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RESEARCH ARTICLE

Liquid egg products characterization during storage as a response of novel phyto-additives added in hens diet

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ABSTRACT

This study aims to assess the effect of novel phyto-additives (bilberry leaves, walnut leaves, sea-buckthorn pomace and a mix of bilberry leaves, walnut leaves and sea-buckthorn pomace) added in hens diet on the liquid egg components (egg albumen, egg yolk and whole liquid egg) during storage. The phyto-additives added to the basic feed remarkably affected almost all the characteristics investigated, colour, pH and rheological behaviour of liquid egg products. The changes of egg yolk and albumen's colour and pH were not uniform. The supplementation of feeding with walnut leaves decreases significantly ($p < 0.01$) the luminosity of egg yolk, whereas the luminosity of egg albumen increases compared to the control. The pH values were influenced by the type of phyto-additive added in the hens diet, but its variations were only slightly dependent on the storage time of eggs. The rheological properties showed a pseudoplasticity of egg components depending on the hens diet and egg storage time. Ostwald-de Waele model fitted well with the rheological data, offering information on the changes of the apparent viscosity in relation to the diet type and storage time. The results indicated that these novel phyto-additives could be incorporated in hens diet for production of improved eggs quality and walnut leaves having a remarkable effect.

Keywords: Colour parameters; Eggs; pH; Phyto-additives; Rheological properties

INTRODUCTION

Eggs, known as unique food material, represent a rich source in high-quality proteins, unsaturated fatty acids, phospholipids, minerals, vitamins and lipophilic pigments including antioxidant carotenoids (Huopalahti et al., 2007). After breaking the shell, the basic components obtained are egg albumen which portion makes up 60% of the egg's weight and yolk egg that represents approximately 35% of the liquid egg. The albumen and yolk egg have many functional properties which mainly depend on the quality of their proteins (Mine, 2002). As multifunctional food materials, due to their nutritional and functional properties, eggs are incorporated in various food systems, increasing their nutritional value, improving color and flavor. Egg is used as ingredient in bakeries, biscuits, pasta e.g., (Stadelmann and Cotterill, 1995). In recent years, egg consumption in the form of egg components has increased compared to marketed shell eggs (Jesús et al., 2013). The egg components characteristics such as colour, pH and viscosity are influenced by the diet administered to laying hens (Lomakina and Mikova, 2006). The carotenoids, particularly lutein and zeaxanthin and xanthophylls with their antioxidant effects influenced the colour of egg yolk (Kopřiva et al., 2014). Also, the housing system has a significant effect on the yolk colour (Sokołowicz et al., 2018).The pH affected the thinning of the thick albumen (Burley and Vadehra 1989; Kato et al., 1970) and is being used to assess thick albumen thinning (Beveridge and Nakai, 1975). The viscosity depends also on the storage time and temperature, water content and egg stress.

Several tests have been made to reestablish the egg consumption by modifying egg composition through feeding nutritionally-changed diets. The supplementations of the hens diet with phytonutrients can remarkable affect on the quality of eggs (Nour et al., 2018; Dvořák et al., 2017). Several reports have described the effect of different feed additives in laying hens fed diet on egg quality (Świątkiewicz et al., 2018; Arpášová et al., 2012). The phytochemicals, like phenolic compounds, are considered beneficial for both human and animal health (Silva et al., 2006).

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Supplementation of the feed diet with bilberry leaves, walnut leaves and sea-buckthorn pomace a to laying hens could modify components of the edible part of eggs, leading to properties favorable to consumers. Bilberry leaves contain high levels of phenolic compounds (Martz et al., 2010). The phenolic compounds content of bilberry leaves depends on the harvest period (Ieri et al., 2013). Bilberry leves can be used as antibacterial, anti-inflammatory, being also helpful in cancer prevention (Mechikova et al., 2010). Walnut leaves are a good source of phenolic compounds (Pereira et al., 2007). The walnut leaves contain high amounts of antioxidants. The most important component is represented by the elagic acid which has anti-cancer effects (Nour et al., 2012). Due to their high antioxidant potential bilberry leaves and walnut leaves could be useful in diseases prevention in which free radicals are implicated. Sea-buckthorn pomace is a valuable by-product which contains high amounts of valuable vitamins, flavonoids and fatty acids. The lipid fraction of sea-buckthorn contains an average of 6 – 13% α-linolenic acid and has a low ratio of n-6/n-3 polyunsaturated fatty acids of 1.1–1.3 and a high concentration of monounsaturated fatty acids (49–52%) (Nuernberg et al., 2015). Sea-buckthorn pomace is valued for its antioxidant properties because of its bioactive substances content (Korekar et al., 2011).

After laying, during storage, eggs components properties are significantly influenced by their storage condition mainly in a time and temperature dependent way (Chung et al., 2014). The changes are complex and affect the functional properties of liquid egg components, albumen, yolk and whole liquid egg. Modifications in the structure of protein can affect the rheological behavior of egg components. In fact, the rheological properties are usually influences by the pH value of liquid egg components. The rheological properties of liquid egg components are remarkable related with their flow transport and rheological properties are connected with continuous thermal processing system. Some of these problems are described by Souza and Fernandez (2013) and Cabral et al. (2011).

The use of liquid egg components in food industry needs rigorous knowledge of their rheological behaviour (Velez-Ruiz, 2002). Egg components viscosity influences a number of their functional and technological properties such as whipping, emulsifying and gelling ability (Kemps et al., 2010; Atílgan and Unluturk, 2008). Most of the studies have been frequently carried out with only egg yolk (Simeonovova et al., 2003). There is a lack of information about the rheological properties of liquid egg albumen and whole liquid egg.

This study aimed to investigate if the addition of novelty phyto-additives to laying hens feed affects liquid egg components pH, colour and whether the addition of phyto-additives to the diet of laying hens affects the rheological properties of liquid egg components, yolk, albumen and whole egg.

MATERIALS AND METHODS

Experimental design

The research was conducted on liquid egg components of egg-laying breeds which were fed 4 weeks with a diet enriched with some phyto-additives. The experiment was performed on 168 140 Tetra SL laying hens (32 week old) which were housed in identical conditions, at temperature of 23.08 ± 0.98°C, moisture of 66.35 ± 5.68% and ventilation/head/hen of 1.70 ± 0.14%. Lighting was provided for 16h of the 24h. Laying hens individually weighed were randomly divided into five experimental groups (C, E1, E2, E3 and E4). The groups of hens were reared in cages (2 hens/cage; 14 cages/experimental group) structured by three levels, with *ad libitum* access to feed and water. For the elaboration of the combined feed formulations used in this experiment, we considered the objective of the experiment, the species, the hybrid, the age and the nutritional requirements of the hybrid Tetra SL laying hens (Tetra-SL LL commercial Layer Management Guide, 2007). Control group (C) consumed a complete feeding mixture without the addition of phyto-additives, while the other four groups consumed a complete feeding mixture with phyto-additives such as, bilberry leaves (E1), walnut leaves (E2), sea-buckthorn pomace (E3) and, mix of bay bilberry leaves, sea-buckthorn pomace and walnut leaves, in equal proportions (E4). Water was administered using automatic feeders. The basic structure of fodder recipes was the same for all five experimental groups, characterized by 2800 kcal/kg metabolizable energy, 17.8% crude protein, 2.98% crude fat, 3.90% calcium, 0.78% lysine, 0.44% methionine and a ratio of the metabolizable energy/crude protein at a value of 157.30.

A total of 12 eggs were collected from each hens group after a 4-week experimental period to evaluate the quality parameters of the liquid egg components. The selected eggs were subsequently stored under temperature of 5°C in unchangeable conditions for a period of one, 2, 3 and 4 weeks. Every time the liquid eggs components were obtained after eggshells were washed with deionized water and hand broken properly. The albumen and yolk were separated, filtered by colander in order to separate chaladza and other membranous impurities from egg yolk, and to eliminate foam formation in egg albumen.

pH measurement

The pH change of liquid egg components, in terms of egg albumen, egg yolk and whole liquid egg was performed

using a HQ30d Portable pH Meter (HACK, Germany). Buffer solutions of pH 7 and pH 4 are used for calibration of pH meter. All measurements were carried out in triplicate.

Colour egg components measurement

The egg yolk, separated from the egg white prior to measurement, was placed on a Petri dish with a diameter of 50 mm. Color analysis was performed using a Konica Minolta CR-400 colorimeter. The color parameters of L^* (lightness), a^* (red-green intensity) and b^* (yellow-blue intensity) of the CIE-Lab system (Commission Internationale de l'Eclaraige) were determined by reflectance CIE - L^* a^* b^* colour coordinates. The total color difference (ΔE^*) were calculated as function of deviation from L^* (ΔL^*), a^* (Δa^*) and b^* (Δb^*) by the Equation 1:

$$\Delta e^{*} = (\Delta a^{*2} + \Delta b^{*2} + \Delta L^{*2})^{1/2} \quad (1)$$

The instrument was calibrated with a white calibration before the measurements. All measurements were performed in triplicate.

Rheological measurements

The rheological measurements of the liquid egg components, in terms of liquid egg yolk, liquid egg albumen and liquid whole egg were carried out using a Haake MARS 40 dynamic rheometer (Thermo Haake Mars, Germany) equipped with a concentric cylinder measurement system (CC 25, 25 mm in diameter, 16.1 mL measuring cell). The flow curves was obtained by increasing shear rate from 0 to 100 s^{-1} The variation of sample apparent viscosity η which is the ratio of shear stress τ and shear rate (Eq. 2) (Steffe 1996) was evaluated in the same analysis interval.

$$\eta = \frac{\sigma}{\dot{\gamma}} \text{ [Pa s]} \quad (2)$$

Ostwald-de Waele rheological model was adjusted to the shear stress - shear rate curve of each sample, in order to determine if the model fits better to the rheological properties of liquid egg components. The RheoWin 4 Data Manager software (version 4.20, Haake) was used for parameters calculations.

The mean calculated from the three measured values was used for further evaluation. All measurements were performed at room temperature (20 ± 1°C).

Data analysis

All data were statistically processed for each quality parameters of the liquid egg components. Analysis of variance (ANOVA) was applied to test the dependence of liquid egg components quality parameters on the addition of phyto-aditives in a feedings dose. The dependence of the feeding of laying hens and eggs storage duration on the evaluated quality parameters were tested using two-factor ANOVA. The level of significance was selected at $p< 0.05$. For viscosity curve fitting of the experimental data a RheoWin 4 Data Manager software (version 4.20, Haake) was used. The suitability of the fitted model was evaluated by the coefficient of determination (R^2).

RESULTS AND DISCUSSION

Effect of hens diet and egg storage time on colour parameters of liquid egg components

The colour of liquid egg components was represented by L^*, a^* b^* and ΔE^* values.Table 1 shows the colour parameters of the egg albumen. Luminosity, L^* was more variable, decreasing or increasing in samples as function of type of phyto-additives added in hens diet.

A high increase of lightness was obtained in E3 and E2 samples when the feeding was supplemented with sea-buckthorn pomace and respectively, walnut leaves. The high increase of lightness in E3 and E2 samples can be related to the compounds from sea-bucktorn pomace and for walnut leaves, such as lutein and zeaxanthin. Lutein, a yellow-orange color pigments, has been used for many years in poultry diets as a mean to pigment egg yolks (Wu et al., 2009). Redness of albumen is given by a^* value, while b^* values suggest the yellowness of albumen. Values of a^* and b^* parameters decrease significantly ($p< 0.05$) in the experimental groups E1, E2, E3 and E4, when the phyto-additives were added in hens diet, compared with the control. With the storage time increase, there was a significant decrease of L^* and an increase of a^*, meaning lower lightness and higher redness of albumen. The total colour difference (ΔE^*) is higher for E2 (12.22) and E3 (12.64), as compared to the control group. The values obtained are very similar to those reported by Dvořák et al., (2012) for pastured laying hens. The increase of ΔE^* with the addition of walnut leaves (E2) and sea-buckthorn pomace (E3) indicated the darker color of albumen which can be due to some bioactive substances and/or natural pigments from these phyto-additives known as xanthophylls.

The average changes of yolk colour as function of diet type of group and during storage is shown in Table 2. The addition of bilberry leaves in feeding caused a significant decrease only in value a^*, the b^* suggesting the yellowness of yolk. This effect may be explained by the active constituents from bilberry leaves, such as quercetin, catechins, tannins and acids which can lead to the yellowness of yolk. A significant decrease ($p< 0.05$)

Table 1:Colour parameters of the liquid egg albumen in relation to the phyto-additives added in hens diet and egg storage time

Storage time (week)	Group	Colour parameters			
		*L**	*a**	*b**	Δ*E**
1	C	57.97±0.51^{aA}	-5.33±0.38^{a}	13.70±0.57^{aA}	0.00
	E1	56.89±0.05^{bA}	-4.72±0.98^{b}	10.08±1.03^{aA}	3.70
	E2	68.87±0.46^{cA}	-4.59±0.71^{b}	9.39±0.52^{bA}	12.22
	E3	69.47±0.74^{dA}	-4.79±0.51^{b}	9.79±0.09^{aA}	12.64
	E4	56.66±0.12^{eA}	-4.37±0.64^{c}	8.80±0.04^{cA}	5.06
2	C	56.90±0.31^{aA}	-4.28±0.22^{a}	9.08±0.78^{aA}	0.00
	E1	57.89±0.47^{bA}	-5.29±0.32^{b}	13.18±1.50^{aA}	4.34
	E2	57.34±0.31^{cA}	-4.76±0.34^{c}	10.99±1.56^{bA}	2.28
	E3	57.45±0.29^{dA}	-4.93±0.29^{c}	12.10±1.28^{aA}	1.40
	E4	57.35±0.71^{eA}	-4.88±0.26^{c}	11.63±1.05^{cA}	0.89
3	C	56.82±0.18^{aB}	-4.39±0.08^{a}	9.68±0.42^{aB}	0.00
	E1	57.26±0.32^{bB}	-4.70±0.52^{b}	12.04±0.09^{aB}	1.27
	E2	57.12±0.08^{cB}	-4.39±0.12^{a}	9.67±0.73^{bB}	1.24
	E3	56.88±0.36^{dB}	-4.51±0.21^{a}	10.23±0.72^{aB}	0.57
	E4	56.82±0.44^{eB}	-4.58±0.33^{a}	10.40±1.31^{cB}	0.20
4	C	56.80±0.78^{aB}	-5.25±0.38^{a}	12.75±0.70^{aB}	0.00
	E1	56.92±0.32^{bC}	-4.58±0.44^{b}	10.62±1.92^{aB}	2.24
	E2	57.04±0.05^{cC}	-4.70±0.25^{b}	10.99±1.14^{bB}	0.45
	E3	57.20±0.17^{dC}	-4.95±0.25^{b}	12.29±1.18^{aB}	1.38
	E4	57.45±0.36^{eC}	-4.85±0.30^{b}	11.74±1.13^{cB}	0.85
Two-way ANOVA *p* value					
Type of phyto-additive		< 0.001	NS	< 0.05	
Storage time		< 0.001	NS	< 0.01	
Type of phyto-additive×Storage time		< 0.001	< 0.05	< 0.001	

*C, control group which consumed a complete feeding mixture without the addition of phyto-additives; E1, feeding mixture with bilberry leaves; E2, feeding mixture with walnut leaves; E3, feeding mixture with sea-buckthorn pomace; E4, feeding mixture with mix of bilberry leaves, sea-buckthorn pomace and walnut leaves, in equal proportions.Different lowercase letters in vertical indicate difference between diets ($p \leq 0.05$); Different uppercase letters in horizontal shown difference between time of storage ($p \leq 0.05$). Data are showed as mean±standard deviation

of *L** and *b** was obtained in E2 group when walnut leaves are used to supplement the feed for laying hens. With the addition of sea-buckthorn pomace in hens feed, there was a significant decrease of *a** and an increase of *b**, meaning lower redness and higher yellowness of egg yolk. Other authors reported a markedly increased of *a** and *b** parameters with the amount of added sea-buckthorn pomace in hens feed (Dvořák et al., 2017). Also, a high yellowness was found in E4 group, whose diet was enhanced with a mix of bilberry leaves, sea-buckthorn pomace and walnut leaves, in equal proportions. An increase of luminosity was obtained in E4 group when a mixture of bilberry leaves, sea-buckthorn pomace and walnut leaves, in equal proportions was added in hens diet. Mansoori et al. (2008) reported that the supplementation of a hens diet with dried tomato pulp led to a more intense egg yolk colour, while Chong et al. (2008) revealed the significantly lighter colour of the yolk when extracted palm kernel pomace was added in the hens diet. The ΔE^* showed very small differences compare to the control. The ANOVA test of differences in yolk colour during storage showed a significant difference ($p < 0.05$) among weeks of storage. It was found that the colour changes on the last week of storage were higher for the control group compared to the experimental groups. The results of two-way ANOVA showed that the interaction between type of phyto-additive and storage time have not a significant effect ($p > 0.05$) on colour of egg yolks.

The addition of novel phyto-additives in the hens feed changes the values of egg yolk colour parameters *L**, *a** and *b** (Table 2). An increase of *L** value was obtained for E1 and E4 groups, compared to control group, while it decreases for E2 group. The decrease of the intensity of egg yolk from E2 group can be due to the walnut leaves from hens diet, yolk pigmentation being mainly due to dietary factors (Minelli et al., 2007). The addition of bilberry leaves leads to an increase of colourity intensity which is related to the *L** parameter. The parameter Δ*E** remarkable increases, depending on the phyto-additive type added to the hens feed. The composition of feeding is reflected in the resultant variation of colour parameters.

Table 2:Colour parameters of the liquid egg yolk in relation to the phyto-additives added in hens diet and egg storage time

Storage time (week)	Group	Colour parameters			
		*L**	*a**	*b**	Δ*E**
1	C	59.38±0.92^{a}	-4.98±0.86^{aA}	54.05±0.73^{aA}	0.00
	E1	59.81±0.72	-5.40±0.34^{A}	54.16±0.26^{A}	0.61
	E2	58.10±2.68^{b}	-5.44±0.59^{bA}	49.87±0.65^{A}	5.02
	E3	59.34±0.75	-2.92±0.69^{cA}	55.33±0.99^{bA}	2.46
	E4	59.69±0.91	-4.75±0.78^{dA}	55.26±0.85^{cA}	3.34
2	C	59.72±0.65^{a}	-6.21±0.82^{aB}	50.52±1.53^{aB}	0.00
	E1	57.92±0.22	-6.38±0.51^{B}	51.14±1.42^{B}	0.62
	E2	57.55±0.91^{b}	-5.74±0.24^{bB}	52.63±0.59^{B}	2.11
	E3	57.21±0.74	-3.71±0.31^{cB}	54.74±0.78^{bB}	4.22
	E4	58.56±0.74	-5.10±0.06^{dB}	51.60±1.11^{cB}	1.08
3	C	58.68±0.65^{a}	-6.78±0.22^{aC}	49.16±0.51^{aC}	0.00
	E1	59.12±1.53	-6.20±0.14^{C}	50.50±0.98^{C}	1.34
	E2	57.92±1.12^{b}	-5.77±1.02^{bC}	51.25±0.38^{C}	2.09
	E3	57.89±1.01	-3.77±0.53^{cC}	55.15±0.52^{bC}	5.99
	E4	59.45±0.23	-4.66±0.07^{dC}	54.96±0.54^{cC}	5.80
4	C	56.64±0.99^{a}	-5.95±0.17^{aD}	51.45±0.96^{aD}	0.00
	E1	57.89±0.95	-5.98±0.22^{D}	51.92±0.70^{D}	0.47
	E2	57.01±0.50^{b}	-4.98±0.63^{bD}	52.15±0.74^{D}	0.70
	E3	57.60±0.83	-4.35±0.62^{cD}	53.27±0.48^{bD}	1.82
	E4	57.28±0.36	-5.09±0.72^{dD}	52.81±0.46^{cD}	1.36
Two-way ANOVA *p* value					
Type of phyto-additive		< 0.001	< 0.001	< 0.001	
Storage time		< 0.001	< 0.01	< 0.001	
Type of phyto-additive×Storage time		< 0.01	NS	< 0.001	

*C, control group which consumed a complete feeding mixture without the addition of phyto-additives; E1, feeding mixture with bilberry leaves; E2, feeding mixture with walnut leaves; E3, feeding mixture with sea-buckthorn pomace; E4, feeding mixture with mix of bilberry leaves, sea-buckthorn pomace and walnut leaves, in equal proportions. Different lowercase letters in vertical indicate difference between diets ($p \leq 0.05$); Different uppercase letters in horizontal shown difference between time of storage ($p \leq 0.05$). Data are showed as mean±standard deviation

Effect of hens diet and egg storage on pH-value of liquid egg components

The results on the effect of feed hens and egg storage time on pH values of liquid egg components measured at each week for a period of 4 weeks are showed in Table 3. Egg albumen pH was dependent on the storage and showed an increase progressively by increasing storage time during the first 3 weeks of storage. After this period, pH value exhibits a decrease, but the values were always superior to pH 9. The results are in disagreement with those obtained by Walsh et al. (1995) which reported that storage time have not an influence on the albumen pH. Studies made by other researchers have reported an increase of pH value by extending the storage time duration (Kumbar et al., 2015). The increase of pH is affected by the ovomucina-lysozyma complex and leds to the liquefaction of albumen. Egg albumen was pseudoplastic at pH values 3, 4 and from 7 to 10, but that it behaves as a Newtonian fluid at pH values 5, 6 and 11 (Gossett et al., 1983).

An increase of pH with the storage length was obtained in all egg yolk samples. The increase of pH observed in yolk was not as large as in albumen, and it was different as function of phyto-additive type added in hens diet. Overall increases of the yolk pH were observed at the end of the storage time. This may be related to the deterioration of the yolk quality as a consequence of moisture evaporation and carbon dioxide loss. These changes lead to an increase of the pH and concomitant structural change in the yolk protein (Chung and Lee, 2014).

A decrease of alkalinity in whole liquid egg even after 3 weeks of storage time was observed in control group. In contrast, in E1, E2, E3 and E4 groups a slightly increase of alkalinity was obtained. The trend was not uniform during all storage periods. The results obtained indicated that pH parameter can be a useful tool for describing the changes in egg components quality diet over time during storage and in function of phyto-additve added in hens.

The increase of pH observed in all egg components was not as large in E1, E2, E3 and E4 groups compared to control. The feeding mixture with phyto-additives didn't affect significantly ($p > 0.05$) egg yolk pH value, except

Table 3: Egg products pH in relation to the dietary supplementation with phyto-additive and storage time

Product	Group*	pH value during storage time			
		1 week	2 week	3 week	4 week
Liquid egg albumen	C	9.11±0.01^{aA}	9.12±0.04^{aB}	9.25±0.08^{aC}	9.20±0.04^{aB}
	E1	9.02±0.04^{aA}	9.21±0.04^{aB}	9.25±0.04^{aC}	9.18±0.03^{aB}
	E2	9.02±0.05^{bA}	9.14±0.08^{bB}	9.18±0.05^{bC}	9.08±0.05^{bB}
	E3	9.02±0.05^{cA}	9.17±0.06^{cB}	9.20±0.03^{cC}	9.12±0.06^{cB}
	E4	9.03±0.01^{aA}	9.12±0.10^{aB}	9.28±0.05^{aC}	9.17±0.02^{aB}
Liquid egg yolk	C	6.04±0.09^{aA}	6.31±0.02^{aA}	6.21±0.09^{aA}	6.35±0.09^{aA}
	E1	6.02±0.09^{aA}	6.24±0.09^{aA}	6.25±0.07^{aA}	6.26±0.03^{aA}
	E2	6.06±0.08^{aA}	6.08±0.09^{aA}	6.17±0.06^{aA}	6.23±0.02^{aA}
	E3	6.04±0.03^{bA}	6.15±0.02^{A}	6.19±0.08^{bA}	6.20±0.09^{bA}
	E4	6.04±0.05^{aA}	6.03±0.04^{aA}	6.23±0.09^{aA}	6.26±0.09^{aA}
Whole liquid egg	C	7.52±0.08^{aA}	7.41±0.08^{aA}	7.39±0.04^{aB}	7.45±0.02^{aA}
	E1	7.38±0.09^{aA}	7.46±0.09^{aA}	7.39±0.08^{aA}	7.33±0.09^{aA}
	E2	7.36±0.02^{aA}	7.37±0.05^{aA}	7.27±0.05^{aA}	7.47±0.06^{aA}
	E3	7.26±0.04^{bA}	7.45±0.07^{bA}	7.51±0.09^{bA}	7.27±0.06^{bA}
	E4	7.21±0.02^{bA}	7.27±0.09^{bA}	7.47±0.07^{bA}	7.38±0.07^{bA}

*C, control group which consumed a complete feeding mixture without the addition of phyto-additives; E1, feeding mixture with bilberry leaves; E2, feeding mixture with walnut leaves; E3, feeding mixture with sea-buckthorn pomace; E4, feeding mixture with mix of bilberry leaves, sea-buckthorn pomace and walnut leaves, in equal proportions. Different lowercase letters in vertical indicate difference between diets ($p \leq 0.05$); Different uppercase letters in horizontal shown difference between time of storage ($p \leq 0.05$). Data are showed as mean±standard deviation

E3 group where the hens diet was supplemented with sea-buckthorn pomace. Regarding the whole liquid yolk pH, significant value ($p < 0.05$) was obtained between C and E3 and, E4 respectively. These results can be related to the synergistic effect of phytochemical compounds from diet. An increase of pH value was obtained in some groups treatment which can be explained by the loss of CO_2 from the egg. During storage, the albumen becomes thinner and loses CO_2, which allows the electrostatic complex between the lysozyme and ovomucin to rupture, which helps increase the pH of eggs (Scott and Silversides, 2000).

At the same time no significant changes ($p > 0.05$) were obtained for the whole liquid egg pH in function of treatment groups of E1 and E2 compared to control. The interaction effect between hen's diet and storage time were significantly ($p < 0.05$) with respect to whole liquid egg pH (Table 3).

All liquid egg components, albumen, yolk and whole egg pH were influenced by the type of phyto-aditive added in hen's diet and egg storage time. The results obtained highlighted that the pH values were only slightly dependent on the storage period. Similar results have been obtained by the Tilki and Inal (2004).

Effect of hens diet and egg storage on the viscosity of liquid egg components

The apparent viscosity of liquid egg components were measured at room temperature (20 ± 1°C) as a function of strain rate with increasing shear rate. The shear rate varied from 0 to 100 $s^{-1}$and de duration of the experiment was set to 60 s.

As it is visible in Fig. 1, the value of albumen viscosity changes with eggs storage time. The dependence shows that albumen exhibit shear-thinning behaviour, the apparent viscosity decreasing with shear rate. Lower viscosity did not change foam density and stability. The E2 group showed a higher viscosity compared to E1, E3 and E4 after 1 week of storage. This behaviour can be related to the constituents from walnut leaves from feeding mixture. The ovomucina-lysozyna complex which is stabilized by electrostatic connections can be responsible for higher viscosity of the albumen in eggs after 1 week. A decrease of albumen viscosity was obtained after 3 week in C group compared to E1, E2, E3 and E4 groups. This decrease can be explained by the liquefaction of albumen due to the increase of pH. Therefore, the diet supplemented with the phyto-additives can improve the albumen viscosity of eggs. An increase of albumen viscosity during storage was obtained for E2 after 3 and 4 weeks; at lower shear rates there is a tendency toward an increase of albumen viscosity.

The viscosity values obtained for egg yolk showed differences between samples due to the varied hens diet (Fig. 1). Compared to the control, a high value for viscosity after 1 week was obtained in egg from hens witch has a diet supplemented with phyto-addditives (Fig. 1). The yolk viscosity values decrease with storage time increase. The decrease of yolk viscosity with storage time can be related to the decreasing number of interactions in yolk structure occurring during storage.

The decrease of gel presence with storage was observed by Sakanaka et al. (2000) which found that the complex modulus

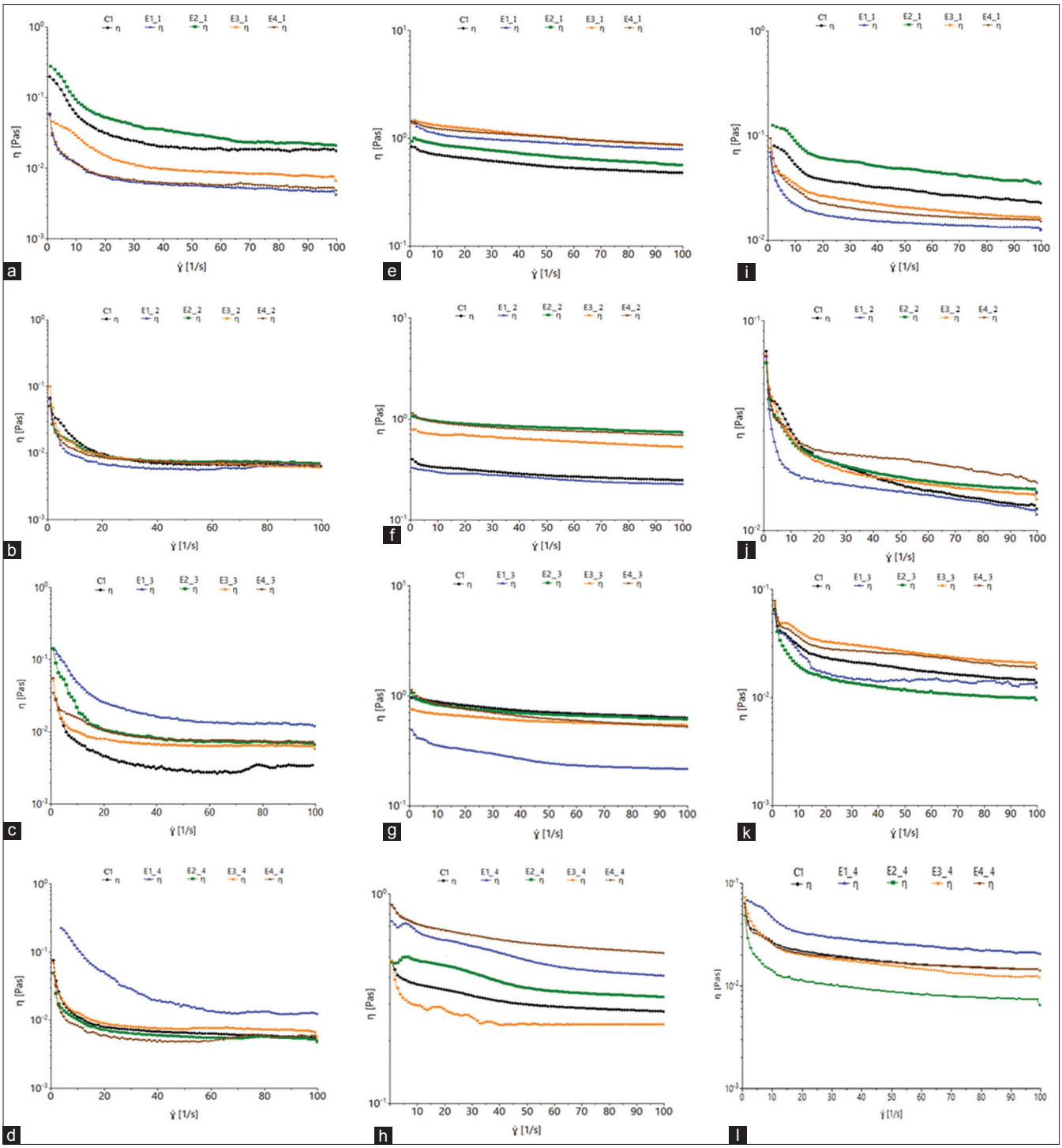

Fig 1. Variation of egg albumen (a-d), egg yolk (e-h), and whole egg (i-l) viscosity during egg storage time: (a, e, i) 1 week; (b, f, j) 2 week; (c, g, k) 3week; (d, h, l) 4 week as a response of a hens diet supplemented with different phyto-additives.

of the yolk decreases in time. The component rearrangement which was in progress throughout the storage period and fewer contacts which are present can be an explanation for this behavior (Severa et al. 2010). It is obvious that viscosity values decreases with shear rate increase, yolk exhibiting shear-thiningbehaviour. This behaviour was expected in yolk because its texture is influenced by weak physical bonds and hydrophobic interactions (Atílgan and Unluturk, 2008). The decrease of the yolk viscosity with shear rate can to be a response of destruction of the interactions between the main components of the egg yolk, triacylglycerols, phospholipids, proteins and carbohydrates.

The effect of hens diet supplemented with novel phyto-additives on the flow behaviour of the whole liquid

egg during storage time is showed in Figure 1. The apparent viscosity value varies in function of type of phyto-additives added in the hens diet, and there is the possibility to identify eggs from hen feeding with phyto-additives by determining whole egg viscosity. The viscosity decreases with storage time increase, a high decrease was obtained in eggs from E2 group after 4 weeks compared to the 1 week. This behaviour can lead to an increased emulsifying ability and whipping performance of the whole liquid egg.

The evaluation of the rheological behaviour of liquid egg components highlighted the non-Newtonian rhologicalbehaviour. Ostwald–De Waele rheological flow model (Eq. 3) based on shear stress was applied (Rao, 2013):

$$\tau = K \cdot \dot{\gamma}^{n} \quad (3)$$

Where τ is the shear stress (Pa), γ is the shear strain rate (s^{-1}), *K* is the consistency index (Pa-s^n) and *n* is the flow behavior index.

The application of Ostwald-de Waele model, also known as power-law, was selected because it presents a technical and practical advantage in the design of piping and pumping systems for food processing, like modeling of continuous pasteurization in pipes (Kumbar et al., 2015a), compared to other rheological models.

The addition of phyto-additives to the feed and storage time of eggs influence the consistency of liquid egg component in various ways. The parameters of the tested model, Eq. (3) for all liquid egg components evaluated are showed in Table 4.

As it can be seen, the coefficient of determination (R^2) revealed goodness of the model fit to the experimental data due to his higher value. The flow behaviour index, *n* of egg albumen suggested a pseudoplasticbehaviour for shear rates from 0 to 100 s^{-1} and did not undergo great variations with added phyto-additives in hens diet. It has tended to increase with the egg storage time. A comparison of *n* values with those reported in other studies showed that the flow behaviour index values is close to those obtained by Lucisano et al. (1996) and Kumbar et al. (2015). The consistency index, *K* decreased with the storage time due to the viscosity decrease. This change can be attributed to water loss by evaporation

Table 4: Liquid egg products parameters of the Ostwald-de Waele model

Group	Storage duration (Weeks)	Egg albumen			Egg yolk			Whole liquid egg		
		K (Pa·s^n)	n	R^2	K (Pa·s^n)	n	R^2	K (Pa·s^n)	n	R^2
C	1	0.0614	0.7061	0.9213	1.3980	0.7620	0.9994	0.0989	0.6549	0.9987
	2	0.0153	0.7168	0.9824	0.5188	0.8317	0.9997	0.0647	0.6629	0.9996
	3	0.0201	0.7450	0.9821	0.5939	0.8540	0.9998	0.0596	0.6603	0.9990
	4	0.0189	0.7816	0.9949	0.9131	0.8437	0.9995	0.0384	0.7161	0.9995
E1	1	0.0227	0.7260	0.9653	2.6715	0.7855	0.9994	0.0450	0.6716	0.9993
	2	0.0161	0.7657	0.9947	0.5359	0.8368	0.9998	0.0531	0.7390	0.9983
	3	0.0591	0.7486	0.9727	1.0460	0.8591	0.9996	0.0608	0.6625	0.9974
	4	0.0287	0.7864	0.9921	1.3070	0.8446	0.9997	0.0521	0.7168	0.9995
E2	1	0.0355	0.7381	0.9824	1.8270	0.7899	0.9995	0.1549	0.6875	0.9990
	2	0.0344	0.7690	0.9866	1.3270	0.8393	0.9998	0.0772	0.7528	0.9973
	3	0.0269	0.7576	0.9801	0.9954	0.8652	0.9998	0.0320	0.6794	0.9996
	4	0.0141	0.8045	0.9963	0.8113	0.8595	0.9997	0.0338	0.7318	0.9993
E3	1	0.0349	0.7417	0.9888	2.4895	0.7918	0.9995	0.0393	0.8483	0.9997
	2	0.0166	0.7725	0.9964	0.6473	0.8505	0.9998	0.0488	0.7554	0.9992
	3	0.0167	0.8065	0.9947	0.7523	0.8899	0.9993	0.0589	0.6919	0.9994
	4	0.0147	0.8548	0.9954	1.1823	0.8754	0.9997	0.0533	0.7406	0.9993
E4	1	0.0358	0.7509	0.9922	2.5310	0.8210	0.9994	0.0490	0.7550	0.9976
	2	0.0320	0.7825	0.9902	0.6959	0.8664	0.9999	0.0732	0.7348	0.9976
	3	0.0152	0.8144	0.9883	1.5140	0.8964	0.9999	0.0721	0.6958	0.9976
	4	0.0061	0.9003	0.9939	0.9738	0.8808	0.9999	0.0462	0.7490	0.9976

*C, control group which consumed a complete feeding mixture without the addition of phyto-additives; E1, feeding mixture with bilberry leaves; E2, feeding mixture with walnut leaves; E3, feeding mixture with sea-buckthorn pomace; E4, feeding mixture with mix of bilberry leaves, sea-buckthorn pomace and walnut leaves, in equal proportions. R^2 is the coefficient of determination

through the pores in the shell and the escape of CO_2 from egg albumen. The effect of these changes can be observed by a decline of egg albumen quality during storage time.

The liquid egg yolk shows more remarkable non-Newtonian behaviour, the values of the flow behaviour index *n* being higher than those obtained for the albumen (Table 4). An increase of *n* value was obtained for E1, E2, E3 and E4 groups compared to the control. This behaviour can be related to the novel phyto-additives added in hens diet, a higher increase being found in E3 and E4 groups. The increase could be attributed the presence of phytochemicals compounds from sea-buckthorn pomace and from the mix of bilberry leaves, sea-buckthorn pomace and walnut leaves which were included in the feed. In according to another study (Abbasnezhad et al., 2015) the non-Newtonian behavior of egg yolk can be due to the phosphate and lipo-proteins or other complex molecules. The high content in lipid in the food system may favor the formation of some protein-lipid complexes which will affect the viscosity (Codina and Mironeasa, 2016; Mironeasa et al., 2012). The egg yolk proteins being high molecular weight soluble polymers can greatly increase the viscosity even at very low concentrations. During storage, the viscosity increases in the experimental groups as a function of phyto-additives added in hens diet.

The whole liquid egg has a similar non-Newtonian behavior with egg albumen and yolk (Table 4), Ostwald-de Waele rheological model being suitable. As it was expected, whole egg behaves as a pseudoplastic, fact suggested by the the flow behaviour and consistency coefficient values. During egg storage, an increase of flow behaviour index was also obvious. This behaviour can be related to the integrity of the ovomucin-lysozyme complex, and particularly on the β-fraction of ovomucin (Kato et al., 1970).

The results of these experiments proved that under controlled feeding conditions, certain changes in liquid egg components, in terms of albumen, yolk and whole egg can be observed. The evaluation of the rheological properties shows the changes of liquid egg components with storage time. Remarkable changes can be noticed on the flow behaviour index and the consistency coefficient in function of the phyto-additive type added in hens diet.

CONCLUSIONS

The properties of liquid egg components as influenced by some phyto-additives incorporated in hens diet and measured during storage can have essential applications in egg industry. According to the results obtained, the best characteristics were recorded for eggs provided by the hens whose diet was supplemented with walnut leaves. The liquid egg components differed significantly in traits, namely colour,pH and rheological properties. The viscosity of the liquid egg components decreases with the time of egg storage. The rheological test allows determining the differences between eggs which provide from a varied diet of hens with phyto-additives according to their values of flow behavior and consistency indexes. The consistency index highlights better the differences between hens diets during storage time and could be used to determine the egg freshness. The properties of liquid egg components determined make possible to improve the diet of hens in order to obtain desired quality for eggs. The monitoring of the rheological behaviour of liquid hens egg components during storage time could be of interest in the design of egg conservation conditions and consequently its quality control. The results obtained could be useful in the design of piping and pumping systems for food processing industry.

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Authors' contributions

Tatiana DumitraPanaite, Silvia Mironeasa, MădălinaIuga, and PetruAlexandruVlaicu contributed equally to th study design, collection of data, development of the sampling, analyses, interpretation of results, and preparation of the paper. All authors read and approved the final manuscript.

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