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Rheological characterization of rhea (*Rhea americana*) eggs subjected to different storage periods

Caracterização reológica de ovos rhea (Rhea americana) submetidos a diferentes períodos de armazenamento

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Abstract
Eggs are used as food industry ingredients due to their functional properties. Eggs from different bird species are used in industrial processing; therefore, rheological experiments were carried out at steady state to obtain the flow curves and also at a dynamic state to study the rheological properties of greater rhea eggs. The viscoelastic behavior of the fluids as a function of the coagulation temperatures for albumen and yolk and of the oscillatory shear frequency was studied. Fifteen rhea eggs stored for up to 28 days at 10 °C were analyzed at 1, 7, 14, 21, and 28 days. The albumen and yolk fractions showed pseudoplastic rheological behavior with small initial flow shear and dependence on shear time, with albumen demonstrating thixotropy and the yolk showing rheopexy. In low shear rheological tests, thermal gelation of albumen was observed, presenting a first change in the elastic modulus around 56 °C, and a second from 78 °C. In the yolk, a change was observed from 66 °C, with more structural variations in the development of the gel at higher temperatures. For rhea eggs, the gel structuring (gelation) temperature of albumen (80 °C) and egg yolk (69 °C) did not undergo variations during the storage periods. Compared to commercial eggs, the higher viscosity and gelation temperatures presented by the rhea eggs can help determine the size of the industrial processing system as they can be submitted to higher temperatures without modifying protein structures and hindering the process.
Keywords: birds; denaturation temperature; flow; functional properties; protein

Resumo
Os ovos são usados como ingredientes da indústria alimentícia devido às suas propriedades funcionais. Ovos de diferentes espécies de aves podem ser usados no processamento industrial. Foram realizados experimentos reológicos em estado estacionário para obtenção das curvas de fluxo e também em estado dinâmico para estudar as propriedades reológicas dos ovos de emas *Rhea americana*. O comportamento viscoelástico dos fluidos em função das temperaturas de coagulação para albúmen e gema e da frequência oscilatória foi estudado. Foram analisados 15 ovos de rhea armazenados por até 28 dias a 10 °C em 1, 7, 14, 21 e 28 dias. As frações de albumina e gema mostraram comportamento reológico pseudoplástico com pequeno cisalhamento de fluxo inicial e dependência do tempo de cisalhamento, com albúmen demonstrando tixotropia e a gema mostrando reopexia. Em testes reológicos de cisalhamento baixo, observou-se gelificação térmica de albúmen, apresentando uma primeira mudança no módulo elástico em torno de 56 °C, e um segundo de 78 °C. Na gema, observou-se uma mudança a partir de 66 °C, com variações mais estruturais no desenvolvimento do gel a temperaturas mais elevadas. Para os ovos de rhea, a temperatura estruturante de gel (gelificação) de albumen (80 °C) e gema de ovo (69 °C) não sofreu variações durante os períodos de armazenamento. Em comparação com os ovos comerciais, as maiores temperaturas de viscosidade e gelificação apresentadas pelos ovos rhea podem ajudar a determinar o tamanho do sistema de processamento industrial, pois podem ser submetidos a temperaturas mais altas sem modificar estruturas proteicas e dificultar o processo.
Palavras-chave: aves silvestres; temperatura de desnaturação; fluxo; propriedades funcionais; proteína

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Introduction

Eggs are essential ingredients in the food industry and are used in the manufacture of mayonnaise, pasta, bakery products, and confectionery [1] because of their polyfunctional properties of emulsification, heat coagulation, and foam formation [2]. Information on their physical and chemical properties is required for processing eggs as they are subjected to various unitary operations, such as storage, pumping, agitation, pipe transportation, heat exchange, and drying [3]. Rheological studies assess the flow properties of these fluids when subjected to the action of external forces [4]. Most food fluids are non-Newtonian. The viscosity of such fluids depends on the applied shear stress at a constant temperature or may also depend on shearing time. Thus, the majority of these fluids are not characterized only by viscosity as this is not constant. More complex models with at least two parameters are required for their characterization [5].

The viscosity of eggs is associated with the behavior of the time-dependent flow shear, and these egg fluids may demonstrate thixotropy or rheopexy. Thixotropy occurs when the structure of the system ruptures, whereas rheopexy indicates a development of the structure. Thixotropic behavior is common in structured foods, and in the case of albumen, can be associated with a network of proteins in solution that breaks with shear. Conversely, rheopexy is not common in foods, wherein increase in viscosity with shearing time is associated more with the effect of heating or system temperature than with shearing with the time of application [6, 7].

The physicochemical characteristics of albumen influence the formation and elasticity of processed products [8]. These characteristics may change with storage time, especially when subjected to heat treatment[7, 9]. The protein concentration, thickness of the formed protein film, ionic strength of these molecules, temperature, and the presence of other components in the food systems affect albumen's viscoelastic and foaming properties. As the protein concentration increases, a thick lamellar film usually forms, resulting in increased stability upon processing [10]. The viscosity of the yolk provides good stability to emulsions; furthermore, a linear relationship between the stability of the emulsion and the square root of the viscosity can be seen [11].

Temperature also has an effect on the majority of egg proteins, which are denatured when exposed to moderate heating between 60 to 100 °C for a one hour. Thermal treatment causes amino acid denaturation, and after moderate heating (below 100 °C), proteins are digested more easily. This allows proteases to act more easily. From a nutritional perspective, partial denaturation improves the digestibility and biological availability of essential amino acids. Excessive denaturation of protein often results in their insolubilization [12]. At thermal treatment above 100 °C, crosslinks may be formed, affecting functionality and preventing protein digestion, thus reducing their nutritional value. At temperatures above 180 °C, destruction of amino acids occurs, reducing digestibility of protein [13].

As rhea eggs are a potential ingredient for the food industry and are a less studied material, the objective of this work was to characterize their rheological behavior. This was done to provide parameters for industrial processing of these eggs and for their development as ingredients in food formulations. The effect of egg storage time on rheological behavior was assessed using steady state tests to obtain the flow curves, and dynamic tests to verify the viscoelastic behavior of the fluids according to heating temperatures.

Material and methods

Fifteen infertile rhea eggs were used from a commercial breeder registered at the Brazilian Institute of Environment (Reg No: 6012603). The eggs were collected from the nest within a 48-hour period, packaged separately, and sent to the laboratory. They were stored at a temperature of 10 °C throughout the experiment period. The eggs were distributed into five lots composed of three eggs (five treatments × 3 replicates) and analyzed at 1, 7, 14, 21, and 28 days in duplicate, with three independent replicates per egg. The rheological tests of the albumen and egg yolk were performed at steady state (high deformations) and oscillatory state (low shear). A controlled Physica MCR 101 rheometer (Anton Paar, Germany) with a stainless steel cone-plate geometry of 60 mm diameter was used.

The flow curves (viscosity) were determined at a shear rate between 0 and 500 s^{-1} (the most common range in food processes) at a fixed temperature of 25 °C, using the controlled shear modulus. Three continuous flow ramps were used to verify existence of dependence on shear time, or the characterization of thixotropy or rheopexy of the system: the first ramp involved increasing shear rate (0 to 500 s^{-1}) (Ramp 1), the second was a decreasing ramp (Descent), and subsequently, the third was a new increasing shear ramp (Ramp 2).

To characterize rheological behavior, the flow curves were adjusted to rheological power law models (pseudoplastic) (equation 1) and the Herschel–Bulkley (HB) model. The latter differs from the pseudoplastic model by taking into account the shear required to initiate flow (equation 2). The adjustment was performed using Excel software taking the best coefficient of determination (R^2).

$$\sigma = \boldsymbol{k}.\ (\dot{\gamma})^{n} \qquad (1)$$

$$\sigma = \sigma_0 + \boldsymbol{k}.\ (\dot{\gamma})^{n} \qquad (2)$$

Where σ is the shear stress, $\dot{\gamma}$ is the shear rate, $\boldsymbol{n}$ is the flow index, $\boldsymbol{k}$ is the consistency index, and σ_0 is the shear required for the initiation of the fluid flow (Yield stress).

The parameters evaluated were the apparent viscosity at 100 and 500 s^{-1}, the flow index, the consistency index, and the values of yield stress. For the dynamic oscillatory tests at low shear, preliminary tests were initially performed of shear scans at 25 °C at a constant frequency of 1 Hz. This was done to verify the range of linear viscoelasticity, i.e., to ensure that the shear tests were not exceeding 5%. Within the linear range, sequential scans were performed to describe the variation of elastic (G') and viscous (G") modules of the albumen and yolk samples according to the heating temperature and oscillation frequency.

The same stainless steel cone-plate geometry of 60 mm diameter was used. The first step consisted of an oscillatory frequency scan test between 0.1 and 10 Hz at 25 °C followed by a heating ramp and subsequent cooling ramp with a fixed heating and cooling rate of 1 °C per minute. After the cooling test, the sample was again subjected to an oscillatory frequency scan between 0.1 and 10 Hz at 25 °C.

The gelation temperature was initially characterized by considering the point of equality between the elastic and viscous modules. However, given that many samples already presented a higher elastic modulus to the viscous modulus at the beginning of the experiment, the significant point of development of the elastic gel structure was taken. This was arbitrarily defined as the point at which the derivative of the elastic modulus' variation as a function of temperature (dG'/dT) was greater than 5 Pa. All assays were performed in triplicate, and the development temperature data was subjected to analysis of variance and evaluated with a Tukey test ($p < 0.05$) using the software R [14].

Results and discussion

The results of the flow curve compared to the shear rate are presented in Figure 1A for albumen and in Figure 1B for egg yolk.

Figures 1 indicate that both the albumen and yolk of rhea eggs displayed shear dependent flow as in both cases, the ascendant and descendant ramps presented significant hysteresis. The fractions of albumen and yolk displayed the same flow at the time of shearing for all storage periods, and the intensity of flow varied (Table 1 and 2).

Albumen (Figure 1A) presents lower shear values than yolk (Figure 1B), indicating that yolk is a more structured material. The first ascendant ramp of the shear rate of 0 to 500 s^{-1} was higher than the second descendant ramp for albumen and was lower for yolk. This result implies that rheological behavior is dependent on the time of thixotropy for albumen and time of rheopexy for yolk.

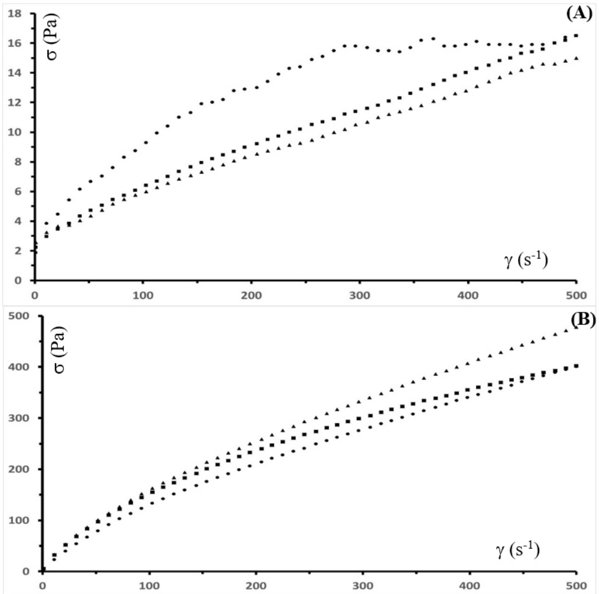

Figure 1. Flow curves for albumen (A) and yolk (B) of rhea's egg. Ascending ramp (●), downward ramp (■), e second ascending ramp (▲) of strain rate.

The rheopectic behavior observed in rhea egg yolk (Figure 1B) can be associated with an emulsifying effect on the system with the application of shear. It should be noted that yolk is a system rich in water, fats, and proteins, which in turn have emulsifying activity. It is likely that the shear stress promoted a homogenization of this complex system, and that the emulsifying action of proteins promoted the formation of a more complex structure, culminating in the increased shear observed (rheopectic behavior). Ohata & Viotto [3] observed thixotropic behavior in rheological tests with chicken egg yolks in conditions of high shear rate and shear stress. The results obtained in this experiment for rhea egg yolk showed different behavior to that obtained for commercial eggs.

After the third shear curves (third ramp), the material passed through an intense shear, culminating in the modification of the structure and, therefore, a withdrawal of the dependence of the rheological behavior with time. Therefore, these curves were adjusted to the power law (pseudoplastic) and HB models obtaining results for albumen (Table 1) and egg yolks (Table 2), with the previous sample storage periods of 1, 7, 14, 21, and 28 days.

Table 1. Rheological behavior (Herschel Bulkley Model and Power Law Model) for rhea egg albumen stored in periods of 1, 7, 14, 21 and 28 days at 10°C

Storage (Days)	n_{100} (Pa.s^{-1})	n_{300} (Pa.s^{-1})	Power law models			Herschel–Bulkley (HB) model			
			k (Pa.s^n)	n	R^2	σ_0 (Pa)	k (Pa.s^n)	n	R^2
1	0.06 **b***	0.05 **a**	1.23	0.34	0.969	3.94	0.11	0.91	0.998
7	0.07 **ab**	0.05 **a**	1.67	0.39	0.999	3.56	0.11	0.81	0.969
14	0.06 **ab**	0.05 **a**	1.52	0.37	0.964	4.01	0.10	0.75	0.980
21	0.05 **b**	0.03 **a**	1.41	0.38	0.984	2.99	0.05	0.94	0.983
28	0.09 **a**	0.04 **a**	1.88	0.44	0.995	5.74	0.07	0.84	0.981
C. V.	27.01	36.16	42.14	21.28	21.28	-	-	-	-
P value	0.34	0.01	-	-	-	-	-	-	-

(n 100; n 300) = Viscosity; (K) = Consistency Index; (n) = Behavior Index; (σ_0 = Residual tension);
(R^2) = Coefficient of determination
* Means followed by different letters in columns differ significantly by Tukey test p<0,05 significance.

Table 2. Characterization of rheological behavior (Model of Power Law and Herschel Bulkley Model) for rhea's egg yolk stored periods of 1, 7, 14, 21 and 28 days at 10°C

Storage (Days)	n_{100} (Pa.s^{-1})	n_{300} (Pa.s^{-1})	Power law models			Herschel–Bulkley (HB) model			
			k (Pa.s^n)	n	R^2	σ_0 (Pa)	k (Pa.s^n)	n	R^2
1	1.42 a	1.01 a	8.61	0.16	0.996	0.21	6.51	0.79	0.999
7	1.76 a	1.24 a	7.65	0.69	1.000	0.25	7.59	0.69	0.999
14	0.55 b	0.44 b	2.15	0.72	0.993	0.01	1.36	0.82	0.998
21	0.74 b	0.45 b	2.71	0.85	0.998	0.01	2.87	0.69	0.999
28	0.69 b	0.41 b	2.42	0.76	0.999	0.41	2.41	0.78	0.999
C. V.	32.49	30.61	41.45	8.28	1.08	-	-	-	-
P value	0.34	0.21	-	-	-	-	-	-	-

(n 100; n 300) = Viscosity; (K) = Consistency Index; (n) = Behavior Index; (σ_0) = Residual tension
(R^2) = Coefficient of determination
*Means followed by different letters in columns differ significantly by Tukey test p<0,05 significance.

The consistency index (k) was significantly higher for yolk, indicating higher viscosity for this egg fraction by both the power law and HB models. This can also be verified by higher values of apparent viscosity at 100 and 300 s^{-1}. For the initial storage period, the viscosity for the yolk at both low shear (n_{100}) and high shear (n_{300}) was higher, undergoing a significant reduction ($p < 0.05$) from 14 days on. For albumen, the viscosity at low shear (n_{100}) displayed differences between the storage periods, and for high shear (n_{300}), no significant differences were found. The viscosity values indicated that albumen displayed different behavior when subjected to high and low shear, whereas yolk showed no such differentiation. The flow index of yolk ranged between 0.16 and 0.81 on different storage days, and between 0.34 and 0.44 for albumen, indicating the pseudoplastic behavior ($n < 1$) of the rhea egg samples.

It can be observed that the viscosity ($n_{100,}$ n_{300}) and the consistency index (k) of yolk decreased from the seventh to the fourteenth day (observed for the two adjusted models), which may be related to a decrease of the sample structure throughout the storage period. In contrast, the yield stress parameter (σ_0) decreased from the seventh to the fourteenth day, increasing again on the twenty-eighth day.

Albumen displayed lower values of consistency index (k) and apparent viscosity at 100 and 300 s^{-1} compared to egg yolk, but the flow variation in these parameters was different for the two fractions. With storage, according to their compositions, the albumen underwent less variation, indicating a stability of consistency with storage time. The yolk, which was initially more consistent, underwent more intense changes in consistency with time. It was also observed that albumen presented lower flow index (n) than yolk. In contrast, the values of initial flow shear were higher for albumen than those observed for yolk. The characterization of the viscoelastic behavior of albumen and yolk fractions in eggs stored for seven days, according to heating and cooling temperature, in Figure 2.

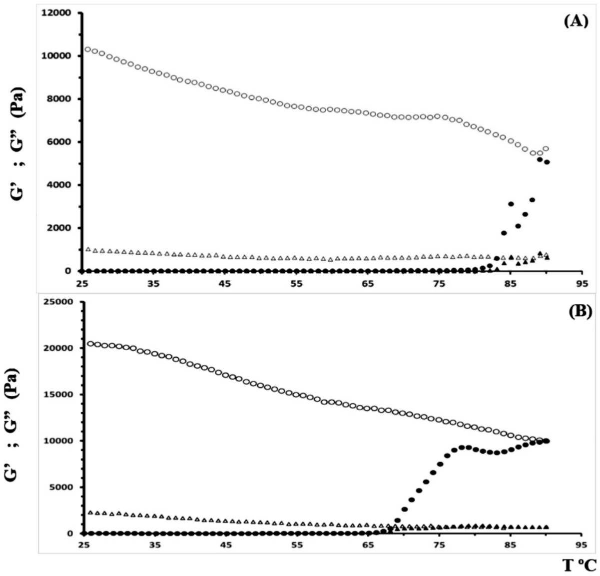

Figure 2. Dependence of elastic modulus (G') and viscous (G") with respect to temperature in the albumen (A) and yolk (B). Heating (●G';▲G"). Cooling (○G' e△G").

With heating, both albumen and yolk showed a change in the viscous and elastic modulus (gel). For albumen, a significant increase of the elastic modulus was observed from approximately 80 °C, while for yolk this variation was initiated at approximately 69 °C. It may be noted that from these temperatures, the increase of the elastic modulus was more pronounced for albumen than yolk, indicating a difference in the development of the elastic structure of the gels obtained by heating for these two systems.

In both systems, a significant increase of the elastic modulus (G') was observed from the cooling cycle, indicating strengthening of the elastic gel structure with the cooling of the system. Additionally, it can be observed that the elastic modulus was higher than the viscous modulus over the whole temperature range, whereas the viscous modulus did not undergo much change. During heating, the variation of the elastic modulus of the systems was analyzed differentially (dG'/dT) to allow better visualization of abrupt changes in the development of the gel structure. The results of the variation of the elastic modulus are presented in Figure 3 (A) for albumen and Figure 3 (B) for yolk.

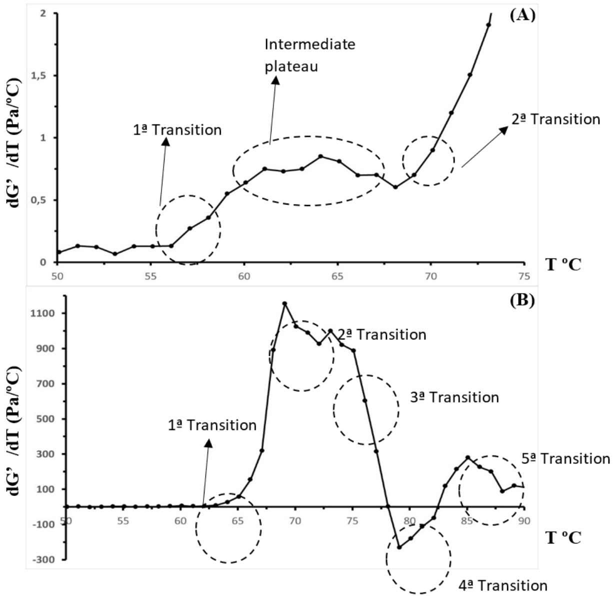

Figure 3. Transitions of behavior of the rate of change of the elastic modulus as a function of the heating temperature (dG'/dT) throughout the albumen heating (A) and yolk (B) of rhea egg stored for seven days at 10ºC

It is important to emphasize that the derivative of the elastic modulus (dG'/dT) allowed the determination of the rate or speed with which changes in the structure of the gel occur with heating. For albumen, changes were characterized in a first range of transition from approximately 56 °C, where an increase in the rate of structural modification was seen. An intermediate zone followed this between approximately 60 and 67 °C, defined as the intermediary plateau, where changes in the rate were smaller. A second range of transition followed from approximately 69 °C, where an increase of the elastic structure occurred.

Such transition zones may be related to thermal denaturation of specific fractions of proteins contained in the albumen that have different initial denaturation temperatures and therefore contribute specifically to development of the gel structure. Such behavior was observed in similar tests performed by Christ [15] in the assessment of albumen gel structure development in hen eggs.

The same heat treatment was applied to yolk, and different patterns were observed. Five bands of transition were observed in yolk; the first at 64 °C, the second at around 68 °C, the third at 76 °C, the fourth around 79 °C, and the last from 85 °C. Unlike albumen, the yolk presented three zones of reduction of the variation rate of the elastic modulus with temperature (second, third, and fifth band). These data, as with albumen, may be related to thermal denaturation of protein fractions in the constitution of the yolk. In addition to reductions in the rate of variation of the elastic modulus, this may lead to a possible structural accommodation with the unfolding of specific protein fractions.

Before being subjected to heat, egg and its fractions have a liquid texture, and the molecules are bound and configured in their three-dimensional structures. With the application of heat, from 60 °C, new covalent bonds are formed, making the gel firmer and stronger, and therefore elastic [7]. The proteins of egg albumen react to heat first, followed by yolk proteins. The gelation process of protein in rhea eggs begins with albumen at a temperature of 57 to 62 °C [16, 17]. The results obtained for albumen and yolk gelation for rhea eggs indicated gelation start temperatures of 80 °C for albumen and approximately 69 °C for yolk. Compared to chicken eggs, rhea eggs displayed a higher gelation temperature, indicating new possibilities for processing the albumen and yolk fractions at higher temperatures than those currently used industrially, while maintaining protein integrity. The gelation temperatures were obtained from the point at which the derivative of the elastic modulus, as a function of temperature (dG'/dT), presented values higher than 5 Pa. The data obtained are listed in Table 3.

Table 3. Developing temperature of the structure of the yolk gel and the albumen of the rhea egg

during sotrage (days)	yolk °C	albumen °C
7	64,9 **ab** ±1,4	78,1 **a** ±2,0
14	66,8 **a** ±1,0	78,2 **a** ±3,3
21	66,6 **ab** ±0,7	80,6 **a** ±3,5
28	63,8 **b** ±1,2	78,9 **a** ±1,6
CV	1,69	3,45

* Averages followed by different letters in the same column, within each variable, differ from each other by the Tukey test p<0.05

It can be observed that for rhea egg yolks, gelation occurred from 68 to 69 °C, not undergoing significant changes ($p > 0.05$) between the first and the twenty-seventh day of storage. For the albumen of rhea eggs, temperatures between 80 to 81 °C were observed with no significant differences ($p > 0.05$) in gelation temperature for different storage periods. Figure 4 shows the oscillatory shear frequency scan values in albumen (A) and yolk (B) samples, respectively.

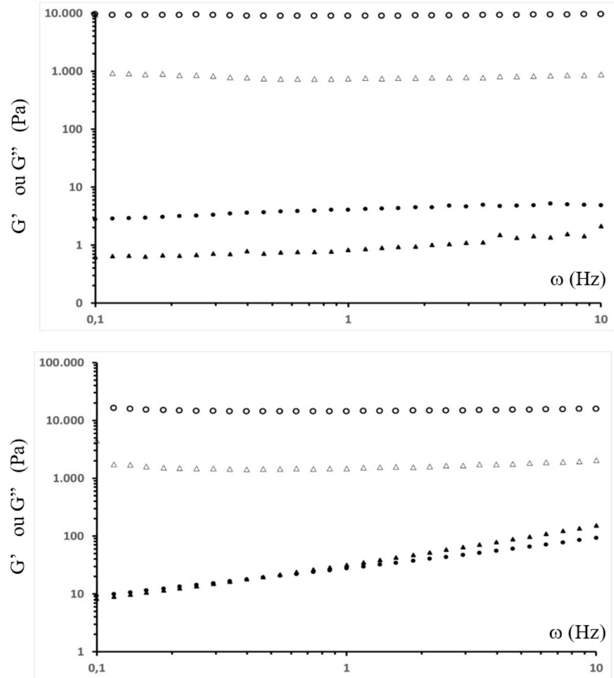

Figure 4. Frequency ramp for albumen (A) and yolk (B) of rhea egg. Before heating closed symbols ●G' e ▲G''; after cooling symbols ○G' e △G''.

Generally, albumen and yolk presented far superior elastic modulus to the viscous modulus in the entire range of oscillatory frequency and were not dependent on that frequency (constant values). This characterizes these systems as strong gels before heating and particularly after cooling. However, yolk demonstrated a different behavior before warming, when there was a strong dependence of viscous and elastic modules on cooling and very close values of the moduli. This can be linked to a weak gel structure before heating.

Conclusion

The albumin and yolk fractions of rhea eggs display rheological behavior dependent on shear time, with thixotropy in albumen and rheopexy in yolk. Both systems also feature pseudoplastic behavior and small initial flow shear for all storage periods. The gelation temperatures of albumen and yolk are higher than those observed in commercial eggs and do not undergo significant variations with storage. The highest values of viscosity and gelation temperatures obtained for the rhea eggs may determine potential changes in the industrial processing system, as this information demonstrates that they may withstand higher temperatures without modifying protein structures and hindering processing.

Conflict of interests

The authors declare no conflict of interest

Author contributions

Conceptualization: R. C. Cervi, M. A. Andrade, A. L. F. Cavallieri, C. J. de Moura and M. B. Café. *Formal analysis*: R. C. Cervi. *Investigation*: R. C. Cervi, M. A. Andrade, A. L. F. Cavallieri, C. J. de Moura and M. B. Café. *Supervision:* M. A. Andrade and M. B. Café. *Writing (original draft):* R. C. Cervi. *Writing (review & editing):* R. C. Cervi and M. B. Café.

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