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Effects of UV-C on physicochemical quality attributes and *Salmonella enteritidis* inactivation in liquid egg products

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ABSTRACT

The perspectives of UV-C radiation as a non-thermal treatment for liquid egg products were evaluated from the point of view of the effects on egg quality attributes and the decontamination efficiency against the main egg contaminant *Salmonella enterica* subsp. *enterica* Ser. Enteritidis. UV-C treated egg fractions (egg yolk, egg white and whole egg) were analyzed for changes in pH, color, temperature-dependent viscosity and TBARS index, and were inoculated with *S. enteritidis* (ATCC 13076). Contrary to heat treatments, UV-C was not affecting viscosity and pH. Browning due to Maillard was perceptible in egg yolk and whole egg at low UV-C doses, but the corresponding browning indexes were always lower than in heat pasteurized egg fractions. Major changes were only due to lipid oxidation. TBARS values at the highest UV-C doses were larger than in pasteurized egg yolk and whole egg; under dynamic conditions and 0.61 J cm^{-2}, results were not significantly different to natural untreated samples. And UV-C was effective to inactivate *S. enteritidis*. In egg white, a load reduction up to 5.3 log_{10} was achieved under dynamic conditions (9.22 J cm^{-2}, 39 min), while 3.3 log_{10} and 3.8 log_{10} reductions were recorded in egg yolk and whole egg. Static treatments were less efficient, but still, load reductions between 1.7 and 2.8 log_{10} were obtained. At 3.94 mW cm^{-2}, time necessary to achieve a 4D reduction of *Salmonella* cells was estimated to be around 7.4 min in egg white.

1. Introduction

Heat pasteurization is the key process to eliminate pathogenic microorganisms during the production of ready-to-use and shelf-stable liquid egg products (LEP), which find extensive use in the industrial production of bakery goods, confectionaries and ice cream. But egg functional properties can be easily impaired by heat treatments; hence pasteurization is conducted on a critical temperature–time regime where the coagulation of egg proteins is prevented. For LEP pasteurization, temperature–time combinations of 56.6 °C/3.5 min for albumen, 60 °C/3.5 min for whole egg and 61.1 °C/3.5 min for egg yolk are required (Muriana, 1997; USDA-ARS 74-48, 1969). Under those conditions, however, heat resistant microorganisms such as *Bacillus* and *Micrococcus* ssp. might survive and spoil liquid egg products, even under refrigeration. Thus, disease outbreaks involving *Salmonella enterica* Ser. Enteritidis in LEP continue to be a major public health concern (FSA, 2008; Little et al., 2007).

Since actual consumers prefer minimally processed and preservative-free products, the need for alternative non-thermal pasteurisation technologies, which do not compromise product quality, is crucial. Contrary to the limitations of thermal treatments, UV-C radiation is known to be extremely effective against most vegetative microorganisms, suggesting that this technology can be an alternative and cost-effective non-thermal process for LEP in order to achieve microbiologically safe and shelf-stable products (Bintsis, Litopoulou-Tzanetaki & Robinson, 2000; Donahue, Canitez & Bushway, 2004). The use of ultraviolet light at germicidal wavelengths has been approved to treat food surfaces and clear fruit juices (US-FDA, 2002). However, the efficiency of UV-C radiation depends on the UV-C absorption: increasing the amount of solids, large suspended particles or microbial populations will reduce the penetration of UV-C (Guerrero-Beltran & Barbosa-Canovas, 2004; Koutchma, Parisi & Patazca, 2007; Lopez-Malo & Palou, 2002).

Especially due to the difficulties of the radiometers to accurately measure the incident UV-C radiation near long tubular lamps (Qualls & Johnson, 1983), alternative calibration methods have been developed. Thus, bench-scale equipments with collimated beams have been extremely useful as a standardized methodology to study UV effects on microorganisms (Bolton & Linden, 2003) and have been extensively applied to calibrate UV-C water disinfection systems. Concerning studies in liquid egg, Unluturk, Atilgan, Baysal, and Tari (2007) used a flat black collimated beam to evaluate the efficiency of UV-C as a non-thermal process for LEP fractions using

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the *Escherichia coli* strain ATCC 8739, among others. The best reduction (>2 log cycles) for this highly UV resistant strain was achieved in liquid egg white (LEW), at 0.153 cm fluid depth and 1.314 mW cm^{-2} UV intensity. But under similar conditions, maximum inactivation ranged 0.675 log_{10} CFU mL^{-1} in liquid egg yolk (LEY), and 0.316 log_{10} CFU ml^{-1} in liquid whole egg (LWE). Ngadi, Smith, and Cayouette (2003) worked on the inactivation of *E. coli* O157:H7 in LEW under a stainless steel tube, and reported a reduction of 5 log cycles with 0.1 cm sample depth at 5 mW min cm^{-2}. Furthermore, Geveke (2008) reported an effective UV treatment of *E. coli* K12 (ATCC 23716) in LEW, using a continuous process with a low-pressure mercury lamp surrounded by UV transparent tubing and a silicon rubber tape. In that work, the population of *E. coli* was reduced by 4.3 log cycles after being exposed to UV at 50 °C for 160 s.

Under collimated beams, however, treatment surface and sample volume are considerably small due to the recommended tube dimensions. Furthermore, industrial designs benefit from higher irradiances and highly reflective surfaces to enhance the UV germicide effects (Keyser, Müller, Cilliers, Nel & Gouws, 2008; Koutchma, Keller, Chirtel & Parisi, 2004). To gain in flexibility, complex irradiation geometries or larger sample amounts are required; in this case, the most accurate measure of incident photons is probably the well defined chemical actinometry (Kuhn, Braslavsky, & Schmidt, 2004; Linden & Darby, 1998). In this paper, the effects of UV-C radiation treatments on relevant quality attributes in liquid egg products were evaluated. The study of pH, colour, viscosity and lipid oxidation, provided an initial assessment of the impact and benefits of UV-C radiation, compared to conventional heat treatments of LEP. In addition, kinetic parameters for the decontamination efficiency of UV-C on the most relevant contaminant in eggs were determined in samples inoculated with *S. enteritidis* (ATCC 13076). Experiments were carried out in a bench scale apparatus where the light intensity was enhanced by a highly reflective surface in order to minimise effects on the quality by reducing UV-C exposure time. A chemical actinometer was used to estimate the incident energy. Experiments were performed separately in egg yolk, egg white and whole egg.

2. Material and methods

2.1. Materials

Fresh eggs were purchased from Avícola Llombay (Valencia, Spain). They were of yellow shell, and weighted between 55 and 61 g. After reception, eggs were inspected for shell integrity and stored under refrigeration at 8 °C. Just before experiments were carried out, the egg content (separately, egg whites and egg yolks) was removed under aseptic conditions, and collected in sterile containers. The pH of the samples was controlled before proceeding with the experiments and eggs were considered to be fresh when pH was around 7.2 (±0.2) for egg white, and 6.2 (±0.2) for the egg yolk.

The chalaza was removed and the separated egg fractions were then homogenized for 1 min using a vortex (MS3 Digital, IKA®, USA) at the maximum speed (3000 rpm). To prepare the whole egg samples, 13.3 mL of egg yolk were mixed with 26.7 mL of egg white.

2.2. Methods

2.2.1. UV-C radiation of egg samples

UV radiation of samples at germicide wavelengths was conducted in batch in a UV chamber constructed by UV-Consulting Peschl® España (Burjassot, Valencia, Spain). The chamber is made of stainless steel and provided with one low-pressure mercury lamp with 7.3 W output and 436 mm length (Heraeus Noblelight GmbH, Hanau, Germany), with maximum peak radiation at 253.7 nm. Chamber dimensions are 60 × 40 × 40 cm, the inner surface is flat black painted to avoid light reflection in the walls; to enhance the amount of light arising the samples, an aluminium reflector surface covered the lamp. After UV emission stabilization, the lamp remained on; a shutter between the lamp and the exposition chamber was used to protect the operator without disturbing the operational conditions of the lamp. The average UV radiation intensity (total UV-C output Units (mW) per Area (cm^{-2})) arising the sample surface was quantified using chemical actinometry. For this, the iodide/iodate method was carried out in bi-distilled water under continuous agitation, as proposed by Rahn (1997), in an area equivalent to the treatment surface. The incident photons were calculated by assuming that, at defined concentrations where the mixture is opaque below 290 nm, all the incident photons are absorbed by the solution (Rahn, 1997). Thus, the average estimated fluence rate at the position chosen was 3.94 mW cm^{-2}, and the dose calculated as fluence rate × time in s (expressed as J cm^{-2}). The excess heat was dissipated by a ventilator installed on the upper part of the chamber.

A magnetic stirrer (Ovan MBG15, Barcelona, Spain) was installed at the central part of the lamp. Samples (12 mL volume; 0.2 cm height) were placed in polystyrene Petri dishes (60.3 cm^2) and they were treated in batch, under static or dynamic conditions. For the dynamic treatment, samples were continuously stirred during irradiation.

Before each test was run, the apparatus was cleaned and sanitized. A series of trials were conducted to evaluate the effect of UV light dose on the population of microorganisms on liquid egg products and on some quality attributes. The experiments were run in a thermostated room at 21 °C, the temperature of untreated samples was around 21 °C and presented an increase below 0.5 °C during the treatment.

2.2.2. Pasteurization

D-values of LEP products have been characterised (Garibaldi, Straka & Liichi, 1969). Aiming at a comparison with conventional pasteurizations, 1 mL ampoules of the analyzed egg fractions were treated using a thermostatic bath (Unitronic OR, Selecta, Spain) set to 56.6 °C, 60 °C and 61.1 °C respectively for egg white, whole egg and egg yolk. The conditions for pasteurisation were chosen in conformity with the requirements of the USDA (USDA ARS 74-48, 1969). The holding time used for the three fractions was 3.5 min when the coldest point of the sample attained the pasteurization temperature.

2.2.3. Effects of UV-C on physicochemical quality attributes

2.2.3.1. pH. The determination of pH was undertaken in triplicate with a calibrated Consort C830 pH meter (Consort, CE, Belgium). Before mechanical homogenization, fresh eggs showed an average pH value of 7.8 in albumen and 6.2 in egg yolk (Table 1). The pH of

Table 1
Effects of sample treatment (homogenization, pasteurization, UV-C) on the pH values of egg fractions: egg white, egg yolk and whole egg. Mean ± S.D., mean values of three replications.

		LEW	LEY	LWE
Natural		$7.81^{a} \pm 0.10$	$6.22^{a} \pm 0.09$	$7.28^{a} \pm 0.04$
Natural homogenized		$9.22^{c} \pm 0.09$	$6.22^{a} \pm 0.17$	$7.85^{c} \pm 0.10$
Pasteurized		$9.90^{b} \pm 0.09$	$6.58^{b} \pm 0.16$	$8.56^{b} \pm 0.10$
UV static	0.612 J cm^{-2}	$9.09^{c} \pm 0.23$	$6.21^{a} \pm 0.20$	$7.73^{c} \pm 0.12$
	3.645 J cm^{-2}	$9.12^{c} \pm 0.26$	$6.19^{a} \pm 0.15$	$7.75^{c} \pm 0.17$
UV dynamic	0.612 J cm^{-2}	$9.20^{c} \pm 0.21$	$6.11^{a} \pm 0.12$	$7.77^{c} \pm 0.12$
	3.645 J cm^{-2}	$9.00^{c} \pm 0.14$	$6.14^{a} \pm 0.11$	$7.65^{c} \pm 0.08$

Values with different superscripts within a column are significantly different ($P < 0.05$), as determined by the Tukey's test at the 95% confidence level.

egg albumen is strongly related to the egg aging process, being CO_2 exchanged through the shell over the storage period: this process increases considerably the pH values. On the contrary, yolk pH values are not influenced by the CO_2 concentration and remain almost unchanged during aging. Thus, the relatively low pH values found throughout this study proved for egg freshness. The pH value of homogenized samples was situated within the expected standard conditions (around 9.2).

2.2.3.2. Color. Color changes were quantified through the CIELAB color space coordinates (L^*, a^*, and b^*) obtained by a spectrocolorimeter (Hunter Labscan II, Minolta, Tokyo, Japan), equipped with D65 as the light source, and using an observation angle of 10°. The spectrocolorimeter was calibrated with standard black and white tiles. The spectrocolorimeter is equipped with a Color Data Software CM-S100w Spectra magic NX (Konica Minolta, Tokyo, Japan).

From L^*, a^* and b^* coordinates, the values of C^* (chroma), h^* (hue) were directly calculated, while ΔE^* (CIE total color difference), and BI (browning index) were calculated using equations (1) and (2), respectively (Palou, Lopez-Malo, Barbosa-Canovas, Welti-Chanes, & Swanson, 1999). Triplicate measurements were carried out for each experiment.

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

$$BI = \frac{100\left[\frac{0.31(a^* + 1.75L^*)}{5.645L^* + a^* - 3.012b^*}\right]}{0.172} \quad (2)$$

2.2.3.3. Temperature dependent viscosity. The temperature-dependent viscosity measurements were carried out using a rheometer (Rheostress RS100, Haake, Karlsruhe, Germany), equipped with a parallel-plate measuring system (rotor 222-1223, 35 mm radius, 1.0 mm gap) and a Thermo Haake C25P refrigerated bath (Karlsruhe, Germany), which allows an accurate temperature control of the plate.

The temperature-dependent viscosity behavior was determined, according to the method of Jaekel and Ternes (2009), by placing the sample in the rheometer, then equilibrating for 10 min and increasing the temperature linearly from 18 °C up to 33 °C using a temperature gradient of 1 °C min^{-1}, and measuring the viscosity at a constant shear rate of 26 s^{-1} following Jaekel, Dautel and Ternes (2008). Low-viscosity silicon oil was chosen as a sealing fluid for all the measurements in order to avoid coagulation of the egg protein and to prevent moisture loss during heating.

2.2.3.4. Lipid oxidation. To evaluate the extension of the lipid oxidation on the samples, the determination of the amount of the formed 2-thiobarbituric acid-reactive substances (TBARS) was undertaken, according to Vynckel (1970) and Ramanathan and Das (1992). Before TBARS analysis was conducted, UV-C treated egg samples were freeze-dried in a Genesis Freeze Dryer (SP Virtis, 35EL Genesis SQEL85, New York, USA). Then 2.5 g of the dry samples were mixed with 17 mL of 7.5% (w/v) trichloroacetic acid (TCA). The samples were allowed to mix up for 10 min, and then they were filtered with a cellulose filter in a 10 mL volumetric flash. Volume (up to 10 mL) was filled up with 7.5% TCA solution if necessary. Five mL of the filtered solution were then mixed with 5 mL of 0.02 M thiobarbituric acid (TBA) thoroughly with a Vortex mixer, and heated in a boiling water bath (Unitronic OR, Selecta, Spain) for 40 min. Afterwards samples were allowed to cool down to room temperature. TBARS index was estimated at 530 nm in a spectrophotometer (Agilent, St. Claire, USA) by comparison with a blank sample containing only TCA/TBA reagent. Concentrations of TBARS were determined using a standard curve prepared using malondialdehyde (MDA) and expressed as mg MDA kg^{-1} of dry sample.

2.3. Effects of UV-C on the inactivation of S. enteritidis

S. enterica subsp. *enterica* Ser. Enteritidis ATCC 13076 was obtained from the Spanish Type Culture Collection (Valencia, Spain). Strain was stored in Tryptone Soy Broth (TSB) with 20% glycerol at −80 °C. Stock cultures were kept by regular subculture on agar Tryptone Soy Agar (TSA) slants at 4 °C. Before inoculation of egg fractions, an overnight culture was prepared, afterwards a loopful of the *Salmonella* strain was transferred to Tryptone Soy Broth (TSB) and incubated at 37 °C for 18 h to obtain early-stationary phase cells. Incubation for 24 h allowed the respective bacteria to approach the stationary phase of growth.

For *Salmonella* inactivation trials, the overnight was homogenised and distributed in ten 1 mL Eppendorf tubes that were centrifuged at 1000 rpm during 2 min and ambient temperature. 0.8 mL of the supernatant phase was discarded and the other 0.2 mL of all ten tubes was spread on 50 mL of fresh samples (LEW, LEY, LWE), to obtain a concentration of cells around 7–8 $\log_{10}$ CFU mL^{-1}. Afterwards, 12 mL of the homogenized mixture were transferred to standard Petri dishes (equivalent to 0.2 cm height). For enumeration, decimal dilutions were made with 0.1% peptone water and samples were surface plated in duplicate on Trypticase Soy Agar (TSA, Scharlau, Germany). The plates were incubated at 37 °C for 24 h and counted. All experimental conditions were tested in triplicate.

2.4. Fitting of kinetic data

To define the time required to achieve a 4D reduction, GInaFiT V1.5 (Geeraerd, Valdramidis & Van Impe, 2005) was used to fit the experimental data on the inactivation kinetics of *S. enteritidis* at a UV light intensity of 3.94 mW cm^{-2} to a Weibull distribution (Mafart, Couvert, Gaillard, & Leguerinel, 2002):

$$\log_{10}\left(\frac{N}{N_0}\right) = -\left(\frac{t}{\delta}\right)^p \quad (3)$$

where N is the number of microorganisms at time t (CFU mL^{-1}); N_0 is the initial number of microorganisms (CFU mL^{-1}), t is the treatment time (min), δ is the time required for the reduction of one $\log_{10}$ cycle in min, and p is the shape parameter describing concavity (when $p = 1$, survival curve is linear; $p > 1$ indicates upwards concavity).

2.5. Statistical analysis

One-way analysis of variance (ANOVA) was performed with the software XLSTAT-Pro (Win) 7.5.3 (Addinsoft, NY). Statistical analysis was run with a confidence level of 95%. Comparisons between treatments were evaluated with the Tukey test.

3. Results and discussion

3.1. pH

LEW and LEY are used by the food industry as food ingredients because of their excellent functional properties, such as foaming, emulsifying and gelling. Among others, pH is an essential attribute to achieve adequate albumen functional properties. Treatments with UV-C of the homogenized samples have not caused an effect on pH directly after treatment. UV-C treated LEY was not significantly

different ($P > 0.05$) to the untreated control at both doses, under static or dynamic conditions (Table 1). Functional properties of the egg yolk are highly dependent on pH, and around pH 6, an acceptable solubility of the yolk proteins can be achieved (Chang & Chen, 2000). The effects of homogenization were evident only in the pH of LEW and LWE, and the pH of the UV-C treated samples was also not significantly different ($P > 0.05$) to the homogenized products (Table 1). But contrary to the negligible effects of UV-C radiation on the pH, the pH of the egg fractions was more impacted by the thermal pasteurization, finding significant differences ($P < 0.05$) for all analyzed egg fractions.

3.2. Color

Customers may not accept discoloration and the change in the shade caused by the UV radiation or the thermal treatments, considering the eggs as being of low-quality. Color perception highly depends on the chemical and physical properties of the egg components (Min, Nam, Lee, Ko, Trampel, & Ahn, 2005). The raw CIELAB L^*, a^* and b^* coordinates are represented in Table 2 and the calculated BI and the ΔE^* for the three fractions of the liquid egg are recorded in Table 3. In general, the browning index increased as a function of the UV-C dose in all the studied fractions, and ΔE^* values also increased as a consequence of the UV treatment. Remarkably, differences with the untreated controls were more evident when the samples were submitted to static UV-C treatments, pointing out for a certain dissipation of the oxidative effects during mixing, probably due to the radical scavenging capacity of the available antioxidants.

In UV treated LEW, there was a slight but not statistically significant tendency of the coordinate b^* to increase, and a significant decrease in L^* at the higher doses. Regarding the parameter a^*, which reflects the changes in the red region of the spectrum (i.e. +120, red color), UV treated egg whites showed a small tendency to increase, if compared with untreated controls. The slight increase in the browning index led to perceptible changes in samples treated under static conditions. But dynamic treatments up to 3.645 J cm^{-2} in LEW caused ΔE^* values which were not expected to be detectable by the naked eye ($\Delta E^* < 3$).

In accordance to the results obtained for LEW, also the parameter a^* of LWE and LEY increased slightly after treatment with UV-C lamps, showing a tendency upon more reddish tones. But in LEY and LWE, the parameter b^* (yellow color, +120; blue-violet color, −80) is predominant due to the presence of carotenoids. In those samples, the parameter b^* increased remarkably after treatments with UV-C, although differences were not significant. That increase is generally associated to the destruction of carotenoids and the formation of Maillard products, which are evident during egg processing or storage (Badr, 2006; Caboni et al., 2005). As in LEW, the effects of the treatment were more evident under static conditions, indicating a buffering effect of the available antioxidants during mixing. The increase in the browning index of the egg yolk was perceptible at the lowest doses applied, since ΔE^* values were above 3. But in LWE, the presence of the egg white minimized the changes in color, and 3.645 J cm^{-2} were not enough to cause a detectable value change since ΔE^* was below 3 under dynamic conditions. The changes observed in UV irradiated samples are opposite to the generally reported for ionizing radiation, where a discoloration (regarding a significant decrease in L^*, a^* and b^*) of the yolk towards a more pale tone has been reported at doses above 2.5 kGy (Dvorak, Kunova, Strakova, Suchy & Kunova, 2005).

Heat treatments accelerated the production of brown Maillard products. Compared to UV-C, the loss in lightness, and the increment in the parameters a^* and b^* were more evident in heat pasteurized samples, pointing out for a more extensive effect of heat on the colour of the liquid egg samples. Those results confirmed a tendency towards more reddish/brownish tones of the heat pasteurized LEP.

Table 3
Total color difference (ΔE^*) and browning index (BI) in egg fractions submitted to homogenization, pasteurization or UV-C. Results are the mean of three independent replications.

		LEW		LEY		LWE	
		ΔE^*	BI	ΔE^*	BI	ΔE^*	BI
Natural homogenized		0.00	129.08	0.00	321.26	0.00	142.28
Pasteurized		12.24	272.78	14.85	785.48	11.97	227.28
UV static	0.612 J cm^{-2}	6.40	175.73	11.02	550.77	6.12	176.21
	3.645 J cm^{-2}	8.41	197.64	12.22	614.42	4.41	165.98
UV dynamic	0.612 J cm^{-2}	1.30	136.73	9.16	465.57	2.65	155.25
	3.645 J cm^{-2}	2.51	144.24	9.47	478.47	2.09	152.39

$\Delta E^* < 2$, minimum differences; ΔE^* between 2 and 3, acceptable differences; ΔE^* between 3 and 5, almost unacceptable; $\Delta E^* > 5$, unacceptable differences.

3.3. Temperature-dependent viscosity

Egg functional properties are highly dependent on rheological attributes. Typically, the temperature dependent flow behavior of egg shows a viscosity minimum, which is close to 65 °C, e.g., in egg yolk (Jaekel & Ternes, 2009). With regards to the UV-C treatments investigated here, dynamic or static conditions have not originated severe differences in the flow behavior compared to the native samples, and a continuous decrease in flow viscosity was observed up to 33 °C (Fig. 1a–c). And among the different processing conditions tested, the static UV-C treatment of the egg white and whole egg fractions provided results closer to the native than the others (pasteurization and dynamic UV-C). Therefore, and contrary to the watery structure observed in eggs irradiated with a linear accelerator (Min et al, 2005), apparent viscosity was highly preserved after UV-C treatments. The loss in viscosity, and the associated watery structure in LEW and LWE, has been related to changes in the configuration of ovomucin (important for the gel-

Table 2
CIELAB L^*, a^* and b^* color coordinates in egg fractions submitted to homogenization, pasteurization or UV-C. Each sample was measured in 5 different positions; results are the mean of three independent replications.

		LEW			LEY			LWE		
		L^*	a^*	b^*	L^*	a^*	b^*	L^*	a^*	b^*
Natural homogenized		$42.33^{a} \pm 1.66$	$0.77^{a} \pm 0.18$	$32.54^{a} \pm 1.45$	$59.28^{a} \pm 1.27$	$27.40^{a} \pm 0.57$	$69.87^{a} \pm 5.90$	$63.32^{a} \pm 0.56$	$19.72^{a} \pm 1.23$	$47.13^{a} \pm 1.01$
Pasteurized		$30.16^{d} \pm 1.37$	$1.22^{b} \pm 0.14$	$33.95^{a} \pm 0.52$	$50.76^{b} \pm 0.78$	$30.65^{b} \pm 1.86$	$81.60^{b} \pm 5.98$	$53.11^{c} \pm 2.11$	$23.29^{b} \pm 2.06$	$52.24^{b} \pm 2.80$
UV static	0.612 J cm^{-2}	$39.90^{ab} \pm 1.49$	$0.84^{a} \pm 0.15$	$32.96^{a} \pm 1.39$	$57.14^{ab} \pm 0.46$	$28.32^{ab} \pm 1.33$	$78.96^{ab} \pm 1.41$	$60.89^{ab} \pm 2.81$	$20.21^{a} \pm 0.73$	$47.86^{a} \pm 1.03$
	3.645 J cm^{-2}	$38.32^{bc} \pm 2.90$	$0.88^{ab} \pm 0.19$	$33.23^{a} \pm 2.73$	$53.55^{ab} \pm 4.53$	$30.15^{ab} \pm 1.84$	$80.13^{ab} \pm 1.82$	$57.71^{bc} \pm 1.30$	$21.25^{ab} \pm 1.73$	$49.05^{ab} \pm 1.88$
UV dynamic	0.612 J cm^{-2}	$41.07^{ab} \pm 0.68$	$0.83^{a} \pm 0.13$	$32.93^{a} \pm 0.41$	$57.62^{ab} \pm 2.75$	$28.11^{ab} \pm 0.77$	$78.79^{ab} \pm 4.41$	$61.45^{ab} \pm 3.72$	$20.18^{a} \pm 1.48$	$47.71^{a} \pm 1.05$
	3.645 J cm^{-2}	$35.90^{c} \pm 1.19$	$0.87^{ab} \pm 0.18$	$33.18^{a} \pm 2.31$	$55.16^{ab} \pm 5.89$	$29.52^{ab} \pm 1.47$	$79.83^{ab} \pm 7.56$	$59.34^{ab} \pm 1.76$	$20.74^{ab} \pm 0.34$	$48.64^{ab} \pm 2.17$

Different superscripts indicate significantly different to the natural homogenized sample at the 95% confidence level.

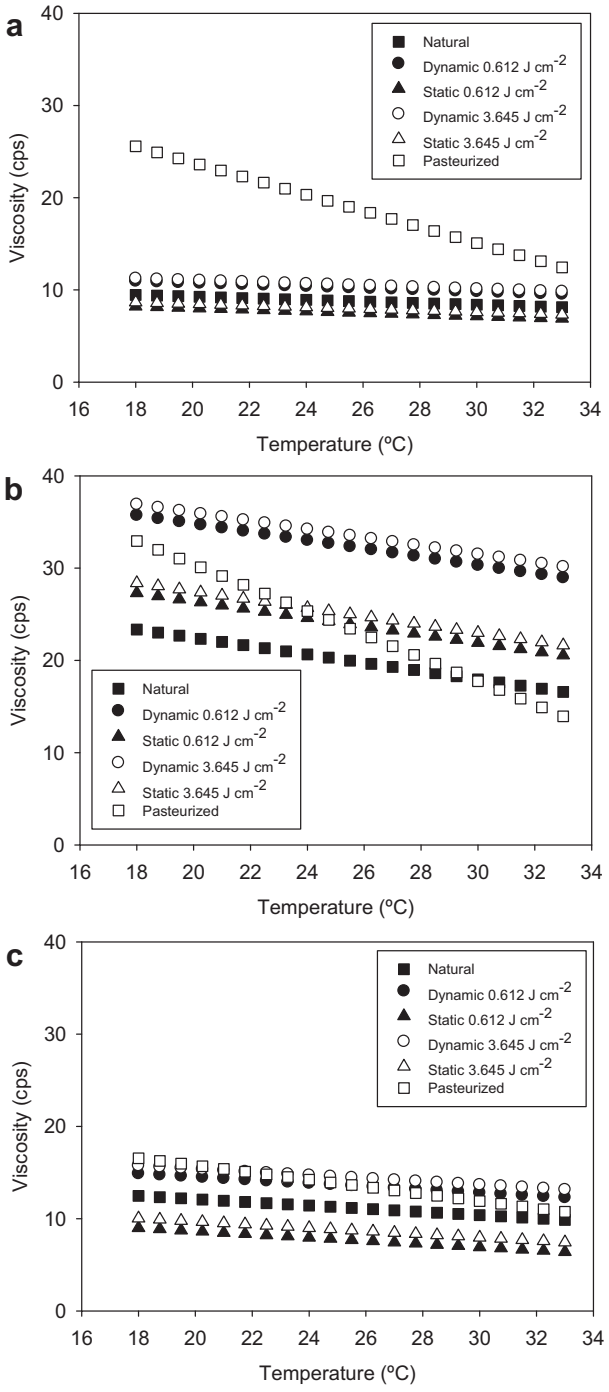

Fig. 1. Influence of UV-C radiation at a fluence rate of 3.94 mW cm^{-2} on the temperature-dependent viscosity of (a) LEW, (b) LEY, (c) LWE, under static and dynamic conditions. Results are the mean of three replicates. Average S.D. was below 5%.

like structure of the egg white), which seems to be highly preserved after UV-C treatments.

However, after conventional pasteurization, a slight increase in the viscosity of the yolk is expected in the low temperature range, at the view of the results of Jaekel and Ternes (2009). In this work, this effect was also evident. Differences were, however, more remarkable in the pasteurized LEW, where protein content is higher than in LEY or LWE. But to obtain a further insight into the flow behavior of the egg fractions, studies over higher temperature ranges have to be done.

3.4. Lipid oxidation

In eggs, quality deterioration due to oxidative processes in cholesterol and unsaturated fatty acids might be originated by UV, since radiation at short wavelengths is an effective promoter of the lipid peroxidation (Spikes, 1981). But natural antioxidants, such as tocopherols, carotenoids and phosvitin, and the structure of the yolk low density lipoproteins (LDLs) might contribute to decelerate the oxidative processes. The evaluation of the presence of the thiobarbituric acid-reactive substances provides a first estimation to the extent of lipid oxidation originated by decontaminating UV-C wavelengths.

The TBARS values of fresh eggs after homogenization ranged approx. 0.594 and 0.791 mg MDA kg^{-1}, respectively for LWE and LEY. The TBARS values obtained in this study are comparable to those obtained by other authors for whole egg (Ren, 2009), showing that the undertaken agitation step has not caused a considerable increase in the concentration of secondary products of the lipid oxidation.

The TBARS values (expressed as mg MDA kg^{-1} of dry sample) for UV-C treated liquid egg products are reported in Fig. 2(a and b), as well as the values obtained for thermally treated egg samples and for the untreated homogenized samples. Contrary to the fair stability of TBARS in chicken breasts shown by Chun, Kim, Lee, Yu and Song (2010) submitted to UV-C radiation, the TBARS values of LEP increased as a function of the UV dose, with results significantly different to the untreated controls at 3.645 J cm^{-2}. Pasteurized LEY also showed a significant increment, and was more similar to UV-C samples than to the untreated controls.

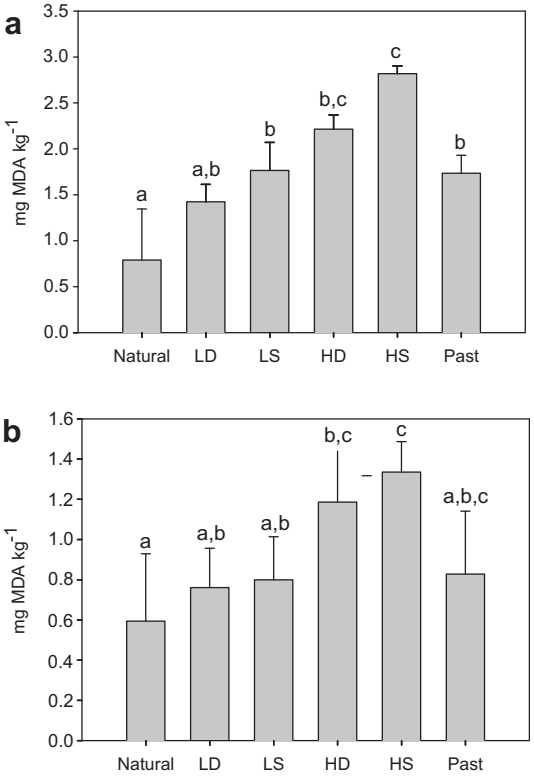

Fig. 2. Influence of UV-C radiation at a fluence rate of 3.94 mW cm^{-2} on lipid oxidation of (a) LEY and (b) LWE, under the conditions LD (0.612 J cm^{-2} dynamic), LS (0.612 J cm^{-2} static), HD (3.645 J cm^{-2} dynamic) and HS (3.645 J cm^{-2} static). Natural are natural homogenized samples, and PAST are pasteurized samples. Results are the mean of three replicates.

Few studies deal with the effects of egg processing on TBARS values. Thermal processing of eggs originated a remarkable increase in the TBARS (Liu, Yang, Lin, & Lee, 2005; Ren, 2009). Formation of hydroperoxides was also accelerated in egg yolk powder submitted to ionizing radiation, with the subsequent degradation of carotenoids (Katusin-Razem, Mihaljevic & Razem, 1992). And spray-drying of LWE originated the formation of furosine through Maillard, and the oxidation of cholesterol (Caboni et al., 2005). In our study, both processes (UV and thermal) significantly increased the TBARS values in all egg fractions, which could have consequences in the organoleptic characteristics. After the short heat treatments, thermally treated liquid egg products presented TBARS values 2–3 fold higher than the untreated controls, but the treatment with UV-C at the highest doses tested seems to be more oxidizing than heat.

3.5. UV-C inactivation of S. enteritidis ATCC 13076 in liquid egg samples

Based on the *Salmonella* counts after serial dilutions, semi-logarithmic survivor curves are represented in Fig.3a–c, relating the viable *Salmonella* population ($\log_{10}(N N_o^{-1})$) to the UV-C dose under static or dynamic conditions.

Salmonella strains in food matrices are more resistant to inactivation than in buffered systems. Inactivation of *S. enteritidis* under UV-C at 3.94 mW cm^{-2} fluence rate and under dynamic conditions was more accurately described by the Weibull distribution than by linear models, meaning that data presented a certain tailing in all liquid egg fractions. This can be mainly attributed to the egg low light transmittance, which protects the bacterial cells during irradiation.

The estimated parameters for the UV-C inactivation under dynamic and static conditions are recorded on Table 4. An effective reduction of 5.2 $\log_{10}$ CFU mL^{-1} was achieved in LEW samples subjected to the highest UV-C doses (9.2 J cm^{-2}) (Fig. 3a) confirming the good results reported by Geveke (2008) and Ngadi et al. (2003); while remarkable 3.34 $\log_{10}$ CFU mL^{-1} (Fig. 3b) and 3.77 $\log_{10}$ CFU mL^{-1} (Fig. 3c) reductions were recorded in LEY and LWE, respectively, also at the maximum dose. Absorbance coefficients, as calculated applying the Beer–Lambert law after absorbance measurements of serial dilutions for LEP fractions, explain the effectiveness of the treatments (egg white > whole egg > egg yolk). They accounted 130 cm^{-1} for LEW, 620 cm^{-1} for LEY and 337 cm^{-1} for LWE, in the range recently commented by Unluturk, Atilgan, Baysal, and Tari (2007) for similar products. Consequently, the dose required to achieve *Salmonella* inactivation in LEP is relatively high, if compared to microorganisms in clarified juices (0.04 J cm^{-2}, as commented by Guerrero-Beltran & Barbosa-Canovas, 2004) or on chicken breast (0.5 J cm^{-2}, as commented by Chun et al., 2010), for example. But results here are presented as a function of the average fluence rate as calculated by actinometry, which is higher than the expected absorbed dose in 0.2 cm deep LEP samples: 0.360 J cm^{-2} in LEW, 0.075 J cm^{-2} in LEY, and 0.140 J cm^{-2} in LWE, estimated following Morowitz (1950).

Due to large amount of variables, comparison of *S. enteriditis* UV sensibility with results for other microorganisms in a similar system is difficult. Unluturk, Atilgan, Baysal, and Tari (2007) described the kinetic results under collimated beams for *E. coli* ATCC 8739 in egg products using a first-order approximation. Using their fit, a decimal reduction time of 10 min is expected in LEW for that microorganism, which is considerably larger than the decimal reduction time found in this work for *Salmonella* (0.01 min in LEW under dynamic conditions). In food systems, *Salmonella* is known to be a highly UV resistant microorganism (Chevrefils et al., 2006; Yaun, Sumner, Eifert, & Marcy, 2003), but for example, UV intensity, being considerably higher in the system tested here, might play a non-negligible role in the inactivation of microorganisms under UV-C. Consequently, models able to accurately describe the microorganism inactivation under UV will have to consider the largest amount of operational variables influencing the UV-C efficiency, and will be the subject of following studies.

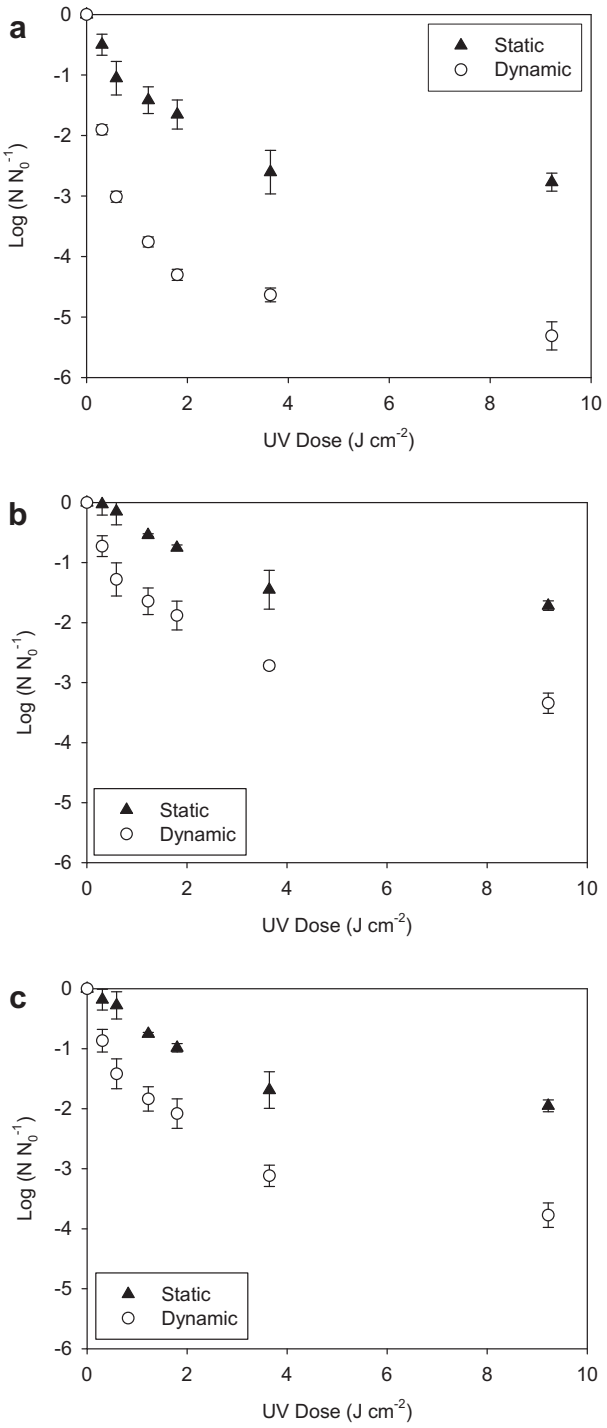

Fig. 3. Influence of UV-C radiation at a fluence rate of 3.94 mW cm^{-2} on *Salmonella enteritidis* inactivation under static or dynamic conditions: (a) LEW; (b) LEY; (c) LWE. Results are the mean of three replicates.

Table 4
Weibull parameters describing the inactivation of *S. enteritidis* under UV-C applied at a fluence rate of 3.94 mW cm^{-2}, under static or dynamic conditions. δ is the characteristic time in min, and p is the shape parameter.

		δ	p	R^2	RMSE	t (min) to achieve 4D
Static	LEW	1.19	0.33	0.88	0.31	>39
	LEY	11.46	0.54	0.92	0.17	>39
	LWE	8.08	0.48	0.92	0.18	>39
Dynamic	LEW	0.01	0.21	0.90	0.39	7.4
	LEY	0.94	0.34	0.92	0.26	>39
	LWE	0.70	0.35	0.92	0.28	38.6

UV-C is already used to decontaminate egg shells because it lowers remarkably the concentration of *S. enteritidis* (Rodriguez-Romo & Yousef, 2005). And, although treatment parameters are not fully comparable, lower effects than the ones reported here for UV-C decontamination have been recorded in LEP treated with other non-thermal technologies. Thus, for example, energy levels up to 250 J g^{-1} could be required to achieve a pasteurization of LWE with pulsed electric fields (Monfort, Gayan, Raso, Condon & Alvarez, 2010), and lower energy levels seem to be ineffective against *S. enteritidis* even if applied at 55 °C; furthermore, high pressure cycles achieved only 2 log cycles reduction around 20 °C (Huang, Mittal, & Griffiths, 2006). And *S. enterica* showed a larger D (over 2.6 min) in raspberries and strawberries (Bialka, Demirci, & Puri, 2008) during treatments with aqueous ozone than observed here for *S. enteritidis* under UV-C treatments (0.01 min in LEW). The influence of other variables in UV-C, especially the fluence rate, or the combination with heat or antimicrobials, remains to be investigated.

4. Concluding remarks

Results obtained confirm UV-C treatment as a promising technology to reduce microbial loads without impairing relevant quality attributes of liquid egg products. The main UV-C radiation-induced chemical changes were related to the lipid oxidation. TBARS values increased as the radiation doses increased; but results were not significantly different to heat pasteurized if dynamic conditions were applied. A perceptible browning in egg yolk and whole egg was stated due to Maillard, but total color differences were acceptable for egg white and whole egg also at high UV-C doses. Under dynamic UV-C treatments at a fluence rate of 3.94 mW cm^{-2}, a reduction of approx. 5.3 log cycles was achieved in the population of *S. enteritidis* in liquid egg white. But UV-C itself, when applied at temperatures below 30 °C, might not be able to pasteurize liquid whole egg or liquid egg yolk. The influence of other treatment parameters, as the fluence rate, or the combination with mild heat or antimicrobials, remains to be investigated.

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