pa), which needed an hour to gel completely. For OS BW oleogels (**Fig. 3**), the liquid-like region was in

the same temperature range as that of OP BW oleogels. However, T_{gel} increased from ≈ 39.2 °C to ≈ 46.3 °C on doubling the BW concentration.

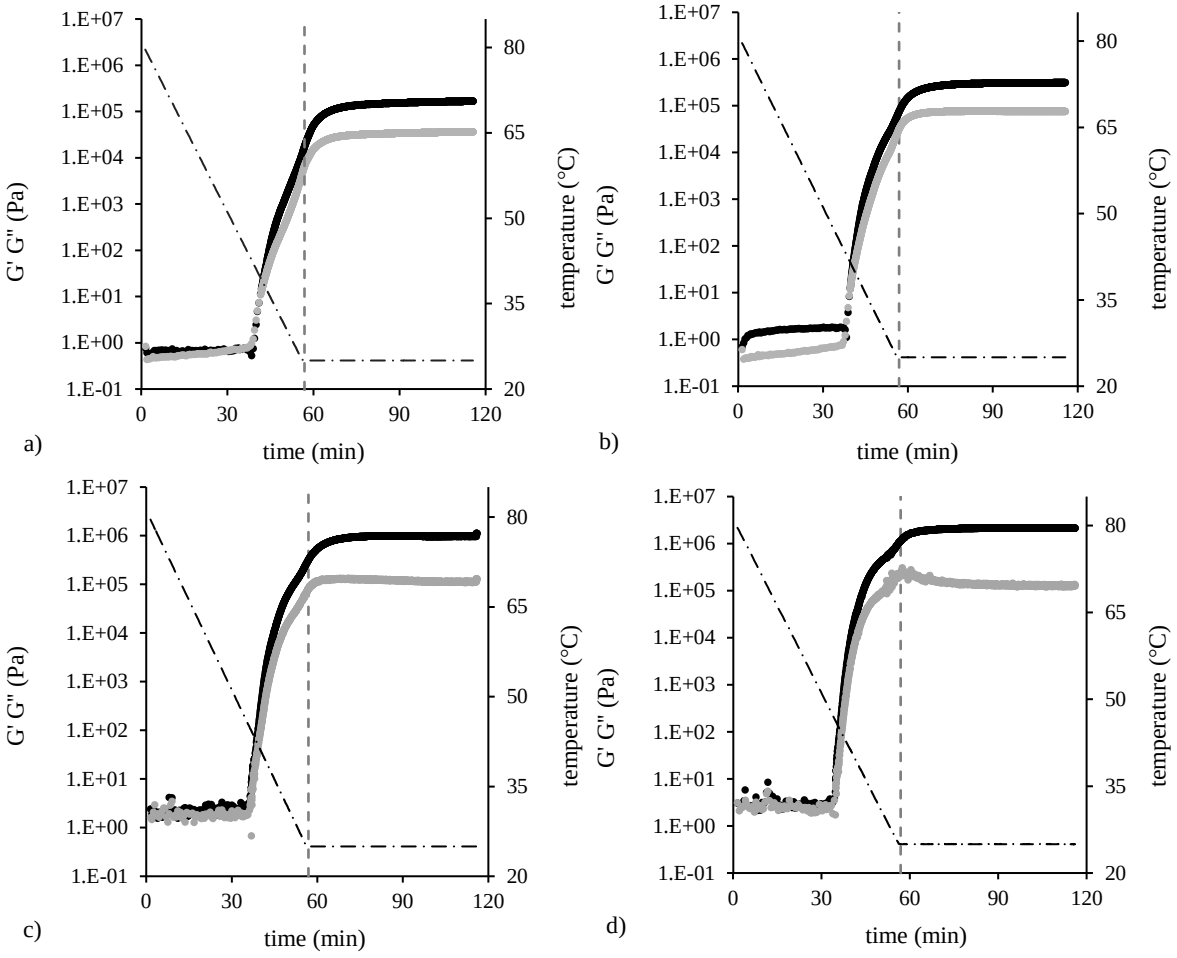

Figure 3 Sol-gel transition, during a cooling stage followed by a setting stage at 25°C, of sunflower seed oil based oleogels at different percentage of beeswax; a) 4%, b) 5%, c) 6% and d) 8%. G′ in black and G″ in grey.

Also in this case at the end of the cooling stage the gel was not completely formed. The percentage increase of G′ before the setting stage was comparable to that of OP BW oleogels (11 % OS BW4; 53.7 % OS BW8), although 45 minutes at 25 °C were enough to obtain a complete network formation of the sample OS BW8. However, OS BW oleogels reached higher G′ and G″ values with respect to OP BW (G′: $2.2 \cdot 10^6$ and $1.7 \cdot 10^5$ Pa, for 4 % and 8 % of BW concentration respectively), indicating a more interconnected structure and harder oleogels at all wax concentrations. The above results could be associated with a longer-chain oil or a lower mobility of the gelator and a strengthened network of gels formed during the crystallization

process (Martins et al., 2016). High G′ moduli are usually associated with the presence of smaller crystals forming a well-structured homogeneous network, with excellent oil-binding properties (Blake et al., 2018). If stronger gels were obtained using sunflower seed oil, it would be challenging to study whether, by changing the wax type, equally strong pumpkin seed oil-based oleogels could be obtained.

The gelation behavior of pumpkin seed oil based oleogels with CW is reported in **figure 4**. The liquid-like region ranged from 95 °C to 60 °C and T_{gel} increased from ≈ 52.6°C to ≈ 57.9°C as a function of CW concentration. The characteristic shape of the rheological curves after the gelation point indicated that the crystalline network was formed in two steps, the second occurring at temperatures ranging from ≈ 35.6 °C, for OP CW4, and ≈ 42 °C, for OP CW8, and showing a lower rate.

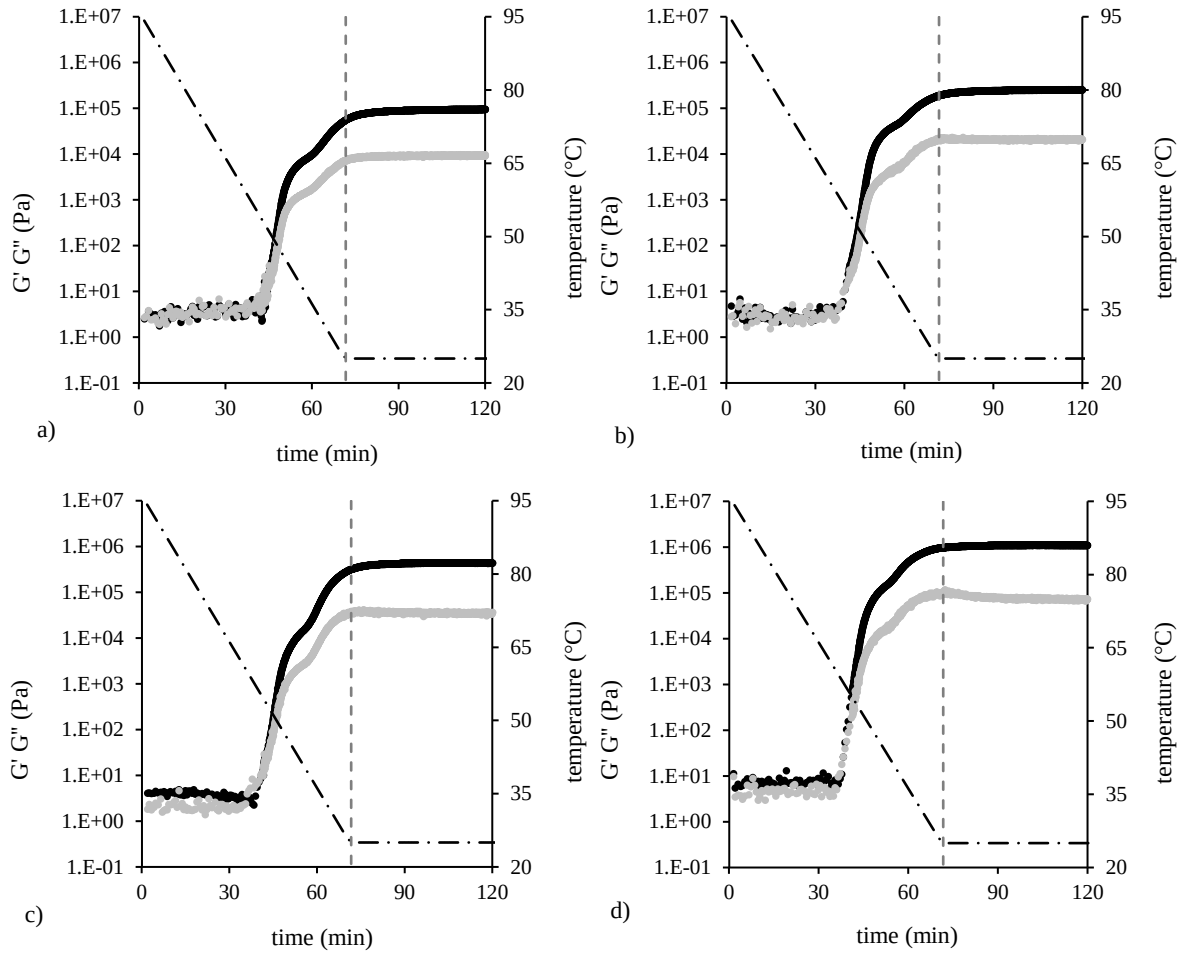

Figure 4 Sol-gel transition, during a cooling stage followed by a setting stage at 25°C, of pumpkin seed oil based oleogels at different percentage of carnauba wax; a) 4%, b) 5%, c) 6% and d) 8%. G′ in black and G″ in grey.

The first stage could be associated with the shelf assembly of the high melting components of CW, while crystallization of the low melting components could explain the second stage of the crystalline network formation. Indeed, the crystallization profiles of OP CW oleogels had two distinct exothermic peaks, and a minimum peak was observed at 37 °C and 44 °C, at 4 % and 8 % of CW, respectively. According to Martins et al. (2016), the low and high melting components of chemically heterogeneous waxes do not co-crystallize.

At the end of the cooling stage, the three-dimensional network was almost complete (G′ reached 55.4 % and 88.1 % of its final value for OP CW4 and OP CW8, respectively). Consequently, a low increase in the moduli was observed during setting at 25 °C. Using CW, OP oleogels presented G′ and G″ values very similar to those of OS BW.

3.2.2 Rheological properties

In addition to delineating the linear viscoelastic region (LVR), strain sweeps were conducted to differentiate ductile or brittle oleogels, depending on the linkage strength in the crystalline network (Doan et al., 2017a). From stress sweep curves (**Fig. 5 a, c, e**), it was evident that all the samples exhibited a gel behavior, with the elastic component (G′) dominating the viscous component (G″). A plateau region at low strain, approximately in the range 0.0001 – 1 %, was observed. The strain at the limit of linearity (γ_0) depends on both the type of oil and the wax concentration. The critical strain amplitudes of OS BW and OP CW were similar to and lower than that of OP BW oleogels, respectively. For all samples, the LVR shrunk as wax concentration increased, indicating that wax-rich oleogels were more rigid and brittle than the low-wax oleogels. Our results agree with those reported by Wijarnprecha, Aryusuk, Santiwattana, Sonwai, & Rousseau (2018) for rice bran oil-based oleogels with rice bran wax, and other investigators (Doan et al., 2018; Patel, Babaahmadi, Lesaffer, & Dewettinck, 2015). Moreover, the yield stress (σ*), defined as the point at which the deforming oleogels begin to show a liquid-like behavior, rose as the wax concentration increased. Yield stress is an important macroscopic property of materials, strongly related to material stability as well as to sensory properties such as hardness and spreadability (Wright, Scanlon, Hartel, & Marangoni, 2001). Upon increasing wax concentrations from 4 % to 8 %, both G′ and G″ moduli increased by 100 times for OP BW oleogels, while by 10 times for OS BW and OP CW oleogels. Similar results were reported by Zhao et al. (2020) for rice bran wax oleogels prepared with two different oils. As expected, OP BW oleogels showed lower G' values with respect to OS BW oleogels, confirming that they are less strong oleogels, while OP CW showed a rheological behavior very similar to that of OS BW oleogels, with slightly lower G″ values.

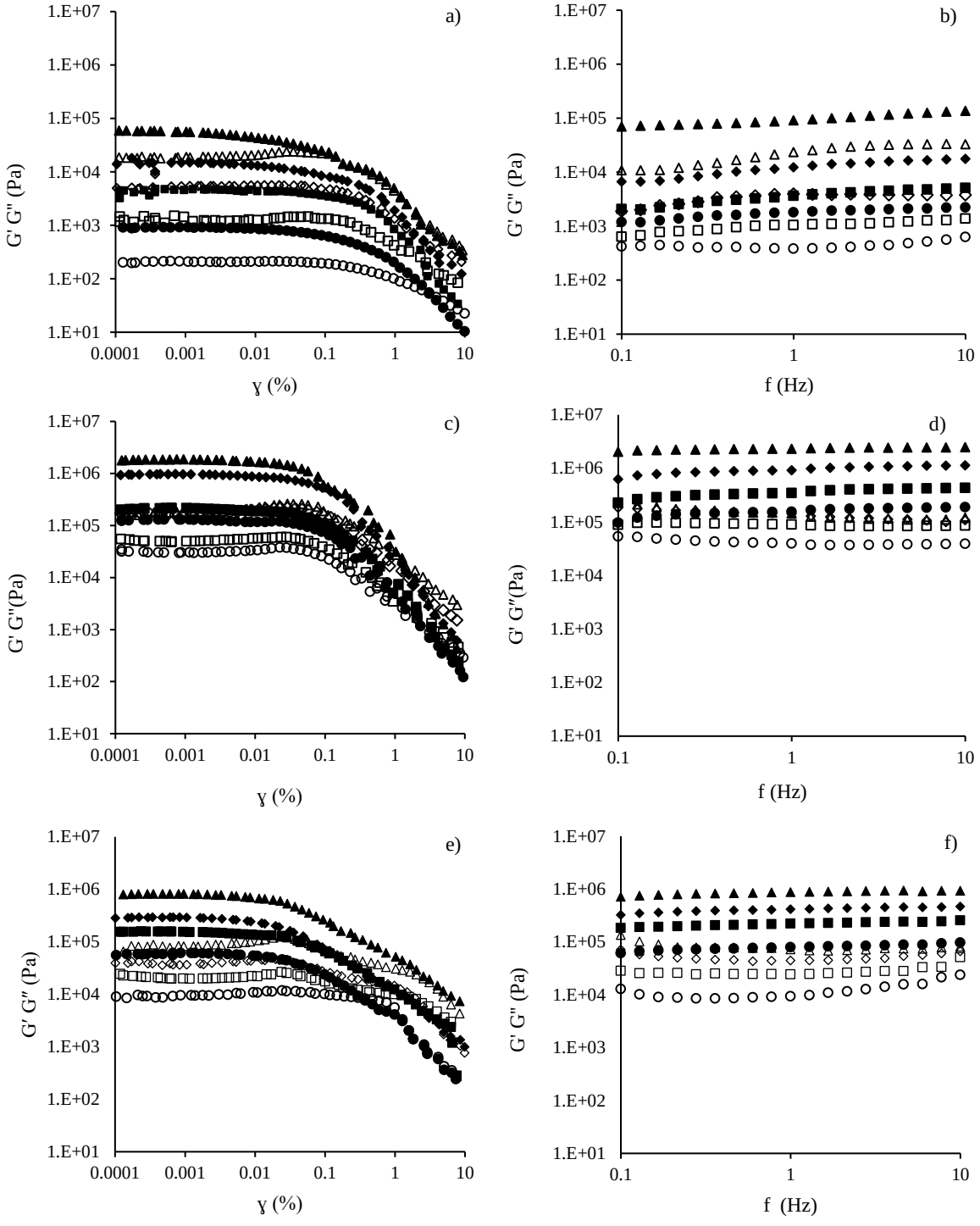

Figure 5 Strain sweeps of OP BW (a), OS BW (c), OP CW (e) and frequency sweeps of OP BW (b), OS BW (d), OP CW (f); at different wax concentration (●) 4, (■) 5, (♦) 6 and (▲) 8%. Fill indicators G'; empty G".

Frequency sweeps were performed to investigate the time-dependent deformation and to classify the samples as strong gels, weak gels, or viscous sols. A clear increase in oleogel strength with increasing wax concentration can be observed from frequency sweep curves (Fig.5 b, d, f); G′ was always greater than G″ and they were only slightly (OP BW oleogels) or not at all affected by oscillation frequency, as well as for viscoelastic solids. The results are in accordance with those reported by Martins et al. (2016) for BW-based oleogels with two types of oil that differ in their fatty acid composition. They not only reported an increase in oleogel strength with increasing wax concentration but also specified that for less concentrated oleogels (<4 % of BW) G′ and G″ moduli depended on frequency, thus confirming our finding that 4 % of BW must be used to obtain a well-structured oleogel with a complete three-dimensional network, able to bind a greater amount of oil. It can be concluded that OP BW samples behaved as weak gels, while OS BW and OP CW as strong gels.

3.3 Physical fractal dimension

For all the oleogels analyzed, a decreasing linear trend of γ_0 as a function of ϕ was observed (data not shown). Thus, based on scaling theory (Shih et al., 1990), it may be stated that the oleogels follow a strong-link regime and the resistance they oppose under applied stress depends on the elastic constant of the intra-floc links. The fractal dimension of the fat crystal network (D) was determined from the slope of the log-log plot of both G' and σ* as a function of ϕ. Both increased with increasing ϕ, showing a linear trend with a very good correlation (R^2> 0.95 in all cases). Estimated D values, considering both x = 1 and x = 1.3, followed this order: OP BW>OS BW>OP CW oleogels (**Table 2**).

Table 3 Slope and Pearson correlation coefficient (R^2) for the linear fitting between logG′ setting and logσ* vs logϕ, fractal dimension (D) considering the minimum and maximum values of x in a strong link regime (1 and 1.3), for OP BW, OS BW and OP CW oleogels.

	logG′ vs logϕ				logσ* vs logϕ			
	slope	R^2	D (x=1)	D (x=1.3)	slope	R^2	D (x=1)	D (x=1.3)
OP BW	6.42	0.96	2.37	2.33	6.40	0.98	2.37	2.33
OS BW	3.72	0.95	1.93	1.85	3.52	0.92	1.86	1.77
OP CW	3.49	0.99	1.85	1.77	4.07	0.89	2.01	1.94

OP BW = pumpkin seed oil and beeswax based oleogels
OS BW = sunflowers seed oil and beeswax based oleogels
OP CW = pumpkin seed oil and carnauba wax based oleogels

In OP BW oleogels, which had a greater fractal dimension, BW was able to form large and wide flocs and the interactions among them were consequentially weak. OS BW and OP CW oleogels were characterized by a smaller fractal dimension corresponding to a stronger network than OP BW, with higher yield stress. The fractal dimension values estimated in this work were in line with D values observed for cocoa butter and palm oil (2.37±4 and 2.82±0.6, respectively) (Narine & Marangoni 1999).

3.4 Physical properties

Firmness (N) and oil binding capacity (OBC) are reported in **Table 3,** while solid fat content (SFC) values are reported in **Table 4**. Wax concentration significantly affected the firmness of three type of oleogels (OP BW p=0.00; OS CW p=0.00; OP CW p=0.00). As the concentration of BW increased from 4 to 8 %, the firmness of OP oleogels linearly increased from 0.14 N to 0.53 N, showing the lowest resistance to penetration.

Table 4 Firmness (N) and oil binding capacity (%) of OP BW, OS BW and OP CW oleogels, at a different wax concentration (4-8%)

	Firmness (N)	Oil binding capacity (%)
OP BW4	0.14 ± 0.00 ^{c}	61.91 ± 1.20 ^{c}
OP BW5	0.16 ± 0.00 ^{c}	65.63 ± 1.55 ^{b}
OP BW6	0.34 ± 0.01 ^{b}	68.22 ± 0.57 ^{b}
OP BW8	0.53 ± 0.05 ^{a}	95.85 ± 3.74 ^{a}
OS BW4	0.49 ± 0.00 ^{d}	85.60 ± 1.86 ^{b}
OS BW5	0.91 ± 0.02 ^{c}	98.41 ± 0.46 ^{a}
OS BW6	1.27 ± 0.06 ^{b}	98.06 ± 0.39 ^{a}
OS BW8	3.79 ± 0.16 ^{a}	99.79 ± 0.29 ^{a}
OP CW4	0.58 ± 0.00 ^{d}	97.10 ± 1.47 ^{a}
OP CW5	1.05 ± 0.02 ^{c}	97.53 ± 1.21 ^{a}
OP CW6	2.37 ± 0.02 ^{b}	97.77 ± 0.66 ^{a}
OP CW8	5.73 ± 0.04 ^{a}	99.35 ± 0.13 ^{a}

Means (in the same column) with different letters are significantly different ($p \leq 0.05$).

OP BW = pumpkin seed oil and beeswax based oleogels
OS BW = sunflowers seed oil and beeswax based oleogels
OP CW = pumpkin seed oil and carnauba wax based oleogels

While, for both OS BW and OP CW oleogels, firmness exponentially increased with oleogelator concentration, which could be explained by the increase in the number of wax clusters that form a strong three-dimensional network. Wax concentration significantly affects

the oil binding capacity of OP BW oleogels, which increased from 62 % to 96 % as the amount of BW increased. Interestingly, OP CW oleogels showed high oil binding capacities (> 97 %), indicating a well-interconnected structure even at low concentrations of wax.

A significant relationship was also found between the oil type and firmness of BW based oleogels with the same wax concentration (p=0.00 at each level of wax concentration). The oil type also influenced the oil binding capacity of BW based oleogels. According with previous results, sunflower oil gave rise to more resistant gels with higher oil binding capacity than those prepared with pumpkinseed oil, when gelled with beeswax. Solid fat content usually decreases as temperatures increase. For OP BW and OS BW, the SFC proportionally decreased as the temperature rose to 50 °C, reaching mean values close to 1.8 % and 0.9 % respectively. Then (at 60 °C) a rapid decline toward values <0.7 % was observed.

The SFC values of OP CW oleogels also linearly decreased up to 80 °C. The melting rate then decreased and SFC values became close to 0.8 %. The above results are in line with crystallization profiles of BW and CW, for which T_{in} was 64.01 °C and 84.35 °C, respectively. Surprisingly, the OS BW oleogels assumed an atypical behavior at temperatures above 80 °C, showing a slight increase in SFC. Similar results were also observed by Winkler-Moser et al. (2019) for soybean oil and sunflower wax oleogels. This result could be associated with greater gelator mobility as the temperature increased, and a consequential aggregation of crystals, thereby increasing the solid fraction. The wax type and concentration also affected the solid fat content. However, for high temperatures (50 and 80 °C upward, for BW- and CW-based oleogels, respectively) the differences decreased. Moreover, the amount of solid fat depends on the fatty acids, as well as on temperature. OP BW showed lower SFC than OS BW, probably due to the presence of higher saturated fatty acid content in sunflower oil compared with pumpkin seed oil. These results were in line with rheological properties and the estimated network fractal dimension of the oleogels investigated. The BW formed a stronger gel in the OS, capable of binding the oil more strongly, than the OP system. CW is a more efficient gelator in pumpkin seed oil since a lower concentration is required to obtain firmer oleogels which retain more oil compared to OP BW oleogels. OP CW oleogels also showed the greatest SFC values, confirming that these oleogels are more resistant to melting. A direct relationship between oleogel microstructure and their oil binding capacity was reported by Blake & Marangoni (2014): as the size of the crystal decreases, the total amount of solid surface increases, providing a greater interfacial area on which the oil can adsorb, with a consequent increase in the ability to retain oil.

Table 4 Solid fat content (%) of OP BW, OS BW and OP CW, at different wax concentration (4-8 %) and temperatures (10-100 °C).

	Temperature (°C)										
	10	**15**	**25**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**
OP BW4	3.29 ± 0.06 ^{d}	3.00 ± 0.11 ^{d}	2.46 ± 0.10 ^{d}	2.22 ± 0.04 ^{d}	1.80 ± 0.28 ^{d}	1.26 ± 0.19 ^{b}	0.52 ± 0.00 ^{b}	0.53 ± 0.01 ^{c}	0.51 ± 0.00 ^{c}	0.40 ± 0.01 ^{c}	0.37 ± 0.01 ^{b}
OP BW5	3.95 ± 0.02 ^{c}	3.41 ± 0.03 ^{c}	3.08 ± 0.05 ^{c}	2.96 ± 0.06 ^{c}	2.39 ± 0.00 ^{c}	1.65 ± 0.02 ^{b}	0.57 ± 0.00 ^{b}	0.55 ± 0.01 ^{bc}	0.53 ± 0.08 ^{c}	0.50 ± 0.01 ^{b}	0.42 ± 0.00 ^{a}
OP BW6	4.49 ± 0.02 ^{b}	4.14 ± 0.04 ^{b}	3.81 ± 0.01 ^{b}	3.44 ± 0.02 ^{b}	3.23 ± 0.01 ^{b}	2.20 ± 0.01 ^{a}	0.63 ± 0.03 ^{a}	0.60 ± 0.02 ^{ab}	0.58 ± 0.02 ^{b}	0.51 ± 0.00 ^{b}	0.47 ± 0.01 ^{a}
OP BW8	5.02 ± 0.05 ^{a}	4.94 ± 0.04 ^{a}	4.65 ± 0.01 ^{a}	4.45 ± 0.01 ^{a}	4.36 ± 0.00 ^{a}	2.24 ± 0.26 ^{a}	0.7 ± 0.01 ^{a}	0.66 ± 0.03 ^{a}	0.63 ± 0.02 ^{a}	0.56 ± 0.02 ^{a}	0.57 ± 0.01 ^{a}
OS BW4	3.48 ± 0.16 ^{d}	3.55 ± 0.03 ^{d}	2.26 ± 0.03 ^{d}	1.84 ± 0.02 ^{d}	1.51 ± 0.00 ^{d}	0.41 ± 0.03 ^{c}	0.48 ± 0.10 ^{a}	0.64 ± 0.00 ^{a}	0.62 ± 0.01 ^{a}	0.70 ± 0.00 ^{c}	0.94 ± 0.03 ^{b}
OS BW5	4.47 ± 0.17 ^{c}	3.96 ± 0.00 ^{c}	2.87 ± 0.03 ^{c}	2.25 ± 0.04 ^{c}	1.87 ± 0.06 ^{c}	0.78 ± 0.03 ^{b}	0.51 ± 0.02 ^{a}	0.52 ± 0.02 ^{a}	0.58 ± 0.02 ^{a}	0.71 ± 0.01 ^{c}	0.98 ± 0.07 ^{b}
OS BW6	5.32 ± 0.08 ^{b}	4.61 ± 0.03 ^{b}	3.43 ± 0.10 ^{b}	2.73 ± 0.06 ^{b}	2.20 ± 0.05 ^{b}	0.90 ± 0.09 ^{b}	0.50 ± 0.04 ^{a}	0.65 ± 0.02 ^{a}	0.53 ± 0.08 ^{a}	1.01 ± 0.01 ^{b}	1.14 ± 0.13 ^{ab}
OS BW8	6.30 ± 0.21 ^{a}	5.05 ± 0.01 ^{a}	3.88 ± 0.01 ^{a}	4.23 ± 0.12 ^{a}	3.48 ± 0.11 ^{a}	1.85 ± 0.02 ^{a}	0.54 ± 0.03 ^{a}	0.67 ± 0.08 ^{a}	0.66 ± 0.13 ^{a}	1.07 ± 0.00 ^{a}	1.28 ± 0.00 ^{a}
OP CR4	3.05 ± 0.03 ^{c}	3.08 ± 0.02 ^{c}	3.07 ± 0.01 ^{d}	3.02 ± 0.04 ^{d}	2.92 ± 0.04 ^{d}	2.54 ± 0.03 ^{d}	2.27 ± 0.04 ^{d}	2.04 ± 0.01 ^{d}	1.81 ± 0.00 ^{b}	0.87 ± 0.07 ^{a}	0.88 ± 0.03 ^{a}
OP CR5	4.00 ± 0.22 ^{b}	3.91 ± 0.18 ^{b}	3.65 ± 0.11 ^{c}	3.83 ± 0.11 ^{c}	3.81 ± 0.11 ^{c}	3.47 ± 0.12 ^{c}	2.96 ± 0.19 ^{c}	2.81 ± 0.21 ^{c}	2.26 ± 0.08 ^{a}	0.9 ± 0.04 ^{a}	0.83 ± 0.08 ^{a}
OP CR6	6.16 ± 0.34 ^{a}	5.88 ± 0.20 ^{a}	4.96 ± 0.17 ^{b}	4.77 ± 0.06 ^{b}	4.74 ± 0.09 ^{b}	4.25 ± 0.14 ^{b}	3.97 ± 0.01 ^{b}	3.57 ± 0.05 ^{b}	2.17 ± 0.08 ^{a}	0.84 ± 0.09 ^{a}	0.82 ± 0.05 ^{a}
OP CR8	6.12 ± 0.17 ^{a}	6.22 ± 0.10 ^{a}	5.74 ± 0.00 ^{a}	5.74 ± 0.02 ^{a}	5.73 ± 0.02 ^{a}	5.52 ± 0.06 ^{a}	4.54 ± 0.03 ^{a}	4.07 ± 0.05 ^{a}	2.18 ± 0.03 ^{a}	0.85 ± 0.08 ^{a}	0.80 ± 0.10 ^{a}

All the oleogel samples, except for OP4, showed firmness values comparable to those of coconut oil under the same experimental conditions (data not shown).

Conclusions

Wax type, wax concentration and oil type affected crystallization and gelation kinetics, and physical properties of the oleogels. Gelation kinetics increased with wax concentration; a complete structured oleogel needed a setting stage at 25 °C. The higher wax concentration provided an oleogel with higher solid fat content, more resistant to melting, more rigid and brittle with a greater yield stress and ability to retain oil. OS BW oleogels showed higher amounts of solid fraction, higher values of the elastic and loss modulus, acting like strong gels compared to OP BW, mainly due to the different fatty acid composition of the oils. Carnauba wax proved to be a more efficient oleogelator in pumpkin seed oil, a lower concentration being required to obtain an oleogel with excellent physical properties. Furthermore, based on fractal dimension theory and rheological behavior, it may be asserted that oleogels follow a strong-link regime. Further, OP CW was characterized by the smallest fractal dimension, corresponding to the stronger network. These results suggest that OP CW oleogels could be used as a fat replacer in many foodstuffs.

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*We suggest the work of Doan et al, (2107a and b) as key references because they extensively deal with the role of different natural waxes in oil structuring.

*We suggest the work of Martins et al., (2016) as a key reference because it is explained as different oil types could affect the beeswax gelation process and thus the physical properties of the oleogels produced.

*We suggest the work of Patel et al., (2015) as a key reference because in this study was reported a detailed rheological characterization of natural waxes and sunflower oil-based oleogel.

CHAPTER 5

Its going to be submitted on Food Research International

NEW HEALTHY SPREADABLE CREAMS WITH PUMPKIN SEED OIL OLEOGEL AND LUCUMA POWDER

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Abstract

This research aimed to understand the effect of fat and sugar replacement on structure and physical properties of new healthy creams at different refining degree. To this purpose, three different creams were prepared in a stirred ball-mill: a reference cream R with cocoa butter and sugar; a cream A where the cocoa butter was totally replaced by a pumpkin seed oil and carnauba wax oleogel; finally, a cream B with both oleogel, as total fat replacer, and Lucuma powder, as a partial substitute for sugar was also prepared. Creams A and R showed very similar unimodal distribution, reaching a similar D90 value (26.4 and 24.4 μm, respectively) at 150 minutes of refining. The oleogel seemed to accelerate the refining, which could also be stopped at 120 minutes for cream A. However, in terms of rheological behaviour, cream A and R greatly differed in yield stress, indicating that strength of the network structure of the creams, so the attractive particle-particle interactions, are affected not only by the particle size, but also the composition of the fat matrix in which the powders are dispersed. On the contrary, sample B showed the highest D90 value at the end of refining, with a PSD and a rheological behaviour less affected by the refining time. Moreover, both cream A and B showed a solid-like behaviour and a good tolerance to deformation rate, exhibiting also a pseudoplastic behaviour, an oil binding capacity, a water activity and a physical stability comparable to those of the reference cream. Thus, viable alternatives for reformulating foods with healthier nutritional profiles were provided.

Keywords: healthy creams; oleogel; pumpkin seed oil; mill ball refining; natural sweeteners.

1 Introduction

Sweet anhydrous creams represent an important ingredient in different confectionery foods such as in wafers, sandwiches and co-extruded biscuits. They have often achieved the greatest success on their own as spreads, whose market is constantly developing. Creams are generally considered unhealthy products due to the large amount of fats and sugar, which strictly affects their physical and sensory properties.

Hard fats commonly used in creams production are rich in saturated fatty acids, whose consumption also leads to negative health implications, including cardiovascular diseases (CVD), high cholesterol, cancer and type II diabetes (Micha & Mozaffarian, 2010). It is not generally suitable to directly replace hard fats with liquid oil, leading to technological problems, such as texture weakness and oil leakage in several food products for their low viscosity (Kim, Lim, Lee, Hwang, & Lee, 2017). To solve this problem, in the last years the oleogelation process has been successfully developed. The oleogelation consists in structuring liquid oil by using an oleogelator, offering to food products the functionality of fats with the nutritional profile of liquid oil, avoiding saturated and trans-fats. Our group has recently developed new oleogels with pumpkin seeds oil and carnauba wax, for potential food applications (Borriello, Masi & Cavella, 2021). Pumpkin seed oil was distinguished from others vegetable oil, such as hempseed and almond oils, by the presence of arachidonic acid (0.27%), which is essential for optimal performance of the nervous system (Tallima & El Ridi, 2018; Borriello, Miele Masi, Aiello & Cavella, 2022). On the other hand, the caloric value of a spreadable cream is largely affected by the sugar, which is a key ingredient for the structure and quality of the product. Sugars daily intake should be less than 10% as stated the WHO guidelines, because its consumption negatively affects health increasing several chronic diseases risk, such as obesity and diabetes (Patel, Moghadam, Freedman, Hazari, Fang & Allen, 2018). Several strategies can be adapted to reduce sugar in foodstuffs, the commonly suggested one is the product reformulation replacing sugar, partially/totally, or reducing its amount (Di Monaco, Miele, Cabisidan, & Cavella, 2018). For sugar substituting natural sweeteners plant-based are usually preferred to artificial sweeteners as they may contain beneficial bioactive compounds, such as polyphenolic compounds with antioxidant properties. Lucuma is Peruvian fruit with similar caramel sweet taste that should be used as natural food sweetener, with a high nutritional value (Banasiak, 2003; Dini, 201; Rojo et al., 2010). It is a source of fiber, iron, niacin and beta-carotene which are useful to improve children's physical development, prevent depression and increase the immunologic system efficiency. Moreover, it is a low glycemic sweetener with a high content of phenol and flavonoid components, thus it could be considered as a superfood

(Taiti, Colzi, Azzarello & Mancuso, 2017). Lucuma was successfully introduced in many foodstuffs such as ice cream, juices, cakes, biscuits, yogurt, chocolate, baby food and pies (Durakova et al., 2019; Ak-Cvitanovìc et al. 2015).

Spreadable cream production generally consists of mixing and refining the ingredients, obtaining the characteristic structure of a concentrated suspension of several solid particles (sugar, cocoa powder, milk whey, milk powder, dehydrated cream, nut solids, etc.) in a continuous liquid (oil) or semi-solid phase (cocoa butter or other fats) (Miele, Borriello, Fidaleo, Masi & Cavella et al., 2020). Refining, also called grinding, aimed to obtain an optimal solid particle size distribution, strictly related to quality and stability of final product. For small-scale production, stirred ball mills represent a valid technology for cream production (Fidaleo, Mainardi & Nardi, 2017; Fidaleo, Miele Mainardi, Armini, Nardi, & Cavella, 2017; Konar and Bingol, 2019; Pajin et al., 2011; Toker, Zorlucan, Konar, Daglıoglu, Sagdic, & Sener, 2017). Ingredients and balls stirring in the mill tank results in impact and shearing actions that provide a progressive reduction of the solid particles as well as their homogeneous dispersion (Cavella, Miele, Fidaleo, Borriello & Masi, 2020). Particle size distribution of creams mainly depends on the amount and type of the solid particles and their size before grinding, but also on the oil/powder ratio, because the lower the oil/powder ratio, the coarser the granulometry of the cream (Armini, Miele, Albero, Sacchi, & Cavella, 2018). The physical properties of refined creams are also affected by the physical state of the fat phase. Comparing the fineness of a hazelnut-and-cocoa-based paste, containing a vegetable oil, and of a white chocolate flavouring paste, containing a blend of vegetable oil and fats, refined in the same ball mill under similar operative conditions and containing powders of comparable initial granulometry, a similar evolution of fineness over refining time was observed (Miele et al., 2020; Cavella et al., 2020). However, those products differed for the type of oil/fat used so presented different PSD of creams at 25°C probably because in one of them there was also a high fraction of several fats that could crystallize when the temperature changed from 45 (processing in the ball mill) to 25 °C (storage).

For confectionary products, few studies reported the usage of oleogels in chocolate products, as summarized in the review of Zhao, Zihao and Xue (2021). Li and Liu (2019) suggested the application of oleogels based on monoglyceril stearate, β-sitosterol/lecithin and ethyl cellulose to replace cocoa butter in dark chocolate. Results reported by Sun et al. (2021) showed that oleogels with β-sitosterol and gamma oryzanol were the best substitute for cocoa butter in dark chocolate. Recently, Bascuas, Espert, Llorca, Quiles, Salvador and Hernando (2021) designed new chocolate spreads with oleogels based on olive and sunflower oil, HPMC and xanthan

gum. Their results showed that if the cocoa butter was replaced at 50% with the suggested oleogel, the final product was like the control one, but a complete replacement determined a very different product, with a less homogeneous spread. To date, very few studies exist regarding using wax oleogels in chocolate product, and never the oleogel have been added at the beginning of refining. Doan, Patel, Tavernier, De Clercq, Van Raemdonck (2016) using rice bran oil oleogel at different beeswax concentration to partially replace the palm oil in hazelnut fillings. Fayaz et al. (2017) studied the use pomegranate oleogels-palm oil mixtures to produce functional chocolate spread. However, cream production involved the refining of only hazelnuts and sugar in 3-roll mill, while the oleogel was later added by mixing with a mixer equipped with "B" flat beater or by hand.

Regarding the natural sweetener Lucuma, its chemical composition and beneficial effect on health have been largely studied (Banasiak, 2003; Dini et al., 2011), but only one study reported its inclusion in a confectionary product, a low-sugar chocolate (Ak-Cvitanovìc et al., 2015). Their results indicated that surface weighted mean D[3,2] should be less than 20 μm and mean particle size D50 less than 90 μm to enhance chocolates textural quality. Moreover, food application of lucuma, from a technological point of view, should be investigated. There are no studies that have investigated the effect of using oleogels, as well as Lucuma on refining in a ball mill. Therefore, this work aimed to investigate the structure and physical properties of new healthy spreadable creams at different refining times. Firstly, it was verified the ability of the oleogel chosen to mimic the cocoa butter behaviour during refining. To do that, a reference cream (cream R), with cocoa butter and sugar, and a cream A, with pumpkin seed oil oleogel at 6% of carnauba wax as 100% cocoa butter replacer, were prepared in a stirred ball mill. Secondly, considering that powder type could affect the structure and the properties of a cream, a third cream B was produced including both the oleogel and Lucuma powder, to partially replace sugar phase and was compared to the other two creams. The effect of replacements on the structure of creams at different refining degree was investigated analysing their granulometric and rheological behaviour. Apparent viscosity, oil binding capacity, water activity, colour and Turbiscan stability were also analysed to assess how the physical properties of the creams, with different fat and/or sugar phase, can be modified during refining. The addition of both oleogels and Lucuma powder could allow to produce new spreadable cream with improved health properties, not only reducing the level of saturated fats and sugar but also enhancing the essential fatty acids and bioactive profiles.

2 Materials and Methods

2.1 Materials

The ingredients (hazelnut, cocoa, sugar, cocoa butter and salt) used to prepare creams were supplied by Me.Pa Alimentari S.r.l (Napoli, Italy). Pumpkin seeds oil was purchased from Baule volante e Fior di Loto (Bologna, Italy). Micronized carnauba wax (CW) was kindly provided by a local candy company.

2.1.1 Cream preparation

A spreadable cream based on cocoa butter and sugar was prepared and used as reference cream (sample R). Two healthy creams were prepared, by changing the fat phase (sample A) and fat and sugar phases (sample B), respectively (**Fig 1**). The formulations of the creams are shown in **table 1**. For creams production, a Roboqbo universal processing system was used (model Qb8-4, Roboqbo s.r.l, Bologna, Italy) equipped with a spherical refinement system (Bilia, designed to be used in conjunction with the Qbo - Universal Processing System series) with 4 kg of 6 mm diameter stainless steel AISI 316L balls. Ingredient weight was set on 3 kg, according to the instrument specifications.

Table 5 Spreadable creams composition (%).

Ingredients	**Formulations**		
	R	**A**	**B**
hazelnut	45	45	45
cocoa powder	4.9	4.9	4.9
sugar	32	32	16
lucuma powder	-	-	16
cocoa butter	10	-	-
pumpkin seed oil	8	8	8
oleogel OPCW6	-	10	10
Salt	0.1	0.1	0.1

Figure 4 Image of spreadable creams with cocoa butter and sugar (R), oleogel and sugar (A), oleogel, sugar and Lucuma powder (B).

Speed rate was fixed (250 rpm) and the temperature controlled (45±2°C). Hazelnuts and sugar cutting, at 25°C for 15 min, was performed to obtain the hazelnut paste. For sample C also lucuma powder was add in this step. Then, the other solid components and melted cocoa butter were add and mixed into the Qbo before starting the refining. An oleogel based on pumpkin seeds oil and 6% of carnauba wax was separately prepared, as reported in Borriello et al. (2021). The oleogelator was added to the oil previously heated at 90 °C and the mixture was stirred at 200 rpm, using a magnetic stirrer, until a clear solution was obtained. Then the mixture was

cooled down to 45°C and finally added into the Qbo. Three productions for each cream were carried out and samples refined for 60, 90, 120 and 150 min were collected.

2.2 Granulometric measurement

A digital micrometer (Metrocontrol Srl, Casoria, NA) was used to measure the fineness of the creams during refining (45°C). Therefore, grinding was stopped at 150 minutes since the solid particle size was close to 30 µm for all samples. The particle size distribution (PDS) was analyzed by using a Mastersizer laser diffraction particle size analyser equipped with Hydro 3000 dispersion unit (Malvern Instruments, Worcestershire, UK). For each cream, three different replicates were analyzed and for each replicate, 20 measurements were performed.

2.3 Rheological measurement

Viscosity of cream samples was determined by a Modular Advanced Rheometer System (Haake MARS, Thermo Scientific, Waltham, *USA),* equipped with a vane tool geometry (diameter 22mm, length 16 mm, gap = 1 mm, ≈ 28 ml of the sample). The viscosity curves were measured as a function of increasing shear rate (0.1-100 s^{-1}) at 25°C. Strain sweep tests (strain ranging from 0.0001 to 10 %, frequency = 1 Hz) were carried out to define the LVR, and to determine the yield stress. Frequency sweep tests (frequency ranging from 0.1 to 10 Hz, γ= 0.0005 %) were performed to investigate the time-dependent deformation behaviour of the creams. The measurements were conducted in triplicate.

2.4 Oil binding capacity, water activity and colour

The oil binding capacity was determined using a centrifuge method. Creams (50 ml) was weighed in a centrifuge tube and were centrifuged at 10000 rpm for 20 min at 25 °C using a centrifuge (HERMLE Z 326 K). The excess oil was then decanted and the weight of the remaining creams was determined. Oil binding capacity was calculated as:

$$\mathrm{OBC}(\%) = \left[1 - \frac{(m_1 - m_2)}{m_1}\right] \times 100 \quad (1)$$

where m_1 is the mass of the initial sample and m_2 is the mass of the cream after centrifugation. Three replicates were performed for each sample. Water activity determinations (Aw) were acquired using an Aqualab-Dew point water activity meter (4 TE, USA).

The colour of the creams was determined using a colourimeter (Minolta Chroma Meter, CR 300, Japan). The Hunter parameters L* (from 0=black to 100=white), a* (- a= greenness to + a= redness), and b* (-b=blueness to +b= yellowness) were measured and averaged from three

randomly positions for each sample the total colour difference (ΔE) was calculated according to the following equation:

$$\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (2)$$

where ΔL*, Δa*, Δb* are the difference of the samples A and B and the reference R colour with respect to each parameter at the same refining time.

2.5 Turbiscan stability

The physical stability of creams was evaluated by measuring the backscattering (BS) of pulsed near infrared light (wavelength of 880 nm) using a Turbiscan Tower stability analyser (Formulaction, France). Cream was placed into cylindrical glass tubes up to the height of 45 mm and scanned for 120 h (≈ 5 days) at 25 °C. Stability of samples was expressed using the Turbiscan Stability Index (TSI), which is defined as follows:

$$TSI = \frac{\sum_i \sum_h |scan_i(h) - scan_{i-1}(h)|}{N_h} \quad (3)$$

where $scan_i(h)$ is the light intensity of the scan acquired at the i-th time instant and at a height of h, and N_h is the number of height positions in the selected scanning zone of the tube (top, center, bottom or global) (Cavella et al., 2020). The data were analysed by using the software package TowerSoft Ver 1.2 (Formulaction, France).

2.6 Data analysis

Results are reported as mean ± standard error of at least three replications. Critical strain value (γ_0) and yield stress (σ^*) were determined from strain sweep curves. The critical strain was determined as the strain where G′ value decreased of more than 10% the values recorded in the LVR, while the yield stress as the stress where the viscous and the elastic contributions are equal (G′ = G″) (Dinkgreve, Paredes, Denn, & Bonn, 2016).

The frequency curves were fitted according to the rheological Power Law model which is expressed by the following equation (4) (Steffe, 1967):

$$G' = a(\omega)^b \quad (4)$$

where G' is the storage modulus (Pa), ω is the frequency (rad/s), and a (Pa·s^b) and b (-) are parameters used to describe rheological behaviour.

Apparent viscosity curves of spreadable creams were described using Casson rheological model, that is adopted by International Office of Cocoa and Chocolate for interpreting chocolate-based product behaviour, represented in the following equation (5):

$$(\eta)^{1/2} = (\frac{\sigma_0}{\dot{\gamma}})^{1/2} + (\eta_\infty)^{1/2} \quad (5)$$

where σ_0 is the Casson yield stress and η_∞ is the infinite-shear viscosity, also called the Casson plastic viscosity. The yield stress can be used to calculate whether a sample is likely to settle in situ, or whether it will be difficult to start pumping or stirring (Steffe, 1996). Multivariate analysis of variance (ANOVA) and multiple comparisons of means (Duncan's test, $p \leq 0.05$) were performed to evaluate whether differences among the samples, due to the different formulation (at the same refining time) and due to different refining time (for each sample), were statistically significant ($p \leq 0.05$) by using SPSS for Windows version 17.0 (SPSS Inc., Chicago, IL, USA).

3 Results and Discussion

3.1 Particle size distribution

Figure 2 shows the particle size distribution (PSD) of spreadable creams refined for 60 (**Fig. 2a**), 90 (**Fig. 2b**), 120 (**Fig. 2c**) and 150 (**Fig. 2d**) minutes. As refining proceeds, the range of size class of solid particles shrunk from 0.52-859 to 0.52-211 μm for cream R, from 0.52-515 to 0.52-211 μm for cream A, and from 0.52-.586 to 0.52-211 μm for cream B. Creams A and R showed very similar unimodal distribution, as also reported in other studies for anhydrous pastes refined in a ball mill refiner (Fidaleo et al., 2017; Miele et al., 2020; Cavella et al., 2020), with some differences at high particle size which became less evident as refining proceeds. Cream B showed a larger particle size distribution curve with a lower particles volume percentage around 11 μm compared to the other samples. Moreover, PSD of cream B changed from bimodal to unimodal during refining, most likely due to the presence of a multicomponent sugar phase. At particle sizes between 0.5-0.8 μm a small left shoulder was observed for all the samples.

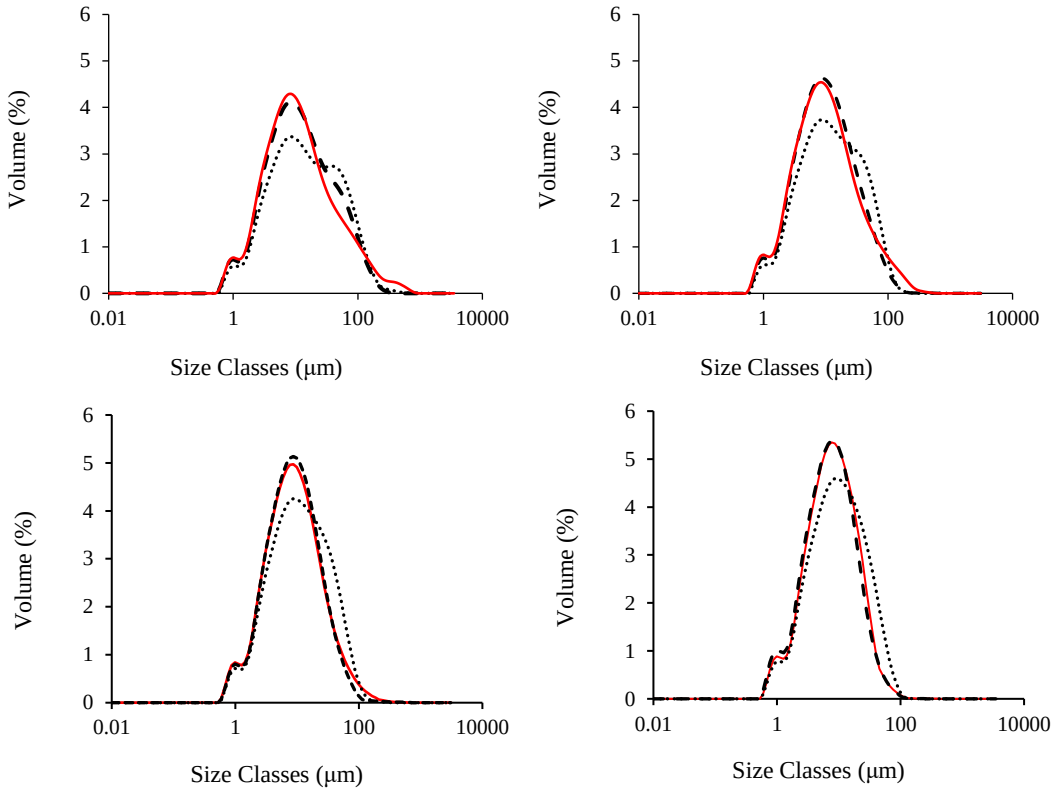

Figure 2 Particle size distribution of spreadable creams R (———), A (– – – –) and B (·····), at different refining time 60 (a), 90 (b), 120 (c) and 150 (d) min.

Table 2 reports the percentiles of the PSD for creams analysed. There were no differences between D10 values of creams R A and B refined for the same time. For medium refining times (90 and 120 min.) creams R and A showed similar D50 values which were lower than those of cream B, while D90 of creams A and B were lower and higher than D90 values of reference cream, respectively. Creams R and A refined for 150 minutes showed similar D90 values (26.4 μm and 24.4 μm) lower than those observed for sample B (37.4 μm). It should be explain considering that all the creams presented the same oil/powder ratio, but the oil/sugar ratio in sample A and R was lower than sample B. Accordingly with Armini et al. (2018), who found an effect of oil/sugar ratio on D90 of RUTF formulation, we found that refining in terms of D90 was affected by the type of powder. Apparently the oleogel did not affect the refining. However, it should be considered that hazelnut past was cut ensuring a particle size of 350 μm of solid particles at the 0 time of refining for all the creams samples (data not shown). Taking into account this result, it can be affirmed that most of the refining takes place in the first hour, and

the oleogels seemed to accelerate the refining since a smaller particle size is achieved after the same refining time. On the other hand, Lucuma flour slows down the refining despite having a smaller granulometry than sugar.

Table 2 Mean values (±standard error) of D10, D50, D90 for creams R, A and B at different refining time.

Cream	Refining time (min)	D_{10} (μm)	D_{50} (μm)	D_{90} (μm)
R	60	2.60±0.01^{b}	10.86±0.14^{d}	86.49±5.76^{d}
	90	2.49±0.04^{a}	9.89±0.11^{c}	51.56±1.00^{c}
	120	2.49±0.03^{a}	9.08±0.13^{b}	35.66±1.40^{b}
	150	2.42±0.00^{a}	8.27±0.12^{a}	26.41±1.43^{a}
A	60	2.71±0.01^{d}	11.62±0.04^{d}	66.64±0.43^{c}
	90	2.64±0.01^{c}	10.12±0.05^{c}	42.37±0.34^{b}
	120	2.55±0.00^{b}	9.04±0.01^{b}	31.37±0.25^{a}
	150	2.21±0.06^{a}	7.76±0.11^{a}	24.38±0.22^{a}
B	60	2.97±0.14a	16.02±2.20^{d}	87.63±0.98^{d}
	90	2.61±0.31a	12.60±0.24^{c}	56.67±0.50^{c}
	120	2.72±0.03a	11.38±0.08^{b}	46.74±0.25^{b}
	150	2.55±0.00a	10.14±0.00^{a}	37.39±0.07^{a}

3.2 Rheological behaviour

Strain sweeps (**Figures 3 a, b, c, d**) were performed to determinate the stability of spreadable creams by means of critical strain value (γ_0) and yield stress (σ^*) determinations, as shown in **table 3**. Critical strain is the onset of nonlinearity corresponding to the structure deformation necessary to initiate flowing (Doan, Van De Walle, Dewettinck & Patel, 2015). The yield stress, related to material stability as well as to hardness and spreadability, represents the point at which the deforming creams begin to show a liquid-like behaviour (Wright, Scanlon, Hartel & Marangoni, 2001). All the samples showed a gel-like behavior with a storage modulus (G') greater than the loss modulus (G'') and linear viscoelastic region (LVR) approximately in the range 0.0001–0.006 %, in accordance with results reported by Palla, Wasinger & Carrín (2021) for filling creams made with monoglyceride oleogels. There were no significant differences in critical strain (γ_0) values observed for R A and B creams refined for the same amount of time, except a slightly lower γ_0 value than the others in cream B at the end of refining. Independently of the refining time, different values of yield stress (σ^*) were observed following this order: R<B<A. However, refining time strictly affects both γ_0 and σ^* parameters ($p > 0.05$), which increased with increasing refining time. Those results seemed to suggest that creams with oleogels were more structured and required higher shear stress to flow, compared to the

reference cream. The yield stress is related to the strength of the network structure, depending on attractive particle-particle interactions. Therefore, particle size and particle concentration, as well as density of the network could affected its magnitude (Larsson, 1999; Coussot & Ancey, 1999). Comparing creams with a similar D90 around 25 μm, e.g. cream R with cream A refined for 150 min (table 2), their σ* values (table 3) were quite different. However, the solid particles, in our case sugar and cocoa powders, could be dispersed in matrix in a different way depending on the fat matrix composition (Babin, Dickinson, Chisholm & Beckett, 2004; Palla et al., 2021) so also particle-particle interactions could vary. It could be hypothesized that in cream A the interactions among solid particles inside carnauba wax and pumpkin seed oil, lead to a strong three-dimensional network. On the other hand, cocoa butter decreases forces between particles, reducing the resistance to flow and leading to lower values of yield stress (Beckett, 2009). Lucuma powder seemed to hinder the affinity between solid particles probably due to its heterogenous composition.

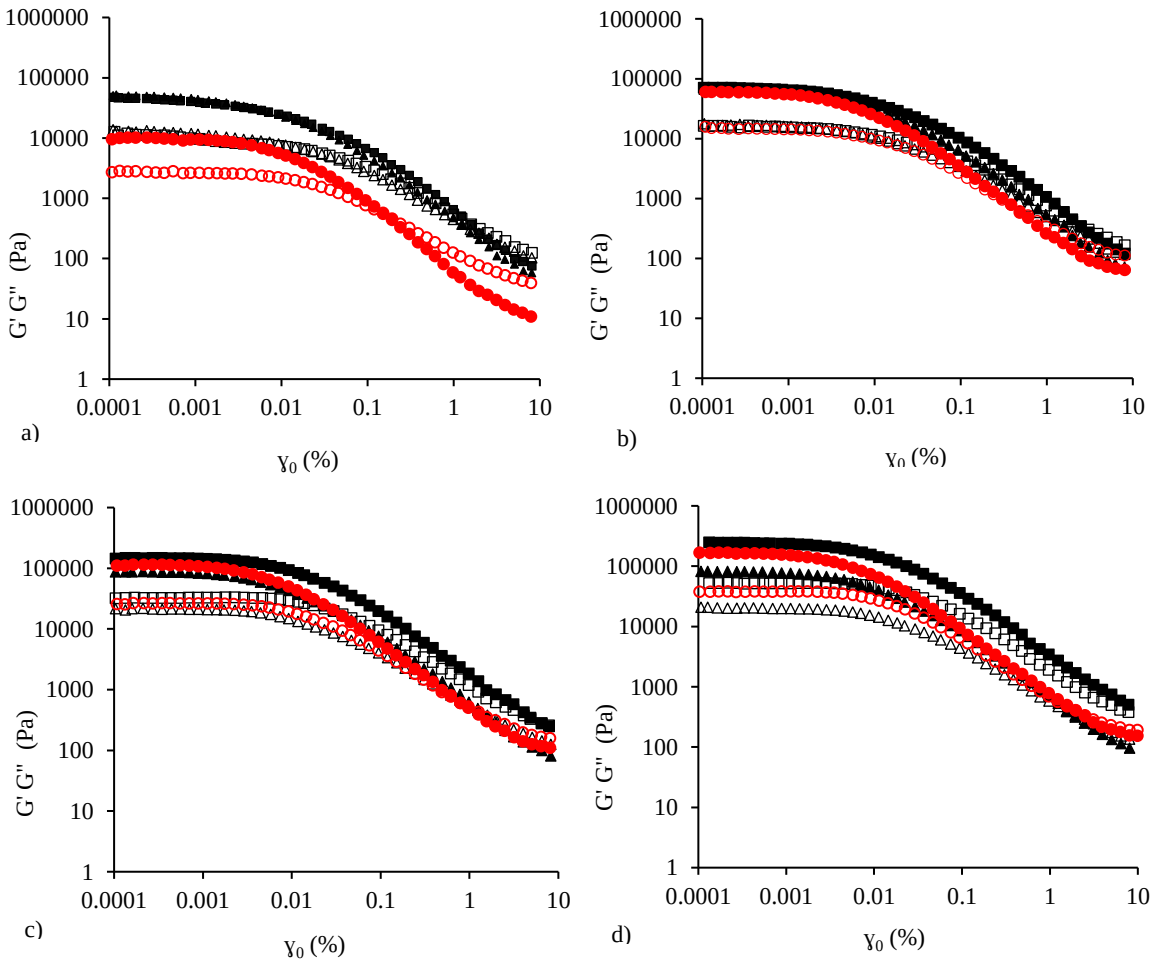

Figure 3 Strain sweeps of spreadable creams R (●), A (■), B (▲) at different refining time 60 (a), 90 (b), 120 (c) and 150 (d) min. Fill indicators G', empty G".

At low refining time, creams A and B showed similar G′ and G″ close to 40000 Pa, values which were higher than those observed for sample R (10000 Pa). As the refining proceeds, G′ values increased with a linearly trend (R^2=0.99; m=1722.4) in sample R, with an exponential trend (R^2=0.99; n=0.019) in sample A. Finally, G′ value of sample B linearly increased (R^2=0.99; m=611.62) up to 120 minutes of refining, then reached a constant value. Sample B refined for 150 minutes showed slightly lower storage modulus than the other sample mainly due to the low sugar amount. A direct relationship between the G′ and sugar amount was also found by Palla et al. (2021) in filling creams for sandwich cookies using monoglyceride oleogel as fat phase. Those results, in accordance with PSD profiles, suggested that for the cream A the refining process should be stopped at 120 minutes, since desired values of both, particle size and yield stress were obtained.

The frequency sweeps were performed to compare the deformation behaviour of three cream formulation during refining. **Figure 4** (**a, b, c, d**) shows the mechanical spectra of frequency sweeps, which were also fitted with the power-law model estimating parameters *a* and *b*, reported in **table 3**.

Table 3 The strain at the limit of linearity (γ_0) and the yield stress (σ^*) determined by strain sweep test; the parameters *a* and *b* estimated fitting frequency curves with the power-law model ($G' = a\,\omega^b$).

Sample	Refining time (min)	γ_0(%)	σ^*(Pa)	*a* (Pa·s^b)	*b*	R^2
R	60	0.002±0.00^a	1.16±0.00^a	15547±1332^a	0.197±0.00^b	0.98
	90	0.001±0.00^a	4.29±0.27^b	20241±1004^b	0.192±0.00^b	0.97
	120	0.001±0.00^a	6.76±0.30^c	22236±968^b	0.188±0.00^b	0.98
	150	0.006±0.00^b	11.76±0.01^d	36448±1452^c	0.115±0.01^a	0.96
A	60	0.002±0.00^a	8.12±0.50^a	39616±4146^a	0.175±0.05^c	0.98
	90	0.002±0.00^a	12.33±0.48^b	62057±4257^b	0.152±0.05^b	0.97
	120	0.003±0.00^b	23.11±0.14^c	141118±2375^c	0.149±0.02^b	0.92
	150	0.005±0.00^c	51.33±0.05^d	219261±5425^d	0.131±0.01^a	0.97
B	60	0.001±0.00^a	6.46±0.09^a	38751±753^a	0.204±0.02^d	0.98
	90	0.002±0.00^b	7.31±0.00^b	53519±875^b	0.193±0.01^c	0.97
	120	0.001±0.00^a	8.19±0.00^c	72452±1008^c	0.177±0.02^b	0.97
	150	0.002±0.00^b	9.18±0.00^d	73870±2265^c	0.169±0.00^a	0.97

The power-law model (equation 4) well described storage modulus data (R^2>0.92). For all refining times, cream R showed the lowest *a* value, which ranged from 15547 to 36448 Pa·s^b, while *b* values were similar to those estimated for sample B (≈0.19).

Cream A always showed the highest *a* value and the lowest *b* value compared to the other samples. Moreover, a relationship between refining time and both parameters was found in cream R (*a* p=0.00; *b* p=0.03), cream A (*a* p=0.00; *b* p=0.001) and B (p=0.00 for *a* and *b*). In detail, *a* increased with refining time, while *b* followed the reverse order. The solid-like behaviour was confirmed for all the samples which showed a slight frequency dependence and a good tolerance to deformation rate. Fayaz et al. (2017) also found a solid-like behaviour in chocolate spreads prepared with palm oil and wax oleogels in ratio 1:1. At low refining time, creams A and B exhibited similar storage modulus, which were higher than those observed in R sample. Similar values of storage and loss moduli were also found by Glicerina, Balestra, Pinnavaia, Dalla Rosa & Romani (2013) in nut creams with different type of fats. In detail, samples A and B could be resembled to a cream with 1:1 and 3:1 palm oil/hydrogenated fat ratio, respectively.

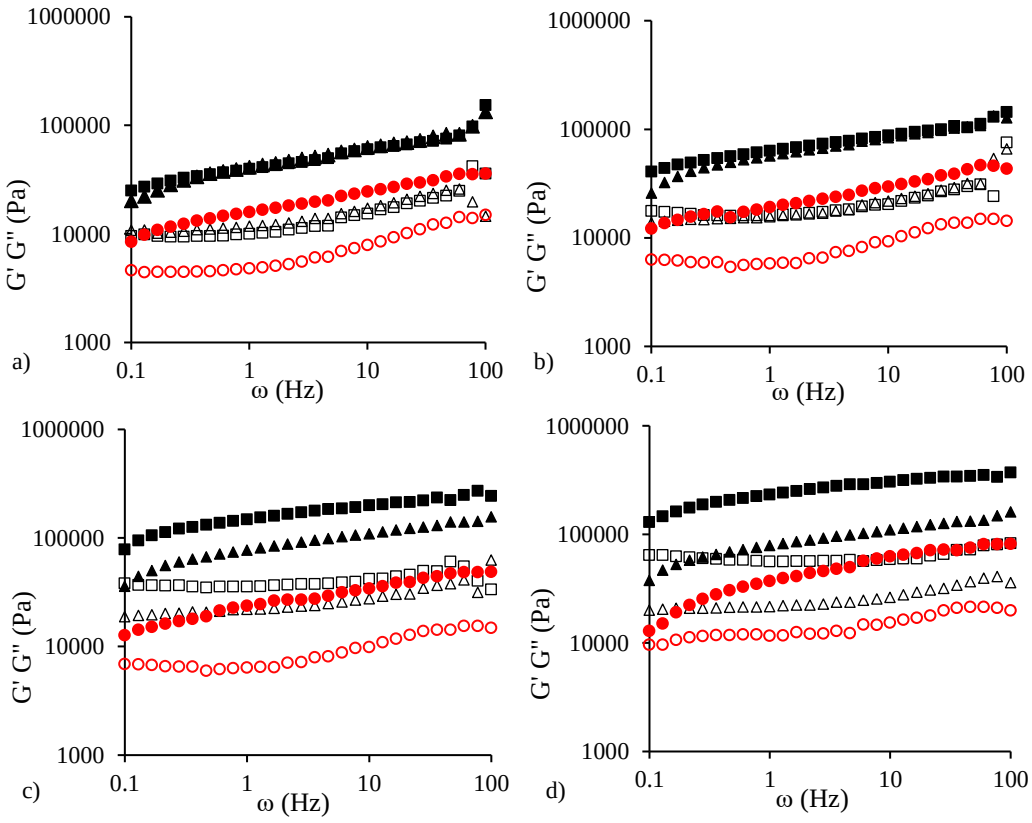

Figure 4 Frequency sweeps of spreadable creams R (●), *A* (■), *B* (▲) at different refining degree 60 (a), 90 (b), 120 (c) and 150 (d) min. Fill indicators G', empty G".

3.3 Creams characterization

3.3.1 Apparent viscosity

The shear viscosities of spreadable creams refined for different times are represented in **Figure 5**. All the spreads showed a pseudo-plastic behaviour since the apparent viscosity (η) decreased as shear rate (γ̇) increasing. Viscosity increased as a function of the refining time for cream R and A (Fidaleo et al., 2017), even if for cream B it seemed that its viscosity increased a lot moving from 60 to 90 min, but not at increasing refining time. At low shear rate (0.1 s^{-1}), the samples R (**Fig. 5a**) and A (**Fig. 5b**) showed very different viscosity values depending on refining time that ranged from 64 Pa·s to 819 Pa·s and from 300 Pa·s to 1700 Pa·s as refining time increasing, respectively. For cream B, its viscosity after 150 min (**Fig. 5d**) seemed to have the lowest viscosity values indicating an effect of the lucuma powder on this cream property, as also observed by Miele et al. (2020) for RUTF formulations in which the oil/powder ratio was the same, but the sugar content was reduced, and the soy flour content increased. However, it also true that at the end of the refining time cream B presented a D90 higher than those of both R and A cream.

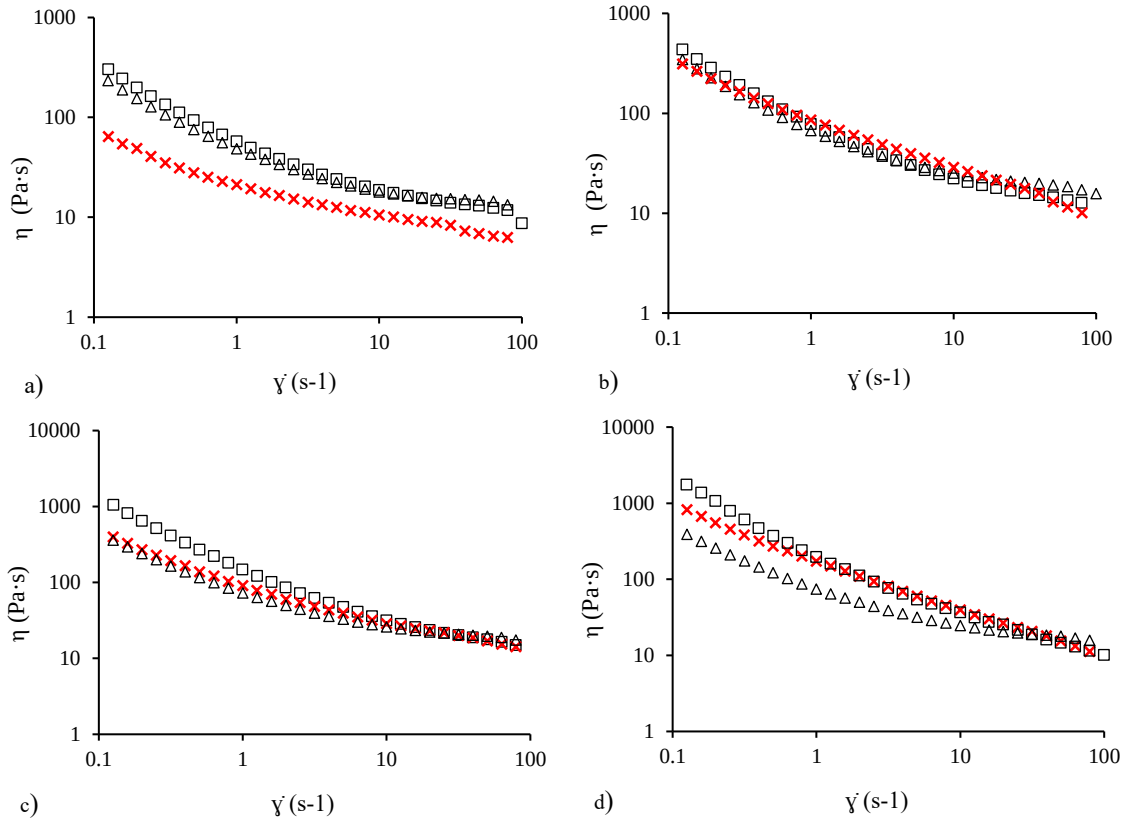

Figure 5 Apparent viscosity (η) as a function of shear rate (γ̇) for spreadable creams R (×), A (□) and B (Δ) refined for 60 (a), 90 (b), 120 (c) and 150 (d) min.

Table 4 Rheological parameters (mean ± standard error) of Casson model (yield stress, σ_0, and viscosity at infinite shear rate, η_∞), for spreadable creams at different refining time.

Cream	Refining time	Casson parameters		
		σ_0 (Pa)	η_∞ (Pa·s)	R^2
R	60	4.15 ± 0.01^{a}	7.85 ± 0.33^{a}	0.99
	90	25.45 ± 2.33^{b}	13.04 ± 2.32^{ab}	0.99
	120	37.00 ± 0.00^{c}	10.23 ± 0.00^{bc}	0.99
	150	76.82 ± 0.07^{d}	14.53 ± 0.86^{c}	0.99
A	60	26.20 ± 3.05^{a}	8.36 ± 0.30^{b}	0.99
	90	37.20 ± 0.00^{b}	7.86 ± 0.02^{b}	0.99
	120	83.10 ± 0.10^{c}	7.72 ± 0.14^{b}	0.99
	150	158.66 ± 0.03^{d}	4.91 ± 0.82^{a}	0.98
B	60	15.40 ± 3.74^{a}	7.72 ± 0.15^{a}	0.99
	90	29.77 ± 0.00^{b}	8.86 ± 0.00^{b}	0.99
	120	29.95 ± 0.00^{b}	9.51 ± 0.00^{b}	0.99
	150	32.11 ± 0.00^{b}	10.36 ± 0.43^{c}	0.99

Casson model well fitted flow curves (R^2 =0.998) and the estimated parameters Casson yield stress (σ_0) and the Casson plastic viscosity (η_∞) are reported in **table 4**. At low refining time, all the samples showed similar η_∞ values close to 8 Pa·s. After 120 minutes of refining, creams R and A showed the highest (≈14 Pa·s) and the lowest (≈5 Pa·s) plastic viscosity, respectively. A higher plastic viscosity can allow problems during process of some types of chocolate formulations (Patel et al., 2014). In samples R and B, the Casson plastic viscosity increased as refining proceeds from 7 to 14 and from 7 to 10 Pa·s, respectively. However, in cream A η_∞ seemed to decrease with particle size reduction. Afoakwa, Paterson, and Fowler (2008) reported an increase of plastic viscosity decreasing particle size for chocolate sample with 25% fat and 0.3% lecithin, mainly due to the increase of points of contact between particles. However, they also noted a reduction in plastic viscosity with increasing lecithin from 0.3 to 0.5%, especially at lower particle size. They attributed this reduction to an association between lecithin with sugar particles. As already stated, fat phase could affect sugar dispersion with an effect on the viscosity and rheological properties of the final product (Palla et al., 2021; Babin et al., 2005), because when sugar crystals were well dispersed in the matrix, viscosity of the product was reduced. In our case the oleogels, or more specifically the carnauba wax, seemed to behave as lecithin reducing plastic viscosity at low particle size. A significant increase of Casson yield values decreasing particle sizes was found, accordingly with many investigators (Afoakwa et al. 2008; Aydemir, 2019). Sample A showed the higher σ_0 values than sample R at all refining times.

A similar result was also found by Patel et al. (2014) in spreads chocolate with shellac oleogels. They found that spreads with oleogel had higher yield stress respect with reference chocolate paste prepared using an oil binder, probably due to the higher interactions between solid sugar particles. Furthermore, Taylor, Van Damme, Johns, Routh, and Wilson (2009) investigated the rheological behaviour of chocolate with crumb and sunflower oil founding σ_0 and η_∞ values in the range 20-70 Pa and 4-15 Pa·s, respectively. Our results are also in line with those reported by Cavella et al. (2020) who studied the effect of ball milling on rheological properties of a white chocolate anhydrous paste with a fat phase made up of a mix of cocoa butter, palm and sunflower oil.

3.4.2 Oil binding capacity, water activity and colour

Oil binding capacity (OBC), water activity (A_w) and colour parameters (L* a* b* *and ΔE*) are reported in **table 5**. The oil binding capacity plays an important role in determining the effectiveness of a new fat formulation (Doan et al., 2016). Sample A and B showed similar OBC values (≈86%), which were slightly lower than those observed for R cream (≈89%). Doan et al. (2016) reported that most of the oil loss occurs in the first day after production and then decreases. Considering that OBC measurements were performed one-week later samples production, and the total replacement of cocoa butter led to an oil loss only 4% greater than the reference cream, it can be concluded that sample contained OPCW oleogel exhibited a high OBC. Moreover, the refining did not affect the OBC of samples, although for creams refined for 120 min slightly higher OBC values were observed. Samples were microbiologically stable since a_w values were close to 0.5 in all spreadable creams. There were no significant differences between the L*, a* and b* values of samples R and A ($\Delta E<1$), while cream B showed lower L* and b* values compared to the others. The lightness increased with the refining time. Thus, the colorimetric differences between cocoa butter (white/yellow) and OPCW oleogel (brown/green) were well masked by the addition of other ingredients such as cocoa powder. On the other hand, Lucuma powder decreased both, lightness and yellowness of the cream, and those differences were visible to the human eye ($\Delta E>3$).

Table 5 Spreadable creams physical characterization. Oil binding capacity (OBC %), water activity (%) and colour parameters (L* a* b* and ΔE) of creams R, A and B at different refining time.

Sample	Refining time (min)	OBC	Aw	L*	a*	b*	ΔE
R	60	86.66±0.77^{a}	0.50±0.00	23.05±0.11^{a}	14.56±0.10	10.53±0.10^{a}	-
	90	90.44±0.22^{c}	0.49±0.00	24.17±0.05^{b}	14.81±0.03	11.36±0.09^{b}	-
	120	90.66±0.00^{c}	0.50±0.01	25.18±0.08^{c}	14.71±0.05	12.36±0.12^{c}	-
	150	88.66±0.38^{b}	0.48±0.01	27.01±0.04^{d}	14.38±0.10	13.37±0.08^{d}	-
A	60	85.77±1.17	0.49±0.02	23.24±0.10^{a}	14.48±0.14	10.31±0.22^{a}	0.63±0.32
	90	85.77±0.44	0.51±0.00	24.14±0.06^{b}	14.51±0.07	11.01±0.08^{b}	0.51±0.01
	120	86.22±1.16	0.45±0.01	26.09±0.42^{c}	13.95±0.22	11.50±0.34^{b}	1.52±0.30
	150	85.77±1.09	0.48±0.01	26.89±0.14^{d}	14.10±0.03	12.61±0.03^{c}	0.85±0.31
B	60	86.50±0.95	0.44±0.02	20.26±0.85	14.23±0.07	7.63±0.93	5.31±0.12^{a}
	90	86.00±1.15	0.42±0.00	19.74±0.02	14.31±0.07	6.67±0.11	6.47±0.11^{b}
	120	87.33±0.38	0.43±0.00	20.66±0.00	14.38±0.10	7.30±0.05	6.79±0.19^{b}
	150	86.66±0.77	0.43±0.00	21.10±0.03	14.25±0.01	8.20±0.11	7.86±0.12^{c}

3.4.3 Turbiscan stability index

Physical stability of spreadable creams could be affected by changing fat or sugar phase. **Figure 6** (**a**, **b**, **c**, and **d**) reports the evolution over time of Turbiscan stability index (TSI) for samples R, A and B at different refining degrees. Samples R and A initially showed a similar TSI trend that rapidly increased in first 7 hours, then the grow rate slowed down. The cream B seemed to follow an opposite trend since initially showed the lowest TSI kinetics which became faster to reach similar TSI value as cream A after 5 days of storage. At low refining degree, TSI curve of sample B showed a characteristic shape indicated that the sedimentation occurred in two steps. It is probably due to a larger particle size population consisting in different sweeteners. Accordingly with PSD curves, bimodal behaviour faded as refining proceeded. TSI value of sample R and B decreasing from 1.32 to 0.89 and from 0.9-0.75 with increasing refining time, respectively. The cream with cocoa butter as lipid phase showed the highest TSI values, mainly due to the sedimentation of solid particles but still acceptable as lower than 1.4. There were no differences between TSI value (0.7) of sample A refined for 90, 120 and 150 min of refining. Moreover, sample A was the only one that seemed to assume a constant trend, suggesting that it will not be subject to significant destabilization phenomena over long storage time. However, all the samples were stable during about 5 days of storage, since TSI values were lower than 10, which is considered the Turbiscan stability threshold. Our results are in line with those reported by Cavella et al. (2020), which investigated the TSI at the top of the

vials of anhydrous pastes with cocoa butter refined in stirred ball mills. They showed that the samples refined for 150 min reach TSI value close to 4 in about 70 h and that the sedimentation occurred mainly for the less refined sample. All the creams analysed in this work showed TSI value lower than 1.4 and a TSI at the top of the vials (data not shown) lower than 1.2, suggesting that both, the particles sedimentation and a consequent separation of oil phase at the meniscus was negligible. Babin et al (2005) reports that sugar crystals differently interact each other depending on the fat phase, determining different sediment volumes, which could explain the small differences observed in TSI values of our samples. They reported that a lower sediment volume was observed in palm kern oil than in cocoa butter, because of crystals were more aggregated in the first fat, while interacted with repulsive forces in the cocoa butter.

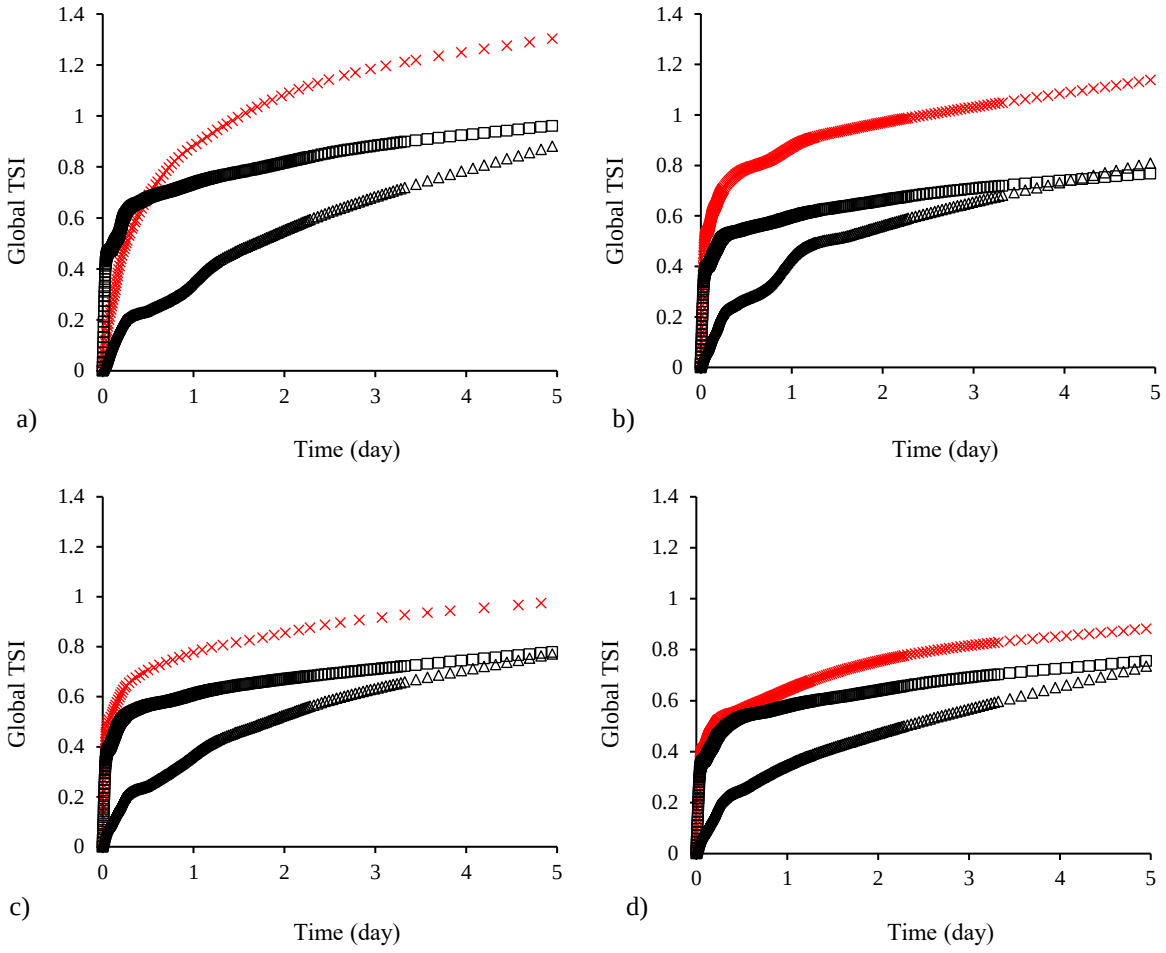

Figure 6. Turbiscan stability index (TSI) as function of storage time (day) for spreadable creams R (×), A (□) and B (Δ) refined for 60 (a), 90 (b), 120 (c) and 150 (d) minutes.

Conclusions

The present work emphasized the impact of a novel oleogel and uncommon sweetener on the structure and physical properties of healthy creams at different refined degrees. Similar creams were obtained by changing only the fat phase, in terms of PSD and rheological properties, with small differences in terms of the strength of the network structure. The oleogel accelerated the refining process and its structure was completely recovered in cream with 100% sugar which showed high shear stress to flow. At low sugar particle size, the carnauba wax, acted as an emulsifier, reducing the Casson plastic viscosity. On the other hand, Lucuma seemed to slow down the refining and to slightly hinder the recovery of the oleogel structure in the cream. A solid like-behaviour and a good tolerance to deformation rate was observed for all the sample showing that the oleogel based on pumpkin seed oil and carnauba wax could be totally replace the cocoa butter, and Lucuma could partially decrease the sugar intake. Moreover, both oleogel and Lucuma did not affect oil binding capacity and water activity ensuring the physical and microbiological stability of creams. Our results confirmed the effect of particle size on rheological and stability properties also in oleogel- based creams. Concluding, these findings could provide useful information in the application of pumpkin seed oil oleogel and Lucuma powder for novel confectionery products with healthier nutritional profile.

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CHAPTER 6

6.1 Final remarks and future perspective

In the present PhD thesis novel approaches for healthy spreadable creams production were investigated and interesting findings were highlighted.

A market online survey especially designed proved to be useful to explore experiences and desires toward a new healthy spreadable cream of consumers with different healthy choice attitudes. New product development, as well as strategies to introduce new healthy foods on the market, should be consumer-driven to be successful. Results showed that a healthy spread is in line with the current trend market. On one hand, it could satisfy the needs of the consumer increasingly aware of the close correlation between nutrition and health. On the other, expanding the market range of healthy products is an effective strategy to encourage eating habits. The strength of this study also lies with the prediction of healthy vs unhealthy food choices using a healthy choice index, exploring a list of food ingredients useful in healthy spreadable cream development. The limitations of this research, including the small number of males and self-reported height and weight, should be considered as well. Those specific results should not be generalized towards the global population. Moreover, the research highlighted how the healthiness concept is strictly related to the perception of the fat and sugar content since consumers proposed healthier alternatives including the use of polyunsaturated vegetable oils and natural sweeteners.

The effectiveness of oleogelation as a novel strategy to structure liquid oils into oleogels was proven by exploring both the effect of oil type and wax concentration on oleogels structure. The work contributed to expanding the knowledge on the oleogelation of polyunsaturated and monounsaturated oils using complex oleogelators such as waxes. The effect of oil type on network formation mechanism and physical properties of oleogels was investigated, explaining how very small differences in terms of fatty acid amounts or viscosity can affect the self-assembling of oleogelators during the gelation. The practical application of this study also lies in providing criteria for choosing the most suitable oil for designing oleogels with desired

physical properties to reduce saturated fatty acids. Furthermore, novel oleogels based on almond, pumpkin and hemp seed oil, that have never gelled, have been proposed.

The effect of wax concentration and type on oleogelation of pumpkin seed oil was explored. This vegetable oil has gained attention because of its beneficial health effects mainly due to the large amounts of polyunsaturated fatty acid and bioactive compounds.

The work highlights the importance of a comprehensive understanding of the crystallization and gelation of novel oleogels, making on their thermal and rheological behaviour. Thus, characterization and optimization of an innovative oleogels based on pumpkin seed oil were useful to understand to better control their food application.

Therefore, innovative approaches were explored in cream production taking into account consumer demands: the oleogels to reduce solid fat and increase the unsaturated fatty acid amount, and Lucuma powder as a natural sweetener to reduce sucrose intake. The oleogel proved to be a valid alternative to the cocoa butter since a total fat replacement slightly affected the cream's structure, rather it improved the physical stability. Lucuma powder allowed obtaining a 50% reduction of sucrose improving the nutritional profile of the cream. Moreover, the research highlights the effect of the new ingredients on the structure of the cream taking into account the real production process of refining, providing useful information for an industrial application. Thus, viable alternatives for reformulating foods with healthier nutritional profiles were proved.

In conclusion, the overall obtained results demonstrate that the rheological and structural investigation was essential for designing healthy and highly innovative food products, providing additional options for tailor-made formulations.

Further investigation on almond and hemp seed oils should be done exploring the best wax concentration to produce oleogels useful to replace the traditional solid fat. Further studies are needed to improve the recipe investigating different substitution levels of sugar phase and/or other natural sweeteners. The oxidative stability of oleogel spreads should be evaluated as well. The effect of using novel ingredients on consumers' behaviour and the acceptability of new products should be also performed.