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ORIGINAL ARTICLE

Effect of citric acid addition on functional properties of pasteurized liquid whole eggs

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Abstract The aim of this study was to examine the influence of different concentrations of citric acid (0, 300, 400 and 500 mg/L) on the physical and functional properties of pasteurized liquid whole eggs (LWE) over 4 weeks of storage. The properties tested include pH, conductivity, colour, particle size, rheological, and textural properties, as well as protein solubility, foaming and emulsification. The 4 weeks of storage had a statistically significant ($P < 0.05$) effect on every tested parameter, while the addition of citric acid had a statistically significant ($P < 0.05$) effect on pH, conductivity, L^* and b^* values, protein solubility, emulsion activity index, emulsion capacity, emulsion stability, and an increase in foaming and texture parameters, but not on rheological parameters. Citric acid addition and a storage period of 4 weeks resulted in a change of pH and an increase in protein solubility. It also led to a lower foaming capacity and a larger drainage of the system, which causes a lower power (work load) requirement to break formed gels. Apparent viscosity did not change significantly in the samples with citric acid.

Keywords Liquid whole eggs · Physical properties · Functional properties · Citric acid · Storage

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Introduction

Eggs and egg products have an important and irreplaceable role in human nutrition due to high protein content, high bioavailability, valuable nutrients, the diverse range of applications, as well as a relatively low cost and high availability in most countries. The majority of eggs are consumed as shell eggs, but significant amounts are further processed into egg products, specifically pasteurized liquid whole eggs (LWE) (Kovacs-Nolan et al. 2005). LWE are primarily used as a food ingredient (bakery products, meat products, confectioneries, etc.). LWE contribute a high nutritional value and alter the functional properties of foods such as emulsifying, gelling, foaming, colouring and flavouring (Monfort et al. 2012; Lee 2002; Koc et al. 2011). For this reason, knowledge of the functional properties of LWE is critical.

Pasteurization is the most important processing step for egg products, and has been thoroughly investigated (Monfort et al. 2012; Lechevalier et al. 2005; Mine et al. 1990). Pasteurisation of LWE assures food safety by eliminating heat sensitive pathogens, but it can also influence egg quality by changing the functional properties through the denaturation, insolubilization and aggregation of proteins (Lee 2002). Egg pasteurisation decreases the foaming ability by denaturing ovotransferrin. The addition of citric and phosphoric acid salts and metallic ions such as Fe, Cu, Al, etc. improves the foaming properties of egg albumen after pasteurisation (Lomakina and Míková 2006). Non-thermal preservation of LWE with pulsed electric fields (PEF) is an attractive alternative to thermal pasteurization (Monfort et al. 2012), along with the incorporation of additives (potassium sorbate and citric acid).

Citric acid is a naturally occurring antioxidant that prevents the green discolouration of egg products and

lowers pH. Citric acid is widely recognized as a safe (GRAS) additive currently used in a variety of commercial liquid egg products to bind iron and prevent colour loss (greening) during cooking (Schuman and Sheldon 2003). Additives such as potassium sorbate and citric acid are commonly used in the production of LWE and other egg products (Lelieveld et al. 2007). Gongora-Nieto et al. (2003) investigated the shelf life of LWE that had been stabilized with 0.15% and 0.5% of citric acid in order to observe its effect on colour darkening, and to increase the effectiveness of pasteurisation treatment. The authors of the study concluded that LWE with 0.5% citric acid addition had a shelf life of 26 days. However, they did not investigate how varying concentrations of citric acid could affect the functional properties of LWE.

The aim of this work was to examine the influence of citric acid addition at different concentrations (0, 300, 400 and 500 mg/L) on the physical properties (pH, colour, electrical conductivity, protein solubility and viscosity) and functional properties (foaming, emulsifying, turbidity, texture and particle size distribution) of LWE during 4 weeks of storage.

Materials and methods

Samples of LWE

Egg samples were obtained from Lukač Ltd. from Zagreb, Croatia. Eggs were pasteurized at 66 °C, at a flow rate of 1000 L/h. For research purposes, samples of pasteurized liquid whole egg with three different concentrations of citric acid were prepared: 300, 400 and 500 mg/L. Control samples were samples with no added citric acid. In all samples, preservative potassium sorbate ($C_6H_7KO_2$), in an amount of 2.7 g/L was added. The samples were packaged in sterile bags (Optopack Ltd.) of 1 kg and packed in bag in box packaging (DS Smith Belišće, Croatia). Samples were stored during 4 weeks in refrigerated storage at 4 °C.

Physical properties

The pH was determined at 20 °C in triplicate with a pH meter (Pye Model 292, Pye Unicam).

A reflectance spectrophotometer Minolta CM-3500d (Osaka, Japan) was used to measure the colour of LWE samples. The L^* (lightness), a^* (redness), and b^* (yellowness) (CIE 1976) were measured. Chroma (C^*) and hue angle (h) was also measured. The average value for each sample parameter was the mean of 10 determinations. Conductivity were measured using a conductivity-meter (HI-2030-edge, Hanna Instruments), and temperature using an infrared thermometer (PCE-777, PCE Instruments).

Protein solubility

The protein solubility was measured by method Lowry et al. (1951). LWE samples were centrifuged at 4 °C for 10 min at 10,000×*g* in a Rotina 380R Refrigerated Centrifuge (Hettich Zentrifugen GmbH, Germany). Supernatant was used for further analysis. In a 10-mL test tube 100 µL supernatant, 500 µL of reactant A (Dc Protein Assay Reagent A) and 4 mL of reactant B (Dc Protein Assay Reagent B) was added. Then the tube was shaken vigorously for 30 s. After 15–20 min standing at ambient temperature, absorbance at 750 nm was measured on Spectrophotometer Helios Beta (Spectronic Unicam, Cambridge, UK).

Viscosity determinations

Torque measurements were carried out on the model systems using a Rheometric Viscometer (Model RM 180, Rheometric Scientific, Inc., Piscataway, USA) with the spindle (no. 3; ∅ = 14 mm; *l* = 21 cm). Shear stress against the increasing shear rates from the lowest value of 0–1290 s^{-1}, as well as downwards, was applied. The volume of the beaker was 36 mL. The samples were kept in a thermostatically controlled water bath for about 15 min before measurements (to obtain temperature of 25 °C). Measurements were done in triplicates. Rheometric computer program was used to interpret the shear rate versus shear stress. The values for n and k were obtained from plots of log shear stress versus log shear rate, according to the following equation:

$$\log \tau = \log k + n \log \gamma \quad (1)$$

where τ is the shear stress (Pa), k is the consistency coefficient (Pa s^n), n is the flow behaviour index and γ is the shear rate (s^{-1}).

Apparent viscosity (η_{app}) was calculated at 1290 s^{-1} using Newtonian law, in addition to linear least square method for regression analysis.

$$\tau = \eta_{app}\gamma \quad (2)$$

Functional properties

Foaming properties

Foaming properties were measured according to the method by Phillips et al. (1987). 100 mL of 15% LWE solution in deionized water was whipped with a beater (Braun Turbo 600 W) at the maximum speed of 12,500 RPM in a 1000 mL graduated glass for 2 min, and then foam volume was measured. Foaming capacity (% FC) was calculated using the following equation:

$$\%FC = [(V_2 - V_1)/V_1] \times 100 \quad (3)$$

where V_1 is the initial LWE volume and V_2 is the obtained foam volume.

The volume of drainage of liquid (V_d) was recorded at 10 min, the foam stability was calculated as follows:

$$\%drainage = V_d/V_1 \times 100 \quad (4)$$

Emulsifying properties

Emulsifying properties were measured according to the method by Zhao et al. (2007) at 20 °C. Fifty (50) mL of 5% LWE solution in deionized water and 50 mL of commercial sunflower oil were mixed using a iKAT18 basic ultraturrax (IKA, Staufen, Germany) for 5 min in order to prepare oil in water emulsions. The mixture was divided in three different plastic tubes and left at room temperature (20 °C) for 90 min. After that, the volume of the emulsified layer was measured. The emulsifying capacity (EC) was calculated using the following equation:

$$\%EC = (V_E/V_1) \times 100 \quad (5)$$

(V_E is the volume of the emulsified layer and V_I is the initial volume of LWE).

After that, emulsion stability (ES) was determined: the tubes with 30 mL of previously prepared emulsions were heated at 80 °C for 30 min in a thermostatic water bath and immediately cooled to 20 °C in ice water. After that, the volume of emulsified layer was measured and the percentage of emulsion (% ES) was calculated:

$$\%ES = (V_{E2}/V_{E1}) \times 100 \quad (6)$$

(V_{E2} is the volume of the emulsified layer that remains after heating and V_{E1} is the initial volume of emulsified layer).

Turbidity

Emulsions were prepared with 3% m/v protein suspension using sunflower oil (Zvijezda d.d, Zagreb, Croatia). Twenty (20) mL of 3% egg suspension and 10 mL of oil, were mixed for 90 secs in a blender. The absorbance of the diluted emulsions was measured by spectrophotometer (Helios-b, Pye Unicam Ltd, Cambridge, UK) at 500 nm in 1 cm path length cuvettes.

The emulsifying activity index (EAI) was determined by the turbidimetric method of (Krešić et al. 2008). The absorbance was read initially, after what turbidity (Nephelometric Turbidity Units, NTU) and EAI were calculated using the following formula:

$$T = \frac{2.303 \cdot A}{I} \quad (7)$$

where T = turbidity, A = absorbance at 500 nm and I = path length of cuvette (m).

The emulsion activity index (EAI) in m^2/g^1 was then calculated as:

$$EAI = \frac{2 \cdot 2.303 \cdot D \cdot A}{I \cdot \Phi \cdot C \cdot 10000} = \frac{2 \cdot T}{\Phi \cdot C} \quad (8)$$

where D = is the dilution factor, A = the absorbance at 500 nm, I = the path length of the cuvette (cm), ϕ = the volumetric fraction of oil; C = the weight of protein per unit volume of aqueous phase before the emulsion was formed (g mL^{-1}) and 10,000 the correction factor for square meters, T = turbidity (calculated from above equation).

For emulsion stability determination the emulsion was held at 4 °C for 24 h and reanalysed for emulsion activity as described previously. An emulsion stability index (min) was calculated by the following formula:

$$ESI = \frac{T \cdot \Delta t}{\Delta T} \quad (9)$$

where T = turbidity value at 0 h, ΔT = change in turbidity during 24 h period, Δt = time interval (24 h).

Textural properties of LWE

To evaluate textural properties gel of LWE was prepared by heating the suspensions placed in 150 mL glass beaker at 80 °C for 15 min in water bath with constant stirring. After that, the samples were cooled to room temperature by immersing the beaker in icy water and then kept at 4 °C for further analysis. Texture was determined 24 h after the gel preparation.

Gel hardness and work applied were determined with a texture analyser (Texture analyser HD+, Stable Micro Systems, Godalming, UK). The speed of the measuring probe was 1 mm/s after contact with gel surface and during penetration. Pull out speed of probe was set at 5 mm/s. The measurement depth was 10 mm. Tool used in analysis of mechanical properties is steel cylindrical probe with 6 mm diameter. Gel hardness was defined as maximal force applied (N) during first compression part of the TPA cycle. Work is analysed as surface under force-distance curve. Samples were assayed in triplicate.

Particle size distribution

Particle size was determined by laser light scattering (Malvern Mastersizer 2000 equipped with a 100 mm lens, Malvern Instruments Limited, Malvern—Worcestershire, UK with Hydro MU sample dispersion unit). Results were analysed with, Mastersizer 2000 software, using a Mie

scattering model for the analysis of the raw data (Jambrak et al. 2014).

Statistical analyses

Experiments were conducted as multifactor categorical design. Descriptive statistics were used to assess the basic information about the experimental dataset (e.g. to obtain sample basic metrics, check for normality of distribution, transform the variables if necessary). Variables were analysed using multivariate analysis of variance (MANOVA), and marginal means were compared with Bonferoni multiple comparison tests. Linear regression was employed to build and compare mathematical models. The significance levels for all tests were $P < 0.05$, while analyses were performed with Statgraphics Centurion software (StatPoint Technologies, Inc, VA, USA).

Results and discussion

Effect of citric acid addition on pH, conductivity, protein solubility and texture parameters

Table 1 shows the effect of citric acid addition (300, 400, 500 mg/L) on pH, conductivity, protein solubility and texture parameters of LWE. The pH value is a critical factors in maintaining the quality and properties of egg products. The pH value of liquid whole eggs ranges from 7.0 to 7.6, averaging 7.2 (Cotterill and McBee 1995). Control LWE's pH value was constant at 7.6–7.7 during the initial 3 weeks of storage, decreasing to 6.5 after 4 weeks of storage. Samples of LWE with a citric acid addition had a pH of 7.6–7.9 after 3 weeks and decreased to 6 after 4 weeks. Giampietro-Ganeco et al. (2012) found that storage at lower temperatures is a more effective way to maintain egg quality characteristics. Rêgo et al. (2012) found that the pH value of commercially pasteurized eggs decreases after 2 weeks of storage. Alleoni and Antunes (2001) investigation showed the pH value of the eggs stored at 5° C for 3 weeks ranged from 7.6 to 7.9, concurring with the results of this study. Microbiological analysis of LWE was continuously performed during storage (data not shown), ensuring that the LWE retained microbiological safety.

The level of conductivity determines many of the internal properties of treated food. The results in Table 1 show that after 3 weeks of storage all samples had an electrical conductivity above 5 mS/cm, with the highest electrical conductivity value was 6.38 mS/cm found in samples with 500 mg/L citric acid. More acidic samples had a higher conductivity: samples with the highest concentration of citric acid had the highest electrical conductivity after 3 weeks of storage. Malek et al. (2006) investigated the relationship between LWE and fruit juice conductivity, finding that LWE had higher conductivity. Compared with fruit juices, the value of conductivity of LWE was higher due to the large amounts of salt and acid concentration acting as electrolytes, conducting electricity (Halden et al. 1990). Studies have shown that the conductivity of a liquid influences the nature of the ion (chemical composition) and ionic fluid movement (Palaniappan and Sastry 1991).

The protein solubility of the control sample (sample with no citric acid) lowers with increased storage time. The protein solubility dropped 17.13% from 113.2 to 93.4%, between the first and second week of storage after which it remained stable until the 4th week of storage. The reduction in protein solubility of LWE was caused by the denaturation of the proteins and by the destruction of their tertiary and quaternary structures, intensifying the aggregation reactions between them and consequently reduces their solubility (Yang and Baldwin 1995; Wang and Tu 2008). The addition of 300 mg/L citric acid showed a positive effect on protein solubility. The concentration of protein solubility after 7 days of storage was 113.2 mg/mL and decreased 0.24% by further storage. However, the addition of citric acid in concentrations of 400 and 500 mg/L did not have the same effect. With the addition of 400 mg/L, the solubility was reduced by 3.40% from the first to the second week of storage (from 110.5 to 106.77 mg/mL) and by 12.68% in the remaining 2 weeks (from 110.5 to 96.5 mg/mL). Better protein solubility was obtained with the addition of 500 mg/L citric acid, where the protein solubility decreased by 2.04% from the 1st week (108.7 mg/mL) to the end of the storage time (106.5 mg/mL). From these results, it can be concluded that the addition of 300 mg/L citric acid caused a mild pH change during the 4 weeks of storage and had a positive effect on the preservation of protein solubility. The addition of citric acid, time in storage (weeks of storage) and interaction between those two factors had a statistically significant increase in protein solubility (Tables 1 and 2).

Table 1 shows the effect of citric acid addition on texture parameters (hardness and work) during 4 weeks of storage. Significant differences ($P < 0.05$) were found when comparing samples with citric acid addition with control samples (Table 2). Hardness (N) considered one of the most important parameters tested is one of the few properties than can also be observed through sensory analysis. Gelling capacity is a functional property essential to obtain viscoelastic gels which act as a food thickener and binder, and for stabilizing emulsions and foams. Gel formation involves protein denaturation and aggregation. Some of the factors affecting gel hardness are: temperature, time of heating and pH increase. Denaturalized protein

Table 1 Effect of citric acid addition (300, 400, 500 mg/L) (A) and control (C) samples on pH, conductivity, protein solubility and texture parameters of LWE

Samples	Storage week (B)			
	1	2	3	4
pH				
C	7.74 ± 0.02[a]	7.41 ± 0.10[a]	7.63 ± 0.11[a]	6.53 ± 0.01[b]
300	7.45 ± 0.05[c]	7.53 ± 0.05[c]	7.39 ± 0.03[c]	6.17 ± 0.02[d]
400	7.37 ± 0.10[c]	7.52 ± 0.01[c]	7.31 ± 0.02[c]	6.19 ± 0.01[d]
500	7.41 ± 0.04[c]	7.43 ± 0.01[c]	7.36 ± 0.14[c]	6.20 ± 0.02[d]
Conductivity (mS/cm)				
C	5.94 ± 0.09[a]	5.10 ± 0.71[a]	5.83 ± 0.49[a]	6.34 ± 0.08[b]
300	6.11 ± 0.03[b]	6.01 ± 0.07[b]	6.14 ± 0.04[b]	6.38 ± 0.08[b]
400	5.84 ± 0.11[a]	5.84 ± 0.08[a]	5.86 ± 0.07[a]	6.20 ± 0.14[b]
500	6.07 ± 0.00[b]	5.96 ± 0.02[a]	6.18 ± 0.04[b]	6.38 ± 0.08[b]
Protein solubility (mg/mL)				
C	113.19 ± 0.09[a]	93.80 ± 0.35[c]	93.43 ± 0.26[c]	95.04 ± 0.17[d]
300	113.12 ± 0.26[a]	112.82 ± 0.07[a]	114.60 ± 0.00[a]	113.37 ± 3.95[a]
400	110.53 ± 3.93[b]	106.77 ± 1.75[b]	97.14 ± 0.96[d]	96.52 ± 3.67[d]
500	108.68 ± 0.09[b]	104.05 ± 0.00[b]	102.75 ± 0.09[b]	106.46 ± 0.17[b]
Hardness (N)				
C	6.88 ± 1.43[a]	7.10 ± 0.46[b]	21.76 ± 0.86[e]	5.12 ± 0.01[h]
300	6.48 ± 0.11[b]	7.57 ± 0.47[c]	16.28 ± 1.80[f]	2.72 ± 0.04[i]
400	6.33 ± 0.13[b]	6.70 ± 0.50[d]	15.93 ± 0.33[f]	1.86 ± 0.15[j]
500	6.79 ± 1.11[a]	6.94 ± 0.17[b]	14.70 ± 0.78[g]	2.65 ± 0.02[i]
Work (mJ)				
C	13,720.69 ± 985.95[a]	12,269.30 ± 54.81[b]	30,947.31 ± 1132.53[c]	21,484.89 ± 1261.01[d]
300	13,115.90 ± 565.00[a]	14,169.18 ± 2984.59[c]	28,652.63 ± 2669.03[f]	12,164.75 ± 307.46[b]
400	11,598.17 ± 895.63[c]	13,764.94 ± 2500.49[a]	29,710.49 ± 437.22[f]	10,430.31 ± 368.59[c]
500	13,607.97 ± 1699.07[a]	9024.38 ± 267.66[e]	24,647.28 ± 2956.36[g]	11,507.17 ± 11.14[b]

*Different letters [a–j] (for each parameter) represents statistically significant difference ($P < 0.05$)

augments and becomes available for incorporation into the gel network, increasing gel hardness (Monfort et al. 2012). The control samples showed higher gel hardness after the 1st, 3rd and 4th weeks of storage. The sample with 300 mg/L of citric acid had the highest hardness after 3 weeks. In the 400 mg/L sample storage gel hardness increased and peaked after 3 weeks, then decreased reaching its minimum after the 4th week of storage. Tunick et al. (1991) investigated the hardness property of LWE throughout storage time and found that higher hardness values may be caused by low humidity. The highest work value was found in the control samples after the 3rd week of storage, while the lowest work value was found in the 400 mg/L citric acid sample after the 4th week of storage. Similar with gel hardness: the highest gel hardness was found in the control samples, while the lowest was found in the 400 mg/L citric acid samples. Tokuşoğlu and Barbosa-Cánovas (2018) found that pH reduction decreased the hardness of prepared egg gels explaing the lower hardness and work in this study, showing samples with citric acid addition had lower hardness and work values than control samples. The LWE gel in this study became significantly harder with increased storage time, especially from second to third week of storage. After 4 weeks of storage the hardness is decreased probably due to structural changes in LWE. Slight changes in pH caused slight changes in charge on the protein molecule, resulting in slightly less water uptake on protein molecules. Also during the formation of gels an increase in temperature causes formation of thermally induced disulphide bonds. The charges measured as the conductivity, are also slightly increased after the 4th week of storage (Nakano et al. 2018).

Effect of citric acid addition on colour parameters of LWE

In Table 3 the colour parameters (L^*, a^*, b^*, C^* and h) are shown. One effect of citric acid addition to LWE is that it

Table 2 Statistical analysis of influence of main effects A: citric acid addition and B: weeks of storage, as well their interactions (AB effects) on investigated properties

Parameter	Source	Main effects		Interactions
		A:citric acid addition	B:weeks of storage	AB
pH	*P* Value	**0.0000**	**0.0000**	0.7107
Conductivity	*P* Value	**0.0164**	**0.0009**	0.2346
L^*	*P* Value	**0.0000**	**0.0000**	0.1138
a^*	*P* Value	0.0580	**0.0000**	0.0990
b^*	*P* Value	**0.0233**	**0.0000**	0.2272
Protein solubility	*P* Value	**0.0000**	**0.0000**	**0.0000**
Emulsion activity index	*P* Value	**0.0000**	**0.0000**	**0.0000**
Emulsion capacity	*P* Value	**0.0003**	**0.0000**	**0.0008**
Emulsion stability	*P* Value	**0.0015**	**0.0000**	**0.0030**
Foaming	*P* Value	**0.0000**	**0.0000**	**0.0000**
Increase in foaming	*P* Value	**0.0005**	**0.0000**	**0.0004**
Flow beahvior index	*P* Value	0.0843	**0.0012**	0.0963
Consistency coefficients	*P* Value	0.0920	**0.0006**	0.0889
Work (texture)	*P* Value	**0.0002**	**0.0000**	**0.0014**
Hardness	*P* Value	**0.0000**	**0.0000**	**0.0002**

*Bolded values are statistically significant, and main effects have significant effect on changes in investigated properties

prevents colour loss (greening) during cooking (Schuman and Sheldon 2003). The results in Table 2 shows a statistically significant influence ($P < 0.05$) of the main factors (addition of citric acid and week of storage) on L^* and b^* values, while a^* value was affected only by 4-week storage period. Interactions between the main factors did not influence L^*, a^*, b^* values. Citric acid addition affected the L^* value of LWE resulting in a lighter colouring in contrast with control samples during the 4 weeks of storage. The L^* value of all LWE samples decreased from the 1st week to the 2nd week, after which it showed a gradual increase during the 3rd and 4th weeks of storage. The a^*, b^* and C^* value showed a similar pattern.

Citric acid addition did not have an effect on the a^* value, which decreased from first to second week of storage indicating diminution in redness of the sample. After 3 weeks of storage the a^* value first increased and then decreased after the fourth week of storage. At the end of the storage period control sample a^* had higher values in comparison with samples with citric acid addition. The increase in a^* value could be explained by the formation of Fe-conalbumin complex. The conalbumin yields a red colour when it forms a complex with Fe^{3+} ions. The egg white in which conalbumin is suspended contains no Fe^{3+} ions, but the Fe^{3+} ions can be supplied from the egg yolk during the preparation of LWE (Tokuşoğlu and Barbosa-Cánovas 2018). The yellow colour of LWE indicated by b^* value, is contributed by the pigments in egg yolk such as xanthophyll, lutein and zeaxanthin (Lee 2002). b^* value increases significantly ($P < 0.05$) from second to fourth week of storage and shows an increase in yellow colour in samples with citric acid addition in contrast with control samples. The highest b^* value was found in the 400 mg/L citric acid samples, while the lowest were in the control samples. Parameter C^* refers to the colour tone. The highest C^* value was found in the 300 mg/L citric acid samples after the 4th week of storage, while the lowest was found in the control sample after the 2nd week of storage. Parameter h refers to the saturation of colour; a lower value means a lighter colour. The lowest saturation value was found in the 400 mg/L citric acid samples after the 3rd week of storage and the highest was found in the 500 mg/L citric acid samples after the 4th week of storage.

The colour of LWE depends on the physical and chemical properties of the egg components. Colour changes observed in this study are due to a variation of the pH which is affected by addition of different concentration of citric acid which acted as a colour stabilizer. Su and Lin (1993) found that protein denaturation which occurs due to thermal coagulation, explains the main colour changes that occurred in treated LWE samples.

Effect of citric acid addition on foaming and emulsifying properties of LWE

Table 4 shows the foaming properties (foaming capacity and drainage), emulsifying properties (emulsifying capacity and stability), turbidity from 0 to 24 h and the emulsion activity index in LWE. The results in Table 2 show a statistically significant influence ($P < 0.05$) of the main

Table 3 Color of pasteurized liquid whole eggs- control (C) and the samples with citric acid (300, 400, 500 mg/L) during the 4 weeks of storage

Samples	Storage week			
	1	2	3	4
L^*				
C	61.41 ± 0.66^{a}	53.23 ± 0.13^{b}	61.50 ± 0.11^{a}	64.05 ± 0.29^{d}
300	62.04 ± 2.07^{a}	56.16 ± 0.04c	64.23 ± 0.05^{d}	68.29 ± 1.36^{e}
400	59.19 ± 1.71^{a}	52.65 ± 0.24^{b}	61.18 ± 0.26^{a}	65.64 ± 0.30^{d}
500	64.65 ± 1.27^{c}	56.39 ± 0.16^{c}	64.19 ± 0.19^{d}	67.81 ± 0.74^{e}
a^*				
C	14.82 ± 1.34^{a}	10.65 ± 0.43^{b}	17.72 ± 0.18^{c}	16.64 ± 0.25^{d}
300	12.06 ± 2.89^{a}	11.38 ± 0.06^{b}	16.72 ± 0.16^{c}	12.62 ± 2.01^{d}
400	13.79 ± 3.04^{a}	11.46 ± 0.01^{b}	19.19 ± 0.65^{c}	14.53 ± 0.60^{d}
500	15.61 ± 2.75^{a}	10.51 ± 0.30^{b}	16.40 ± 0.45^{c}	11.26 ± 0.98^{d}
b^*				
C	43.52 ± 1.36^{a}	33.59 ± 0.44^{b}	45.02 ± 0.08^{c}	46.97 ± 0.21^{d}
300	43.01 ± 3.59^{a}	37.80 ± 0.05^{e}	48.15 ± 0.08^{f}	51.14 ± 3.09^{g}
400	42.40 ± 3.06^{a}	34.97 ± 0.24^{b}	47.39 ± 0.41^{f}	51.84 ± 0.77^{g}
500	44.53 ± 2.26^{c}	34.96 ± 0.21^{b}	45.39 ± 0.40^{c}	48.25 ± 1.27^{f}
C^*				
C	45.98 ± 1.73^{a}	35.24 ± 0.54^{b}	48.38 ± 0.14^{c}	49.83 ± 0.28^{d}
300	44.69 ± 4.24^{a}	39.47 ± 0.03^{e}	50.97 ± 0.12^{f}	52.67 ± 3.48^{g}
400	44.61 ± 3.85^{a}	36.80 ± 0.23^{b}	51.13 ± 0.63^{f}	53.83 ± 0.91^{g}
500	47.20 ± 3.03^{c}	36.50 ± 0.28^{b}	48.77 ± 0.53^{c}	49.54 ± 1.46^{f}
h				
C	71.22 ± 1.04^{a}	72.42 ± 0.45^{b}	68.52 ± 0.18^{c}	70.49 ± 0.20^{d}
300	74.46 ± 2.34^{a}	73.26 ± 0.11^{b}	70.85 ± 0.07^{c}	76.19 ± 1.32^{d}
400	72.10 ± 2.51^{a}	71.87 ± 0.11^{b}	67.96 ± 0.49^{c}	74.35 ± 0.38^{d}
500	70.76 ± 2.26^{a}	73.27 ± 0.35^{b}	70.35 ± 0.34^{c}	76.88 ± 0.77^{d}

**Different letters $^{a-g}$ (for each parameter) represents statistically significant difference ($P < 0.05$)

factors (addition of citric acid and week of storage) on the foaming and emulsifying properties as well as the interactions of the main factors influencing the emulsifying and foaming properties. The 300 mg/L sample showed an increase in the foaming capacity after 3 weeks of storage. This is caused by the interaction between citric acid and the proteins in LWE causing better incorporation of air. It can also be explained by the increased propagation of proteins through the air–liquid interface (Jambrak et al. 2008). Higher concentrations of citric acid and lengthened storage times cause a change in pH and an increase in protein solubility. They also lead to a lower foaming capacity and an increase in drainage of the system, causing a lower required power (work load) to break formed gels. The addition of citric acid slightly changes the pH value. At near-alkaline pH levels protein molecules have negative net charges (COO^{-}) on their surfaces and repulsion between them increases. This decreases the protein–protein interactions and increases the protein–water interactions (Foegeding et al. 2006). Emulsion capacity is stable during the first 3 weeks of storage and increases afterwards. The samples with citric acid have higher emulsion stability after 3 weeks of storage than the control samples. The 300 mg/L sampled showed a significant effect on the emulsion activity index after 2 weeks of storage compared to the control samples. This is caused by the ability of egg proteins to stabilise a higher surface area (after heating) and act as an emulsifier (O'Sullivan et al. 2016), however the initial emulsion capacity is lower. Turbidity (0 h) decreases with the addition of citric acid and after 4 weeks of storage.

Effect of citric acid addition on viscosity of LWE

Table 5 shows the rheological values of the citric acid and control LWE samples. Rheological values were determined by the Ostwald-de Waele law since the coefficient of regression was exceptionally high (from 0.995 to 0.999). All samples were evaluated according to the flow indexes obtained in time independent non-Newtonian liquids, where the LWE exhibit a dilatant character because their flow behaviour index is $n > 1$. The results are in accordance with other studies (RaDványi et al. 2012). Dilatant

Table 4 Foaming, turbidity and emulsifying properties of pasteurized liquid whole eggs-control (C) and the samples with citric acid (300, 400, 500 mg/L) during the 4 weeks of storage

Samples	Storage week			
	1	2	3	4
Foaming capacity, FC (%)				
C	445.00 ± 7.07^{a}	415.00 ± 14.14^{b}	420.00 ± 7.07^{c}	390.00 ± 56.57^{d}
300	410.00 ± 7.07^{b}	400.00 ± 0.00^{f}	475.00 ± 7.07^{h}	410.00 ± 7.07^{b}
400	455.00 ± 0.00^{e}	455.00 ± 35.36^{e}	410.00 ± 14.14^{b}	330.00 ± 28.28^{i}
500	440.00 ± 0.00^{a}	375.00 ± 14.14^{g}	400.00 ± 14.14^{f}	280.00 ± 0.00^{j}
Drainage (%)				
C	14.86 ± 0.33^{a}	15.05 ± 0.07^{b}	15.19 ± 0.27^{c}	16.94 ± 0.29^{d}
300	15.88 ± 0.16^{e}	15.80 ± 0.00^{e}	13.76 ± 0.60^{g}	15.50 ± 0.43^{c}
400	14.86 ± 0.19^{a}	14.42 ± 0.44^{a}	15.79 ± 0.30^{e}	19.20 ± 0.80^{h}
500	15.26 ± 1.34^{f}	16.74 ± 0.70^{d}	16.34 ± 1.35^{d}	21.97 ± 0.19^{h}
Emulsifying capacity (%)				
C	13.33 ± 0.00^{a}	13.33 ± 0.00^{a}	13.33 ± 0.00^{a}	21.11 ± 1.92^{b}
300	13.89 ± 0.96^{c}	10.00 ± 0.00^{f}	10.00 ± 0.00^{f}	16.11 ± 0.96^{d}
400	16.67 ± 0.00^{d}	11.11 ± 0.92^{f}	10.00 ± 0.00^{f}	24.44 ± 0.96^{g}
500	16.11 ± 0.96^{e}	13.33 ± 0.00^{a}	10.00 ± 0.00^{f}	17.78 ± 1.92^{h}
Emulsifying stability (%)				
C	125.00 ± 0.00^{a}	104.17 ± 7.22^{b}	125.00 ± 0.00^{a}	158.73 ± 13.75^{c}
300	120.37 ± 8.02^{d}	155.56 ± 9.62^{c}	161.11 ± 9.62^{c}	207.41 ± 12.83^{g}
400	120.00 ± 20.00^{d}	147.22 ± 20.97^{e}	177.78 ± 19.25^{f}	169.84 ± 28.70^{h}
500	117.04 ± 5.13^{d}	125.00 ± 0.00^{a}	177.78 ± 19.25^{f}	141.67 ± 14.43^{e}
Turbidity 0 h				
C	875.72 ± 0.49^{a}	844.74 ± 2.61^{b}	851.65 ± 3.91^{c}	827.24 ± 4.89^{d}
300	856.60 ± 1.47^{c}	906.81 ± 1.79^{e}	845.89 ± 4.23^{b}	846.12 ± 3.26^{b}
400	854.41 ± 0.00^{c}	876.41 ± 0.49^{a}	861.32 ± 1.63^{f}	805.47 ± 3.09^{g}
500	852.69 ± 2.44^{c}	832.07 ± 2.28^{d}	856.03 ± 2.93^{c}	827.47 ± 5.86^{d}
Turbidity 24 h				
C	842.21 ± 3.58^{a}	836.45 ± 2.93^{b}	829.08 ± 1.30^{c}	829.77 ± 0.98^{c}
300	856.37 ± 4.07^{d}	858.79 ± 2.61^{d}	865.93 ± 2.28^{e}	866.39 ± 1.63^{e}
400	813.53 ± 4.07^{f}	850.61 ± 3.10^{f}	827.81 ± 0.81^{c}	831.73 ± 1.47^{c}
500	651.98 ± 1.95^{g}	835.53 ± 1.63^{b}	830.12 ± 7.98^{c}	834.72 ± 5.05^{b}
Emulsion activity index (m^2/g)				
C	0.45 ± 0.00^{a}	0.42 ± 0.00^{b}	0.43 ± 0.00^{c}	0.41 ± 0.00^{d}
300	0.44 ± 0.00^{c}	0.49 ± 0.00^{e}	0.42 ± 0.00^{b}	0.42 ± 0.00^{b}
400	0.43 ± 0.00^{c}	0.46 ± 0.00^{f}	0.44 ± 0.00^{c}	0.38 ± 0.00^{g}
500	0.43 ± 0.00^{c}	0.41 ± 0.00^{d}	0.43 ± 0.00^{c}	0.41 ± 0.00^{d}

*Different letters $^{a-g}$ (for each parameter) represents statistically significant difference ($P < 0.05$)

liquids behave identically to Newton's until reaching a critical value of shear force, after which viscosity increases with the shear rate. This type of behaviour is rarer than pseudoplastic (Jambrak et al. 2008). On the other hand a study by Scalzo et al. (1970) showed flow index (n) values of liquid whole eggs range from 0.929 to 0.988, which indicates that LWE exhibits pseudoplastic character ($n < 1$). The consistency coefficient and flow behaviour index for control samples were highest after 2 weeks of storage. Table 2 shows a statistically significant influence of weeks of storage on rheological values. The citric acid samples show a larger decrease in the consistency coefficient and flow behaviour index values compared to the control samples. The largest decrease was found in the sample with the highest citric acid concentration, 500 mg/L. It is important to note that the type of liquid was

Table 5 Rheological properties of pasteurized liquid whole eggs-control (C) and the samples with citric acid (300, 400, 500 mg/L) during the 4 weeks of storage

Sample	Storage week	Apparent viscosity* μ (mPa s)	Consistency coefficient k (Pa s^n) $\times 10^{-5}$	Flow behaviour index n	Regression coefficient R^2
C	1	0.013 ± 0.001^a	1.678 ± 0.022^a	1.74 ± 0.01^a	0.997
300		0.013 ± 0.001^a	1.759 ± 0.011^a	1.76 ± 0.02^a	0.997
400		0.011 ± 0.001^a	2.066 ± 0.031^b	2.07 ± 0.03^b	0.997
500		0.012 ± 0.001^a	1.736 ± 0.024^a	1.74 ± 0.03^a	0.998
C	2	0.011 ± 0.001^a	2.483 ± 0.022^c	2.48 ± 0.04^c	0.997
300		0.011 ± 0.001^a	2.180 ± 0.032^b	2.18 ± 0.02^b	0.998
400		0.011 ± 0.001^a	2.171 ± 0.041^b	2.17 ± 0.04^b	0.998
500		0.011 ± 0.001^a	1.893 ± 0.024^a	1.89 ± 0.03^a	0.998
C	3	0.012 ± 0.001^a	2.018 ± 0.041^b	2.01 ± 0.04^b	0.995
300		0.012 ± 0.001^a	1.948 ± 0.025^b	1.95 ± 0.04^b	0.997
400		0.012 ± 0.001^a	1.947 ± 0.024^b	1.95 ± 0.03^b	0.998
500		0.012 ± 0.001^a	1.926 ± 0.026^b	1.93 ± 0.04^b	0.997
C	4	0.010 ± 0.001^b	2.059 ± 0.024^b	2.05 ± 0.03^b	0.997
300		0.011 ± 0.001^a	2.074 ± 0.026^b	2.07 ± 0.02^b	0.997
400		0.011 ± 0.001^a	2.031 ± 0.036^b	2.03 ± 0.03^b	0.999
500		0.012 ± 0.001^a	1.952 ± 0.033^b	1.95 ± 0.02^b	0.998

*At 1290 s^{-1}

***Different letters $^{a–c}$ (for each parameter) in same column represents statistically significant difference ($P < 0.05$)

maintained as a dilatant. The lowest apparent viscosity of 0.010 mPa s at 1290 s^{-1} was found in the control samples after 4 weeks of storage, while the highest value (0.013 mPa s) was found in the control samples after 1 week of storage. Samples with 300, 400 and 500 mg/L of citric acid showed no significant change ($P > 0.05$) in the apparent viscosity value. The consistency coefficient varied from 1.678 to 2.483 (Pa s^n). Singh et al. (2011) investigated LWE stored at room temperature and at 6 °C. LWE at room temperature showed more pseudoplastic characteristics after storage time elapsed than samples stored at 6 °C. That is concluded based on the decrease of flow behaviour index in investigated samples. In all samples the R value was above 0.99. The highest rheological consistency coefficient was found in the control samples after the 2 weeks of storage and the lowest value was found in the samples with 500 mg/L citric acid at the beginning of storage. Increasing viscosity with an increase in shear rate characterizes the dilatant fluid.

Effect of citric acid addition on particle size of LWE

The particle size distribution in LWE samples was analysed in order to determine the particle size changes and the possible existence of agglomerates which further influence the properties of LWE. The particle size is reduced by the free surface effect of the LWE samples. In this case, the particle size is reduced due to protein denaturation resulting from egg pasteurization (Jambrak et al. 2008). Table 6 shows the results of the particle size distribution, specific surface area, mean surface value, mean volume value of the control (C) and treated samples (300, 400, 500 mg/L) of citric acid during the 4 weeks of storage. The particle size did not change significantly ($P > 0.05$) in any sample during the 1st week of storage. The mean volume value in the control sample was 3.3 and the lowest value (0.75) was found in the 300 mg/L samples. This indicates that the particle volume decreased the most with the lowest concentration of citric acid. The most common mean value was observed when using laser diffraction is the volume mean or D4.3. The D4.3 is very sensitive to the presence of large particles in the distribution. The addition of citric acid firstly affects large aggregates formed. Therefore, slight addition of citric acid with agitation and mixing procedure has an impact in reducing aggregates in the samples. After the 2nd week of storage, the specific surface area and mean surface area did not show any difference in any sample. The particle size did not change significantly in any samples during 4 weeks of storage. The mean volume of the control sample was 10.65 while the lowest value (0.62) was found in the 500 mg/L sample. The particle volume decreased the most in the sample with the highest concentration of citric acid. After the 4th week of storage, the specific surface area and the mean surface area did not

Table 6 Particle size distribution, specific surface area, mean surface value and mean volume value of the control (C) and treated samples (300, 400, 500 mg/L) of citric acid during 4 weeks of storage

Samples	specific surface area (m^2/g)	10% less than*	50%less than**	90% less than***	Mean surface value D [3.2]	Mean volume value D [4.3]
1 week particle size (μm)						
K	38.25 ± 0.23^{a}	0.08 ± 0.01^{a}	0.19 ± 0.01^{a}	1.38 ± 0.01^{a}	0.16 ± 0.01^{a}	3.30 ± 0.01^{a}
300	37.75 ± 0.21^{a}	0.08 ± 0.01^{a}	0.19 ± 0.01^{a}	1.36 ± 0.01^{a}	0.16 ± 0.01^{a}	0.75 ± 0.01^{c}
400	39.25 ± 0.08^{a}	0.07 ± 0.01^{a}	0.18 ± 0.01^{a}	1.41 ± 0.01^{a}	0.15 ± 0.01^{a}	0.92 ± 0.01^{c}
500	37.00 ± 0.07^{a}	0.08 ± 0.01^{a}	0.19 ± 0.01^{a}	1.71 ± 0.01^{a}	0.16 ± 0.01^{a}	1.23 ± 0.01^{b}
2 week particle size (μm)						
K	36.40 ± 0.17^{a}	0.08 ± 0.01^{a}	0.20 ± 0.01^{a}	1.74 ± 0.01^{a}	0.17 ± 0.01^{a}	6.40 ± 0.12^{b}
300	37.70 ± 0.14^{a}	0.08 ± 0.01^{a}	0.19 ± 0.01^{a}	1.39 ± 0.01^{a}	0.16 ± 0.01^{a}	0.77 ± 0.01^{c}
400	38.75 ± 0.15^{a}	0.07 ± 0.01^{a}	0.18 ± 0.01^{a}	1.28 ± 0.01^{a}	0.16 ± 0.01^{a}	0.57 ± 0.01^{c}
500	35.85 ± 0.13^{a}	0.08 ± 0.01^{a}	0.20 ± 0.01^{a}	1.64 ± 0.01^{a}	0.17 ± 0.01^{a}	2.80 ± 0.14^{a}
3 week particle size (μm)						
K	32.45 ± 0.15^{a}	0.08 ± 0.01^{a}	0.24 ± 0.01^{a}	1.77 ± 0.01^{a}	0.19 ± 0.01^{a}	10.65 ± 0.35^{c}
300	33.95 ± 0.16^{a}	0.08 ± 0.01^{a}	0.22 ± 0.01^{a}	1.82 ± 0.01^{a}	0.18 ± 0.01^{a}	5.30 ± 0.01^{b}
400	35.00 ± 0.16^{a}	0.08 ± 0.01^{a}	0.21 ± 0.01^{a}	1.61 ± 0.01^{a}	0.17 ± 0.01^{a}	5.16 ± 0.01^{b}
500	38.75 ± 0.17^{a}	0.08 ± 0.01^{a}	0.18 ± 0.01^{a}	1.24 ± 0.01^{a}	0.15 ± 0.01^{a}	0.62 ± 0.01^{a}
4 week particle size (μm)						
K	28.50 ± 0.21^{b}	0.09 ± 0.01^{a}	0.37 ± 0.01^{b}	2.59 ± 0.01^{b}	0.21 ± 0.01^{b}	1.63 ± 0.01^{c}
300	29.50 ± 0.18^{b}	0.09 ± 0.01^{a}	0.31 ± 0.01^{b}	2.11 ± 0.01^{b}	0.20 ± 0.01^{b}	1.22 ± 0.01^{c}
400	31.10 ± 0.19^{b}	0.08 ± 0.01^{a}	0.27 ± 0.01^{b}	1.73 ± 0.01^{a}	0.19 ± 0.01^{b}	0.89 ± 0.01^{b}
500	29.55 ± 0.18^{b}	0.09 ± 0.01^{a}	0.31 ± 0.01^{b}	1.99 ± 0.01^{a}	0.20 ± 0.01^{b}	1.24 ± 0.01^{c}

*Using Mie's theory it is determined that 10% particles have diameter less that stated

**Using Mie's theory it is determined that 50% particles have diameter less that stated

***Using Mie's theory it is determined that 90% particles have diameter less that stated

***Different letters $^{a-c}$ (for each parameter) in same column represents statistically significant difference ($P < 0.05$)

show statistically significant differences ($P > 0.05$) in any sample. The mean volume was highest in the control sample (1.63) and lowest in the 400 mg/L citric acid sample (0.89).

Conclusion

The addition of 300 mg/L of citric acid caused a mild pH change and had a positive effect on the preservation of protein solubility in LWE throughout the 4-week storage period. The storage time had a statistically significant ($P < 0.05$) effect on every tested parameter, including pH, conductivity, L^*, a^* and b^* values of colour, protein solubility, emulsion activity index, emulsion capacity, emulsion stability, foaming, flow behaviour index, consistency coefficient, foaming and texture parameters. The addition of citric acid had a statistically significant ($P < 0.05$) effect on pH, conductivity, L^* and b^* values of colour, protein solubility, emulsion activity index, emulsion capacity, emulsion stability, foaming and texture parameters, but not on rheological parameters. Higher amounts of citric acid cause greater changes in pH and larger increases in protein solubility. It also led to a lower foaming capacity and increased system drainage, which caused a lower power (work load) requirement to break formed gels. The lowest apparent viscosity of 0.010 mPa s at 1290 s^{-1} was found in the control samples after 4 weeks of storage, while the highest value (0.013 mPa s) was found in the control samples after 1 week of storage. Samples with 300, 400 and 500 mg/L of citric acid showed no significant difference ($P > 0.05$) in the apparent viscosity value.

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