LWT - Food Science and Technology 169 (2022) 114029

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Effect of freezing on the rheological characteristics of protein enriched vegetable puree containing different hydrocolloids for dysphagia diets

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ARTICLE INFO

Keywords:
Rheology
Texture
Casein
Plant proteins
Thickeners

ABSTRACT

This study optimized the formulation of protein-enriched vegetable purees designed for people with swallowing difficulties and investigated the effect of different storage conditions (freezing and refrigeration) on their nutritional, rheological and textural properties. Two proteins (casein and pea protein) and four hydrocolloids (guar gum, tara gum, xanthan gum and carboxymehtylcellulose) were combined and their behavior analyzed. The formulations were a good source of protein and antioxidants ensuring a significant supply of protein (6.1–6.7%) and antioxidant capacity (43–53 mg phenolic compounds/100g). The addition of an optimum amount of hydrocolloids allowed the target "pudding" viscosity to be achieved. The formulations containing pea protein were more affected by freezing, which decrease their viscosity, and showed a weaker internal structure and a lower firmness after freezing/thawing when compared with casein formulations. Samples with xanthan gum in general showed better stability. These results indicate that freezing can be a good alternative for preserving casein-enriched vegetable puree elaborated with xanthan gum designed for people with swallowing difficulties.

1. Introduction

Dysphagia is a medical disorder defined by the difficulty or the inability to form or to transport safely the food bolus from the mouth to the stomach. It affects over the 8% of the world's population with a higher prevalence in the elderly(IDDSI, 2022) People with dysphagia have a higher risk to suffer from choking, aspiration pneumonia, malnutrition or even death (Andersen et al., 2013). In these circumstances and, particularly in older individuals, achieving adequate protein intakes is challenging (Putra et al., 2021). These authors concluded that higher dietary protein, of up to 1.2 $g/kg_{bodyweight}$/day may contribute to prevent sarcopenia and maintain musculoskeletal health in older individuals. Although milk proteins, such as whey and casein, have been mainly used for dietary protein supplementation due to their good digestibility and complete amino acid profile, over the last few years plant-based proteins have been gaining popularity (Banaszek et al., 2019; Hertzler et al., 2020; Laguna et al., 2017; Paulina & Lesauskait, 2020; Putra et al., 2021). Different studies reported that pea protein provides a high-quality alternative to milk proteins, with similar protein digestibility (Babault et al., 2015; Overduin et al., 2015; Paulina & Lesauskait, 2020; Yang et al., 2012).

On the other hand, Homem et al. (2020) concluded that oropharyngeal dysphagia can lead to poor general nutritional status, lower serum levels of antioxidant vitamins such as beta carotene and vitamin C, and an elevated inflammatory response. Therefore, diets rich in fruits and vegetables should be included in dysphagia management because of their relevant concentrations of vitamins, minerals, antioxidants and fibers (Slavin & Lloyd, 2012). Several products, such as vegetable pureed food (Sharma & Duizer, 2019; Sharma et al., 2017; Strother et al., 2020; Talens et al., 2021), pureed desserts (Lee et al., 2021; Suebsaen et al., 2019) or various meat pasta dishes (Dick et al., 2020, 2021; Pematilleke et al., 2020) designed for people with dysphagia have been developed, but to our knowledge, to date no study on vegetable puree enriched with protein has been carried out far.

One of the most important aspects when designing dysphagia foods is to guarantee safe swallowing and the main strategy applied is the use of hydrocolloids to optimize food texture properties (Giura et al., 2021). Hydrocolloids are a heterogeneous group of long chain polymers

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https://doi.org/10.1016/j.lwt.2022.114029
Received 2 June 2022; Received in revised form 5 September 2022; Accepted 24 September 2022
Available online 27 September 2022

(polysaccharides and proteins), characterized by their thickening and water-binding capacity and their ability to modify the rheology of food systems (Saha & Bhattacharya, 2010; Salvador & Sanz, 2020).

The physical properties of hydrocolloids, such as solubility, rheological properties (viscosity), and gelling properties, that are directly linked to structural features such as monosaccharide composition (simple versus complex), linkage patterns, chain shapes (linear versus branched), functional groups, and conformations can change the structure of the sample when they interact with other components, giving rise to different viscosities (Goff & Guo, 2020). Hydrocolloids also improve product consistency and cohesiveness and reduce syneresis in foodstuffs (Nishinari et al., 2019).

Gum-based thickeners such as xanthan gum, guar gum, tara gum and carboxymethyl cellulose, which were chosen for our study, have been used in recent years for dysphagia management (Dick et al., 2021; Pematilleke et al., 2020; Sharma et al., 2017; Talens et al., 2021). Xanthan gum is the most common hydrocolloid used to prepare dysphagia dishes, principally due to its stability over time, its resistance to temperature and amylase (Methacanon et al., 2021). Guar gum can be easily hydrated in cold water and also is able to form viscous solutions in low concentrations. It has been demonstrated that both, xanthan gum and guar gum reduce oropharyngeal residue ensuring safe swallowing (Methacanon et al., 2021; Rofes et al., 2014; Theocharidou et al., 2022).

Carboxymethyl cellulose (CMC), like guar gum, is soluble in either cold or hot water and as in the case of xanthan, guar and tara gum, have the capacity to form a safe-swallow bolus (Sharma et al., 2017; Talens et al., 2021; Zarim et al., 2021).

Freezing is a practical method of preserving foods, as it maintains the nutritional characteristics of fresh products, while extending their shelf life. However, in the case of vegetables, it has been reported that the freezing/thawing processes can decrease their elastic properties (Ohnishi et al., 2004). Moreover, freezing/thawing can affect the viscoelastic and textural characteristics of foods because of crystallization or re-crystalization (Cao et al., 2003), leading to syneresis and other technological problems. In order to counteract these issues, the use of cryoprotectant mixtures such as pectin, sucrose, xanthan gum, inulin, starch and kappa-carrageenan has been studied in mashed potatoes, pineapple pulps or carrots (Alvarez et al., 2011; Conceição et al., 2012; Nugmanov et al., 2018). No studies on the effect of freezing on the viscoelastic and textural properties of frozen vegetable purees enriched with proteins adapted for dysphagia patients have been reported to date.

In this study, the rheological and textural properties of protein-enriched vegetable puree thickened with several hydrocolloids and designed for dysphagia management were evaluated. Their behavior during freezing was tested in order to determine their stability and to select optimized formulations. The nutritional aspects of the dish were also evaluated.

2. Materials and methods

2.1. Ingredients

Carrots, zucchini and extra virgin olive oil were purchased from local supermarkets (Calahorra, La Rioja, Spain). Calcium caseinate (CC) (protein content: 96 g/100g) was purchased from the HSN store (Granada, Spain) and deodorized pea protein (PP), NUTRALYS® F85M, (protein content: 84 g/100g) was purchased from Roquette (Valencia, Spain). Four hydrocolloids were bought from Dayelet, Barcelona (Spain): guar gum (GG), tara gum (TG), xanthan gum (XG) and carboxymethyl cellulose (CMC).

2.2. Sample preparation and storage conditions

The percentages of the ingredients in the puree are presented in Table S1 (Supplementary material. Vegetable puree was prepared from 25% zucchini, 25% peeled carrots and 50% water, that were boiled for 30 min in a cooking pot. The mixture was pureed at speed 7 (over 4400 rpm) during 3 min using a Thermomix® (TM5, Vorwerk España M.S.L., S.C., Madrid, Spain). Then, the oil (5%), the protein (7% - calcium caseinate or pea protein) and the different concentrations of each hydrocolloid (guar gum, tara gum, xanthan gum or carboxymethyl cellulose) were added, and also pureed, during 3 min at speed 5 (over 2000 rmp) at temperature of 40 °C in order to achieve uniform dispersion.

The experiments were conducted in two phases: a first screening phase was carried out in which, for each type of protein, different concentrations of each hydrocolloid were tested (from 0.3%, to 1.5%), in order to obtain, with the minimum amount of hydrocolloid, formulations with a viscosity of >1750 mPa.s (pudding-like level) at $50s^{-1}$, according to the NDD (National Dysphagia Diet) (American Dietetic Association, 2002). The viscosity of 22 different formulations was measured by performing a peak hold test at 50 s^{-1} in this first trial. The formulations were maintained under refrigeration conditions (4 °C) during 24h in order to reach the maximum viscosity level with the hydrocolloids used.

Once the required viscosity was achieved for every protein-hydrocolloid combination (n = 10), the second phase of the experiment was carried out: ten different types of samples were formulated, five with each protein (no hydrocolloid, and the selected amount for GG, TG, XG and CMC). The samples were divided into two batches (2 kg/batch) that were stored under two different conditions before their analysis: one was stored at 4 °C during 24 h and the other one was stored at −18 °C during 15 days. Prior to analysis, the frozen samples were thawed in a refrigerator at 4 °C for 24 h.

In both experiments, the first trial for the screening and the second phase with the selected formulations, samples were heated in a microwave at medium high potency 750 W during 1 min until the measurement temperature (40 °C) was reached. After heating the corresponding measures of rheological and textural properties were taken.

2.3. Nutritional composition and antioxidant properties

2.3.1. Nutritional composition

Nutritional composition in the two control formulations was determined according to Regulation 1169/2011 of the European Parliament and of the Council. Protein was determined by the Kjeldhal method (AOAC 2000a), fat was analyzed by the Soxhlet extraction method (AOAC 2000b), ash by gravimetric method (AOAC 2000c), fiber by the enzymatic gravimetric method (AOAC 2000d), moisture content by microwave drying (AOAC 2000e) and carbohydrates were determined by difference.

2.3.2. Extract preparation for antioxidant evaluation

Pureed samples were lyophilized with a freeze-dryer-cryodo (Cryodos, Telstar Industrial S.L. Terrassa, Spain). For each lyophilized sample, 2g were weighed and added to 40 ml of methanol (80%). Then, the mixture was homogenized and centrifuged (3000 rpm, 20 °C, for 15 min) and the supernatant was collected. The methanol was then evaporated, and the precipitates were freeze-dried. The resulting extract was diluted with 10 ml of methanol (80%) and subsequently used for the evaluation of antioxidant capacity and total phenolic content.

2.3.3. DPPH radical scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was performed according to the method described by Blois., (1958), 2 mg of DPPH solution was diluted in methanol in order to obtain an absorbance of 0.8 at 516 nm (working solution) (BLOIS , 1958). The sample solution consisted of adding 750 μL of extract and 750 μL of DPPH solution (Sigma-Aldrich Química S.A., Madrid, Spain) to each Eppendorf tube and was left to react for 30 min at room temperature. The final absorbance was measured at 516 nm using a spectrophotometer (FLUOStar Omega spectrofluorometric analyzer, BMG Labtechnologies, Offenburg, Germany). The radical scavenging activity was calculated as percent of

inhibition (% I) and calculated according to the formula:

$$\%I = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} * 100$$

where $Abs_{control}$ and the Abs_{sample} was the absorbance of the control and the sample, respectively, after 30 min of reaction. For calculating the antioxidant capacity, a calibration curve with Trolox was used. Final results were expressed as μg Trolox/100g of puree sample. All measurements were made in duplicate for each solution.

2.3.4. Total polyphenol content

Phenolic content was measured using the Folin–Ciocalteu method (Singleton & Rossi, 1965). Puree extracts (15 μL) were mixed with 1185 μL of distilled water, and 0.075 mL of Folin Ciocalteu's phenol reagent (Panreac, Quimica, S.A, Barcelona, Spain) was then added. After 2 min at room temperature, 225 μL of a saturated sodium carbonate solution (Panreac, Quimica, S.A, Barcelona, Spain) were added. The mixture was allowed to stand for 2 h in the dark at room temperature. The absorbance was measured at 765 nm using a spectrophotometer (FLUOstar Omega, BMG Labtech). Measurements were compared with a gallic acid standard curve and were expressed as mg of gallic acid equivalents/100 g puree (mg GAE/100 g).

2.4. Flow and viscoelastic properties

The rheological properties were carried out at 40 °C in a Discovery Hybrid Rheometer HR-1 (TA Instruments Ltd., New Castle, DW, USA) using a concentric cylinder geometry at 6 mm gap. The rheometer was equipped with a Peltier heating system to ensure the temperature control. After loading, the samples (22 ml approximatively) were released for 5 min to recover from the stress produced by the geometry lowering and to equilibrate the temperature.

2.4.1. Flow rheological properties

The viscosity of the samples was obtained by performing a peak hold test at a constant shear rate of 50 s^{-1} and the viscosity was recorded. The flow rheological properties of the samples were characterized by performing a continuous shear rate ramp from 0.1 to 400 s^{-1} at 40 °C following the method used by Wei et al. (2021) with some modifications. All tests were carried out in triplicate.

The obtained data was fitted to the Ostwald-de Wale (Power-law) model.

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

where η is the apparent viscosity (Pa.s), k is the consistency index ($Pa.s^n$) which describes the overall range of viscosities across the part of the flow curve that is being modelled, (γ) is the shear rate (s^{-1}) and n is the flow behavior index (dimensionless).

2.4.2. Viscoelastic properties

Oscillatory tests including the oscillation time sweep test, oscillation amplitude sweep test and frequency sweep test were performed. The oscillation time sweep test was performed at a constant strain of 0.01% and at a frequency of 1 Hz in order to observe if any change such as drying or sedimentation might occur in storage or loss modulus during the test. To determine the linear viscoelastic region (LVR) of each sample an amplitude sweep test was performed at a strain from 0.01% to 200% and at a frequency of 1 Hz following the method used by Sharma et al. (2017) with minor modifications.

The LVR indicates the range in which the test can be performed without destroying the structure of the sample. The limit of the LVR was considered as the data point at which the G' value deviated 5% from the LVR plateau (according to the standards ISO 6721–10 and EN/DIN EN 14770). Storage modulus (G'_{LVR}, Pa), yield $strain_{LVR}$ (%), yield $stress_{LVR}$ (Pa) and flow point (Pa) were also measured.

The yield $strain_{LVR}$ and the yield $stress_{LVR}$ were considered as the value of the shear strain and shear stress, respectively, at the limit of LVR region. The crossover point where G' = G'' in this case was taken as the flow point.

Frequency sweep tests were performed according to the method used by Vieira, O, et al. (2020) from 0.1 to 10 Hz at a strain inside the linear viscoelastic region obtained for each sample from the large amplitude oscillatory sweeps. Complex modulus (G*, Pa), storage modulus (G', Pa), loss modulus (G'', Pa) and loss tangent (tan δ, G''/G', dimensionless) were determined. All the tests were conducted in duplicate at 40 °C.

2.5. Textural properties

Textural characteristics were evaluated at 40 °C using a texture analyzer (TA.XT2i Plus Texture Analyzer, Stable Micro Systems, Texture Technologies Corporation, Scarsdale, NY, USA) by performing the back extrusion test following the method used by Yong (2017). One hundred grams of each sample were placed into a 60 mm diameter container and a back-extrusion disc (A/BE40, 40 mm diameter) was attached to the texture analyzer, positioned centrally over the container. The disc was dipped into the sample at the following settings: 1 mm/s test speed, 1 mm/s pre-speed, 10 mm/s post-speed, distance 20 mm and return distance 85 mm. The parameters measured were: the maximum positive peak force representing firmness (N), obtained as a result of compression, the higher value the firmer the sample; the positive area of the curve indicating consistency (N.s) of the sample, the higher the value, the thicker the sample; the maximum negative peak force or the cohesiveness (N), this indicates the sample's cohesion and resistance to separation (flow off) from the disc, and the negative area of the curve, which is known as the viscosity index (N.s) or adhesiveness (the amount of energy needed to break probe sample contact). Each analysis was run in triplicate for every sample.

2.6. Statistical analysis

A statistical data analysis was performed by using the STATA 15 program (Stata Corp LLC, TX, USA). Differences between refrigerated and frozen samples were identified using a paired *t*-Student test ($p \leq 0.05$). One-way analysis of variance (ANOVA) was performed to evaluate statistical significance ($p \leq 0.05$) among all formulations on the same storage conditions. The Tukey post hoc procedure was applied to detect statistically significant differences ($p \leq 0.05$).

3. Results and discussion

3.1. Nutritional aspects of the designed dishes

Protein-enriched purees (casein and pea protein) elaborated with vegetables were developed.

The protein enrichment was based on nutritional considerations, trying to maximize its presence, but also on sensory aspects. In this sense, pea protein isolates are known for their bitter taste (Cosson et al., 2022), so their concentration should be carefully chosen. In our case, a deodorized isolated was used, minimizing this effect. Moreover, a sensory test made with laboratory staff demonstrated that the sensory properties (taste, color, palatability) were adequate (data not shown). The nutritional aspects and antioxidant capacity for the baseline formulations (vegetables + oil + water + protein = control) are shown in Table 1.

Both formulations were good sources of protein, showing around 7 g of protein in 100 g of puree. Considering that a serving of pureed vegetables weighs about 250–300 g, each serving of this protein-enriched vegetable puree would provide about 20 g of protein per serving. As the recommended daily dose of protein for the elderly is about 1.2 $g/kg/_{bodyweight}/day$, one serving would account for approximatively a quarter of these needs. Casein and pea protein, as previously stated, are

Table 1
Energy content, nutritional composition, antioxidant activity and total phenolic compounds of 100 g of the baseline puree formulations prepared with the two proteins (casein or pea protein).

Characteristics	Casein (control)	Pea protein (control)
Energy (KJ/Kcal)	358 KJ/82 Kcal	352 KJ/84 Kcal
Protein (g)	6.8 ± 0.3	6.1 ± 0.2
Fat (g)	5.4 ± 0.4	5.1 ± 0.41
Carbohydrate (g)[a]	1.8	3.1
Fiber (g)	1.7 ± 0.3	0.9 ± 0.2
Ash (g)	0.5 ± 0.1	0.61 ± 0.1
DPPH (μg trolox/100 g)	106.6	98.4
Total phenolic compounds (mg GAE/ 100 g)	43	53

[a] Calculated by difference.

high biological value proteins that are crucial health maintenance, particularly in dysphagia patients (Banaszek et al., 2019; Hertzler et al., 2020; Laguna et al., 2017; Paulina & Lesauskait, 2020; Putra et al., 2021).

The purees showed a low content of saturated fatty acids, thanks to the addition of extra virgin olive oil. Extra virgin olive oil is principally composed of monounsaturated (oleic acid) and polyunsaturated (linoleic acid) fatty acids, which provides important benefits in nutrition, such as the: prevention of cardiovascular diseases, improvement of lipid profile, prevention and minimization of the effects of diabetes, and beneficial effects in diseases related to inflammatory and autoimmune responses (Mangas-Cruz et al., 2004; Rubert et al., 2020). The supply of fiber, 3 g/serving, representing around 12% of the recommended daily intake, is also not negligible.

The good lipid profile, the relevant content of protein the low caloric intake, and the significant amount of fiber shown in both formulations make them high nutritional value dishes.

In addition to their significant supply of macronutrients, their contribution with antioxidant bioactive compounds is also relevant, with a total phenolic content of 43 and 53 mg GAE/100 g and the antioxidant capacity of around 100 μg Trolox/g sample. Lee et al. (2021) studying the antioxidant properties of more complex purees for senior elaborated with vegetables, fruits, milk and soymilk found values of total polyphenols content that ranged from 1.05 to 1.22 mg GAE/g sample. The beneficial health effects of vegetables, and particularly of their phenolic compounds, have been well known for some decades. Moreover, recent research has suggested that they exert prebiotic effects (Alves-Santos et al., 2020; Filosa et al., 2018; Moorthy et al., 2020) modulating the gut microbial profile and positively affecting to health (Mithul Aravind et al., 2021). Thus it is clear that it is highly interesting from the health point of view to ensure an adequate intake of these type of compounds in vulnerable groups.

Once the nutritional aspects of the dishes were ensured, it was necessary to adapt their textural properties by adding different hydrocolloids in order to guarantee their availability for dysphagia diets and for freezing. In addition to modifying the textural properties of the puree, protein-polysaccharide complexes formed as a consequence of the addition of hydrocolloids operate as delivery systems for many bioactives or sensitive molecules in food compositions since they can encapsulate a number of active components (Ghosh & Bandyopadhyay, 2012).

3.2. Selection of optimum hydrocolloid concentrations

To determine the required viscosity for each protein-hydrocolloid combination, a screening study was performed by formulating 22 puree sample types, with different concentrations of each hydrocolloid (Fig. S1 a, b; Supplementary material). The baseline formulations elaborated without hydrocolloids were taken as control, showing very low viscosity values at a shear rate of 50 s^{-1} (235 and 692 mPa.s, for casein and pea protein samples, respectively). The hydrocolloid concentrations were different from each other, and were also adjusted for every protein used. The viscosity measurements of the 22 puree samples were taken after 24h of refrigeration following the methodology used by other authors when measuring dysphagia foods (Kim & Yoo, 2018; Sharma et al., 2017). For each protein, the minimum hydrocolloid concentration showing a viscosity higher than 1750 mPa.s was chosen, in order to ensure an adequate consistency (pudding) according to NDD (National Dysphagia Diet) (American Dietetic Association, 2002). Although this criterion of NDD corresponds to measurements at 25 °C, and our measurements were made at 40 °C it seems reasonable to consider that, with viscosity values of 1750 mPa.s, samples have a pudding consistency when ingested. Other authors have also provided results at temperatures different to 25 °C, as there are many foods that are served at higher temperature. Sharma et al. (2017) provided results at a consumption temperature, 55 °C when measuring pureed carrots for dysphagia patients. Hadde et al. (2015) stated that 40 °C is a reasonable temperature, since individuals with swallowing difficulties have a longer oral transit time and a higher temperature can cause them burns. In any case, additional measurements of viscosity at 25 °C were taken in order to ensure that the viscosities remained in the range of pudding consistency also at this temperature (Table S2; Supplementary material).

The selected concentration of each hydrocolloid for both casein and pea protein samples were: 0.95% GG, 0.8%TG, 1.2% XG, 0.6% CMC and 0.6% GG, 0.5%TG, 0.8% XG and 0.6% CMC, respectively. Once the formulation was defined for each protein and type of hydrocolloid a sufficient quantity of chosen samples was newly prepared in order to analyze all the rheological and textural properties under the selected storage conditions. Selected samples containing hydrocolloids, and casein and pea protein control samples (without hydrocolloids) were divided into two batches before testing, one stored at 4 °C during 24 h and the otherat −18 °C during 15 days.

No syneresis was detected in any of the samples with hydrocolloids during thawing whatever the protein was used. This fact may be due to the protein-polysaccharide interaction, which can help to improve the texture and stability of food products. In the presence of polysaccharide, proteins that are surface active can significantly contribute to the stabilization of emulsions by interacting electrostatically or through hydrophobic-hydrophobic interactions. In addition, polysaccharides typically remain in the aqueous phase and help to regulate its rheology by thickening, gelling, and acting as stabilizing agents (Ghosh & Bandyopadhyay, 2012). No color changes were detected in any of the samples during both storage conditions (Fig. S2 a, b; Supplementary material).

3.3. Flow and viscoelastic properties

3.3.1. Flow rheological properties

The apparent viscosities at 50 s^{-1} for selected samples under both storage conditions (refrigeration and freezing/thawing) are shown in Table 2. Control formulations (without hydrocolloids) showed very low viscosities under refrigeration and freezing conditions. The slight difference in the viscosities of the samples measured for the screening study (Table S2; supplementary material) and those measured in the selected samples (Table 2) was due to different fresh batches of vegetables used for the puree elaboration. In order to ensure that no significant differences occurred between the different batches, the viscosity of the puree without proteins and hydrocolloids was measured every time the formulations were elaborated (data not shown).

Casein formulations with hydrocolloids showed great stability after freezing/thawing, displaying similar viscosity values between both storage conditions in all samples, except for the samples thickened with TG, that showed a 18% decrease in viscosity after freezing/thawing.

In contrast, a statistically significant decrease in viscosity was noted

Table 2
Viscosity (mPa.s) at 50 s^{-1} of casein and pea protein formulations with the different hydrocolloids: Control, GG (guar gum), TG (tara gum), XG (xanthan gum) and CMC (carboxymethyl cellulose) under refrigeration and freezing storage.

	Hydrocolloids	*Concentration (%)*	*Refrigeration*	*Freezing*
Casein	*Control*	*0*	203 ± 3^{C}	198 ± 7^{E}
	GG	*0.95*	1857 ± 102^{A}	1913 ± 70^{C}
	TG	*0.8*	2047 ± 33^{E}	1651 ± 48^{AB}*
	XG	*1.2*	2174 ± 41^{B}	2207 ± 38^{D}
	CMC	*0.6*	2290 ± 36^{B}	2263 ± 93^{D}
Pea protein	*Control*	*0*	597 ± 21^{D}	356 ± 21^{F}*
	GG	*0.6*	2591 ± 24^{F}	1999 ± 16^{C}*
	TG	*0.5*	2274 ± 32^{B}	1557 ± 17^{A}*
	XG	*0.8*	1820 ± 16^{A}	1687 ± 6^{B}*
	CMC	*0.6*	1919 ± 26^{A}	1635 ± 30^{AB}*

Note: Data are presented as mean ± standard deviation of three independent experiments. For each formulation the (*), in the same row, indicates significant differences ($P < 0.05$), between both storage conditions refrigeration and freezing/thawing. Different capital letters, in the same column, indicate significant differences ($P < 0.05$) based on the post hoc Tukey test.

in all pea protein samples after freezing/thawing reaching, in the case of formulations with TG, XG and CMC, values slightly lower than the minimum required for a pudding texture (1750 mPa.s). The lowest value was reached for the TG (1577 mPa.s) and the highest for XG (1687 mPa.s). These results highlight that, during freezing, samples elaborated with pea protein are a great risk of undergoing a decrease viscosity than those elaborated with casein. As the time of freezing in this study was 15 days, this fact should be taken into account for longer storage times.

Sim et al. (2021), suggested that, in comparison to animal proteins, plant proteins have inferior nutritional and functional qualities such as poor solubility, foaming, emulsifying, and gelling properties. Our finding, indicating the instability of all pea protein formulations when stored frozen, supports the idea of their worse technological behavior as compared to casein.

As for hydrocolloids, as previously pointed out, structural features determine their behavior. In this sense, xanthan gum is a microbial heteropolysaccharide of high molecular weight with a wide range of applications in the food industry due to its good physical properties and rheological characteristics. Guar gum and tara gum are both galactomannans, with guar containing more galactose chains than tara, making it more water soluble at room temperature, and also more soluble than xanthan as well (Kongjaroen et al., 2022). Due to its branching structure, guar gum it is quickly hydrated and produces viscous solutions, making it suitable for texture modification and product stabilization.

The viscosity curves of the casein and pea protein samples are shown in Fig. 1. (a) and (b), respectively. A shear thinning behavior is shown for every sample, slightly more pronounced in the case of formulations elaborated with pea protein. Viscosity data versus shear rate were successfully adjusted to the power law model. The parameters obtained (flow behavior index *n*, and the consistency index *k),* for all samples are summarized in Table 3. The high correlation coefficients (r) indicate the suitability of the model to describe the flow behavior of the samples. The flow behavior index, *n,* and the consistency index, *k,* were different within the same protein depending on the type of thickener used.

All samples showed a shear-thinning behavior ($n < 1$), with *n* values ranging from 0.168 to 0.438 for the casein and from 0.163 to 0.348 for the pea protein formulations under refrigerated storage. The n values were similar to those obtained from other authors when analyzing dysphagia oriented foods such as milk, orange juice, pea cream, black fungus-based 3D printed foods (Talens et al., 2021; Vieira, Oliveira, et al., 2020; Xing et al., 2022). For both proteins, the sample thickened with XG showed the most pseudoplastic properties and casein sample containing guar gum the least pseudoplastic properties with the lowest correlation coefficient of 0.976 meaning that those formulations decreased their viscosity with increasing shearing stress. A similar behavior for XG was obtained by Zarim et al. (2021) when developing a chicken rendang for the elderly. Additionally (Kongjaroen et al., 2022), pointed out that XG chains could begin to disentangle even at $0.1 s^{-1}$, earlier than other gums (TG and GG) that they presented a Newtonian plateau in water-based products at shear rates below $1 s^{-1}$. Wei et al. (2021) reported that gums with a low *n*-value have a less slimy texture and therefore may be the key to the development of dysphagia foods with a more pleasant texture. Taking into account the considerations made by these authors and also our results, we suggest that samples thickened with xanthan gum may be more suitable for people with swallowing difficulties. After freezing/thawing, the shear thinning behavior was maintained for every sample with a slight increase for all formulations except for the casein samples thickened with GG and XG, which exhibited a slight decrease in *n* values.

The consistency index, *k* values under refrigeration, increased considerably with the addition of hydrocolloids for both types of protein formulations. Cho and Yoo (2015) obtained similar *k* values which ranged from 5.22 ± 0.02 to 39.4 ± 0.78 $Pa{\cdot}s^{n}$ when developing a thickened sport drink for people with swallowing problems. The highest

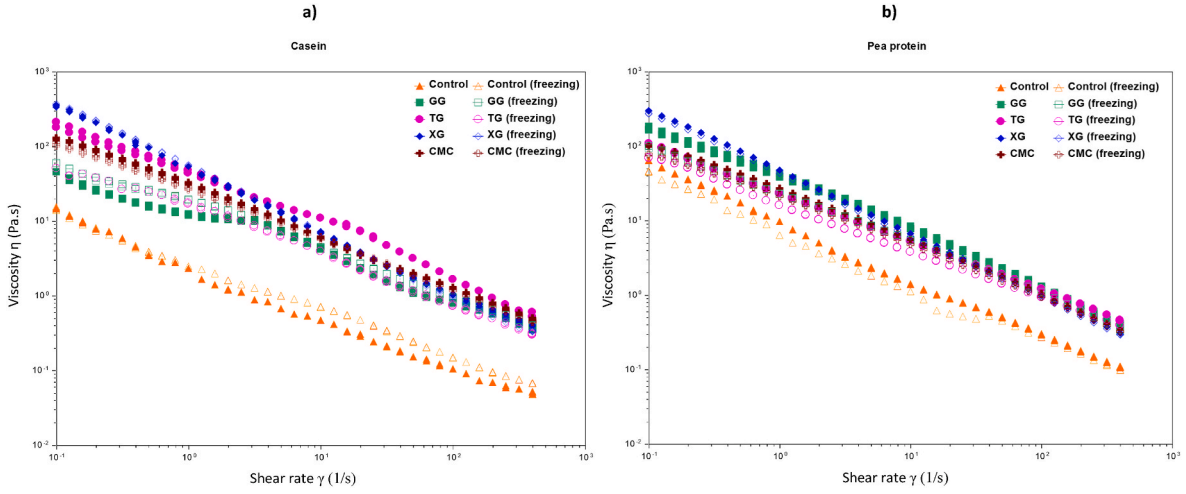

Fig. 1. (a, b). Shear thinning behavior of refrigerated (closed symbols) and frozen (open symbols) casein and pea protein formulations thickened using different hydrocolloids.

Table 3
Flow rheological behavior (n shear thinning behavior, k consistency index, r correlation coefficient) of casein and pea protein formulations with the different hydrocolloids: Control, GG (guar gum), TG (tara gum), XG (xanthan gum) and CMC (carboxymethyl cellulose) under refrigeration and freezing storage.

	Hydrocolloids	n		K(Pa.s^n)		r	
		Refrigeration	Freezing	Refrigeration	Freezing	Refrigeration	Freezing
Casein	Control	0.327 ± 0.011^{A}	$0.373 \pm 0.008^{B*}$	2.238 ± 0.150^{F}	$2.771 \pm 0.080^{B*}$	0.995	0.997
	GG	0.438 ± 0.018^{E}	$0.391 \pm 0.006^{F*}$	12.191 ± 0.923^{B}	$16.961 \pm 0.438^{A*}$	0.976	0.992
	TG	0.330 ± 0.044^{A}	0.368 ± 0.007^{B}	42.561 ± 6.561^{CD}	$14.582 \pm 0.754^{D*}$	0.993	0.991
	XG	0.168 ± 0.013^{B}	$0.133 \pm 0.006^{C*}$	49.866 ± 2.569^{E}	53.720 ± 0.878^{I}	0.999	0.999
	CMC	0.311 ± 0.003^{A}	$0.330 \pm 0.006^{A*}$	30.016 ± 0.905^{A}	$26.464 \pm 1.159^{G*}$	0.999	0.999
Pea protein	Control	0.243 ± 0.009^{C}	$0.271 \pm 0.006^{E*}$	9.203 ± 0.330^{B}	$6.602 \pm 0.705^{C*}$	0.997	0.989
	GG	0.263 ± 0.005^{CD}	$0.332 \pm 0.003^{A*}$	38.512 ± 2.220^{C}	$23.117 \pm 0.340^{F*}$	0.996	0.997
	TG	0.348 ± 0.002^{A}	$0.371 \pm 0.002^{B*}$	23.503 ± 0.462^{A}	$16.647 \pm 0.064^{A*}$	0.999	0.999
	XG	0.163 ± 0.001^{B}	$0.168 \pm 0.001^{D*}$	46.569 ± 0.106^{DE}	$42.904 \pm 0.098^{H*}$	0.999	0.999
	CMC	0.306 ± 0.002^{AD}	$0.334 \pm 0.004^{A*}$	25.693 ± 0.187^{A}	$20.603 \pm 0.297^{E*}$	0.997	0.997

Note: Data are presented as mean ± standard deviation of three independent experiments. For each formulation the (*), in the same row, indicates significant differences ($P < 0.05$), between both storage conditions refrigeration and freezing/thawing. Different capital letters, in the same column, indicate significant differences ($P < 0.05$) based on the post hoc Tukey test.

k values corresponded to the casein and pea protein samples containing XG. Regarding the effect of freezing, significant decreases were observed in all cases, except for the samples elaborated with casein and GG, where an increase was observed, and with XG in which no modification was detected in *k* values. In a study into the effect of a cryoprotectant mixture on the rheological properties of fresh and frozen/thawed mashed potatoes shear stress-shear rate data obtained by Alvarez et al. (2011) indicated that samples thickened with XG showed a good stability during the freeze/thawing process. This conclusion supports the good stability found in the case of samples elaborated with casein + XG.

3.3.2. *Viscoelastic properties*

3.3.2.1. Oscillation amplitude sweep tests. To determine the limit of the linear viscoelastic region (LVR), in which the tests can be performed without destroying the structure of the sample, and to identify the flow point, amplitude sweep tests were carried out. Table 4 shows the viscoelastic parameters such as G'_{LVR}, Yield $strain_{LVR}$, Yield $stress_{LVR}$, and the flow point.

G'_{LVR} is considered an indicator of the material stiffness, namely, the higher the G'_{LVR} value, the stiffer the material is. It is worth to noting the great difference for G'_{LVR} between the controls elaborated with casein and pea protein. These data indicate that pea protein gives the puree greater stiffness or elastic modulus as compared to casein. The pea protein sample thickened with XG exhibited the highest G'_{LVR} followed by the control and those with GG. A similar behavior was also observed by Hidalgo et al. (2016) when studying the effect of xanthan gum on the rheological behavior of sodium caseinate gels. This author suggested that this could be due to the presence of this polysaccharide which changes the kinetics of the gelation process and causes the formation of more compact gels with a high elastic component (Hidalgo et al., 2016).

Moreover, the formulations with casein, which in general displayed lower values than those with pea protein, showed the highest value of G'_{LVR} throughout the LVR in samples with XG.

Freezing caused a significant reduction in G'_{LVR} only in the GG and TG casein samples. In contrast, G'_{LVR} decreased significantly for all the pea protein samples after freezing/thawing, but the decrease in the case of samples with XG was much lower than in the rest of samples (15% and 34–51%, respectively). As we mentioned previously, the instability of the pea protein samples after freezing/thawing could be related with their worse technological behavior as compared to casein samples. Another hypothesis that could explain the decrease of G'_{LVR} in all pea protein samples might be cold denaturation, which may occur in the structure of proteins during the freezing/thawing process (Cao et al., 2003).

In the case of pea protein, samples thickened with XG and GG had a significantly higher yield strain and yield stress, indicating greater flexibility and elasticity than the rest of the samples. On the other hand, the lowest flexibility and elasticity were obtained by the casein control sample and the sample thickened with GG and for the pea protein, samples containing TG and CMC.

Freezing/thawing exerted diverse effects on the samples both for yield strain and yield stress, with the formulations with XG maintaining the highest values for both parameters for each type of protein. As the

Table 4
Viscoelastic parameters obtained from amplitude sweep test of casein and pea protein formulations with the different hydrocolloids: Control, GG (guar gum), TG (tara gum), XG (xanthan gum) and CMC (carboxymethyl cellulose) under refrigeration and freezing storage.

	Hydrocolloids	G'_{LVR} (Pa)		Yield $Strain_{LVR}$ (%)		Yield $stress_{LVR}$ (Pa)		Flow point (Pa)	
		Refrigeration	*Freezing*	*Refrigeration*	*Freezing*	*Refrigeration*	*Freezing*	*Refrigeration*	*Freezing*
Casein	*Control*	170 ± 24^{AB}	176 ± 7^{A}	0.295 ± 0.018^{E}	$0.162 \pm 0.002^{C*}$	0.560 ± 0.024^{C}	$0.278 \pm 0.011^{B*}$	31.8 ± 2.8^{E}	32.7 ± 4.8^{C}
	GG	192 ± 0^{A}	$175 \pm 1^{A*}$	0.179 ± 0.006^{D}	$0.108 \pm 0.011^{A*}$	0.357 ± 0.010^{D}	$0.203 \pm 0.024^{AB*}$	80.2 ± 1.9^{BC}	82.3 ± 2.4^{B}
	TG	308 ± 10^{CD}	$103 \pm 4^{B*}$	0.971 ± 0.018^{G}	$0.107 \pm 0.001^{A*}$	3.243 ± 0.049^{B}	$0.128 \pm 0.004^{A*}$	129.4 ± 8.2^{D}	$60 \pm 2.3^{A*}$
	XG	314 ± 4^{D}	323 ± 1^{F}	1.401 ± 0.021^{H}	$1.508 \pm 0.004^{H*}$	4.401 ± 0.003^{G}	$4.867 \pm 0.024^{H*}$	69.2 ± 1.1^{AB}	$65.5 \pm 0.1^{A*}$
	CMC	135 ± 2^{B}	134 ± 2^{C}	0.561 ± 0.006^{B}	$0.982 \pm 0.003^{G*}$	0.85 ± 0.001^{A}	$1.603 \pm 0.000^{E*}$	66.9 ± 0.1^{A}	$89.2 \pm 0.4^{B*}$
Pea protein	*Control*	437 ± 2^{F}	$257 \pm 9^{D*}$	0.249 ± 0.006^{A}	0.254 ± 0.008^{D}	1.073 ± 0.026^{E}	$0.644 \pm 0.04^{C*}$	53.9 ± 2.2^{F}	$31 \pm 1.6^{C*}$
	GG	385 ± 7^{E}	$195 \pm 4^{A*}$	0.608 ± 0.003^{C}	$0.394 \pm 0.001^{B*}$	2.513 ± 0.052^{F}	$1.286 \pm 0.018^{F*}$	132.2 ± 0.6^{D}	$81.3 \pm 0^{B*}$
	TG	274 ± 3^{C}	$181 \pm 1^{A*}$	0.249 ± 0.001^{A}	$0.419 \pm 0.004^{E*}$	0.727 ± 0.014^{A}	$0.776 \pm 0.000^{D*}$	88.6 ± 0.3^{C}	$50.9 \pm 1.1^{D*}$
	XG	523 ± 7^{G}	$444 \pm 13^{G*}$	0.586 ± 0.001^{BC}	$0.571 \pm 0.003^{F*}$	3.131 ± 0.071^{B}	$2.517 \pm 0.080^{G*}$	73.5 ± 0.2^{AB}	$64.2 \pm 0.4^{A*}$
	CMC	192 ± 3^{A}	$124 \pm 3^{BC*}$	0.387 ± 0.006^{F}	0.396 ± 0.006^{B}	0.823 ± 0.025^{A}	$0.553 \pm 0.002^{C*}$	75.9 ± 0.6^{AB}	$51.2 \pm 2.1^{D*}$

Note: Data are presented as mean ± standard deviation of three independent experiments. For each formulation the (*), in the same row, indicates significant differences ($P < 0.05$), between both storage conditions refrigeration and freezing/thawing. Different capital letters, in the same column, indicate significant differences ($P < 0.05$) based on the post hoc Tukey test.

yield stress denotes the minimum force required to cause structural breakdown, it seems that XG is the hydrocolloid supplying the highest stability even during freezing.

The flow point or the crossover point, where G' = G' is the point at which the structure of the sample is destroyed, and can provide information about the breakdown of the internal structure. After freezing/thawing all samples with hydrocolloids showed values around 50–89 Pa, which in general were a little bit lower than those obtained for refrigerated samples.

3.3.2.2. Oscillation frequency sweep tests. When the frequency sweep curves (between 0.1 and 10 Hz) were obtained, similar behaviors across the different frequencies were observed for all samples analyzed (Fig. S3, a-f; Supplementary material). Data obtained for a frequency of 1Hz are shown in Table 5.

The loss modulus G'' was much lower than storage modulus G' in every sample giving rise to tan δ values < 0.6, indicating a weak gel structure which is considered adequate for people with dysphagia (Ishihara et al., 2011). Similar values were obtained by Talens et al. (2021), Herranz et al. (2021) and Sharma et al. (2017) when characterizing thickened pea cream commercial dysphagia foods and thickened carrots purees. The lowest values for tan δ were obtained when using XG both for the casein (0.248) and pea protein (0.212) formulations, and these did not significantly change with freezing/thawing. Thus, these results seemed to indicate that the evolution for storage and loss modulus during freezing/thawing when XG was used were minimal and did not affect the weakness of the gel. The rest of the samples with hydrocolloids showed differences in tan δ with freezing/thawing. Casein samples containing TG and CMC were the most affected by the storage conditions, with higher tan δ values, indicating a more liquid structure after freezing/thawing than refrigeration.

3.4. Textural properties

The rheological properties were completed with the textural parameters (Table 6) in order to support the adequacy of the samples. Although firmness and consistency of the control samples elaborated with casein were much lower than those elaborated with pea protein, the addition of hydrocolloids had different effects, giving rise, in general, to higher values in both parameters in samples elaborated with casein. The effect of freezing/thawing, in general, yielded a significant but slight decrease in both parameters.

The highest firmness and consistency were obtained for the casein and pea protein samples thickened with xanthan gum under both storage conditions. These data agree with those obtained for yield stress, which showed the highest values for samples with XG. Yong (2017) in a study into the evaluation of the rheological and textural properties of texture-modified rice porridge containing tapioca also found that samples with high firmness values required more force to flow.

Cohesiveness is defined as the extent to which the food deforms when compressed. High cohesiveness may be favorable for dysphagia foods to avoid aspiration or bolus break-up of the food product during the deglutition phase (Ishihara et al., 2011; Sharma et al., 2017). The addition of hydrocolloids increased the cohesiveness and the index of viscosity by 8–14 fold in samples with casein and by 1.8–3 fold in samples with pea protein.

Freezing/thawing increased the values of the cohesiveness parameters only in the casein control products but maintaining low values. For the casein samples thickened with GG, TG and XG and the pea protein samples containing XG and CMC, non-relevant decreases were observed after freezing/thawing. The index of viscosity showed a decrease for the casein samples thickened with XG and CMC and an increase for the control, GG and TG samples. In contrast, in the case of the pea protein control and samples with hydrocolloids, significant but not quantitatively relevant decreases in index of viscosity were observed in most of the samples. Samples with XG were again those with the highest values for each type of formulation.

4. Conclusions

In the present study a high nutritional value dish has been developed. The formulations supply a significant amount of proteins (6.1–6.7%) and also a significant amount of antioxidant bioactive compounds, ensuring a highly nutritional dish for dysphagic people.

The results of the rheological and textural analysis showed that even though all puree had been prepared to obtain a pudding-like viscosity their viscoelastic parameters were different. Pea protein sample thickened with XG showed the highest G' followed by the control and those with GG indicating a stiffer structure. After freezing/thawing, flow, viscoelastic and textural parameters were different for some of the formulations. Sample containing pea protein were more susceptible to changes after freezing/thawing than the casein samples, especially regarding viscosity at 50 s^{-1}. In contrast, all the casein formulations, except the casein + tara gum, presented good stability after freezing/thawing when measuring flow and viscoelastic properties.

The formulations containing xanthan gum, in both the casein and pea protein samples, showed a slightly higher stability than the rest when their viscoelastic properties were measured. In conclusion, xanthan gum and casein could be considered the best option to maintain this type of dish when submitted to freezing conditions.

Funding

This work was supported by Gobierno de Navarra, DEGLUSEN

Table 5
Viscoelastic parameters at 1 Hz obtained from frequency sweep test of casein and pea protein formulations with the different hydrocolloids: Control, GG (guar gum), TG (tara gum), XG (xanthan gum) and CMC (carboxymethyl cellulose) under refrigeration and freezing storage.

	Hydrocolloids	G* (Pa)		G' (Pa)		G'' (Pa)		Tan δ	
		Refrigeration	*Freezing*	*Refrigeration*	*Freezing*	*Refrigeration*	*Freezing*	*Refrigeration*	*Freezing*
Casein	*Control*	110 ± 18^{D}	$193 \pm 2^{AB*}$	109 ± 18^{A}	$191 \pm 2^{A*}$	14 ± 1^{F}	$25 \pm 1^{E*}$	0.130 ± 0.008^{A}	0.128 ± 0.002^{A}
	GG	205 ± 1^{B}	189 ± 7^{A}	195 ± 2^{B}	$172 \pm 6^{D*}$	63 ± 1^{A}	$78 \pm 4^{C*}$	0.324 ± 0.009^{E}	$0.451 \pm 0.010^{E*}$
	TG	421 ± 15^{A}	$108 \pm 8^{C*}$	383 ± 12^{D}	$90 \pm 9^{C*}$	175 ± 8^{E}	$60 \pm 2^{A*}$	0.457 ± 0.007^{B}	$0.668 \pm 0.039^{F*}$
	XG	337 ± 15^{C}	326 ± 3^{H}	327 ± 15^{C}	317 ± 3^{F}	81 ± 4^{B}	78 ± 1^{C}	0.248 ± 0.001^{D}	0.245 ± 0.002^{B}
	CMC	160 ± 6^{E}	169 ± 3^{E}	139 ± 5^{A}	136 ± 2^{B}	80 ± 3^{B}	$101 \pm 1^{D*}$	0.573 ± 0.005^{H}	$0.743 \pm 0.001^{G*}$
Pea protein	*Control*	433 ± 4^{A}	$294 \pm 1^{G*}$	430 ± 5^{G}	$292 \pm 1^{E*}$	49 ± 4^{A}	$37 \pm 1^{F*}$	0.114 ± 0.010^{A}	0.126 ± 0.004^{A}
	GG	397 ± 3^{A}	$227 \pm 2^{F*}$	360 ± 2^{CD}	$201 \pm 2^{A*}$	167 ± 2^{E}	$105 \pm 0^{D*}$	0.464 ± 0.002^{B}	$0.520 \pm 0.004^{C*}$
	TG	304 ± 3^{C}	$208 \pm 0^{B*}$	281 ± 4^{F}	$197 \pm 0^{A*}$	114 ± 2^{D}	$66 \pm 1^{AB*}$	0.407 ± 0.013^{F}	$0.336 \pm 0.004^{D*}$
	XG	536 ± 5^{F}	$458 \pm 8^{I*}$	524 ± 5^{H}	$449 \pm 8^{G*}$	111 ± 3^{CD}	$92 \pm 1^{G*}$	0.212 ± 0.003^{C}	0.206 ± 0.002^{B}
	CMC	219 ± 0^{B}	$145 \pm 0^{D*}$	196 ± 0^{B}	$127 \pm 0^{B*}$	99 ± 1^{C}	$68 \pm 1^{B*}$	0.509 ± 0.005^{G}	$0.538 \pm 0.004^{C*}$

Note: Data are presented as mean ± standard deviation of three independent experiments. For each formulation the (*), in the same row, indicates significant differences ($P < 0.05$), between both storage conditions refrigeration and freezing/thawing. Different capital letters, in the same column, indicate significant differences ($P < 0.05$) based on the post hoc Tukey test.

Table 6
Textural parameters of casein and pea protein formulations with different hydrocolloids: Control, GG (guar gum), TG (tara gum), XG (xanthan gum) and CMC (carboxymethyl cellulose) under refrigeration and freezing storage obtained from Back extrusion test.

	Hydrocolloids	Firmness (N)		Consistency (N.s)		Cohesiveness (N)		Index of viscosity (N.s)	
		Refrigeration	Freezing	Refrigeration	Freezing	Refrigeration	Freezing	Refrigeration	Freezing
Casein	*Control*	0.265 ± 0.010^{C}	0.286 ± 0.006^{A}	4.586 ± 0.152^{D}	4.823 ± 0.059^{A}	0.19 ± 0.004^{H}	0.235 ± 0.003^{C*}	0.206 ± 0.013^{H}	0.343 ± 0.005^{B*}
	GG	1.043 ± 0.030^{G}	0.944 ± 0.021^{E*}	18.536 ± 0.589^{G}	16.122 ± 0.630^{F*}	1.688 ± 0.049^{A}	1.651 ± 0.045^{A}	2.370 ± 0.066^{A}	2.409 ± 0.041^{D}
	TG	1.111 ± 0.005^{B}	1.154 ± 0.023^{G*}	19.806 ± 0.266^{C}	20.112 ± 0.268^{H}	2.06 ± 0.018^{C}	2.066 ± 0.022^{D}	2.869 ± 0.102^{D}	2.977 ± 0.071^{C}
	XG	1.311 ± 0.018^{H}	1.236 ± 0.022^{H*}	23.165 ± 0.421^{H}	22.137 ± 0.351^{I}	1.762 ± 0.042^{A}	1.601 ± 0.018^{A*}	2.416 ± 0.108^{A}	2.244 ± 0.050^{E}
	CMC	0.778 ± 0.015^{F}	0.553 ± 0.040^{D*}	14.251 ± 0.276^{F}	9.801 ± 0.730^{D*}	1.447 ± 0.024^{D}	0.998 ± 0.088^{C*}	2.081 ± 0.033^{B}	1.455 ± 0.107^{G*}
Pea protein	*Control*	0.508 ± 0.011^{A}	0.372 ± 0.006^{B*}	8.762 ± 0.266^{AB}	6.461 ± 0.061^{C*}	0.428 ± 0.016^{G}	0.303 ± 0.003^{B*}	0.642 ± 0.034^{G}	0.453 ± 0.013^{B*}
	GG	0.701 ± 0.012^{E}	0.435 ± 0.013^{C*}	12.598 ± 0.327^{E}	7.709 ± 0.330^{B*}	1.332 ± 0.030^{B}	0.784 ± 0.030^{F*}	1.979 ± 0.064^{BC}	1.179 ± 0.053^{A*}
	TG	0.451 ± 0.008^{D}	0.303 ± 0.011^{A*}	8.088 ± 0.132^{A}	5.296 ± 0.222^{A*}	0.759 ± 0.014^{F}	0.414 ± 0.018^{G*}	1.145 ± 0.025^{F}	0.620 ± 0.021^{H*}
	XG	1.124 ± 0.018^{B}	1.063 ± 0.012^{F*}	19.451 ± 0.214^{C}	18.417 ± 0.163^{G*}	1.304 ± 0.020^{B}	1.235 ± 0.022^{E*}	1.902 ± 0.015^{C}	1.777 ± 0.025^{F*}
	CMC	0.51 ± 0.011^{A}	0.478 ± 0.007^{C*}	9.144 ± 0.161^{B}	8.485 ± 0.139^{B*}	0.881 ± 0.013^{E}	0.818 ± 0.007^{B*}	1.36 ± 0.033^{E}	1.213 ± 0.047^{A}

Note: Data are presented as mean ± standard deviation of three independent experiments. For each formulation the (*), in the same row, indicates significant differences ($P < 0.05$), between both storage conditions refrigeration and freezing/thawing. Different capital letters, in the same column, indicate significant differences ($P < 0.05$) based on the post hoc Tukey test.

project (PhD grant: Ayudas para la contratación de doctorandos y doctorandas por empresas, centros de investigación y centros tecnológicos: Doctorados industriales 2020).

Author contributions

Larisa Giura: Formal analysis, Data curation, Methodology, Investigation, Writing—original draft, Writing—review and editing. Leyre Urtasun: Funding acquisition; Investigation; Resources; Supervision; Writing - review & editing. Diana Ansorena: Conceptualization; Funding acquisition; Investigation; Resources; Visualization; Supervision; Writing—original draft; Writing - review & editing. Iciar Astiasarán: Conceptualization; Funding acquisition; Investigation; Resources; Supervision; Writing—original draft; Writing - review & editing.

Declaration of competing interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

Data availability

No data was used for the research described in the article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2022.114029.

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