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Investigation of the effects of reverse osmosis and ultrafiltration treatments on physicochemical, techno-functional, and rheological characteristics of liquid egg albumen and prepared meringue cookie batter

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ABSTRACT

Membrane filtration has a promising advantage in the processing of egg products. In this study, liquid egg whites (LEW) were separately concentrated by using reverse osmosis (RO) and ultrafiltration (UF) techniques. The first aim of this research was to determine the effects of the concentration pre-treatments on the physico-chemical quality criteria (pH, relative whipping capacity, foaming stability, water holding capacity-WHC, colour) and the rheological behaviour (viscosity, oscillation) of LEW samples. The second aim of this research was to investigate the impact of the membrane pre-treatment on meringue's functional quality attributes such as meringue batter density and meringue batter colour values (L^*, a^*, b^*). The average dry matter of LEW was increased from 12% to 23% by the concentration pre-treatment process (RO and UF), and water was removed from the LEW. In addition, the batter density of meringue cookie samples was 0.37 ± 0.01 g mL^{-1} in UF and RO. The differences between the pH and dry matter values of the ultrafiltration and reverse osmosis treated groups were found to be statistically similar, and it was observed that the elastic modulus (G′) increased with the increase in frequency in the rheological measurements. It has been determined that G′ is higher than G″ in all samples. An elastic/solid-like (G′ > G″) structural behaviour was determined while increasing the frequency value. It was determined that the

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WHC of LEW treated with RO was higher than of the group treated with UF. The results of the study showed that while reducing transportation and storage costs, the water content of >80% of LEW (raw material-the treated liquid egg white) removed by UF and RO applications can be suitable to produce meringue batter cookies (semi-finished product).

KEYWORDS

egg, liquid egg, membrane filtration, meringue, quality criteria, functional properties and rheological characterisation

1. INTRODUCTION

Eggs have been consumed by humans for centuries. Industrially processed egg products (liquid, frozen, powder) are considered as important ingredients for the food industry. Liquid egg white (LEW), known as egg protein (albumin), is the best protein source and is used for different purposes such as foaming, gelling, and binding in the production of many processed foods (Stefanova et al., 2021; Lv et al., 2022). Because of its excellent foaming ability, LEW is a popular aerating agent for baking products such as meringue cookies. The concentration of liquid egg products (whole egg and LEW) to obtain the highest product quality is usually applied before the dehydration process (Pei et al., 2020). Membrane filtration techniques for processing concentrates are attractive due to maintaining the natural nutritional and organoleptic characteristics of eggs (Márki et al., 2012). Ultrafiltration or reverse osmosis is a LEW concentration process used before drying in egg white powder production. The reverse osmosis (RO) and ultrafiltration (UF) are applied for concentration (dewatering) of LEW to reduce volumes by half (from 11% to 23 of dry matter) and liquid whole egg (from 24% to 36% of dry matter) to reduce drying process energy costs and increase the capacity of egg spray drying process (Froning et al., 1986). Concentration of eggs (LEW and whole egg) focuses on increasing the total solids by removing water without heat transfer and reducing energy costs. Dewatering of egg products may alter gel strength and functional properties, which affect the baked products such as cake, meringue, etc. By using a semipermeable membrane filter, nearly two times of solids can be transported by concentrating LEW up to 20%. During a reverse osmosis process operation, the LEW is forced towards a membrane filter that allows only the water to pass without any product loss. In the ultrafiltration process, the LEW flows towards a membrane that allows not only water, but also certain sugars (glucose), sodium, potassium, and salts to pass through the membrane filter. The concentration of LEW by membrane filtration method has been studied by several researchers (Froning et al., 1986; Conrad et al., 1993; Girton et al., 1999; Ould Eleya and Gunesekaran, 2002; Márki et al., 2012; Asaithambi et al., 2022). Ultrafiltration is mainly used for the concentration of LEW and whole egg, while reverse osmosis is used for the concentration of LEW before drying in the egg industry.

Membrane filtration has a promising advantage in the processing of egg products and application in industrial meringue manufacturing. In the study, LEW samples were processed by reverse osmosis (RO) and ultrafiltration (UF) techniques, and the treated eggs were used in model food (meringue cookie), which has not been researched before. The first aim of the study was to remove the water from LEW by RO and UF while increasing the level of dry matter to 23 from an average of 12. The second aim of this research was to investigate the impact of the membrane pre-treatment on functional quality attributes of meringue, such as meringue batter density and meringue batter colour values.

2. MATERIALS AND METHODS

2.1. Materials

The LEW samples were provided by an industrial-scale liquid egg production and processing company (Dibako Sterile Egg Co., Afyonkarahisar, Turkiye), the continuity of the cold chain (+4 °C) was ensured during the transport to the laboratory for analysis and treatments.

2.2. Liquid egg treatments - concentration of by membrane filtration techniques

After egg breaking and separation of the albumen (LEW) from egg yolk, the chalaza was removed from the albumen. The ultrafiltration treatment of LEW was conducted at 10 bar and the reverse osmosis was applied at 30 bar using the laboratory scale membrane filtration module (Sterlitech CF016A Cell Membrane Filtration and High-Pressure Pump and its connected units). The operation was carried out to protect the denaturation of LEW under thermal or mechanical stress during processing. In both treatments, the LEW samples reached 23 °Brix solid content (dry matter: °Brix, Ref%) as a target concentration value. Concentrated LEW samples were taken into flexible packages of 10 kg PE/EVOH bag-in-box.

2.3. Meringue preparation from treated LEW

Meringue cookies, a simple model food system, were made by combining sugar with LEW. To 200 mL LEW 350 g sugar was added and it was mixed using a Hobart N50 mixer at speed 3 for 5 min (Yüceer, 2020a). The specific density of the meringue batter was determined according to Yüceer and Caner (2022c).

2.4. pH determination of LEW

Measurements of pH values in LEW samples were done with a pH meter at 20 ± 2 °C (Yüceer, 2020b).

2.5. Foaming characteristics of LEW

A 75 mL of LEW samples were whipped in a Hobart mixer (N50CE, Hobart Foster Scandinavia A/S, Denmark) at 281 r.p.m. for 3 min and then 580 r.p.m. for 3 min at room temperature. The relative whipping capacity (RWC) was measured using a graduated cylinder as described by Yüceer and Caner (2022a).

$$\text{RWC }(\%) = (\text{volume of prepared foam} - \text{volume of liquid drainage}) \,/\, \text{original volume of liquid} \times 100 \qquad (1)$$

Foam stability was measured after the foam had been allowed to rest for 60 min (Yüceer, 2020b).

2.6. Colour analysis of LEW and meringue batter

Measurements in LEW and LEW foam samples were performed by measuring L^*, a^*, b^* values with a colorimeter (CR-400, Konica Minolta Sensing, Japan) (Yüceer and Caner 2022b). The total colour difference (ΔE), Metric Chroma (magnitude - C^*), Metric Hue Angle (h^*), and WI (Whiteness Index) values were calculated according to Equations (2), (3), and (4).

$$\Delta E^*_{ab} = \left[(L^*_2 - L^*_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2\right]^{1/2} \quad (2)$$

$$\text{Chroma} = C^*_{\text{ab}} = \left[a^{*2} + b^{*2}\right]^{1/2} \quad (3)$$

$$Hue = h^*_{ab} = \arctan(b^*/a^*) \quad (4)$$

2.7. Determination of LEW water holding capacity

LEW gels were prepared by heating the treated and non-treated samples at 45 °C for 45 min in a glass tube using a hot plate (FOUR E's Scientific Co, Ltd, Guangzhou, China) according to Yuceer and Caner (2022). The water holding capacity (WHC) of LEW samples were determined as described by Sun et al. (2019) and (Gao et al., 2022) with slight modifications. The 2 mL of treated and non-treated samples were centrifuged (Hettich/Mikro 185, Tuttlingen, Germany) for 30 min at 5,000×g at 25 °C. The mass of the sample before (m_1) and after (m_2) centrifugation was weighed and calculated according to Equation (5).

$$\text{WHC}(\%) = m_2 \ / \ m_1 \times 100 \quad (5)$$

2.8. Rheological behaviour of LEW

The rheological behaviour of LEW was performed with HR-2 (TA Instruments, USA) rheometer 40 mm SS plate-plate geometry measurement sensor at 1 mm intervals. In the measurement, 1.3 mL of albumen sample was placed between the rheometer plates. Flow ramp test was conducted at 25 ± 0.01 °C for 150 s from a low shear rate (0.01 s^{-1}) to a high shear rate (100 s^{-1}). The oscillation amplitude scanning test was performed to obtain the optimal % strain in the linear viscoelastic region. An angular frequency of 20 rad s^{-1} was applied, and the analysis was performed at values varying from % strain between 0.01 and 100. The oscillation frequency test was performed at 25 °C between 0.01 and 10 Hz. The oscillation temperature ramp scanning was conducted between 37 and 80 °C with a heating rate of 1 °C min^{-1}. Each sample was renewed and measured 3 times (Yüceer, 2022).

2.9. Batter density of meringue

The density measurement of meringue batter was calculated by dividing the weight by the volume of the LEW sample with sugar using a fixed volume of solids to weight. The batter volume was presented as a density (weight per volume) and expressed as g cm^{-3}, according to Mizu and Nagao (2010). Analyses were performed on day 0 (initial day) using an LEW and sugar mixture (freshly whipped meringue batter). Results between 0.35 and 0.40 (g cm^{-3}) meringue batter density were accepted as success (Yüceer and Asik, 2020).

2.10. Statistical analysis

The statistical analysis was performed by LSM-PROG GLM (SAS 2003). The data was calculated using a one-way analysis of variance (ANOVA). The significant level was set at $P < 0.05$ in the study.

3. RESULTS AND DISCUSSION

The changes in pH values of treated LEW samples are presented in Fig. 1. The pH values of different pre-treated LEW samples varied between 9.32 and 9.34. The effects of ultrafiltration and reverse osmosis applications on the pH values were found to be statistically similar ($P > 0.05$).

LEW foams can be incorporated into a variety of foods and are used in many recipes (Damodaran, 2007). LEW as basically a protein colloid solution, can expand to 6 to 8 times its volume and form stable foams when whipped. Foaming stability increased significantly with applied force and time due to the conformational modifications of the LEW protein increasing the surface hydrophobicity. Foam is a colloidal system in which small air bubbles are dispersed in an aqueous continuous phase. LEW proteins, mostly globular proteins, are expected to increase surface hydrophobicity and flexibility by partially opening the proteins, making them

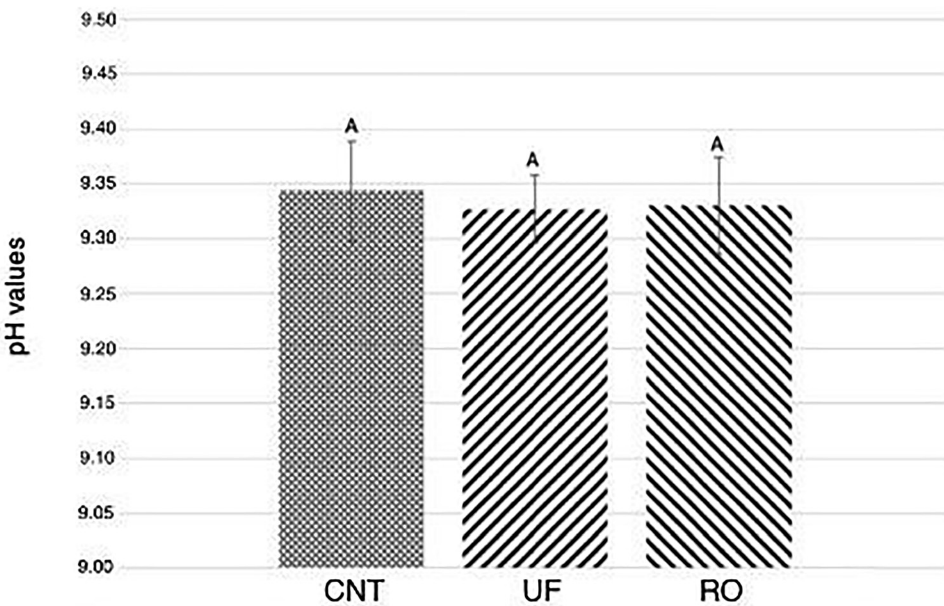


Fig. 1. The effect of membrane ultrafiltration and membrane reverse osmosis filtration on the pH values of liquid egg white (LEW) samples at the initial day (CNT-control, UF-ultrafiltration, and RO-reverse osmosis treated samples). Results are presented as means ± standard deviations of triplicate measurements. [A]: Different capital letters mark significant difference between treatments ($P < 0.05$)

better surfactants (eg foam-forming agents) and improving foaming ability. Structural modification of LEW proteins can be achieved by partially unfolding the proteins (Mine, 1995). According to the foam properties results, the treatment of UF and RO decreased the RWC values in LEW samples. The RWC values of different pre-treated and non-treated LEW samples varied between 473 and 646. The foam values were found to be significantly similar for RO (540 ± 65) and UF (473 ± 150) applications ($P > 0.05$). In Fig. 2a the RWC values of LEW samples are provided. The membrane filtration treatments had no significant adverse effect on the foaming ability of LEW. Molecular flexibility plays a role in protein foaming ability, which is associated with interfacial tension-lowering properties.

Foam stability depends on surface viscosity of albumen. The change in foam stability values of treated LEW samples are presented in Fig. 2b. The foam stability values of different treated and non-treated LEW samples varied between 28 and 46. Changing the pH of a protein medium leads to the unfolding of the proteins. The treatment of UF (28 ± 7) and RO (36 ± 5.2) decreased the foam stability values in LEW samples compared to the control group (46 ± 7.6).

The effect of membrane ultrafiltration and membrane reverse osmosis filtration on LEW's colour values (L^*, a^* and b^*) are presented in Table 1a. It was determined that L^* brightness values decreased statistically significantly in the treatment of LEW samples with ultrafiltration and reverse osmosis. The L^* values of different pre-treated and non-treated LEW samples varied between 52.44 and 61.29. During the reverse osmosis process operation, LEW is forced towards a membrane filter that allows only the water to pass without any product loss in mass. In the ultrafiltration process, the LEW flows towards a semipermeable membrane that allows not only

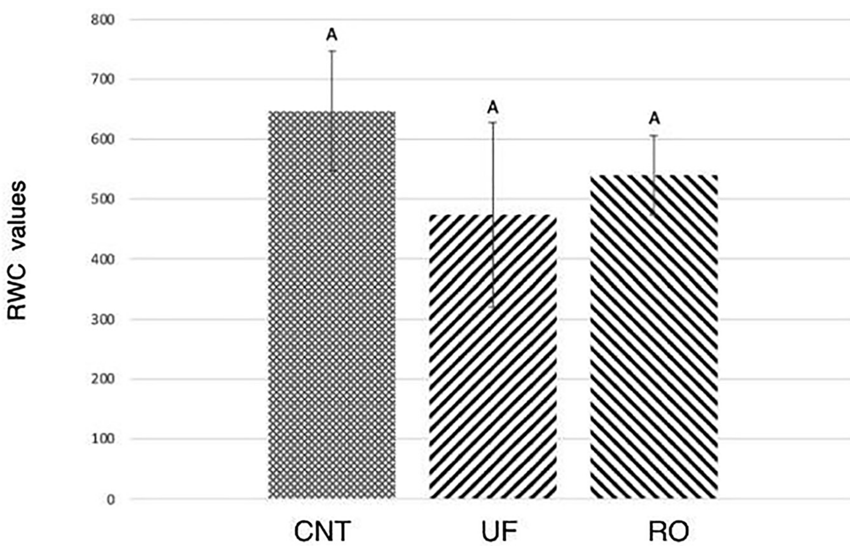


Fig. 2a. The effect of membrane ultrafiltration and membrane reverse osmosis filtration on Relative Whipping Capacity (RWC) values of liquid egg white (LEW) samples at the initial day (CNT-control, UF-ultrafiltration, and RO-reverse osmosis treated samples). Results are presented as means ± standard deviations of triplicate measurements. [A]: Different capital letters mark significant difference between treatments ($P < 0.05$)

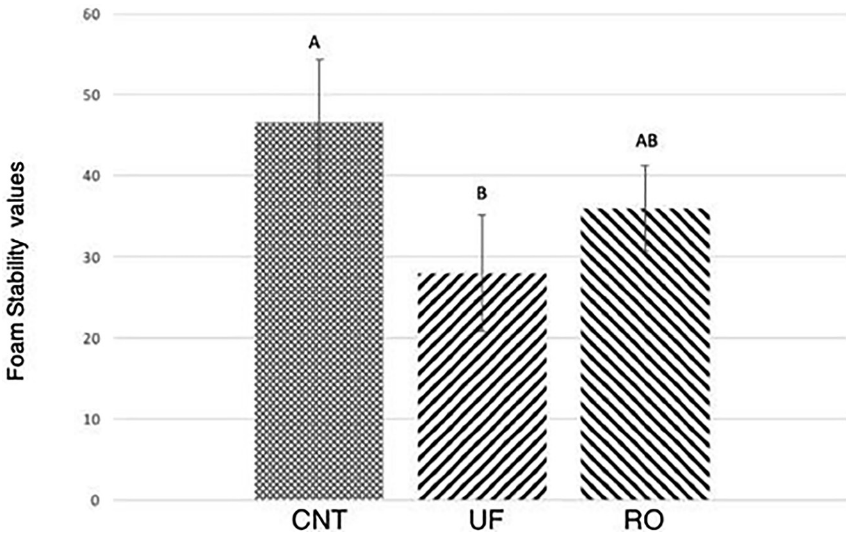


Fig. 2b. The effect of membrane ultrafiltration and membrane reverse osmosis filtration on foam stability values of liquid egg white (LEW) samples at the initial day (CNT-control, UF-ultrafiltration, and RO-reverse osmosis treated samples). Results are presented as means ± standard deviations of triplicate measurements. [A, B]: Different capital letters mark significant difference between treatments ($P < 0.05$)

Table 1a. The effect of membrane ultrafiltration and membrane reverse osmosis filtration on colour values of liquid egg albumen at the initial day

Liquid egg white colour values						
Treatments	L^*	a^*	b^*	ΔE	*Chroma*	*Hue*
CNT	61.29 ± 0.68^a	-2.35 ± 0.02^a	8.87 ± 0.11^a	0	9.17	3.77
UF	52.44 ± 0.67^b	-2.40 ± 0.06^a	9.38 ± 0.02^b	8.86	9.68	3.90
RO	53.35 ± 0.70^b	-2.35 ± 0.05^a	9.24 ± 0.05^c	7.95	9.53	3.93

CNT: control; UF: ultrafiltration and RO: reverse osmosis treated samples.
Results were presented as means ± standard deviations of triplicate measurements ($n = 3$).
[a-c]: Means in the same column with different lowercase letters are significantly different in each colour parameter ($P < 0.05$).

water, but also certain sugars (glucose) and salts to pass through. The effect of membrane UF and membrane RO filtrations on the prepared meringue's batter colour value changes are presented in Table 1b. The L^* values of different meringue batter samples varied between 77.25 and 77.50. The colour indicators are close to the original colour of meringue batter colours and ΔE has the lowest value ($\Delta E < 3.0$). However, the colour difference index of the LEW treated with UF (8.86) and RO (7.95) treated samples shows a significant colour change, probably due to the high pressure of membrane treatment, which causes denaturation in protein structure during processing and the colour changes that occur are caused by water loss due to the dewatering process (Table 1).

Table 1b. The effect of membrane ultrafiltration and membrane reverse osmosis filtration on colour values of prepared meringue's batter at the initial day

Meringue batter colour values						
Treatments	L^*	a^*	b^*	ΔE	*Chroma*	*Hue*
CNT	77.50 ± 0.06^a	-0.73 ± 0.01^a	1.91 ± 0.05^a	0	1.39	−1.63
UF	77.25 ± 0.08^b	-1.16 ± 0.07^b	3.55 ± 0.08^b	2.41	3.73	−3.96
RO	77.38 ± 0.04^c	-1.20 ± 0.09^b	3.30 ± 0.10^c	2.17	3.51	−2.75

CNT: control; UF: ultrafiltration and RO: reverse osmosis treated samples Results were presented as means ± standard deviations of triplicate measurements ($n = 3$).
[a-c]: Means in the same column with different lowercase letters are significantly different in each colour parameter ($P < 0.05$).

It was determined that the water holding capacity values of LEW samples significantly increased due to the treatments of ultrafiltration and reverse osmosis (Fig. 3). The gel strength and the microstructural changes in LEW during membrane filtration treatment process could be one of the key factors affecting the water holding capacity. The coarser the gel structure, the weaker the gelling ability to bind moisture (mainly during baking of meringue cooking), and the

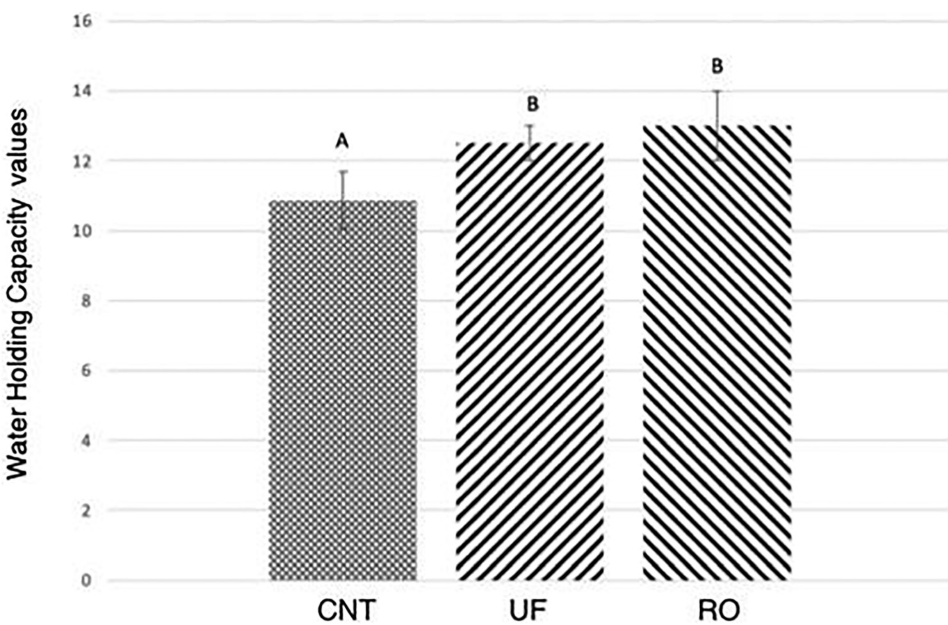
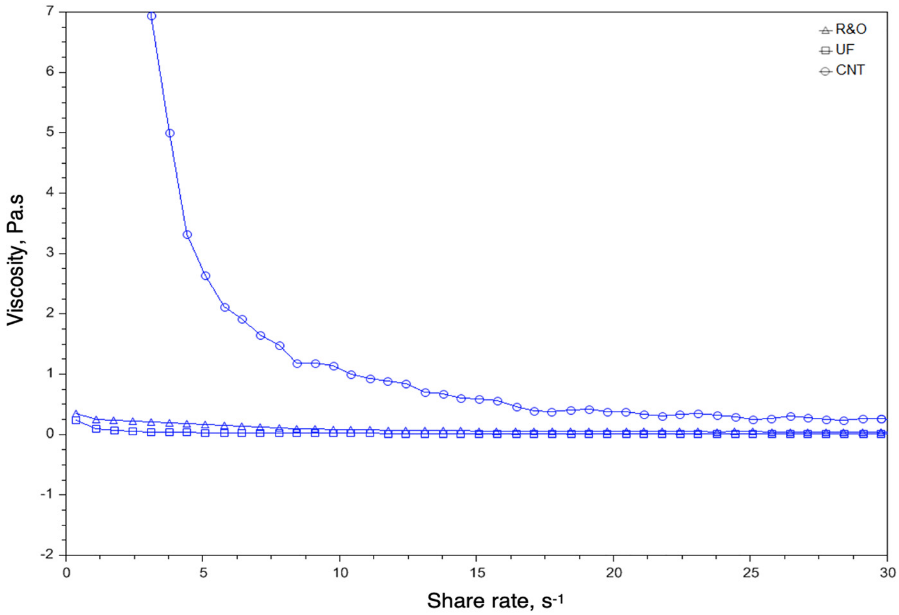
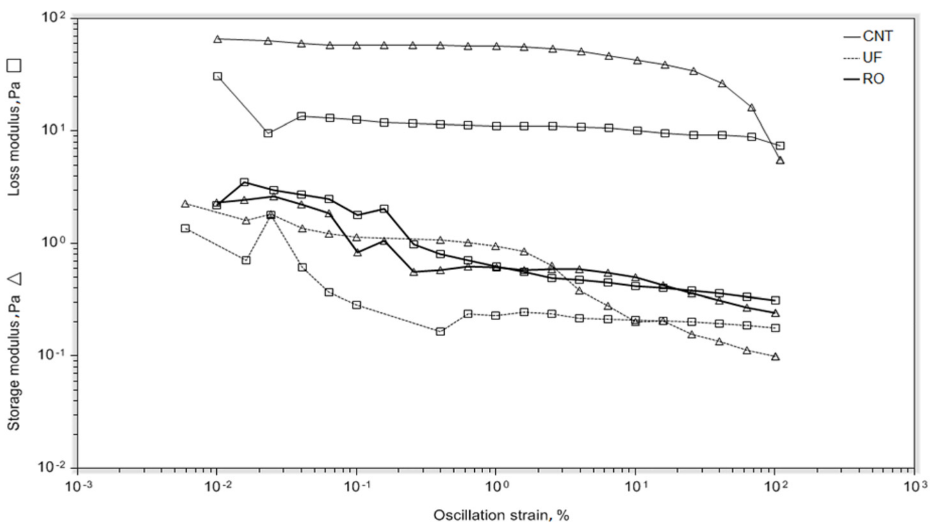


Fig. 3. The effect of membrane ultrafiltration and membrane reverse osmosis filtration on Water Holding Capacity (WHC) values of liquid egg white (LEW) samples at the initial day (CNT-control, UF-ultrafiltration, and RO-reverse osmosis treated samples). Results are presented as means ± standard deviations of triplicate measurements. [A, B]: Different capital letters mark significant difference between treatments ($P < 0.05$)

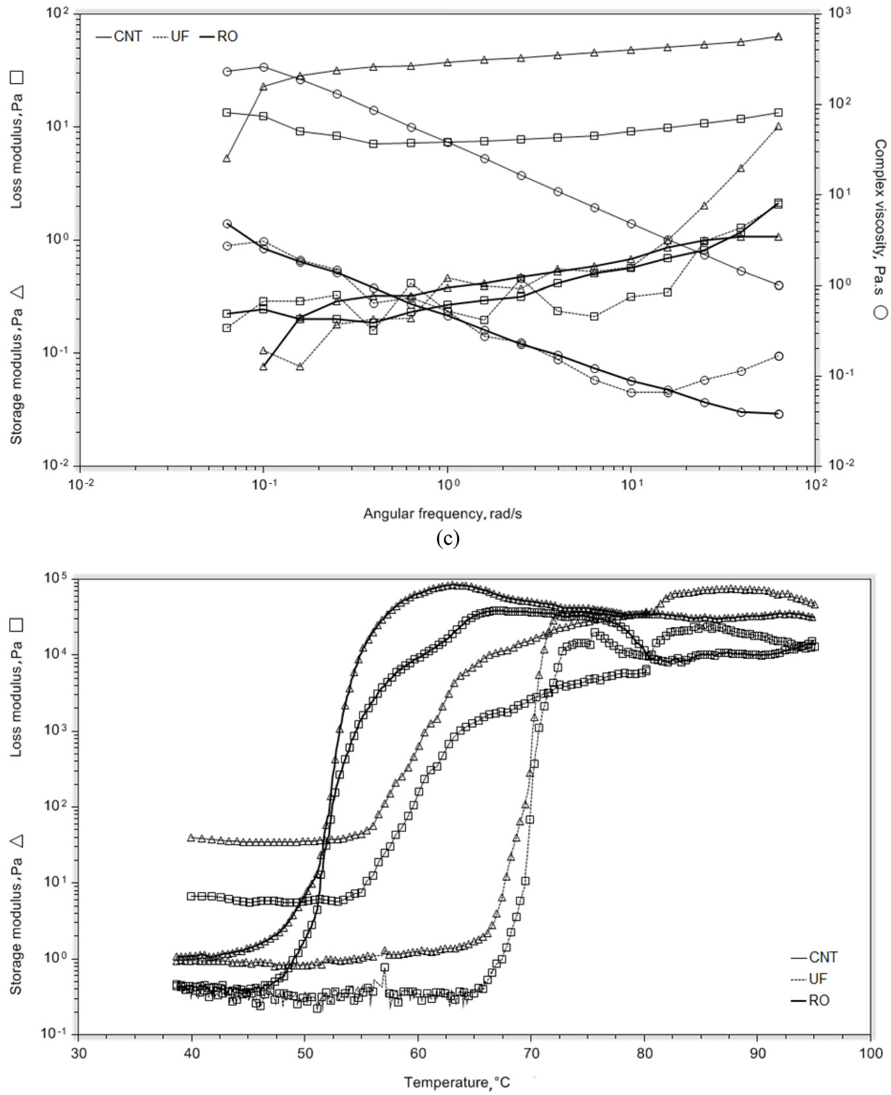


(a)



(b)

Fig. 4. The effect of membrane ultrafiltration and membrane reverse osmosis filtration on (a) viscosity curves (variation of viscoelastic behaviour), (b,c,d) oscillation curves (variation of viscoelastic behaviour with frequency and temperature) of liquid egg white (LEW) samples at the initial day (control and treated with UF: ultrafiltration and RO: reverse osmosis)



(c)

(d)

Fig. 4. continued

easier it is for water/moisture to be squeezed out in the final product (Wang et al., 2022). Rheology is a field of research used in a wide range of scientific studies. From the point of view of the food industry, its importance is that it determines the texture of food and also helps to develop product and process parameters. Rheology is introduced as a definitive quality control tool. LEW seemed to have a decreasing viscosity at increasing shear rates, and it could be said all LEWs showed shear thinning behaviour. The viscoelastic behaviour of the LEW samples was analysed by the yield stress value (Pa), which is among the quality parameters for liquid like products. The membrane process operation may lead to shear stress causing an irreversible deterioration in albumen proteins. The unique whipping properties of albumen proteins decreased after membrane ultrafiltration/reverse osmosis (Figs 2a and b). The correlation coefficient ($R^2 > 0.98$) was best fitted for the Herschel–Bulkley model in all LEW samples. The high elastic modulus indicates an extensive linear elastic region. LEW maintains its structural integrity against the increased shear stress. The non-linearity of the shear stress versus shear rate data indicates pseudoplastic, non-Newtonian behaviour. The apparent viscosity of LEW samples stabilised after UF and RO treatments probably due to the disulphide bonds between interfacial proteins. It was determined in the study that the elastic modulus (G′) increased with the increase in frequency, while the viscous modulus (G″) tended to increase first after being stable (Fig. 4). It was determined that G″ was higher than G″ in all LEW samples. In this case, it was observed that the LEW samples, which showed viscoelastic behaviour, exhibited a liquid-like structure. When the samples were examined, a liquid-like (G″ > G′) character was observed at low angular frequency values, while an elastic/solid-like (G′ > G″) structural behaviour was determined at increasing frequency values (Fig. 4). The LEW coagulation temperature was carried out by extrapolating the rapidly rising storage modulus (G′) to intercept the temperature axis. LEW loses its fluidity around 55 °C with loss modulus values increased depending on the increase in temperature, corresponding respectively to the denaturation temperatures of ovotransferrin and ovalbumin. The UF and RO treatments reduced the coagulation of LEW probably due to non-covalent interactions between interfacial proteins, which are responsible for droplet aggregations. The findings of Pei et al. (2020) showed similarity in the rheological results to our research.

The batter density of meringue batter is related to the air-retaining capacity that is incorporated in the batter structure. Meringue batter was prepared from the filtrates obtained with membrane ultrafiltration and membrane reverse osmosis (Table 2). The membrane filtration process reduced the meringue batter values significantly ($P < 0.05$). The batter density values were determined as 0.37 ± 0.01 g cm^{-3} for both applications (UF and RO). The lower the batter density, the crispier the texture and larger the volume of the meringue (Yüceer and Caner, 2021). It is observed that UF and RO treated samples decreased the batter density of the meringue samples, which may be related to whipping ability and the amount of air that is absorbed as a gas in the foam structure. Larger air bubbles in the LEW-sugar mixture system result in a decrease in the batter density and an increase in baked product yield.

Table 2. The effect of membrane ultrafiltration and membrane reverse osmosis filtration on the batter density values of meringues at the initial day

Treatments	Batter density values of meringues (g cm^{-3})
CNT	0.40 ± 0.01^a
UF	0.37 ± 0.01^b
RO	0.37 ± 0.01^b

CNT: control; UF: ultrafiltration and RO: reverse osmosis treated samples.
Results were presented as means ± standard deviations of triplicate measurements ($n = 3$).
$^{a-b}$: Means in the same column with different lowercase letters are significantly different ($P < 0.05$).

4. CONCLUSIONS

When the final meringue products prepared from liquid egg white treated with ultrafiltration reverse osmosis are compared, it has been determined that products with higher functionality are obtained with reverse osmosis. This has shown that it is possible to obtain egg products suitable for transportation, storage or export, especially due to the >80% water content of LEW. This result suggested that membrane filtration treatments could be used in egg dewatering to decrease the typical batter density of final product such as meringue to obtain low-density foams and increase physical stability. Additional process optimisation for membrane treatment of LEW will need to be investigated for complementary effects.

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