UNIVERSITAT POLITÈCNICA DE VALÈNCIA
Escola Tècnica Superior d´Enginyeria Agronòmica i del Medi Natural

UNIVERSITAT POLITÈCNICA DE VALÈNCIA

DEVELOPMENT OF THE PHASE DIAGRAM OF COLLAGEN-GEL AND ITS MONITORING SYSTEM TO FOOD 3D PRINTING

TRABAJO FIN DE MÁSTER:
CIENCIA E INGENIERÍA DE LOS ALIMENTOS

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ABSTRACT

Food industry is a fundamental pillar of global economy. Because of its economic importance and high level of competitiveness, it is necessary to innovate in the development of new technologies and products to attract consumer's attention. From the search of new technologies, the idea of 3D printing emerges with the aim to build and cook complex food structures and allow the consumer to obtain a high quality ready-to-eat food. In this work several determinations of different collagen formulations were performed, because it is a product with several applications in food industry. First the cooling curve of collagen gel was obtained; its transitions were determined by differential scanning calorimetry (DSC). Parallel double compression tests (TPA) were performed at different temperatures in order to characterize the viscoelastic properties of each gelation state of the different formulations. Moisture and water activity were measured to each one of the samples. Furthermore, the gelling system required a monitoring system to ensure the elastic gel state, for this purpose the gelling process was studied by dielectric spectroscopy in the radiofrequency and microwaves range, yielding relaxations which characterize each type of gel. Finally, a system to measure the thermal diffusivity was developed in order to model the collagen cooling process. In addition, mechanical properties of different aggregation states determined by electrical conductimetry were obtained by TPA. From all measurements made during this work it has been possible to complete a phase diagram of collagen gel with the mechanical properties of each solid state.

Keywords: collagen gel, phase transitions, 3D printers, dielectric properties

RESUMEN

La industria alimentaria es un pilar fundamental de la economía mundial. Debido a su importancia a nivel económico y a la elevada competencia, es necesario innovar en el desarrollo de nuevas tecnologías y productos para captar la atención del consumidor. De la búsqueda de nuevas tecnologías surge la idea de la impresión 3D con el objetivo de construir y cocinar estructuras alimentarias complejas y de permitir al consumidor la obtención de un alimento listo para su consumo. En este trabajo se realizaron varias determinaciones sobre diferentes formulaciones de colágeno, debido a que es un producto que tiene diversas aplicaciones dentro de la industria alimentaria. En primer lugar se obtuvo la curva de enfriamiento del gel de colágeno, se determinaron sus transiciones por calorimetría diferencial de barrido (DSC). Paralelamente se realizaron ensayos de doble compresión

(TPA) a diferentes temperaturas para así caracterizar las propiedades viscoelásticas de cada estado de gelificación de las distintas formulaciones. A cada una de las muestras se les midió la humedad y la actividad de agua. Además, el sistema de gelificación requirió de un sistema de monitorización para asegurar el estado elástico del gel, para ello el proceso de gelificación se estudió por espectroscopia dieléctrica en el espectro de la radiofrecuencia y microondas, obteniéndose las relajaciones que caracterizan cada tipo de gel. Finalmente se ha desarrollado un sistema de medida de la difusividad térmica capaz de modelizar el proceso de enfriamiento del colágeno. Además, se han obtenido las propiedades mecánicas por TPA de los distintos estados de agregación determinados por conductimetría eléctrica. A partir de todas las medidas realizadas durante este trabajo ha sido posible completar un diagrama de estado del gel de colágeno con las propiedades mecánicas de cada una de los estados sólidos.

Palabras clave: gel de colágeno, transiciones de fase, impresoras 3D, propiedades dieléctricas

RESUM

La indústria alimentària és un pilar fonamental de l'economia mundial. A causa de la seua importància a nivell econòmic i a l'elevada competència, és necessari innovar en el desenvolupament de noves tecnologies i productes per a captar l'atenció del consumidor. De la recerca de noves tecnologies sorgeix la idea de la impressió 3D amb l'objectiu de construir i cuinar estructures alimentàries complexes i de permetre al consumidor l'obtenció d'un aliment llest per al seu consum. En este treball es van realitzar diverses determinacions sobre diferents formulacions de col·lagen, pel fet que és un producte que té diverses aplicacions dins de la indústria alimentària. En primer lloc es va obtindre la corba de refredament del gel de col·lagen, es van determinar les seues transicions per calorimetria diferencial de rastreig (DSC). Paral·lelament es van realitzar assajos de doble compressió (TPA) a diferents temperatures per a així caracteritzar les propietats viscoelàstiques de cada estat de gelificació de les distintes formulacions. A cada una de les mostres se'ls va mesurar la humitat i l'activitat d'aigua. A més, el sistema de gelificació va requerir d'un sistema de monitorització per a permetre assegurar l'estat elàstic del gel, per a això es va analitzar per espectroscòpia dielèctrica en l'espectre de la radiofreqüència i les microones, obtenint-se les relaxacions que caracteritzen cada tipus de gel. Finalment s'ha desenvolupat un sistema de mesura de la difusivitat tèrmica capaç de modelitzar el procés de formació del gel de col·lagen. A més, s'han obtingut les propietats mecàniques per TPA dels distints estats d'agregació determinats per conductimetria elèctrica. A partir de totes les mesures realitzades durant este treball ha sigut possible completar un diagrama d'estat del gel de col·lagen amb les propietats mecàniques de cada una dels estats sòlids.

Paraules clau: gel de col·lagen, transicions de fase, impressores 3D, propietats dielèctriques

INTRODUCTION

Gelatin is a popular biopolymer which is obtained by a controlled degradation of the collagen which is widely present in nature as the major constituent of bones, connective tissue and skin. Collagen gelatin is widely used in food because of its unique functional and technological properties (Karim & Bhat, 2009). The most remarkable characteristics of collagen gelatin are its solubility in water and the ability to form thermoreversible gels (Bell, 1989). The use of gels has significantly increased within the food industry due to its use in food formulation. In recent years new substances have been discovered which have useful functions in foods, among which gums and gels stand out due to their gelling and thickening power in food (Velázquez, 2014). Gelatin is the main gelling agent used in foods. This is because gelatin can form gels over a wide range of concentrations (Fonkwe et al, 2003).

The basic unit of collagen is tropocollagen, which consists of three polypeptide chains with the same size and identical composition or not, depending on the type of collagen (López et al, 2008). The structure of collagen molecule is a triple-helix that is composed of two α1 chains and one α2 chain, each containing repetitions of (Gly-X-Y), where Gly is glycine, and X and Y are usually amino acids such as proline and hydroxyproline (Bhattacharjee & Bansal, 2005; Brodsky & Persikov, 2005). Chemical degradation and thermal or physical denaturation of collagen involve the breaking of the triple helix structure into random coils to give gelatin (Bigi et al., 2004; Haug et al.; 2004).

Collagen gel has a colloidal structure; a colloid is a two-phase system in which one of the phases is dispersed as discrete particles in a second continuous phase. The dispersed particles could be solid, liquid, or gas and the continuous phase could also be a liquid, gas, or solid (Trados, 2013). In collagen gel, the continuous phase is solid (protein phase) and the liquid dispersed phase (aqueous phase) is trapped in the three dimensional network.

When collagen is heated in water the triple helix is broken and it separates into single coils, forming a solution. During gelation, the collagen chains try to regenerate the collagen triple-helix structure but can line up only in sections (Figure 1). The unaligned parts of the coils then align with parts of other coils. The helices interact to form a network, where the strands are held together by hydrogen bonded junction zones and in some cases by chemical linkages. Collagen gels formed are thermoreversible gels because they are held together by weak bonds (Gomez-Guillen et al, 2011; von Endt & Baker, 1991).

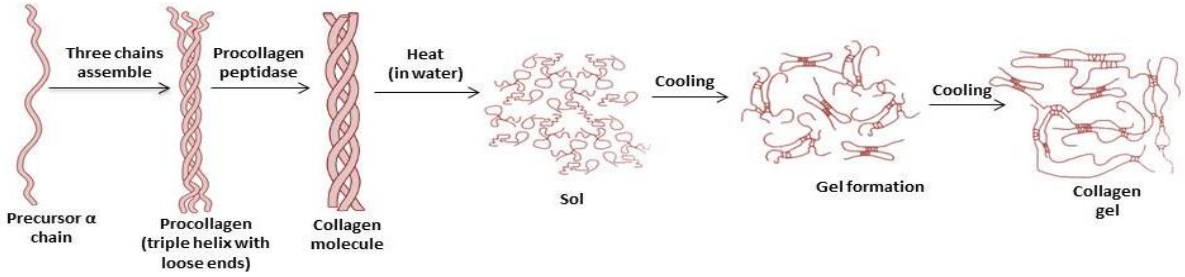

Figure 1. Gelification of collagen gel

Viscoelastic food like these gels can be studied by testing texture. The texture of a product provides information about descriptive parameters related to sensations perceived in the food intake moment such as hardness, elasticity, rigidity, etc. In food industry, to evaluate food texture is very important because it is one of the factors influencing the acceptance of the product by the consumer. One of the most used compression tests in food for characterizing viscoelastic properties is the Texture Profile Analysis (TPA). During this test, a piston compresses twice the sample, simulating the movement of the jaw during chewing. The analysis of force-distance or force-time curves allows obtaining different textural parameters well correlated with sensory evaluation. In addition, they are useful to characterize gel structures and get a glimpse of the microstructural interactions (Duran et al., 2001; Chiralt et al., 2007).

The knowledge of collagen thermal behavior is of great importance, and can be achieved by using some physical-chemistry techniques, especially thermal analysis methods (Budrugeac & Cucos, 2013; Liu & Li, 2010). It is therefore necessary to study phase transitions in order to obtain the phase diagram of collagen gel. Phase transitions in food consist in conformational changes, leading to important variations in the physical properties of the system due to changes in molecular mobility associated with the transition. These changes significantly affect to the quality and stability of food and can be decisive in the processing conditions (Martinez-Navarrete et al., 1998). One of the most used tools to study phase transitions is differential scanning calorimetry (DSC). It is one of the best techniques to analyze the collagen denaturation, by using the estimation of amount of transition energy.

In order to understand the transformation phenomena occurred in collagen gels, other innovative technological techniques are required. In this sense, the dielectric spectroscopy constitutes an ideal non-destructive measurement tool to provide fast and accurate information in real time (Castro-Giraldez et al., 2010; Traffano-Schiffo et al., 2015; Velázquez, 2014).

Dielectric spectroscopy determines the dielectric properties of a medium as a function of frequency. It is based on the interaction of an electric external field with the sample; complex permittivity (ε_r) is the dielectric property that describes this interaction (Metaxas & Meredith, 1993). The real part of complex permittivity is called dielectric constant (ε') and the imaginary part is called loss factor (ε''). The dielectric constant is related with the ability of the food to store energy, and the dielectric loss factor is related to the dissipation of the electric field energy in other kinds of energy such as the

thermal one. Several studies reveal that changes in physical state of foods affect to the dielectric properties (Castro-Giráldez et al., 2012). In radiofrequency (RF) and microwaves (MW) ranges, it is possible to distinguish different dispersions along the electromagnetic spectra, being α, β, and γ the most relevant (Schwan, 1957). The alpha dispersion (α), also called counterion, is induced by the orientation of mobile charges in the dielectric medium. The β dispersion covers a broad spectrum range from kHz to MHz and describes all interactions with fixed charges or with low mobility found in the dielectric medium. This dispersion was subdivided in two sections, the interactions in the kHz range, in which the interactions are included the charges belonging to structural macromolecules that comprise the solid phase of the system, such as proteins. In the part of higher energy, MHz, interactions include charges associated to the surface tension of the solid surface in contact with the medium fluid; this phenomenon is called Maxwell-Wagner. The interaction of the electric field with foods in the range of microwaves produces the so-called γ-dispersion which is closely related with the water content and its motion (Castro-Giráldez et al., 2010). Thus, phase transitions probably can cause changes in dielectric properties at γ-dispersion.

Three dimensional printing (3D) food, also known as Layered Food Manufacture (Wegrzyn et al., 2012), is an automated and digitally controlled process that can build layer by layer 3D foodstuffs (Huang et al., 2013). Thanks to this technology, food can be designed and prepared by controlling the amount of material to be printed in 3D.

The aim of this work is to develop collagen gel transitions diagrams and to adapt them to the 3D printing extrusion system. To achieve this aim, it is necessary to obtain the kinetics of collagen gelation (cooling curves), to determine the phase transitions that collagen gel suffers by using dielectric, textural and calorimetry measurements and to obtain the phase diagram of gel within the rheological properties (first and second order transitions).

MATERIALS AND METHODS

Raw material

All the measurements were carried out on collagen gels. The samples were made up with a commercial collagen from porcine 100% (Edible gelatin, type A, 240°Bloom, from Tradissimo®, Spain), and bidistilled water.

Sample preparation and work plan

Samples were prepared with different collagen contents ranged from 4 to 42 % ratio of protein/water. First, the collagen lamina was cut and weighed on a precision balance, then the required quantities of collagen were mixed with bidistilled water; a homogeneous solution was formed by heating the dispersion at 90°C under agitation using a magnetic stirrer.

Once the homogeneous solutions were formed, several determinations were made during gel formation. First the homogeneous solutions were placed in a silicone container in which thermocouples and conductimeter were coupled to obtain the cooling curve during formation collagen gel. After obtaining the cooling curves, moisture and water activity were obtained for each gel.

Furthermore, thermal transitions of collagen gel were determined by differential scanning calorimetry, first the collagen solution was heated to 90ºC, after reaching this temperature a small amount (15 mg) was poured into an aluminum crucible and was applied in a sweep of cooling followed by a sweep of heating. At the same time, hot collagen solutions were poured into ice buckets and the temperature was measured with a thermocouple in order to perform the double compression test (TPA) at different temperatures and then characterize the viscoelastic properties of each gelation state.

Dielectric properties were also measured during gel formation in the radiofrequency (RF) and microwave (MW) range to characterize the phase transitions of the collagen gels. In addition, the pH was measured and in parallel the volume of each sample was determined by image analysis (in order to obtain the density of each collagen gel), both determinations were made during cooling gel formation.

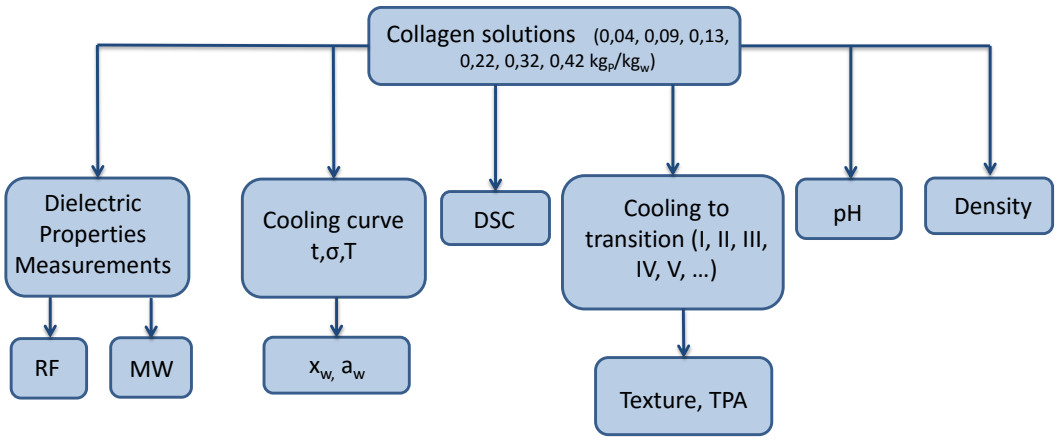

Figure 2. Diagram of the experimental procedure

Physical-chemical analysis

Surface water activity was measured in the structured samples with a dew point hygrometer Aqualab® series 3 TE (Decagon Devices Inc., Washington, USA) with a precision ± 0,003. Moisture content was determined by drying in a vacuum oven at 100ºC till constant weight was reached (AOAC method 935.46, 1997). pH was measured using a pH-meter (Mettler Toledo®) seven easy, with puncture electrode Inlab ® Solids Pro. Mass measurements were made using a precision analytical balance (±0,001) Mettler Toledo, AB304-S.

Volume determination was made using an image analysis technique using the Adobe Photoshop CS5 software (Adobe Systems Inc., San Jose, CA,

USA). Finally, from the ratio between mass and volume, density was obtained.

Dielectric Properties Measurements

The system used to measure the dielectric properties in the RF range consists on a sensor manufactured in collaboration by the Institute of Food Engineering for Development (IuIAD, UPV) and the Institute of Instrumentation for Molecular Imaging (I3M, UPV) connected to an impedance analyzer Agilent 4294A. The frequency range was from 40 Hz to 1 MHz. In the MW range, the equipment used consists on an Agilent 85070E open-ended coaxial probe connected to an Agilent E8362B Vector Network Analyser. All determinations were registered from 500 MHz to 20 GHz.

Test cooling curve

This technique was based in the measures associated with conductivity changes, by using type K thermocouples and conductivity meter connected to a data acquisition system Agilent 34972A (Figure 3) from which the electrical conductivity at 40 Hz and temperature during collagen gelation was measured.

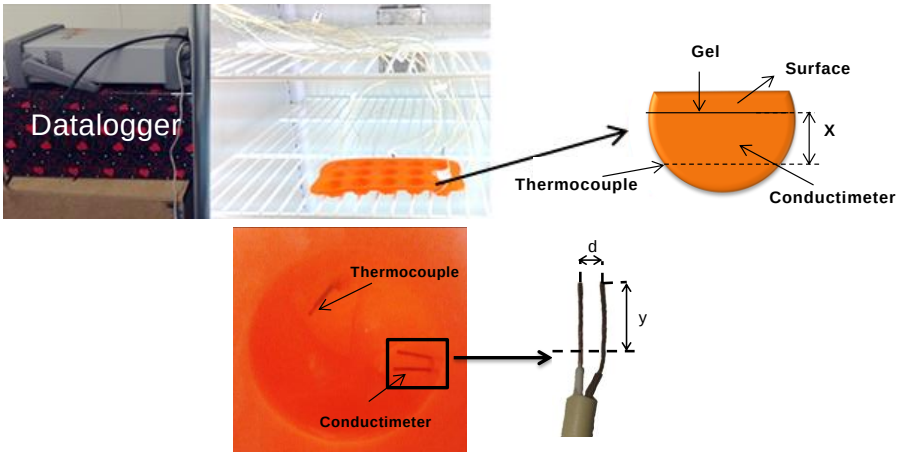

Figure 3. Assembly for test cooling curve.

Texture profile analysis (TPA)

TPA was measured using the Texture analyzer (TA.XT Plus). The homogeneous solution formed by heating with different collagen contents was poured into ice buckets until gel formation. The temperature was measured with a thermocouple in order to perform the compression test at different temperatures and then characterize the viscoelastic properties of each gelation state. The gels were compressed by an aluminum probe (75 mm diameter plate) until the deformation reached 30% at a speed of 0.5 mm/s. The pause between the first and second compressions was 5 s. Four measurements were performed for each formulation of collagen. From the

force-time curve of the texture profile, textural parameters including hardness, springiness, cohesiveness, gumminess and chewiness were obtained.

Differential Scanning Calorimetry (DSC)

Collagen transition temperature was determined by differential scanning calorimetry (DSC) using a Mettler Toledo DSC equipment 1 (Mettler Toledo, Spain). Once the solutions were formed, they were weighed in aluminum crucibles and were sealed hermetically. An empty crucible was used as reference. First, a cooling sweep was performed from 60 to 4 °C and then a heating sweep was performed from 4 to 90 °C, both at a rate of 3°C/min. From the curves obtained and the mass of the samples, specific heat (C_p) was calculated.

Statistical analysis

Statistical analysis was carried out with the Statgraphics Centurion XVI Software (Statgraphics, Virgina, U.S.A.). Statistical analysis was a non-linear regression by using three equations of Gompertz.

RESULTS AND DISCUSSION

Cooling curves for obtaining collagen gel phase transitions

Protein gels show multiple aggregation states associated with their structural order. The different structural levels depend on the system state variables and their kinetic transitions. In this paper the determination of the collagen gel transitions and their characterization are studied by combining different thermodynamic properties.

First, an assembly was designed to determine the cooling curve and the variation of electrical conductivity of each of the formulations described in the Materials and Methods section.

Figure 4 shows the evolution of the electrical conductivity to 40 Hz as a function of temperature during the cooling process performed (80°C to 4°C) for protein mass ratio of 0,09 kg_P/kg_w. In the figure, three curves are shown that correspond with the three replicates for that formulation. Also, a detail of the curve is shown in which it is possible to appreciate that different transitions are produced at different temperatures (each transition is marked by arrows).The changes in the ordering of proteins (continuous phase) cause changes in electrical conductivity in a conductive medium such as collagen (Steinhardt and Reynolds, 1969).

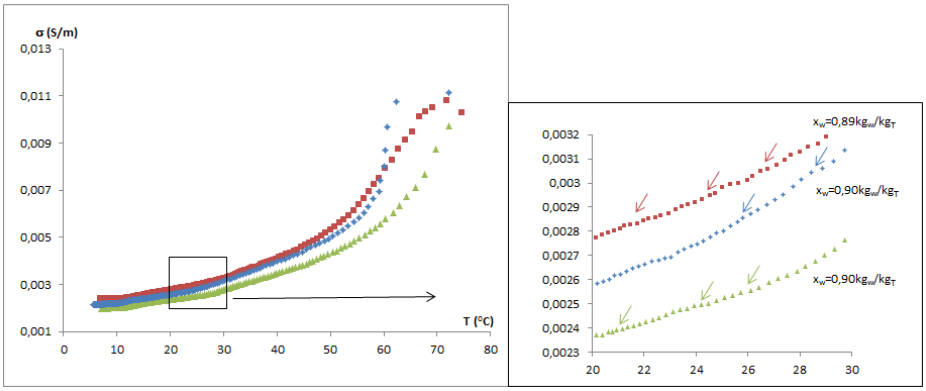

Figure 4. Cooling curve obtained for the formulation 0,09 kg_P/kg_w.

The cooling curves are the first step to perform the phase diagram of collagen gel.

The thermal diffusivity is a parameter arising from development of the second Fourier equation, from the decomposition of the internal energy, and which includes the physical properties: thermal conductivity, density and specific heat (equation 1). This parameter provides the parameterization of thermal processes.

$$\alpha = \frac{k}{\rho \cdot C_p} \qquad \text{(Equation 1)}$$

where: k is the thermal conductivity (W/m K)

C_p is the specific heat (kJ/kg K)

ρ is the density (kg/m^3)

To calculate the thermal diffusivity, the montage shown in the Materials and Methods was used (Figure 3). In that figure, it is possible to observe that the sample had all faces isolated except the top, thus heat transport was considered only by this face, being the geometry an infinite sheet having a thickness equal to the bottom of the receptacle and a distance (x) to the thermocouple described in the scheme. The Fourier development model chosen was the simplification of Crank (1975) for flat geometries with rapid convergence (Equation 2). It has been considered that there is no external resistance to heat transfer.

$$1 - \left(\frac{T_{ext} - T_t}{T_{ext} - T_0}\right) = 2\left(\frac{\alpha t}{\pi x^2}\right)^{1/2} \qquad \text{(Equation 2)}$$

Where: T is the temperature (°C), α is the thermal diffusivity (m^2/s) t is the time (s), x is the distance from the sample surface to the thermocouple (m), and the subscript "ext" refers to environment, "t" is the time at which each temperature was measured and "0" is the initial time.

In Figure 4 the variation of thermal diffusivity with regard to temperature is shown, during the cooling process, for each of the protein/water ratios studied.

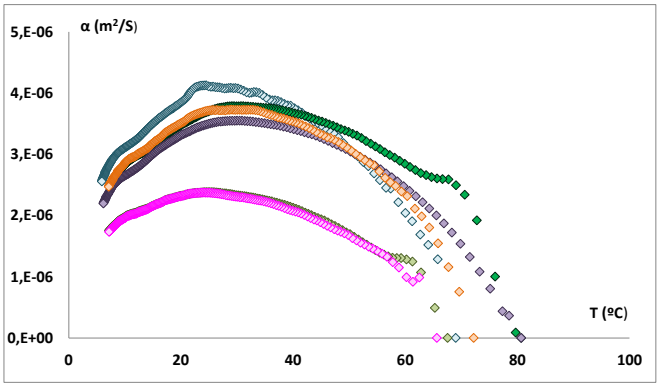

Figura 4. Variation of thermal diffusivity with regard to the temperature, where: ♦ 0,04 kg_P/kg_w, ♦ 0,09 kg_P/kg_w, ♦ 0,13 kg_P/kg_w, ♦ 0,22 kg_P/kg_w, ♦ 0,32 kg_P/kg_w, ♦ 0,42 kg_P/kg_w.

As Figure 4 shows, the thermal diffusivity increases during the cooling process reaching a maximum from which decreases, this maximum changes the behavior of the system with regard to the thermal energy storage, indicating a conformational change of the structure, this value may mean the transition from liquid to solid.

If the value of the gel temperature at maximum diffusivity of each of the formulations is taken, the second step for developing the phase transition diagram of the collagen gel is obtained (Figure 5). In this figure, the temperature value corresponding to the maximum gel diffusivity at each formulation was integrated, where it is possible to observe that the value (max alpha) coincides with the transition IV, transition which showed a sharp change in the electrical conductivity.

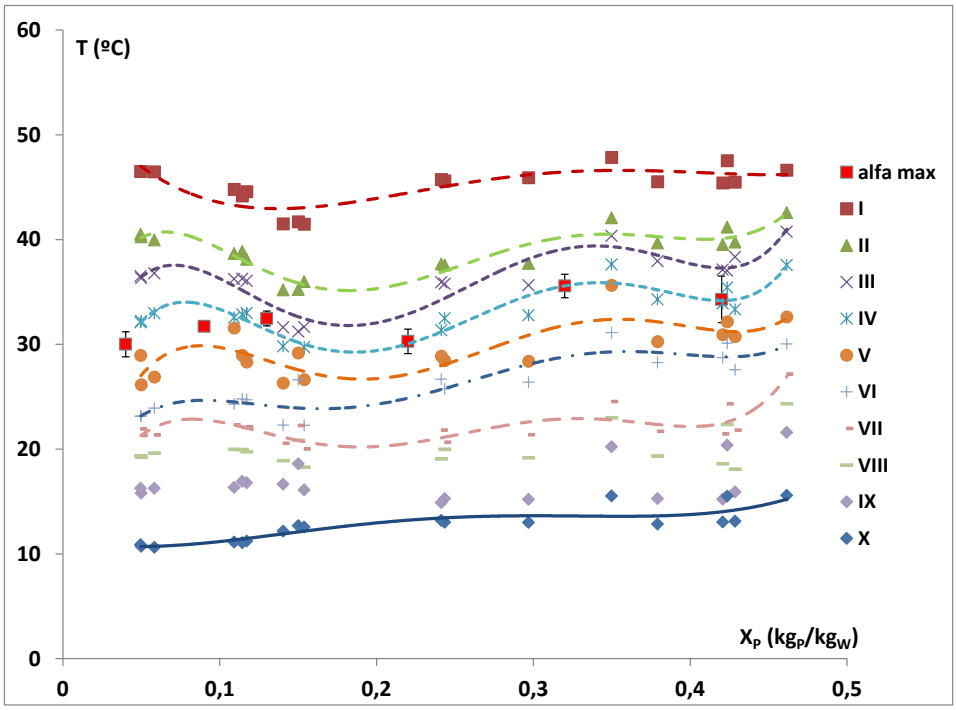

Figure 5. Collagen gel phase transition diagram

Figure 6 shows the evolution of the thermal diffusivity with regard to protein content at different temperatures. In the figure it is possible to observe that the formulations with higher or lower protein content accumulate better the heat than the formulations with intermediate protein content.

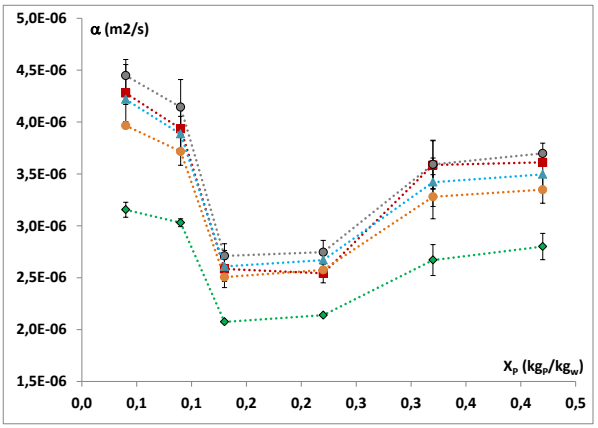

Figure 6. Variation of the thermal diffusivity respect to the protein during gel formation, where: ♦ 12 ºC, ● 21ºC, ▲ 24ºC, ● 30ºC, ■ 42ºC.

In order to explain the physical sense of thermal diffusivity behavior, it is necessary to analyze the physical properties that compose it.

Phase transitions obtained by differential scanning calorimetry; Thermal properties.

As was mentioned in the introduction, one of the most used tools to study phase transitions of collagen is the differential scanning calorimetry. In this research work the different formulations of water and collagen were prepared by heating the solution to 80°C. After sample preparation, a cooling scanning from 60ºC to 4°C was made. After that, a heating scanning from 4 to 90°C, at 3°C/min was performed.

Table 1 shows the values obtained from calorimetry assays, both cooling and heating, where a single transition appears in each scanning. To properly explain calorimetry data, Figure 7 shows the evolution of the phase transition energy of each of the scanning with regard to protein concentration. This figure shows that the transitions are a function of gel protein content, being the liquid-solid transformation (first order transition) the main transition, able to cover up the remaining second order transitions.

Table 1. Data obtained from the cooling calorimetry (TRANS. 1) and heating (TRANS. 2), where the energy of state change (ΔG^0) and the point where it starts (T1) is displayed and transition end (T2) for each of the formulations.

	T_1 (ºC)		T_2 (ºC)		ΔG^0 (mJ/mg)	
X_P (P/W)	TRANS. 1	TRANS. 2	TRANS. 1	TRANS. 2	TRANS. 1	TRANS. 2
0,04	22,1 ± 0,4	23,19 ± 0,14	10,9 ± 0,13	31,73 ± 0,16	0,2 ± 0,113	-0,150 ± 0,014
0,09	25,7 ± 0,6	25 ± 3	14 ± 6	32,5 ± 0,3	1,04 ± 0,03	-0,63 ± 0,06
0,13	26,6 ± 0,4	24 ± 4	9,7 ± 1,7	35 ± 4	1,52 ± 0,03	-1,0 ± 0,3
0,22	28,3 ± 0,3	18,8 ± 0,7	16 ± 7	34,3 ± 0,3	2,1 ± 0,4	-3,0 ± 0,5
0,32	29,5 ± 0,5	18,9 ± 0,6	10,5 ± 0,5	35,0 ± 0,2	3,21 ± 0,05	-3,8 ± 0,4
0,42	30,59 ± 0,18	18,62 ± 0,15	10,9 ± 0,2	35,3 ± 0,8	3,92 ± 0,08	-4,7 ± 0,7

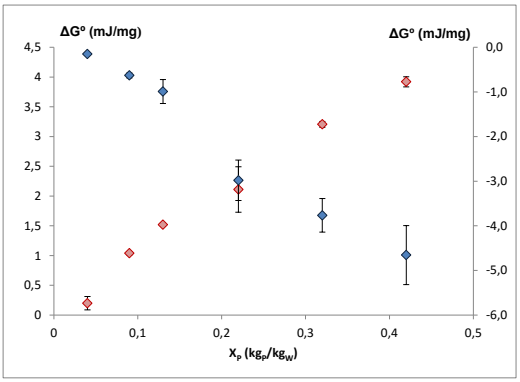

Figure 7. Variation of the phase transition energy with regard to gel collagen formulation, where: ♦ TRANS. 1 (main shaft) y ♦ TRANS. 2 (countershaft).

Figure 8.A shows the variation of specific heat with regard to the protein content of the gels at different temperatures within the transitions range exposed above. This figure shows how the lowest specific heats, and therefore with less energy by temperature variation, are gels at high temperatures (42 to 30°C) while the topmost are in intermediate temperature

(21 to 24ºC), being the lowest temperatures, i.e. the most stable gels, at intermediate values. The behavior of the specific heat is different from the thermal diffusivity, so it is not possible to describe the behavior of the thermal diffusivity with the evolution of the heat capacity of the medium.

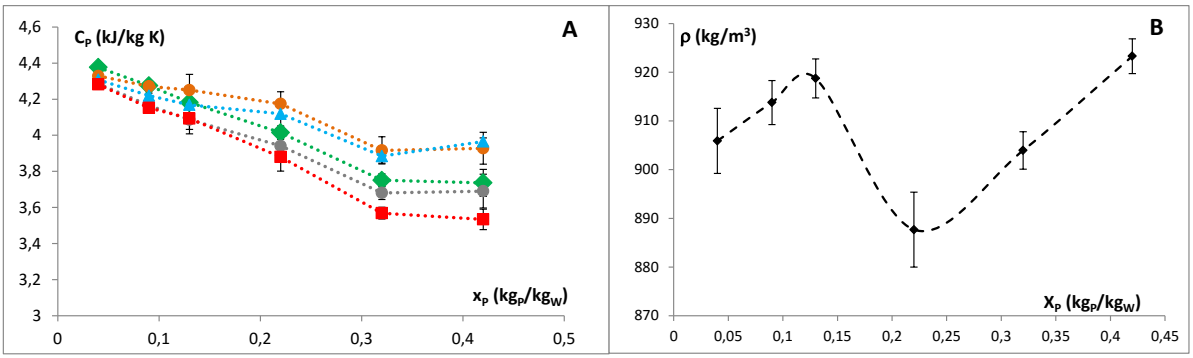

Figure 8. A. Variation of specific heat with respect to the protein, where: ♦ 12 ºC, ● 21ºC, ▲ 24ºC, ● 30ºC, ■ 42ºC. **B.** Variation of the average density of collagen gel in solid state with regard to the mass ratio of protein.

Figure 8.B shows the variation of the average density of collagen at temperatures between 18°C and 4ºC at intervals of 2°C, where it can be observed a uneven behavior between different formulations, being the lowest that of 22% of protein/water.

From the value of the diffusivity (Figure 6), specific heat (Figure 8.A) and density (Figure 8.B), it is possible to calculate the thermal conductivity using equation 1. Figure 9 shows the variation of the thermal conductivity with regard to the formulation of the gels at different temperatures associated with phase transitions.

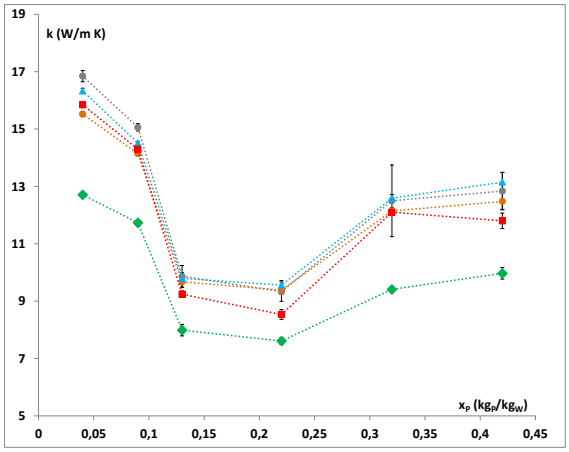

Figure 9. Variation of the thermal conductivity during gel formation with regard to the mass ratio of protein, where: ♦ 12ºC, ● 21ºC, ▲ 24ºC, ● 30ºC, ■ 42ºC.

Thermal conductivity shows the lowest values at intermediate concentrations (13 and 22% protein), and the highest values at low and high

concentrations. At the same time, the lower conductivity occurs in the final gel transition at 12°C (X transition temperature or last transition), while the behavior at higher temperatures is uneven, being higher at 30°C (L-G or IV transition) and 24ºC (VI transition) and lower at 21ºC (VII transition) and 42ºC (non-Newtonian fluid).

MECHANICAL PROPERTIES

During the gelation process, it was observed that the transition IV (as the variation of thermal diffusivity showed) represents the change from liquid to gel. However, such transition can not be measured by TPA test because the rheological properties of the medium did not let the sample removal from the ice bucket.

TPA assays showed high repetitiveness and therefore low deviations. Table 2 shows the hardness texture parameter with regard to the mass fraction of protein for each transition described in Figure 5.

Table 2. Hardness texture parameter (N) with respect to the mass fraction of protein for each transition described in Figure 5, where "L" refers to liquid, "G" refers to solid gel state.

x_p (kg_p/kg_T)	0,038	0,083	0,115	0,180	0,242	0,296
0-I	L	L	L	L	L	L
I-II	L	L	L	L	L	L
II-III	L	L	L	L	L	L
III-IV	L	L	L	L	L	L
IV-V	G	G	G	G	G	G
V-VI	G	G	G	1,11 ± 0,13	0,9 ± 0,1	0,774 ± 0,096
VI-VII	0,46 ± 0,04	0,59 ± 0,04	1,27 ± 0,17	4,4 ± 0,5	25,4 ± 0,2	21,0 ± 0,3
VII-VIII	0,87 ± 0,04	1,40 ± 0,11	1,74 ± 0,05	12,6 ± 0,6	30,95 ± 0,21	26,3 ± 1,2
VIII-IX	1,46 ± 0,05	2,91 ± 0,18	7,8 ± 0,5	28,93 ± 1,11	43,5 ± 1,7	36,6 ± 0,2
IX-X	2,73 ± 0,05	9,25 ± 0,20	15,4 ± 0,7	35,90 ± 1,96	53,5 ± 0,3	76,4 ± 2,6
X-G	3,09 ± 0,23	10,47 ± 0,20	17,5 ± 0,8	35,85 ± 1,06	54,8 ± 0,8	78,6 ± 2,1

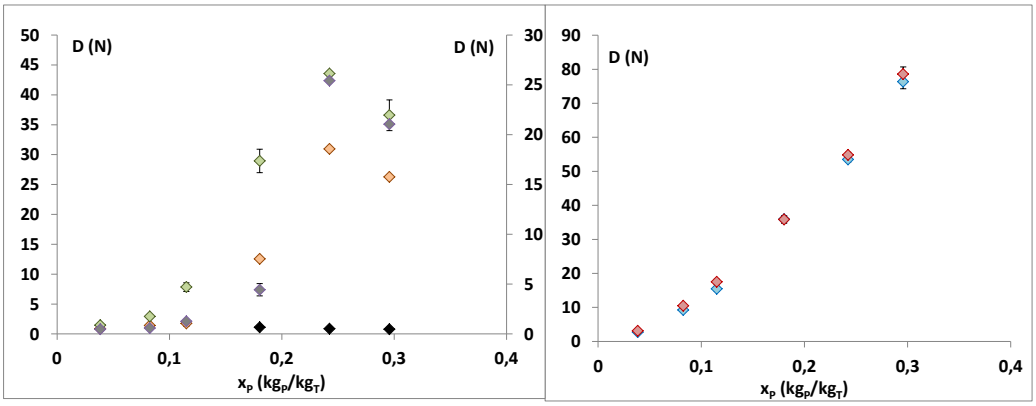

Figure 10. Hardness texture parameter (N) with regard to the mass fraction of protein, where: ♦ transition V-VI, ♦ transition VI-VII (secondary axis), ♦ transition VII-VIII, ♦ transition VIII-IX, ♦ transition IX-X, ♦ transition X-G.

Table 3. Adhesiveness texture parameter (g·s) with respect to the mass fraction of protein for each transition described in Figure 5, where "L" refers to liquid, "G" refers to solid gel state.

x_P (kg_P/kg_T)	0,038	0,083	0,115	0,180	0,242	0,296
0-I	L	L	L	L	L	L
I-II	L	L	L	L	L	L
II-III	L	L	L	L	L	L
III-IV	L	L	L	L	L	L
IV-V	G	G	G	G	G	G
V-VI	GEL	GEL	GEL	-1,1 ± 0,3	-1,1 ± 0,6	-0,84 ± 0,12
VI-VII	-0,005 ± 0,005	-0,23 ± 0,14	-0,46 ± 0,05	-1,83 ± 0,65	-1,20 ± 0,88	-2,20 ± 0,08
VII-VIII	-0,38 ± 0,18	-0,51 ± 0,30	-0,86 ± 0,17	-0,85 ± 0,64	-2,63 ± 0,17	-2,14 ± 0,96
VIII-IX	-0,89 ± 0,02	-1,70 ± 0,32	-2,48 ± 0,42	-1,79 ± 1,11	-1,09 ± 1,47	-2,49 ± 0,15
IX-X	-0,53 ± 0,13	-1,52 ± 0,40	-1,20 ± 1,09	-1,85 ± 0,99	-0,71 ± 0,89	-2,54 ± 0,18
X-G	-1,72 ± 0,63	-3,05 ± 0,54	-2,88 ± 0,32	-1,95 ± 1,68	-2,94 ± 0,50	-2,54 ± 0,20

Table 4. Elasticity texture parameter (s/s) with respect to the mass fraction of protein for each transition described in Figure 5, where "L" refers to liquid, "G" refers to solid gel state.

x_P (kg_P/kg_T)	0,038	0,083	0,115	0,180	0,242	0,296
0-I	L	L	L	L	L	L
I-II	L	L	L	L	L	L
II-III	L	L	L	L	L	L
III-IV	L	L	L	L	L	L
IV-V	G	G	G	G	G	G
V-VI	GEL	GEL	GEL	0,83 ± 0,03	0,76 ± 0,03	0,75 ± 0,01
VI-VII	0,91 ± 0,03	0,86 ± 0,04	0,91 ± 0,02	0,89 ± 0,03	0,96 ± 0,01	0,95 ± 0,01
VII-VIII	0,87 ± 0,03	0,92 ± 0,04	0,88 ± 0,03	0,91 ± 0,04	0,90 ± 0,01	0,92 ± 0,03
VIII-IX	0,85 ± 0,02	0,88 ± 0,02	0,88 ± 0,04	0,95 ± 0,07	0,96 ± 0,03	0,912 ± 0,012
IX-X	0,95 ± 0,03	0,95 ± 0,03	0,95 ± 0,03	0,93 ± 0,06	0,95 ± 0,02	0,92 ± 0,01
X-G	0,89 ± 0,03	0,83 ± 0,05	0,85 ± 0,03	0,91 ± 0,06	0,86 ± 0,06	0,93 ± 0,02

As was aforementioned, the main objective of this research is the adaptation of collagen transitions to 3D printing. Due to this, 3 parameters have been selected in order to characterize the collagen behavior during the deposition and cooling processes. The first parameter chosen was the hardness (Table 2) because of the need of low hardness during the deposition process but a high hardness in the finished product. The second parameter studied was the adhesiveness (Table 3) because the 3D printing process requires deposition of layers that requires a certain ability of the gel to meld in order to achieve the desired geometry. Finally, the elasticity (Table 4) was selected for many reasons, first, the needed of an elastic medium in deposition and second the needed of certain elasticity in the deposited medium when the 3D printing required the combination of different materials.

Hardness texture parameter was represented with regard to the protein content (Figure 10). In the figure, it was possible to observe that in the IX-X and X-G transitions the gel shows higher hardness, which increases with the amount of collagen. With regard to the adhesiveness variation (Table 3), the

cooling transitions (VIII-IX, IX-X y X-G) have higher values, growing with regard to the proportion of protein in the gels VIII-IX and IX-X, and being constant in the X-G state. Regarding the variation of the elasticity (Table 4), the transition IX-X showed the highest elasticity, however the X-G state suffers a sharp decline conferring rigidity to the gel making it unsuitable for deposition but optimum for the final state of the printed product.

Figure 11 shows the different transitions of collagen during gel formation with the most important mechanical properties to characterize the deposition of the gel in a 3D printing.

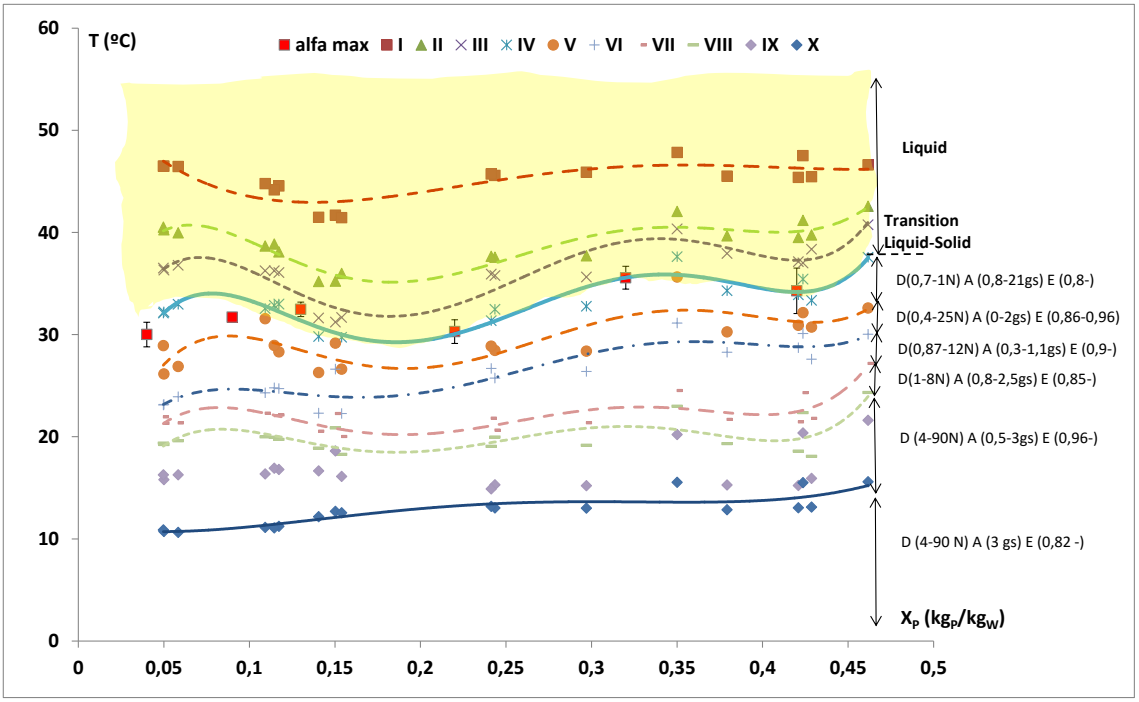

Figure 11. Collagen gel phase transition diagram. Where, D represents the hardness, E elasticity and A adhesiveness.

Gompertz model

With the aim of obtaining the physic parameters which describe the three relaxations phenomena involved in RF and MW dispersions (frequency and dielectric constant of relaxation), a modified Gompertz model with a transformed quadratic in the denominator, coupling three models of this type, one for each relaxation was used (Eq. 3):

$$l\varepsilon'(\omega) = l\varepsilon'_{\infty} + \sum_{n=1}^{3} \frac{\Delta l\,\varepsilon'_n}{1+e^{\left(\left(l\omega^2 - l\omega_n^2\right)*\alpha_n\right)}} \qquad \text{(Equation 3)}$$

Where: lε"represents the decimal logarithm of the dielectric constant, lω represents the decimal logarithm of the angular velocity (obtained from the frequency), Δ represents the amplitude of the dispersion and α are the three

dispersions slopes. The subscripts ∞ and n represent the minimum value of the dielectric constant and the specific relaxation (α, β or γ), respectively.

Figure 12 shows the proposed model and adjustment to the experimental data, also relaxation parameters are represented.

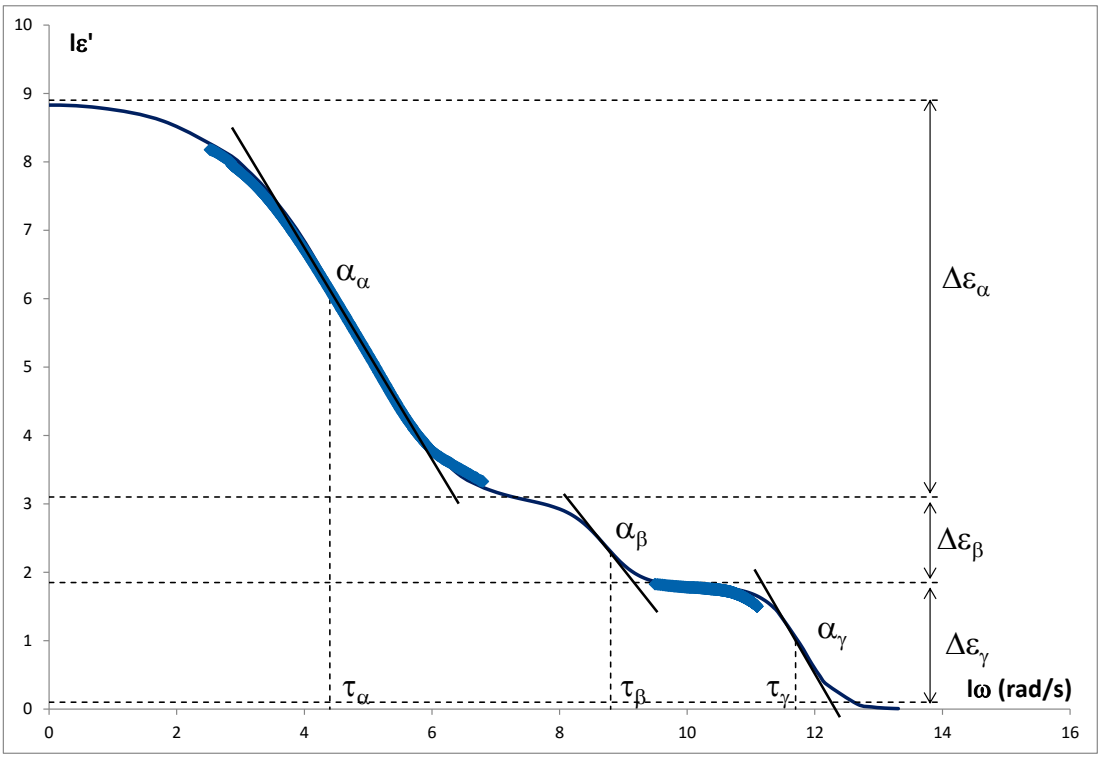

Figure 12. Representation of the dielectric constant with regard to angular frequency, for the formulation 0,13 kg_P/kg_w at the temperature of 65ºC. Where: (—) are the values of mathematical model and (♦) experimental data.

A multiple regression analysis was made in order to determine the protein concentration in a collagen gel using the relaxation parameters obtained by Gompertz adjustment. In Table 5, it is possible to observe the statistically significance of those parameters. The relaxation frequency in the α dispersion and the temperature are both non-significant, while the relaxation frequencies in the β and γ, and also the dielectric constants at the relaxation frequencies in β and γ dispersions are statistically highly significant ($p<0.001$); the dielectric constant at the relaxation frequency in α dispersion is statistically significant ($p<0.05$). Figure 13 shows the predicted protein content versus the observed, where it is possible to observe the good prediction obtained with the dielectric properties independently of the temperature. Thus, dielectric measurements can be a useful monitoring method to determine the protein content of the collagen gel in a 3D printer in order to ensure that the required collagen gel qualities are obtained.

Table 5. Multiple regression analysis to determine the protein concentration by using dielectric properties

Parameter	Estimate	Error	Statistic	p-value
CONSTANT	37,535	3,305	11,358	0,0000
f_β	0,000	0,000	7,173	0,0000
ε'_α	0,000	0,000	-3,251	0,0015
ε'_β	0,045	0,004	10,123	0,0000
ε'_γ	-2,277	0,211	-10,793	0,0000
f_α	0,001	0,001	1,069	0,2871
f_γ	0,000	0,000	-7,239	0,0000
T	0,049	0,044	1,101	0,2727

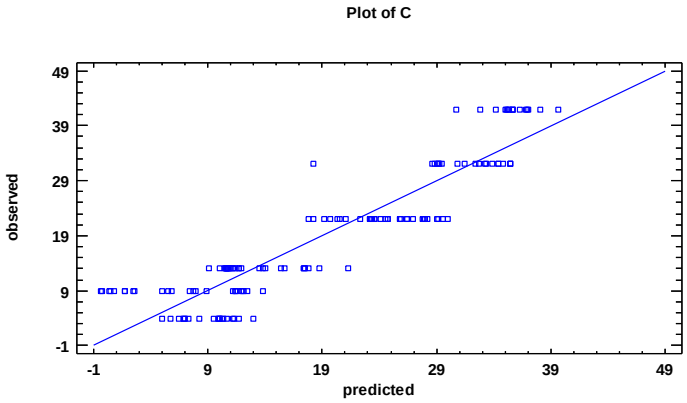

Figure 13. Predicted protein content of the collagen gel versus the real content of protein by using dielectric properties.

Model transitions and heat transfer; 3D print adjustment

Figure 14 shows a scheme for printing with the selection of the most suitable states of the gel for printing and also the thermal properties necessary to calculate the height of deposition.

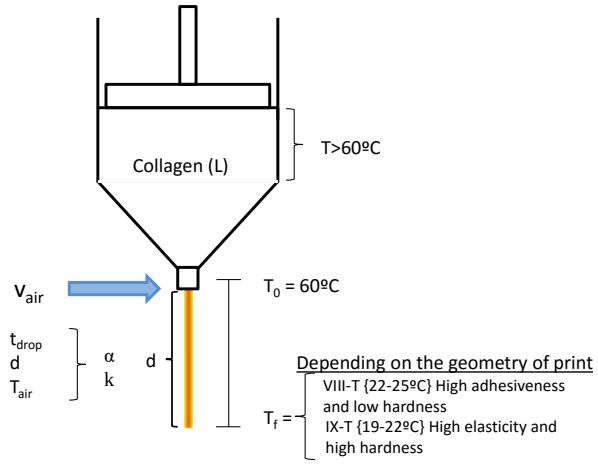

Figure 14. Scheme of the operation of the extruder and the effect of the parameters studied in this research work.

CONCLUSIONS

It has been developed a system for obtaining electrical conductivities in cooling processes of liquid collagen in order to determine the changes in electrical behavior during the formation of collagen gel.

It has developed a system for measuring the thermal diffusivity from a Fourier model capable of modeling the cooling process of collagen. The evolution of this parameter was also obtained, determining a first order transition for each of the formulations.

The physical properties necessary to obtain the thermal conductivity of the gel during the cooling process (density and specific heat) have been obtained.

The mechanical properties of the different states of aggregation determined by electrical conductimetry were obtained by TPA, determining as the most important for 3D printing: hardness, adhesiveness and elasticity.

From all the measurements made during this work, it has been possible to complete a phase diagram of the collagen gel with the mechanical properties of each of the solid states.

By analyzing all the thermal and mechanical measurements, the aggregation state VIII and in some specific geometries the IX were defined as optimal at the point of deposition of the gel.

Dielectric measurements can be a useful monitoring method to determine the protein content of the collagen gel in a 3D printer in order to ensure that the required collagen gel qualities are obtained.

REFERENCES

Bell, A.E. 1989. Gel structure and food biopolymers. In T.M.Hardman, *Water and food quality* (pp. 251-275). London: Elsevier Applied Science.

Bhattacharjee A. & Bansal M. 2005. Collagen Structure: The Madras Triple Helix and the Current Scenario. *Taylor & Francis, Life,* 57(3): 161 – 172.

Bigi A.; Panzavolta S. ;Rubini K. 2004. Relationship between triple-helix content and mechanical properties of gelatin films. Biomaterials 25, 5675–5680.

Brodsky B. & Persikov A. 2005. Molecular structure of the collagen triple helix, Adv. Protein Chem. 70, 301–339.

Budrugeac, P., & Cucos, A. 2013. Application of Kissinger, isoconversional and multivariate non-linear regression methods for evaluation of the mechanism and kinetic parameters of phase transitions of type I collagen. Thermochimica Acta, 565, 241-252.

Castro-Giráldez, M., Fito, P. J., Prieto, J.M., Andrés, A., & Fito, P. (2012). Study of the puffing process of amaranth seeds by dielectric spectroscopy. Journal of Food Engineering, 110, 298–304.

Castro-Giráldez, M., Fito, P.J., Toldrá, F., Fito, P., 2010. Physical sensors for quality control during processing. In: Toldrá, F. (Ed.), Handbook of Meat Processing. Wiley-Blackwell Publishing, U.S.A, pp. 443–456.

Chiralt, A.; Martínez, N.; González, C.; Talens, P.; Moraga, G. 2007. Propiedades físicas de los alimentos. Editorial Universitat Politècnica de València.

Durán, L., FIszman, S.M., & Benedito, C. 2001. Propiedades mecánicas empíricas. En: Métodos para medir Propiedades Físicas en Industrias de Alimentos. Alvarado, J.D. y Aguilera, J.M. (Eds.). Acribia, S.A. Zaragoza (España). 147-187.

Fonkwe, L.G., Ganesan, N., & Cha, A.S. 2003. Characterization of gelation time and texture of gelatin and gelatin–polysaccharide mixed gels. *Food Hydrocolloids*, 17(6), 871–883.

Gomez-Guillen, M. C., Gimenez, B., Lopez-Caballero, M. E., & Montero, M. P. (2011). Functional and bioactive properties of collagen and gelatin from alternative sources. Food Hydrocolloids, 25, 1813-1827.

Haug, I.; Kurt I & Smidsrød O. 2004. Physical and rheological properties of fish gelatin compared to mammalian gelatin. *Food hydrocolloids* 18.2, 203-213.

Karim, A. & Bhat R. 2009. Fish gelatin: properties, challenges, and prospects as an alternative to mammalian gelatins*. Food Hydrocolloids* 23, 563–576.

Kuang, W., Nelson, S. O. (1998). Low-frequency dielectric properties of biological tissues: a review with some new insights.Transactions of the ASAE, 41(1), 173-184.

Liu, W. & Li, G. 2010 Non-isothermal kinetic analysis of the thermal denaturation of type I collagen in solution using isoconversional and multivariate non-linear regression methods Polym. Degrad. Stab. 95, pp. 2233–2240.

López, M.C., Amaral, R., & Kalil, S. 2008. Proteolisis enzimática del colágeno dentinario. *Consicientiae Saúde*., 7(4): 477-486.

Martínez-Navarrete, N., Andrés-Grau, A.M., Chiralt-Boix, A.; Fito-Maupoey, P. 1998. Termodinámica y Cinética de Sistemas Alimento entorno. Editorial: Servicio de publicaciones Universitat Politècnica de València, España.

Metaxas, A. & Meredith, R. 1993. Industrial microwave heating. IEE power engineering series 4. London, UK: Peter Peregrinus LTD.

Schwan, H. P. (1957). Electrical properties of tissues and cell suspensions. Advances in biological and medical physics, 5, 147−209.

Steinhardt, J., Reynolds, J.A. (1969). The measurement of complex formation. En “Multiple Equilibria in Proteins”. Academic Press, Inc, Nueva York

Trados, T. 2013. Encyclopedia of Colloid and Interface Science. Springer Heidelberg New York Dordrecht London.

Traffano-Schiffo, M. V.; Castro-Giraldez, M.; Colom, R. J., & Fito, P. J. (2015). Study of the application of dielectric spectroscopy to predict the water activity of meat during drying process. Journal of Food Engineering, 166, 285-290.

Velázquez, J. 2014. Estudio de los espectros dieléctricos en alimentos con estructura coloidal. Tesis doctoral, Universitat Politècnica de València, España.

von Endt, D. & Baker, M. 1991. The chemistry of filled animal glue systems.