SUPERVISION OF OVOPRODUCTS SPOILAGE WITH RED LED LIGHT

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Abstract

Heat-treatment of ovoproducts is often required to ensure microbial safety. However, it has been shown that in most microbial species slow heating, or heat shocks may induce a higher heat resistance, that means that it is not possible to remove the microbial flora completely. These microorganisms produce on ovoproducts spoilage especially when the cold chain is broken along the transportation and/or storage. As a result, the life span for the product is shortened. The microbial activity inside the product causes changes in several physical properties, which can be supervised using optical methods. The aim of this work is to monitor the sigmoid behaviour underlying the ovoproduct evolution and spoilage by means of red LED light. For two commercial types of liquid and pasteurized egg white, storage at 9ºC, an average correlation of r=0.94 has been found between microorganism growth and images mean grey level of LED light passing though the sample. The results show that it is possible to develop very simple predictive models taking into account only one optical parameter corresponding to a single LED.

Introduction

Ovoproducts show very high sensitivity to microbiological spoilage which makes necessary to apply a thermal treatment to ensure its innocuity. Industrial pasteurisation of liquid egg (white, yolk and whole egg) typically consists of heating at temperatures between 57 and 72 ºC for times ranging from a few seconds to about 10 min, depending on the temperature, the product and the pasteurisation system. This treatment is designed to inactivate pathogenic microorganisms such as Salmonella without damaging the physico-chemical and functional properties of ovoproducts. However, such thermal treatment does not ensure complete eradication of the microbial flora (Guilmineau and Kulozik 2007). Slow heating, or heating for short periods of time at temperatures above the optimum for growth (heat shocks) may induce to most microbial species a higher heat resistance (Mañas et al. 2003),

which leads to limited shelf life of the ovoproduct and the need for storing at 4 ºC maximum (Guilmineau and Kulozik 2007).

Spanish producers recommend 2ºC as optimal storage temperature for every step in the cold chain. One of the main consumers of ovoproduct, the catering trade industry, is not able to ensure such conditions due to frequent doors opening which leads to storage temperatures of 8 or 9 ºC in cold rooms.

The reference method for addressing the microbiological load of one sample is the determination of the concentration of mesophilic microorganisms counting the amount of colonies forming units (CFU). A sigmoid function can be used as primary model to describe the growth curve of microorganisms (Cairé et al 2007). On the other hand, several studies show the effect of heat treatment and or microbiological spoilage on some properties of ovoproducts as turbidity, colour and viscosity (Hou et al. 1996) and gel properties (Coimbra et al. 2006). These physical properties can be supervised by means of optical techniques.

At present time there are some instruments that make use of light emitter diodes (LEDs) to the automatic supervision of coagulation processes as i.e. the cheese clotting process (Castillo et al 2006). **The aim of this work is to carry out a prospective study for the supervision of ovoproduct spoilage with red Light-Emitting Diodes (LEDs) light.**

Materials and methods

The study was carried out at summer time, critic season for preservation of perishable goods. Liquid egg white pasteurized ovoproduct was transported directly from the producing industries in refrigerate trucks and later it was stored in industrial refrigeration cameras at the School of Agricultural Technical Engineering of the UPM for its conservation at temperatures of 2ºC (control) and 9ºC. Two kinds of commercial products were used: the first one, named in this work TP, is produced under traditional pasteurization parameters (57-58ºC during 3 minutes), the second one corresponds to a HTST product (high temperature, 60-62ºC, and short time, 150s) combined with aseptic packaging, which implies that the life span of HTST product is longer than TP product.

The optical test was carried out three or four times along the life span of ovoproduct depending on the storage temperature and product. For storage at 2ºC, TP was tested at 0, 14 and 40 days and HTST at 0, 21 and 63 days from reception. For storage at 9ºC, TP was tested at 0, 6 and 9 days and HTST at 0, 9, 14 and 29 days (see Table 1).The samples were characterized by determination of their microbiological load by counting the CFU/ml along the life span of each type of ovoproduct as it is shown in Table 1.

The optical system consisted of a black chamber where a transparent vat, filled with ovoproduct 1cm deep, is placed directly on top of the LEDs´ array. A methacrylate plate with eight 650 nm LEDs mounted linearly on it was used. Fig. 1 shows how the detector used, a 3CCD IRRB camera (MS3100 Duncantech ®) with a sensibility window of ±20nm at 650 nm, takes the image of red light passing through the sample.

Table 1: Design of the experiment

Test type	Product	Samples by product	Repetitions by sample	Storage temperature (ºC)	Storage times (days)	Total number of tests
Optical	TP	3	3	2	0, 14, 40	27
				9	0, 6, 9	27
	HTST	3	3	2	0, 21, 63	27
				9	0, 9, 14, 29	36
Microbiological	TP	3	2	2	**0**, 6, **14**, 20, 27, 34, **40**, 48	48
				9	**0**, 3, **6, 9**, 12, 19	36
	HTST	3	2	2	**0**, 7, 14, **21**, 28, 35, 42, 49, 56, **63**, 70, 78, 85	78
				9	**0**, 3, 6, **9**, 12, **14**, 16, 18, 21, 23, 25, **29**	42

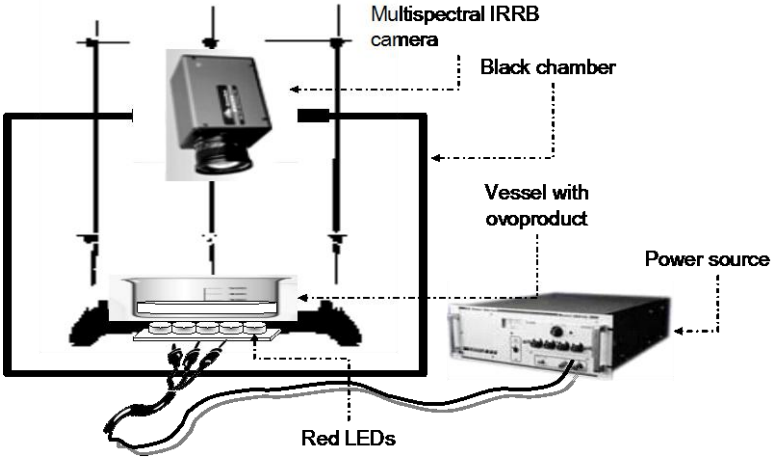

Fig. 1: Scheme of optical test procedure

The images obtained were analysed using denoted Matlab programs. A treatment of the raw image was implemented using image segmentation to convert an intensity image to a binary image, each image was segmented twice. Once by automatically generated segmentation threshold (Fig. 2) based on Otsu's method (Grey Level 1). It chooses the threshold to minimize the intraclass variance between pixels classified as black or white and to maximize the variance inter black and white classes. The second segmentation (Fig. 3, right) is based again on Otsu´s method, but applied on a modified histogram performed by the adjustment of pixels with a

low grey level (between 0 and 50) in the raw histogram, to the full range of grey intensity from 0 to 255. The Matlab program performs morphological operations on the binary image in order to remove isolated pixels, this allows using Grey level 1 to individualize each LED (Fig. 3, left) while Grey Level 2 includes the red scattered light area.

The variables extracted for each segmented image are: Illuminated area (number of pixels above level), Mean grey level (MGL) for the illuminated area (256 grey levels were considered) and Standard deviation (SD) of grey levels in the illuminated area (± grey level).

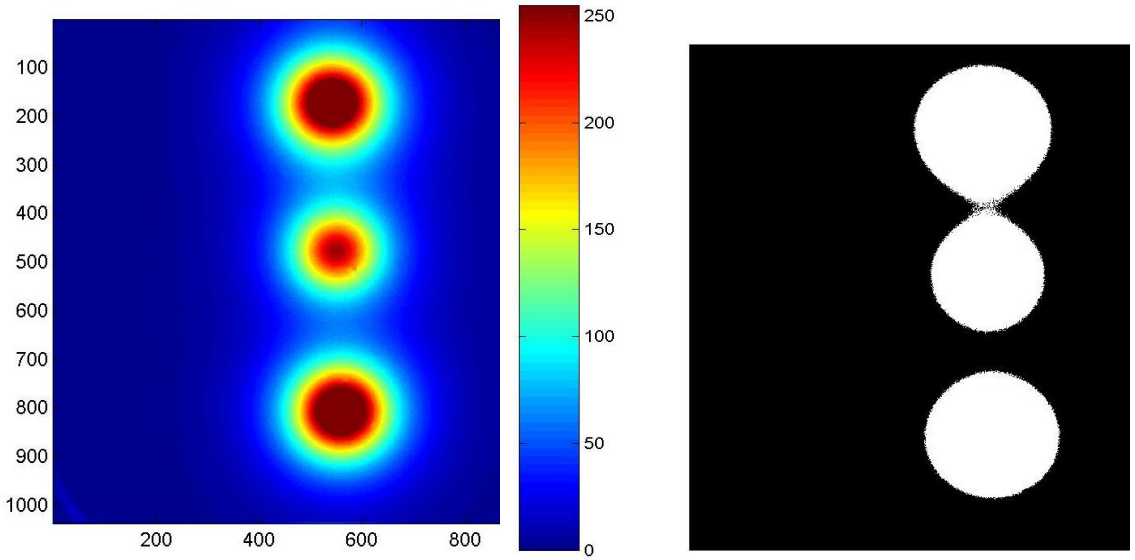

Fig. 2: On the left, raw auto-scaled image corresponding to red channel of 3CCD IRRB camera, on the right the corresponding image after applying segmentation level 1.

Fig. 3: On the left, individualized LEDs subtracting pixels placed in the middle areas and on the right global area illuminated by three LEDs after apply segmentation level 2 on the raw image shown in Fig.2.

Significant differences between treatments of ovoproducts were determined using analysis of variance (one-way ANOVA, Statistica 6.1 StatSoft). Fisher's least significant differences test was used to determine the significant differences between group means ($p<0.05$).

Results and discussion

Experiment at storage temperature of 9ºC

Fig. 4 shows the increase of the number of microorganism regarding storage time, with a fit to the sigmoid model of $r^2 = 0.99$ for both types of ovoproducts. Also some optical parameters can be fitted ($r^2_{TP} = 1$ and $r^2_{HTST} = 0.96$) to such sigmoid behaviour using equation 1, where *MGL* is de mean grey level at time *t*, MGL_{min} and MGL_{max} are the minimum and maximum values respectively of full grey level range registered in that test, and *a* and *b* are the adjusted parameters of the model. The analysis of variance carried out shows that the MGL increases significantly with higher storage times (for MGL emitted by LED1 F_{TP}=13.18 and F_{HTST}= 26.69).

$$MGL(t) = MGL_{\min} + \frac{MGL_{\max} - MGL_{\min}}{1 + e^{-(a+bt)}} \qquad (1)$$

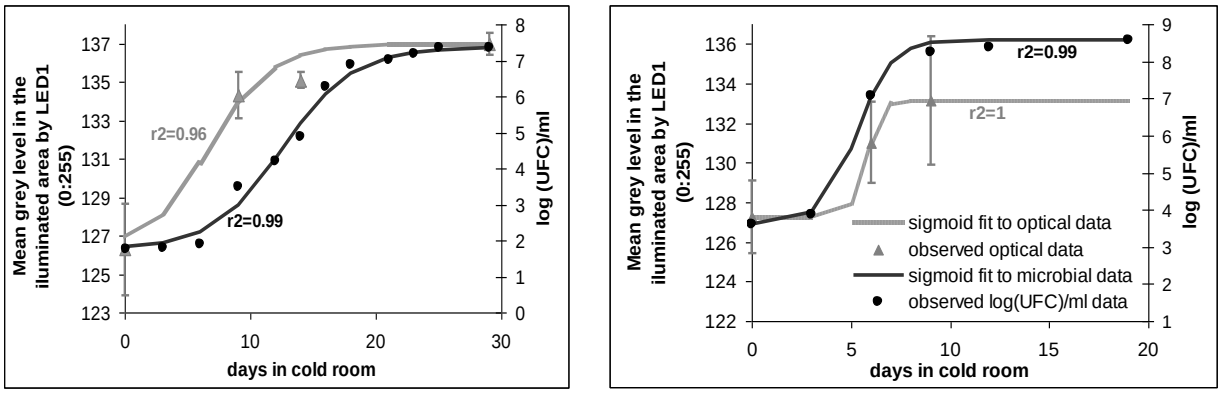

Fig. 4: Sigmoid increase of optical parameters (mean grey level) and microorganism number (log(UFC/ml)) along days in cold room at 9ºC for HTST product (on the left) and TP product (on the right).

Egg white liquefaction along storage time is one of the main product alterations described. The microorganisms' growth causes the denaturalization of proteins, which constitute the 10% of egg white composition. Taking into account that other 85% of egg white is water, that proteins´ denaturalization would explain a decrease in product viscosity (Thapon 1994),

lower optical density of the ovoproduct with not only significant higher values of red light intensity registered by the IRRB camera but also significant bigger illuminated areas by each LED (for parameter illuminated area by LED1 F_{TP}=98.76 and F_{HTST}=10.95).

The coefficients of correlation between the MGL optical parameter and the microorganism growth, range from 0.9 to 0.98 for HTST and TP ovoproducts respectively. Fig. 5 shows an estimation of biomass concentration based on optical properties of the samples. Taking into account only one optical parameter corresponding to a single LED (LED1) it is possible to develop very simple predictive models. An exponential model defined by equation 2 for HTST product (r^2=0.91) and a linear model defined by equation 3 for TP product (r^2=0.95).

$$\log \frac{UFC}{ml} = 2.73 \cdot 10^{-13} \cdot e^{0.225 \cdot MGL_{LED1}} \quad (2)$$

$$\log \frac{UFC}{ml} = 0.724 \cdot MGL_{LED1} - 87.88 \quad (3)$$

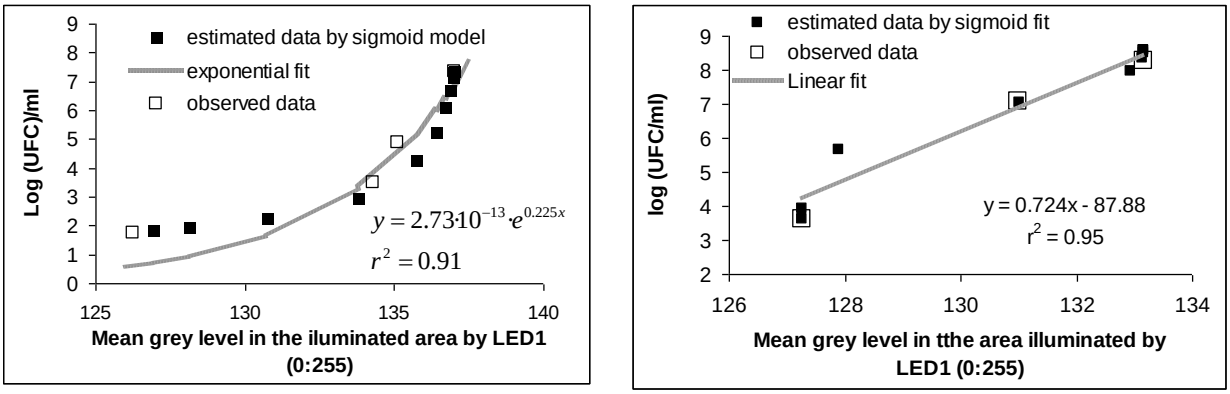

Fig. 5: Models fit to predict number of microorganism (log(UFC/ml)) from observed optical parameter “mean grey level”. On the left, HTST product at 9ºC, on the right TP product at 9ºC.

Experiment at storage temperature of 2ºC

Lorient and Matringe (1994) described that storage of egg product at low temperature induces viscosity increases due to gelation process of proteins, which is more important at lower storage temperatures. This effect could explain in Fig. 6 the decrease in the optical parameters studied especially for the values of the illuminated area by the LEDs at the end of a long storage in cold room at 2ºC. Any way, the lack of optical tests between the end of lag phase and the end of exponential phase in growth microorganism curve prevents any

conclusion about the behaviour of optical parameters with respect the microorganism growth. It is necessary to design and carry out a new experiment to study this case.

On the other hand, Fig. 7 shows how the HTST ovoproduct, made under more restrictive pasteurization conditions, maintains along cold storage at 2ºC approximately 100 times less UFC/ml than samples corresponding to TP product. The significantly lower value registered for the parameter "illuminated area" shown in Fig. 6 for HTST product at 2ºC, allows far a clear differentiation between both types of ovoproducts (F_{LED1}=254.4).

Along cold storage at 2ºC the optical parameter MGL tends to be bigger, while "illuminated area" tends to be smaller for HTST product than TP products. This tendency is also found in cold storage at 9ºC. It seems that the lower microorganism concentration decreases the turbidity of HTST samples being the red LED scattering lower.

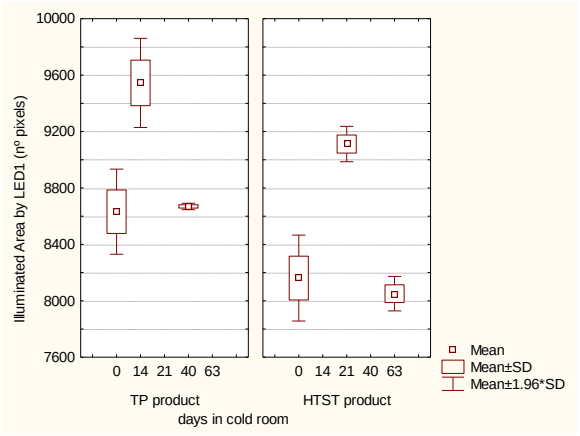

Fig. 6: box and whiskers plot of the optical parameter "illuminated area" by LED1 categorized by days in cold room at 2ºC and kind of ovoproduct.

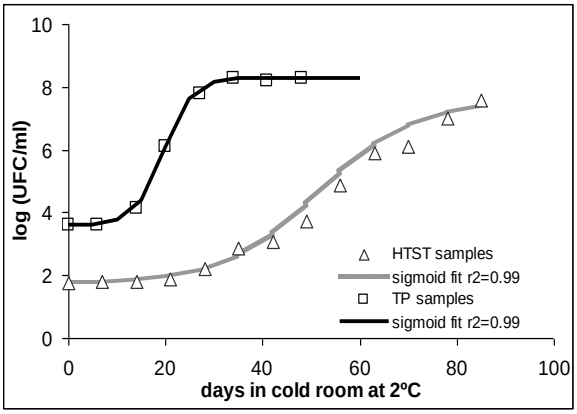

Fig. 7: microorganism growth (log UFC/ml) curve corresponding to TP and HTST products along storage time, at 2ºC.

Conclusions

In egg white samples storage in cold rooms at 9ºC is possible to fit a sigmoid model to the samples´ evolution along storage time using the optical parameter MGL, extracted from segmented images of red light emitted by LEDs passing trough the ovoproduct sample. The high correlation found between the sigmoid evolution of MGL corresponding to one LED of the optical device and the microbial growth, allows to build predictive models. Taking into account only one optical parameter corresponding to a single LED (LED1) it is possible to develop very simple predictive models: exponential for high temperature and short time

pasteurized egg white ovoproduct (r^2=0.91) and linear for traditionally pasteurized ovoproduct (r^2=0.95).

Acknowledgements

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