DISINFECTION OF LIQUID EGG PRODUCTS BY USING UV LIGHT

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ABSTRACT

DISINFECTION OF LIQUID EGG PRODUCTS BY USING UV LIGHT

The application of UV treatment to continuous flow of liquid egg products is the initial study among the others. The objective of this study was to investigate the efficiency of UV-C radiation as a non-thermal pasteurization process for liquid egg products (LEPs). The outcomes of this study were evaluated from the rheological, biodosimetric and inactivation efficiency points of view.

The results pertaining to rheological characterization, physical and optical measurements of the fresh and pasteurized LEPs indicated that liquid whole egg (LWE) and liquid egg white (LEW) showed Newtonian behavior within the range studied. Liquid egg yolk (LEY) exhibited pseudoplastic and time independent behavior in temperature ranges from 4 ºC to 25 ºC. But its rheological behavior was affected at pasteurization temperature and showed thixotropy and time dependent.

The biodosimetric study based on the UV treatment of LEPs by using collimated beam apparatus pointed out that the best reduction (> 2-log) was achieved in liquid egg white (LEW) when the fluid depth and UV dose were 0.153 cm and 98 mJ/cm^2, respectively. Maximum inactivation was 0.675-log CFU/ml in liquid egg yolk (LEY) and 0.316-log CFU/ml in liquid whole egg (LWE). The kinetics of UV inactivation of *E.coli* in LEPs was found nonlinear. Our results emphasize that UV-C radiation can be used as a pre-treatment process or combined with mild heat treatment to reduce the adverse effects of thermal pasteurization of LEPs.

The efficacies of short wave ultraviolet light (UV-C) as a non-thermal process for LEW were investigated in the continuous flow UV reactor based on the result derived from the biodosimetric study. Maximum 0.2 log reduction was achieved after 5 cycle of operation. The efficiency of the UV light treatment in continuous flow UV reactor was found to be lower compared to bench-top studies. This is attributed to the problems caused by inefficient design of UV chamber. Results of these work showed that the UV reactor was designed poorly to supply enough UV irradiation exposure for all the fluid elements in the system. Redesigning of the system was suggested.

ÖZET

SIVI YUMURTA ÜRÜNLERİNİN ULTRAVİOLE (UV) IŞIK İLE DEZENFEKSİYONU

Sıvı yumurta ürünlerinin ultraviole dezenfeksiyonun sürekli akış sistemlerinde uygulaması, diğer UV uygulamalarıyla kıyasla bir ilk teşkil etmektedir. Bu çalışmanın amacı, ısıl işlemden farklı olarak, UV-C ışınlarının sıvı yumurta ürünlerindeki mikrobiyal azalmayı incelemekti. Bu çalışmanın alt başlıkları olarak sıvı yumurta ürünlerinin reolojik, biyodozimetrik ve mikrobiyal azalma verimliliğinin ayrı ayrı karakterizasyonu gösterilebilir.

Taze ve pastörize sıvı yumurta ürünlerinin reolojik, fiziksel ve optik karakterizasyon sonuçlarına gore, yumurta beyazı ve karışık yumurtanın, sıcaklığa bağlı kalmaksızın Newtonian akış özelliği sergilediği görülmüştür. Yumurta sarısının 4 ºC ve 25 ºC lerde psüdoplastik davranış gösterdiği, bununla birlikte pastörizasyon sıcaklığında reolojik davranışının zamana bağlı olarak tiksotropik yapı kazandığı bulgulanmıştır.

Paralel ışın demeti aparatı ile UV dezenfeksiyon baz alınarak yapılan biyodozimetrik çalışmalar sonucunda, sıvı derinliğinin 0.153 cm, UV dozun 98 mJ/cm^2 olarak ölçüldüğü koşullarda en iyi logaritmik mikrobiyal azalmanın (> 2-log) yumurta beyazında sağlandığı farkedilmiştir. Yumurta sarısındaki maksimum inaktivasyon miktarı 0.675-log CFU/ml, bütün yumurtadaki maksimum inaktivasyon oranı da 0.316-log CFU/ml olarak bulunmuştur. Patojen olmayan *E.coli* suşunun sıvı yumurta ürünlerinde UV inaktivasyonunun, doğrusal olmayan bir davranış gösterdiği görülmüştür. UV-C ışınlarının, ön dezenfeksiyon aşamasında kullanılabilirliği veya ısıl olmayan başka bir dezenfeksiyon metoduyla seri bir şekilde kullanılarak yumurta ürünleri üzerindeki negatif etkilerinin en aza indirilebilirliği incelenmiştir.

UV-C ışınlarının devamlı akış UV reaktöründeki etkinliği, biyodozimetrik çalışmaların sonuçları baz alınarak incelenmiş ve maksimum 0.2 log CFU/ml azalma sağlanabilmiştir. Devamlı akış UV reaktörünün inaktivasyon verimliliğinin, biyodozimetrik denemelere göre çok daha düşük olduğu değerlendirilmiştir. Bu verimsizlik, UV reaktörün uygun olmayan tasarımına bağlanmıştır. Sonuç olarak, UV reaktöründen kaynaklanan dizayn probleminin, yeterli UV ışığının akışkan ürüne aktarılmasını önlediği ve sistemin yeniden tasarlanmasının gerektiği görülmüştür.

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LEPs:	Liquid egg products
USDA:	United States Drug Administration
UV-C:	Ultraviolet-C
CFU:	Colony forming unit
LEW:	Liquid egg white
LEY:	Liquid egg yolk
LWE:	Liquid whole egg
CMC:	Carboxylmethylcellulose
R_c:	Radius of container (cm)
R_b:	Radius of spindle (cm)
x:	Radius at which shear rate is being calculated (cm)
M:	Torque input by instruments (dyn / cm)
L:	Effective length of spindle (cm)
τ:	Shear stress (Pa)
τ_0:	Yield stress (Pa)
γ:	Shear rate (s^{-1})
μ:	Newtonian viscosity (Pa.s)
K:	Consistency index ($Pa.s^n$)
n:	Flow behavior index
η_a:	Apparent viscosity (Pa.s)
η_0:	Parameter considered as the viscosity at infinite temperature (Pa.s)
E_a:	Activation energy (J/mol)
R:	Molar gas constant (J/mol K)
T:	Temperature (°K)
I_{avg} :	The average UV intensity (average fluence rate, mW/cm^2)
I_o:	Incident intensity (mW/cm^2)
A_e:	Absorbance per centimeter
L:	UV light path length (cm)
d:	UV dose (mJ/cm^2)
t:	Time (min)

I:	Irradiance (mW / cm^2)
N:	Microbial population after UV exposure (CFU)
N_0:	Initial inoculation level (CFU)
k:	Inactivation rate constant (cm^2/mJ)
Re:	Reynolds Number
Re_g:	Generalized Reynolds Number
D:	Diameter of pipes (m)
υ:	Average flow velocity of fluid (m/s)
ρ:	Density of fluid (kg/m^3)
TSA:	Tryptic Soy Agar
VRBA:	Violet Red Bile Agar
PCA:	Plate Count Agar
EMB:	Eosin Methylene Blue Agar
A:	Titrant for sample in chlorine determination (ml)LEPs
N:	Normality of Sodium Thiosulfate ($Na_2S_2O_3$)
PL:	Power Law model of viscosity
HB:	Herschel Bulkey model of viscosity
S_{est}:	Estimated standard error
L*:	Brightness
a*:	Redness-greenness
b*:	Yellowness-blueness
df:	Dilution factor
DF:	Degrees of freedom
SS:	Sum of square
MS:	Mean of square

CHAPTER 1

INTRODUCTION

The term "egg products" refers to eggs that are removed from their shells for processing and convenience forms of eggs for commercial, foodservice and home use. These products can be classified as refrigerated liquid, frozen and dried products. Liquid egg products (LEPs) are valuable due to its high protein content, low cost, being used throughout the kitchen, both by serving alone or by using as ingredients in a prepared meal in order to provide texture, flavor, structure, moisture and mostly developed from poultry animals (Samimi and Swartzel 1985, Punidadas and McKellar 1999).

Food-borne disease outbreaks involving *Escherichia coli* O157:H7 and *Salmonella enteritidis* in liquid egg products are the major public health concern (Lee et al. 2001, Mañas et al. 2003). As a result, these products must be processed in sanitary facilities under continuous inspection and pasteurized before distributed for consumption. In the production of ready to use and shelf stable LEPs, pasteurization is the fundamental process to eliminate food pathogenic microorganisms from the product. The most common pasteurization method for LEPs is the thermal treatment, having the principle of inactivation of microorganisms by application of heat for certain periods of time (Muriana 1997). USDA requires liquid egg pasteurization (conventional thermal processing) to be conducted on a critical temperature-time condition where egg protein coagulation may not occur. Minimum temperature and holding time requirements for the egg yolk is 60 ºC and 6.2 min. For the egg white and whole egg, minimum temperature and holding time requirements are 55.6 ºC and 6.2 min., 60 ºC and 3.5 min, respectively (USDA-ARS, USA). Although thermal pasteurization still represents the most available and best understood technique, it may affect the coagulation, foaming and emulsifying properties and degrade the quality and functional properties (both technological and nutritive) of egg products (Gongora-Nieto et al. 2003, Hermawan et al. 2004).

Alternative pasteurization methods including ultrasonic wave treatment, high electric field pulses, high hydrostatic pressure or ultrapasteurization combined with aseptic packaging are explored to extend the shelf life and minimize disadvantages of

thermal processing of LEPs (Ball et al. 1987, Ma et al.1997, Ponce et al. 1998, Wrigley and Llorca 1992). Most of these methods cause substantial changes in the structure of liquid egg products by causing coagulation and denaturation of proteins. In response to these limitations, UV-C radiation can be an alternative non-thermal process for LEPs in order to achieve microbiologically safe and shelf stable products (Bintsis et al. 2000). UV-C radiation does not only eliminate the harmful effects of thermal treatment but also decreases the high operating and separation cost of the other pasteurization methods (Garibaldi et al. 2003). In this process, microorganisms are eliminated by penetration of UV-C light to outer membrane of the cell and damaging the DNA due to formation of thymine dimers, which prevent the microorganism from DNA transcription and replication, and eventually leading to cell death (Bank et al. 1990, Bintsis 2000, Miller et al. 1999).

UV-C light treatment has been used in the food industry for different purposes including air sanitation in the meat and vegetable processing, reduction of pathogen microorganisms in red meat, poultry and fish processing (Wong et al. 1998, Liltved and Landfald 2000). UV-C treatment of heat resistant yeasts, moulds, *Bacillus subtilis* and *Bacillus pumilus* spore is also common application in the orange juice processing (Tran and Farid 2004). Additionally, UV-C radiation has been successfully applied for pasteurization of certain liquid foods such as milk and fruit juices (Koutchma et al. 2004, Matak et al. 2005).

There are many research cited in the literature about the efficacy of UV-C light for the reduction of different microorganisms by using either bench top collimated beam apparatus or continuous flow reactors (Lage et al. 2003, Sommer et al. 1998). Most of these works were conducted with drinking and wastewater samples and the microbial inactivation achieved in those studies with lower UV doses than those required for microorganisms suspended in liquid foods such as apple cider and milk. For example, Lage treated *E.coli* suspension with UV exposure of 12 mJ/cm^2 UV dose and achieved 3-log reduction (Lage 2003), whereas Sommer inoculated water samples of 0.4 cm in depth with three different *E.coli* strains (ATCC 25922, ATCC 11229 and isolated from sewage) and applied 10-50 mJ/cm^2 UV dose to obtain maximum 6-log reduction (Sommer 1998). On the other hand, UV dosages applied by Wright et al. ranged from 9.4 to 61.5 mJ/cm^2 and the mean log reduction of *E.coli* O157:H7 strain for treated apple cider was found to be 3.81 log CFU/ml in flow through UV reactor (Wright et al.

2000). 5-log reduction of *L. monocytogenes* in milk was achieved when the milk samples treated with UV dose of 15.8 ± 1.6 mJ/cm^2 in continuous flow reactor.

The application of UV treatment to continuous flow of liquid egg products is the initial study among the others. The objective of this thesis is to investigate the efficiency of UV-C radiation as a non-thermal pasteurization process for liquid egg products (LEPs). It covers four work packages:

- Determination of some physical properties important for the efficiency of UV-C treatment including pH, absorbance, turbidity, and color and rheological parameters of fresh and pasteurized liquid egg products.
- Development of model solutions simulating the rheological behavior of fresh and pasteurized liquid egg products that will be used to adjust the volumetric flow rate in UV-C continuous flow studies.
- UV Dose determination in bench scale UV-C studies with UV-resistant strain of *E.coli* (NRRL B-253) as the target microorganism (Biodosimetric study).
- Non thermal processing of liquid egg white by using continuous flow UV reactor.

CHAPTER 2

LITERATURE REVIEW

2.1. Liquid Egg Products (LEPs)

2.1.1. Structure and Chemical Properties

Egg can be described as the food source developed from most poultry animals, commonly chicks. Eggs, particularly chicken eggs, are known as a perfect food for humans due to their high protein content, low cost and being readily availability in most of the countries (Hamid-Samimi and Swartzel 1985, Punidadas and McKellar 1999). They are highly versatile and are used throughout the kitchen, both by serving alone or by using as ingredients in a prepared meal in order to provide texture, flavor, structure, moisture and nutrition for much prepared foods, from soups and sauces to breads and pastries (Punidadas 1999).

Hen's egg contains three main parts. The egg shell, which is a composition of calcium carbonate, is the tough, outermost covering membrane of the egg. Main purpose of shell is to prevent microbial action from entering and moisture from escaping, and also to protect the egg during handling and transport. Shell color is determined by the breed of the chicken. Color of the shell can change from bright white to brown genetically. On the other hand, shell color has no effect on quality, flavor or nutrition of egg contents (USDA Egg-Grading Manual, 2000).

Egg white (also known as albumen) substitutes for most of an egg's liquid weight, which is about 67% in a whole egg. Egg white, which contains niacin, riboflavin, lysozyme, chlorine, magnesium, potassium, sodium and sulfur, is more opalescent than truly white. Carbon dioxide makes the structure cloudy. But in contrast, carbon dioxide is disappeared in aged eggs, causing more transparent structure of albumen than that of fresher eggs. When egg albumen is beaten vigorously, it foams and increases in volume 6 to 8 times. Consequently, egg foams are essential for making several meals such as soufflés, meringues, puffy omelets, and angel food and sponge cakes (Foregeding et al. 2006).

The term “egg yolk” is defined as the yellow part of the egg that forms approximately one third of the egg (33 % by volume) and includes three fourths of the calories, most of the minerals and vitamins and all the fat content in the whole egg (high nutritive properties exist). The yolk protein lecithin, termed as the compound is responsible for emulsification in products such as hollandaise sauce and mayonnaise (Telis-Romero et al. 2006). Increasing in size and becoming less viscous due to the inner flow of water from the white is the result of higher concentration of solids in the yolk. Hence, egg yolk solidification (coagulation) occurs at temperature range between 65 ºC – 70 ºC. Although the color of a yolk may vary depending on the chicken's feed, color does not affect quality or nutritional content. The yolk also includes such the essential elements that iron, phosphorus, sulphur, copper, potassium, sodium, magnesium, calcium, chlorine, and manganese (USDA, Egg-Grading Manual, 2000). Main components and their weight percentages of LEPs are listed in Table 2.1.

2.1.2. Physical properties

Process properties of liquid egg products are not only necessary for proper design of process instruments, but also fundamental in order to identify product development, quality determination, sensory evaluation, and determination of process instruments (Punidadas 1999, Rao et al. 1999). These properties can be categorized as the rheological, physical and optical properties of LEPs.

In a rheological analysis, the viscosity of a product determines the flow behavior of LEPs, varied from Newtonian to time-dependent non Newtonian, in different processing steps of LEPs, industrially (conversion step, pasteurization, holding and transport from one unit to another in the same process flow). Besides, physical properties, such as density and total solid determination, contribute the estimation of flow regime parameters and composition of LEPs. Finally change in sensory evaluation before and after pasteurization step are dependent on optical parameters like turbidity, absorbance and color standard of LEPs for each. Therefore, understanding the rheological and physical properties of liquid egg products (LEPs) with a wide range of temperature have great importance for the application of new processes in the egg-products industry.

Table 2.1. Components of LEPs.

(Source: Lee 2002)

	Component	Whole Egg	Egg Yolk	Egg White
Proximate (g/100g)	Water	75.33	48.81	87.81
	Protein	12.49	16.76	10.52
	Total Lipid	10.02	30.87	0.00
	Carbohydrate	1.22	1.78	1.03
	Ash	0.94	1.77	0.64
Minerals (mg/100g)	Calcium	49.00	137.00	6.00
	Iron	1.44	3.53	0.030
	Magnesium	10.00	9.00	11.00
	Phosphorus	178.00	488.00	13.00
	Potassium	121.00	94.00	143.00
	Sodium	126.00	43.00	164.00
	Zinc	1.10	3.11	0.01
Vitamins (mg/100g)	Ascorbic Acid	0.00	0.00	0.00
	Thiamin	0.06	0.17	0.01
	Riboflavin	0.51	0.64	0.45
	Nicotinic Acid	0.07	0.02	0.10
	Pantothenic Acid	1.23	3.81	0.12
	Pyridoxal	0.14	0.39	0.00

Rheological and physical properties of LEPs depend on several factors including temperature, density, and total solid content. There are several works available about the rheological properties of LEPs (Kaufman et al. 1968, Hamid Samimi 1984, Hamid Samimi 1985, Ibarz and Sintes 1989, Ibarz 1993, Punidadas 1999, Telis-Romero 2006).

For example, Hamid Samimi et al. and Hamid-Samimi and Swartzel studied the rheological behavior of whole egg (Hamid Samimi et al. 1984, Hamid-Samimi and Swartzel 1985). They found out that whole egg shows shear-thinning (pseudoplastic) behavior at temperatures above 60 ºC while at temperatures below 60 ºC it behaves as a Newtonian fluid. Punidadas et al. investigated the rheological properties and density of liquid egg products at pasteurization temperatures (Punidadas et al. 1999). They reported that all the products show shear-thinning behavior, which are described by the Power-Law model. Telis-Romero studied the rheological behavior of egg yolk at a range of 4-60 ºC using a concentric cylinder viscosimeter (Telis-Romero 2006). They showed that egg yolk is pseudoplastic and dependent on temperature. These studies

revealed that the rheological behavior of all the egg products (except salted ones) showed shear-thinning (pseudoplastic) behavior, which can be described by Power-Law model. The rheological parameters such as K and n values were dependent on dry content and temperature.

2.1.3. Manufacturing of Liquid Egg Products (LEPs)

Instead of traditionally produced and marketed shell eggs, processed liquid egg products have been consumed, increasingly. For example, 30 % of the whole eggs sold in the USA in 2001 were processed (Anonymous). Since processed liquid egg products are handled easily in distribution to the food manufacturing processes and marketing, egg processing plays a fundamental role in addition to the high shelf life and safety for human body. Processing of LEPs constitues several steps to achieve high quality and safety product within high shelf life until consuming.

Before processing, shell eggs are usually held in refrigerated storage no longer than seven to ten days (USDA, 2000). In the production line, shell eggs are holded and received up to their size and shape and divided into quailty groups, then washed and rinsed completely to remove outer faeces coming from hen. This section is essential since great amount of foodborne microorganisms are sourced from faeces after ovulation. Shell eggs are sanitized by highly diluted chemicals as sprayed. In order to determine and remove the imperfections, shell eggs are then candled by quartz halogen light (USDA, 2000).

Shells are broken and liquid eggs are either separated to produce egg white and yolk or mixed to form whole egg. Whole or separated eggs are mixed uniformly to homogenize and filtered to remove shell fragments, membranes, and chalazae. Egg products are sent to pasteurization unit to inactivate foodborne pathogens. Various times and temperatures are used for effective pasteurization, depending on the product. All pasteurized liquid egg products is to contain less than 1,000 microorganisms per gram. Moreover, Only *Salmonella*-negative products are allowed to be sold (USDA, 2000). Pasteurized egg products are finally refrigerated, dried, plained or salted according to the consumers' needs before packaging. Egg production line is shown in Figure 2.1. In the production of ready to use and shelf stable liquid egg products (LEPs),

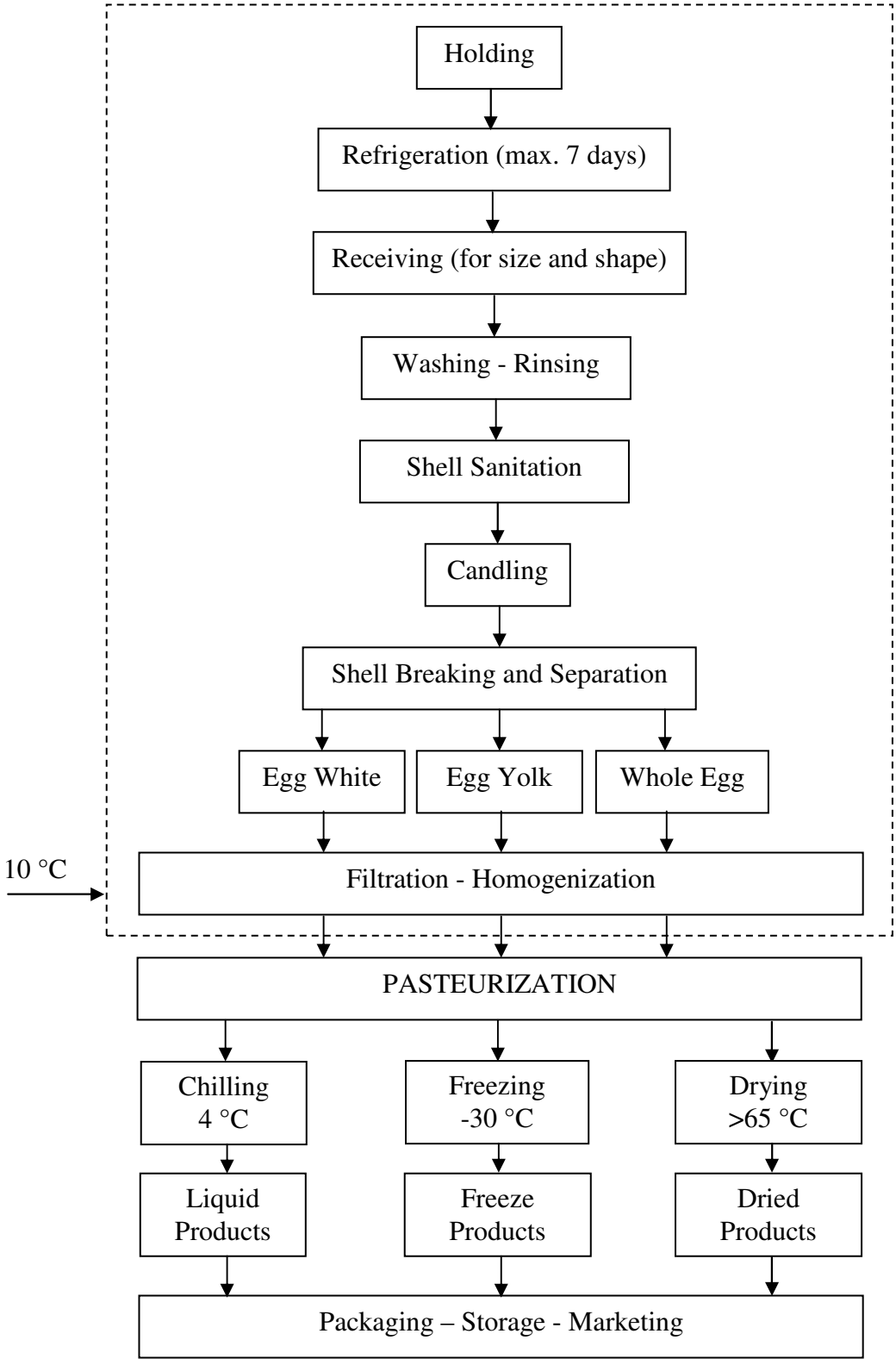


Figure 2.1. Liquid egg products' processing line.

(Source: USDA 2000, Lee 2002)

pasteurization is the most important process in order to inactivate harmful microorganisms and undesirable enzymes from liquid egg products to prevent health risk in human body (Muriana 1997, Gut et al. 2005, Daugrthy et al. 2005). Main pathogen microorganisms, which cause several foodborne ilnesses in liquid egg products are *Salmonella enteridis*, *Salmonella seftenberg, Listeria monocytogenes* and *Escherichia coli* (Ferreira et al. 1998, Lee et al. 2001, Ngadi et al. 2003, Manăs 2003). Most common pasteurization method is defined as thermal pasteurization, having the principle of inactivation of microorganisms at proper pasteurization temperature and residence time without affecting the quality and characterization of products. In this technique, heat is directly applied to LEPs in a plate heat exchanger in a definite period of time according to the product properties. Consequently, heat sensitive food pathogenic microorganisms are inactivated. Moreover, the process parameters is to be designed for the desired level of thermal treatment with minimum damage to the lipoprotein ingredients of the LEPs (Gut 2005).

Since thermal treatment has the potential to degrade the nutritive quality and functional properties (both technological and nutritive) of egg products, negative effects of high temperature, as denaturation of high protein structure should be eliminated (Gongora-Nieto et al. 2003, Hermawan et al. 2004). Two main parameters of LEPs (liquid egg products) are fundamental for thermal pasteurization processes: pasteurization temperature and residence time, which depends directly on the flow type (either in laminar or turbulent) (Pottier et al, 2006, Schuerger et al. 2005). USDA requires liquid egg pasteurization (as a conventional processing) to be conducted on a critical temperature-time condition where egg protein coagulation may not occur (Anonymous).

Minimum temperature and holding time requirements for LEPs without affecting the quality and characteristics are shown in Table 2.2. Instead of thermal treatment, several pasteurization methods have been developing for LEPs, such as ultrasonic wave treatment (Wrigley and Llorca 1992), high electric field pulses (Ma et al. 1997), high hydrostatic pressure (Ponce et al. 1998) or ultrapasteurization combined with aseptic packaging (Ball 1987).

Sanitation method is used by additional chemicals, such as hydrogen peroxide, especially in egg white in spite of its negative effect on quality and taste in the presence of high concentration (Muriana 1997).

Table 2.2. Thermal Pasteurization Conditions of LEPs.

(Source: USDA-ARS, USA)

Liquid Egg Product	Minimum Holding	
	Temperature (°C)	Time (min)
Egg White (Without chemicals)	55.6	3.10
Whole Egg (Without chemicals)	60.0	3.50
Egg Yolk (Without chemicals)	60.0	3.10
Whole Egg Blends (2 % ingredient)	60.0	3.10
Salted Whole Egg (2 % or more salt)	62.2	3.10
Sugared whole egg (2 to 12 % sugar)	60.0	3.10
Salted yolk (2 to 12 % salt)	62.2	3.10
Sugared yolk (2 % or more sugar)	62.2	3.10

2.2. Ultraviolet Light Treatment (UV-C Radiation) of Liquid Foods

Ultraviolet light can be described as a spectrum of light, remaining below the range visible to the human eye (Sosnin et al. 2006, Shama 2007). UV light can be divided into four distinct spectral areas upto their effectiveness, such as Vacuum UV (between 100 to 200 nanometers), UV-C (between 200-280 nanometers), UV-B (between 280-315 nanometers), and UV-A (between 315-400 nanometers). The part of an electromagnetic spectrum, which is responsible for germicidal effect to microbial structure in wavelengths between 200 nm and 300 nm, is called UV-C (Shama 2007). The UV-C spectrum (200-280 nanometers) is the most lethal range of wavelengths for microorganisms. This 253-264 nanometers range having the peak germicidal wavelength is known as the “germicidal spectrum”, shown in Figure 2.2.

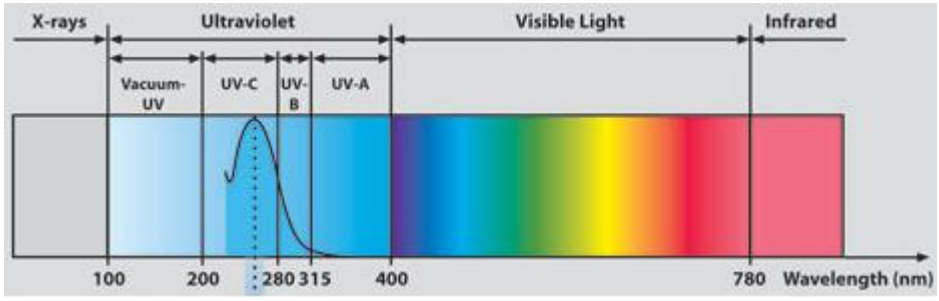


Figure 2.2.UV radiation range groups.

(Source: WEB_1 2003)

UV treatment is used to eliminate microorganisms from several food materials such as fruit juice, apple cider and milk. It is described as a non thermal disinfection method having no undesirable effect on the organoleptic and nutritional properties on food materials (Bintsis et al. 2000). Photochemical damage to nucleic acids in cellular structure is the main result of the absorption of germicidal UV-C light. The DNA of most living organisms is double stranded, including the adenine in one strand is opposite thymine in the other, and linked by one hydrogen bond, and guanine is paired with cytosine by one hydrogen bond (Bank 1990, Moan 1998, Miller 1999, Bintsis 2000, Tornaletti 2005, Cadet et al. 2005). The purine and pyrimidine combinations are called base pairs. When UV light of a germicidal wavelength is absorbed by the pyrimidine bases, the hydrogen bond is ruptured (Cieminis et al. 1987, Tornaletti 2005). New bonds between adjacent nucleotides are structured with the help of high energy, utilized by UV wavelengths ranging in UV-C region forms. This phenomena creates double molecules or dimers (Tornaletti 2005), shown in Figure 2.3. Dimerization of adjacent pyrimidine molecules is the most common photochemical damage. But in contrast, cytosine-cytosine, cytosine-thymine, and uracil dimerization can be identified. Hence, cell replication is interrupted by formation of numerous dimers in the DNA and RNA of microbial structure with the effect of other types of damage such as cosslinking of nucleic acids and proteins resulting cell death (Tornaletti 2005).

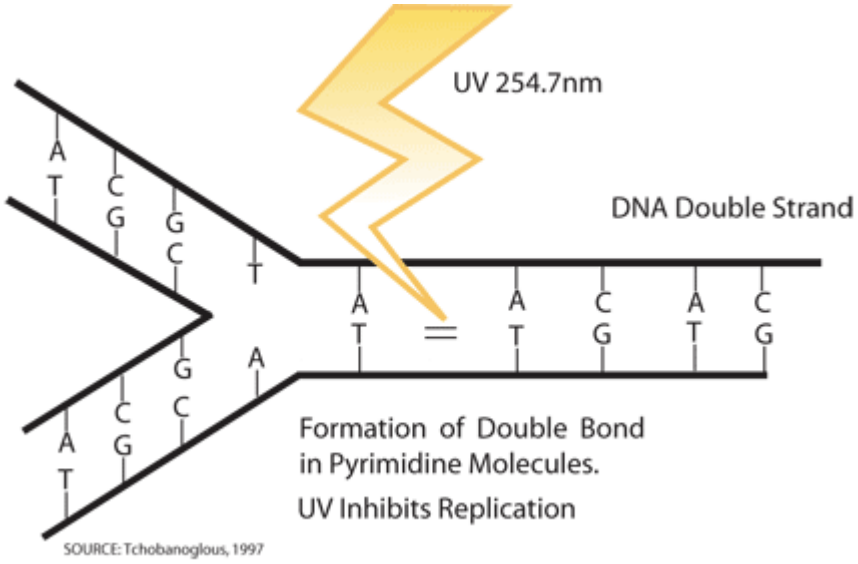


Figure 2.3. Effect of UV-C light on DNA double strand.

(Source: WEB_2 2003)

The amount of cell damage depends on the dose of UV energy absorbed by the microorganisms and their resistance to UV. Requirement of UV doses for inactivation of most bacteria and viruses are low. UV dose is a product of UV intensity and exposure time and is expressed as milli Joules per square centimeter (mJ/cm^2) or milli Watt seconds per square centimetre (mWs/cm^2). UV doses required for 99.9% destruction of various microorganisms are represented in Table 2.3.

Table 2.3. Destruction Levels of UV Doses on Main Microorganisms.
(Source: WEB_3 2006)

Organisms	**Energy ($mW\ sec / cm^2$)**
Salmonella enteritidis	7.6
Bacillus subtilis	11
Bacillus subtilis spores	22
Escherichia coli	6.6
Pseudomonas aeruginosa	10.5
S. typhimurium	15.2
Shigella paradysenteriae	3.2
Staphylococcus aureus	6.6
Saccharomyces cerevisiae	13.2
Baker's yeast	6.6
Brewer's yeast	8.8
Aspergillus niger	330
Influenza	6.6
Paramecium	200
Penicillium expansum	22

Use of ultraviolet irradiation was allowed by Food and Drug Administration (FDA) to inactivate foodborne pathogens and other microorganisms in juice products by regulation effective since November 29, 2000. UV disinfection is mainly used in air and water purification and sewage treatment of food and beverages (Blume and Neis 2004, Chmiel et al. 2002, Green et al. 1995, Hassen et al. 2000) processing of drinking water (Peldszus et al., 2003, Lehtola et al., 2004), milk and fruit juice production (Koutchma 2004, Matak 2005)

Various advantages of UV disinfection process are present in the meaning of low total operation cost, environmentally friendly applications without hazardous chemicals and safe to use. UV systems are universally accepted disinfection processes especially in food and water disinfection. UV systems are not only operated

automatically without special attention, but maintenance is also made with easy re-installation (Tchobanoglous et al. 1996, Lazarova et al. 1998, Elyasi and Taghipour 2006). Overall advantages of UV can be represented as the following.

- UV systems are environmentally friendly and no need to dangerous chemicals to handle or store.
- Low initial capital cost is a characteristic of UV systems as well as reduced operating expenses according to the similar technologies, such as thermal disinfection of ultrasonic treatment, etc. Installation is easy (Lazarova et al. 1998).
- Depending on the food materials, treatment process may be immediately operated without any holding tanks or long retention times (Oppenheimer et al. 1993, Masschelein et al. 1989).
- UV operation is extremely economical that great amount of food material may be treated for a little operating cost with low power consumption (Green et al. 1995).
- Comparing the thermal treatment, no or hardly any change in taste, odor, pH or conductivity is differentiated in food.
- UV system may somehow be used as a compatible device with other treatment equipments or systems when complete destruction of microbial population is needed (Blume and Neis 2004, Koivunen and Heinonen-Tanski 2005).

UV treatment method not only dominates the harmful effects of denaturation of biochemical structure when consuming, also decreases the high operating and separation cost of the other pasteurization methods. (Garibaldi et al. 2003). It is expected that an efficient UV operation must destroy the targeted microorganism in a food material (Lazarova 1998). Hence, there are several parameters affecting design, performance, and operating principals in using an UV system as a disinfector or a pasteurization device (Harris et al. 1987, Qualls et al. 1985, Parker and Darby 1995). Main factors that will help determine the ability and efficiency of a UV disinfection device are as follows:

- The type of lamp used in the treatment (low-pressure or medium/high-pressure) (Harris et al. 1987, Hassen 2000) .
- The length of the lamp being used (Hassen et al. 2000).
- Physical, chemical and optical properties of food material being treated (density, viscosity, turbidity, absorbance coefficient) (Parker and Darby 1995).

- The flow rate of food material through the UV's exposure chamber (Hassen et al. 2000).
- The physical design of the UV's exposure chamber.
- The chemical composition and condition of the food material being treated (temperature, pressure, etc.).

Nevertheless, UV biodosimetric and UV inactivation studies have been present. Koutchma described and compared the performance of three designs of flow-through UV reactors in turbulent flow, thin film and annular laminar systems for the disinfection of a solid-liquid media (apple cider) for UV disinfection of *E.coli* (Koutchma 2006). Triassi et.al. studied ultraviolet disinfection of 30 environmental Legionella strains from the paediatric and cardiac surgery units (CSU) in hospital environment, hospital water supply and medical devices to prevent Legionnaries' disease (Triassi et.al. 2005). Templeton et.al. analyzed the protection against UV light of two viral surrogates (MS2 coliphage and bacteriophage T4) in three types of particles (kaolin clay, humic acid powder, and activated sludge), two coagulants (alum and ferric chloride), two filtration conditions (none and 0.45 mm), and two UV doses (40 and 80 mJ/cm^2 for MS2 coliphage, 2 and 7 mJ/cm^2 for bacteriophage T4) considering in a series of bench-scale UV collimated beam studies at 254 nm (Templeton et.al. 2005). Particle diameter and UV absorbing organic content were critical parameters for this type of protection.

Pottier applied the two-flux approach to the *Chlamydomonas reinhardtii* growth in a photobioreactor of torus shape using daylight fluorescent tubes (Pottier 2006). Instead of the destruction of pathogens, the aim of this study was differed from inactivation processes of UV light in specialization of constructing the necessary m.orgns. It can be predicted that not only UV light is used in food pasteurization, but also applied on the growth of organism in biotechnology. Schuerger improved the UV simulations in order to create an inactivation model for spore-forming *Bacillus* species, used as the benchmark for assessing the cleanliness of spacecraft surfaces prior to launch, on sun exposed surfaces of spacecraft on Mars (Schuerger 2005). Logan et al. studied on the prevention of biofouling on glass and quartz surfaces with photocatalytical materials, like active metal oxides (Logan et al. 2004). Fauquet et al. determined the inactivation procedure for foodborne pathogens in blood derived therapeutic material transferred blood, used in clinic operations from unknown donors (Fauquet et al. 2004).

Lehtola experienced the effectiveness of pipeline modifying on UV and chlorine disinfection (Lehtola 2004). Biofilm formation decreased the effect of UV-light on bacteria and higher chlorine remove the biofilm and microbial population from copper pipes than that of plastic ones. Drinking water was disinfected with UV-radiation (70mWs/cm^2) produced by a low-pressure UV-lamp and the drinking water was disinfected with both UV-radiation and chlorine (NaOCl). Pozos et.al. operated two model distribution systems on water for biofilm and microbial population by UV disinfection (Pozos et.al. 2004). It was seen that, opportunistic pathogen attachment was not affected by the UV treatment, but was correlated to the biofilm density of heterotrophic bacteria having a non-consistent impact of UV treatment on the biofilm community. UV annular system was irradiated with a low-pressure UV lamp, delivering a dose of 106 mJ/cm^2. The UV dose delivered was determined with a bioassay using a collimated beam test. Koutchma developed a mathematical models such as Dispersed Phase model to describe particle phase flow pattern and particle residence times and the UV intensity distribution model to simulate processing of apple cider in a thin-film UV reactor under 253.7 nm UV light at 60 mW.s/cm^2 UV dose (Koutchma 2004).

Unluturk et.al. studied the effects on UV light efficiency on the destruction of *Escherichia coli* K-12 bacteria in the presence of model fluids using laminar and turbulent flow treatment systems by the single factor of UV absorbance on fresh apple juice/cider (Unluturk et.al. 2004). Continuous thin-film laminar and turbulent flow UV reactors were used on this study. Ngadi et.al. evaluated the effect of pH, depth of food medium and UV dose on *E.coli* O157:H7 in egg white and apple juice at 0.315 mW/cm^2 between 0-16 minute in 2 min interval (Ngadi et.al. 2003). Peldszus et.al. investigate the bromate removal from drinking water on irradiation with medium-pressure UV lamps for drinking water disinfection with UV fluences up to 718 mJ/cm^2 (Peldszus et.al. 2003). 19% bromate removal was achieved. Chmiel determined the reduction of highly contaminated spent process water contamination on food and beverage industries using the combination of membrane bioreactor, UV pre-disinfector and UV disinfection apparatus (Chmiel 2002). Wright studied UV inactivation for reducing *E.coli* O157:H7 in apple cider in continuous flow UV reactor in the UV dose range of 9.4-61 mW.s/cm^2 (Wright 2000). UV pathogen treatment was reduced with a mean reduction of 3.81 log CFU/ml. Lage et al. treated *E.coli* suspension with UV exposure of 12 mJ/cm^2 UV dose and achieved 3-log reduction (Lage et al. 2003). Matak

achieved 5-log reduction of *L. monocytogenes* in milk when the milk samples were UV exposured in a dose range of 15.8±1.6 mJ/cm^2 in continuous flow reactor (Matak 2005).

Giese defined the responses of three coliform bacteria species (*Citrobacter diversus*, *Citrobacter freundii* and *Klebsiella pneumoniae*) to three wavelengths of UV light (254, 280 and 301 nm) with a collimated beam apparatus having medium pressure UV lamps at 29 to 575 second under 2.3-39 mW s/cm^2 and achieved 3 log inactivation level (Giese 2000). Hence, germicidal efficiency determined for one species of bacteria or virus may be used to represent the relative responses of all bacteria and viruses to medium pressure UV irradiation. Tosa and Hirata determined the susceptibility of enterohemorrhagic *E.coli* O157:H7 to UV radiation at 254 nm and photoreactivation in a batch disinfection device at 1.5 and 3.0 mWs/cm^2 UV dose (Tosa and Hirata 1999). Liltved identified the significant difference of both photoreactivating and photoinactivating process for two fish pathogenic bacteria (*Aeromonas salmonicida* and *Vibro anguillarium*) after exposing to sunlight and artifical (UV) light in their natural river waters in bench top systems under 254 nm and 2-3 mWs/ cm^2 UV dose (Liltved 2000).

Several researches on the efficiency of UV light for the reduction of different microorganisms by using either bench top collimated beam apparatus or continuous flow reactors were reported in the literature (Sommer 1998, Lage 2003). Bolton calculated the fluence rate distribution and the average fluence rate in an annular UV reactor with either a monochromatic (254 nm) low pressure mercury lamp or a broadband medium-pressure mercury UV lamp, placed at the center (Bolton 2000). Wright examined the efficacy of UV light for reducing *E.coli* O157:H7 in unpasteurized apple cider using thin film UV disinfection unit at 254 nm and 0.94-6.1005 mW s/cm^2 3.81 log reduction was achieved (Wright 1999). Yaun et.al. defined the UV light treatment and reduced the numbers of multistrain coctails of *Salmonella* and *E.coli* O157:H7 on agar surfaces using a 1 m long UV chamber having 253.7 nm wavelength and 100 mW s/cm^2 dose, then achieved 5 log reduction under >8.4 mW/cm^2 and >14.5 mW/cm^2 irradiance average (Yaun et.al. 1999). Wong et.al. determined the >5 log reduction of *E.coli* on the surface of tryptic soy agar with doses of >12 mW/cm^2 (Wong et.al. 1998). Sumner et.al. achieved the 7-log reduction of *Salmonella typhimurium* on brain heart infusion agar plates with doses of 36 mW/cm^2 (Sumner et.al. 1995).

CHAPTER 3

MATERIALS AND METHODS

3.1. Sample Preparation and Handling

3.1.1. Preparation and Handling of Shell Eggs

Shell eggs (maxi sizes, A3 quality) were washed with chlorinated water in order to eliminate "Avian Influenza" virus and other foodborne pathogenic organisms, which could be seen in poultry animals and faeces on shell egg. After the water is drained, shell eggs were washed with deionized water and finally broken properly and separated into 2 of 1000 ml beaker. In order to separate chaladza and other membraneous impurities, egg yolk was filtered by colander. Egg white was double filtered by colander and gauze to separate foam layer. In order to remove foam from egg white completely, egg white was centrifuged at 800 rpm speed for 1 minutes at 4 ºC. Whole egg was prepared by mixing approximately 13-14 eggs (egg white and egg yolk together) in the same container. Then separated from impurities (chaladza, membranes). Finally, 600 ml of samples for each LEPs were stored under refrigeration conditions (T=4-6 ºC) before measurement.

3.1.2. Preparation and Handling of Pasteurized LEPs

Pasteurized products for biodosimetric studies, obtained from the egg product manufacturer (IPAY Izmir Pastörize San. And Tic. A.Ş., İzmir, Turkey), were selected in order to make sure that samples did not contain significant levels of indigenous microflora. 1 liter of LEPs' boxes were handled and stored at 4 – 6 ºC in refrigerator.

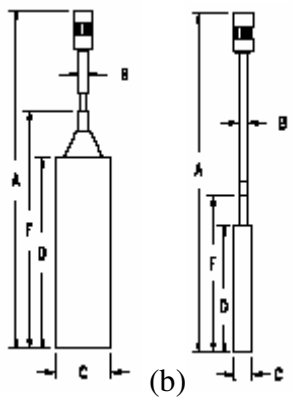
3.1.3. Preparation and Handling of Carboxylmethylcellulose Model Solutions

Before continuous flow UV reactor studies, great amount of LEPs were needed to be used in the system for maximum effectiveness of operations. In order to define flow conditions, including total cycling time and UV residence time per cycle for a given volumetric flow rate for each LEPs, Carboxylmethylcellulose (CMC) is used as one of the rheological modifiers, most commonly employed in the food industry. Different concentrations (0.7 %, 1.25 % and 3.5 % wt/v) of CMC were used as model fluids CMC obtained from Kimetsan Inc, Ankara, Turkey was in powdered form and initially dissolved in small amount of distilled water, then proper concentrations of CMC solutions were prepared in 1 liter of distilled water. Solutions were finally stored under refrigeration conditions before measurements.

3.2. Rheological, Physical and Optical Measurements of LEPs

3.2.1. Rheological Measurements of LEPs

Rheological parameters define the flow behavior of fluids in a defined temperature range. By applying several viscosity tests with respect to temperature and time gives some information about the type of fluid and its flow behavior in the processing equipment. In these studies, up-down rate ramp, time sensitivity test and temperature sensitivity test were done for each LEPs. These tests were carried out by choosing one parameter that is kept constant and changing the remaining factors. Viscosity measurements were conducted for liquid egg products at different temperatures by using concentric cylinder viscosimeter (Brookfield DV II+Pro, Brookfield Engineering Lab. Inc., MA, USA) equipped with a cylindrical spindle (LV-1) (cylinder diameter 18.84 mm, length 115 mm, beaker diameter 86.30 mm and 600 ml of sample volume) as seen in Figure 3.1a. Since egg yolk samples were more viscous than other samples, LV-2C type spindle (cylinder diameter 10.25 mm, length 115 mm) was used for this product (Figure 3.1b). Rotational speeds of these spindles ranged between 5 (1.02 s^{-1}) and 200 rpm (53.7 s^{-1}).



(a) (b)

Figure 3.1. (a) LV-1 cylindirical spindle: A=115 mm, B=3.2 mm, C=18.84 mm, D=65.1 mm, F=80.97 mm. (b) LV – 2C cylindirical spindle: A=115 mm, B =3.2 mm, C=10.25 mm, D=53.95 mm, F=66.25 mm.

Liquid egg white (LEW) and whole egg (LWE) were less viscous and egg yolk (LEY) exhibited considerably high viscosity. Therefore LEW and LWE samples were subjected to a programmed shear rate linearly increasing from 8 to 53.7 s^{-1} (30-200 rpm), followed by a steady shear at 53.7. s^{-1} for 10 minutes and a finally a linearly decreasing shear rate from 53.7 s^{-1} to 8 s^{-1} (hysteresis loop). LEY samples were sheared in a similar way but shear rate was changed from 1 to 34 s^{-1} (5- 160 rpm). During the shear, torque was recorded every 60 sec as a function of rotational speeds by a software program (Rheocalc32, Brookfield Eng., MA, USA) attached with the instrument and connected to a personal computer. At each rotational speed, care was taken to ensure that the torque has attained its steady state value. Measured torque values at different agitation speeds were converted to shear stress–shear rate data by using proper conversion equations (WEB_4 2006). Ranges of shear rate for LEPs were selected to simulate the typical processing conditions. Shear rates at the wall within a commercial UV system under typical processing conditions (data calculated according to the study of Telis-Romero et al. 2006) have been 8 to 30 s^{-1}. Therefore, shear rate ranges were chosen to cover this range. The onset of turbulent flow (Rao 1999) was also evaluated to ensure that the flow remained laminar. Three replicates were made for each sample at a given temperature and new samples were used for each test.

Viscosity measurements were done for LEPs at storage temperatures, room temperature and pasteurization temperature by using different spindle. Spindles were created a shear near the metalic surface of cylinder. This shear rate data were collected

and made an algorithm with respect to viscosity-shear stress-shear rate relationship. Viscosity values were directy displayed on screen. Calibrations of viscosimeter with LV-1 and LV-2C spindles were done by using silicone oil standards (Brookfield viscosity standards, Brookfield Eng., MA, USA). Temperature adjustment was controlled by the help of cooling water bath.

Rheological measurements of LEPs were carried out at different temperatures and summarized as follows:

- For egg white: 4 ºC (storage conditions), 25 ºC (room temperature) and 55,6 ºC (optimum pasteurization temperature).

- For egg yolk: 4 ºC (storage conditions), 25 ºC (room temperature) and 60 ºC (optimum pasteurization temperature).

- For whole egg: 4 ºC (storage conditions), 25 ºC (room temperature) and 60 ºC (optimum pasteurization temperature).

Several rheological tests were applied on LEPs and CMC solutions under the same conditions. These tests were explained in the following sections.

3.2.1.1. Up-Down Rate Ramp

Using the time versus RPM or shear rate curve, flow behavior of fluid at different RPM or shear rate were estimated. Methods were shown in Figure 3.2. A starting and a maximum rotational speed were chosen for each LEPs and CMC solutions. Speeds were increased and decreased with equal intervals. Rheocalc32 software program was used to write an algorithm in order to make data display after each measurement with equal time intervals automatically. Using the method defined above, controlled rate ramp creates the up ramp. Upon reaching the maximum rotational speed or shear rate, direction was reversed and returned to the direction of starting speed to create down ramp.

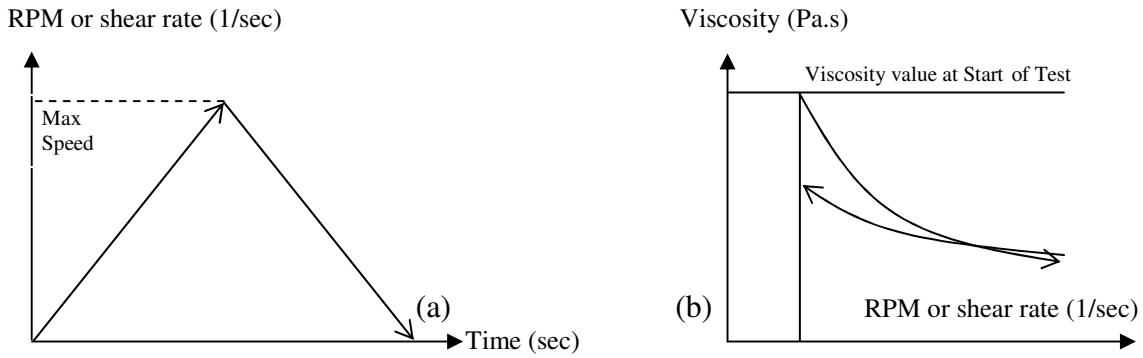


Figure 3.2. Up-down rate ramp method (a) defines the method of rate ramp model and (b) represents the application of rate ramp model with respect to viscosity versus RPM curve.

Viscosity and torque data were recorded at each speed. Torque readings were kept greater than 10 %. For each specific speed or shear rate, hysterisis was observed among the viscosity value on the up ramp and down ramp. Different viscosity value indicate that the material is time sensitive to shearing action. Time interval was defined as 60 seconds for up-down rate ramp viscosity measurements. In addition to the time interval, 10 minutes of resting period was applied for the viscosimeter readings reached at max point in rheological tests of CMC solutions.

Viscosity of liquid egg white and whole egg were measured by # 1 LV cylindirical spindle since viscosity range of liquid egg white is lower at given temperature than that of egg yolk. Following equations were represented to estimate shear rate and shear stres values for measured liquid egg product by # 1 LV spindle:

$$SHEAR\ RATE\ (\sec^{-1}) = \gamma = \frac{2\,\omega\,R_c^{\,2}\,R_b^{\,2}}{x^2\,(R_c^{\,2} - R_b^{\,2})} \tag{3.1}$$

$$SHEAR\ STRESS\ (dynes/cm^2) = \tau = \frac{M}{2\,\pi\,R_b^{\,2}L} \tag{3.2}$$

where: ω=angular velocity of spindle (rad / sec) [= $\frac{2\,\pi}{60}N$] N=RPM

R_c=radius of container (cm)=4.315 cm

R_b=radius of spindle (cm)=0.942 cm (for LV-1) and 0.512 cm (for LV-2C)

x=radius at which shear rate is being calculated (cm)

M=torque input by instruments (dyn / cm)

L=effective length of spindle=7.493 cm (for LV – 1) and 6.062 (for LV-2)

$$torque\ (dynes/cm) = \frac{torque\ (\%) * 673.7}{100} \tag{3.3}$$

where, 673.7 dynes/cm identifies the common spring torque available from Brookfield for LV adapters. Besides, it was not necessary to use R_b, R_c and effective length of LV-2C spindle, since shear stress and shear rate levels could be directly read on Rheocalc32 software during the measurement.

3.2.1.2. Time Sensitivity Test

Time sensitivity test was applied by using viscosity (or torque) versus time curve. Method was shown in Figure 3.3. A starting and a maximum speeds were chosen as appropriate. Time interval was adjusted as 30 seconds for time sensitivity test at storage, room and pasteurization temperatures. For CMC solutions, 20 seconds of time interval was applied. It was observed as whether the viscosity or torque values changed as a function of time.

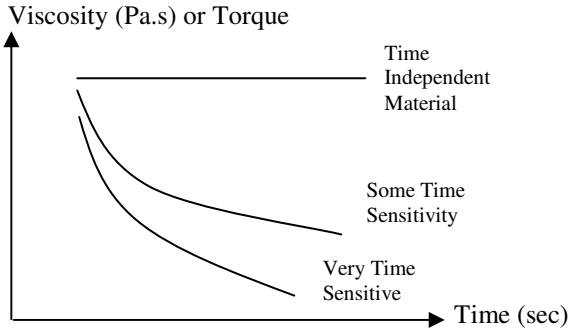


Figure 3.3. Time sensitivity test method.

3.2.1.3. Temperature Profiling with Up-Down Rate Ramp Test

This method combines the approaches in temperature sensitivity test and up – down rate ramp test. Time sensitivity test can be done by choosing a rotational speed or shear rate by choosing a starting and maximum temperature. Viscosity values are recorded at defined time intervals, while temperature ramps up. Method was represented in Figure 3.4.

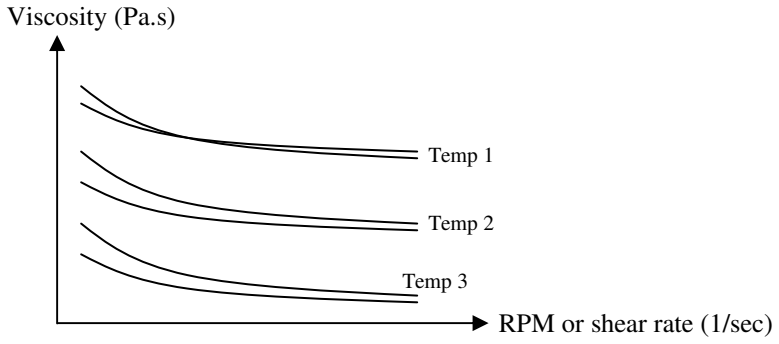


Figure 3.4. Temperature profiling with up-down rate ramp test method.

Temperature profile was defined between maximum and starting temperature. The procedure for choosing the appropriate time interval were the same as in up – down rate ramp and time sensitivity tests.

3.2.1.4. Dynamic Yield Test

Different rotational speed values between the acceptable torque values (10 %-100 %) are chosen after the determination of time interval for specific product. Viscosity, shear stress and shear rate measurement are done in small sample adapter with constant temperature for each test. Shear stress or torque values at defined time intervals are recorded. Data are rewieved and a best fit straight line through the data is determined as shown in Figure 3.5.

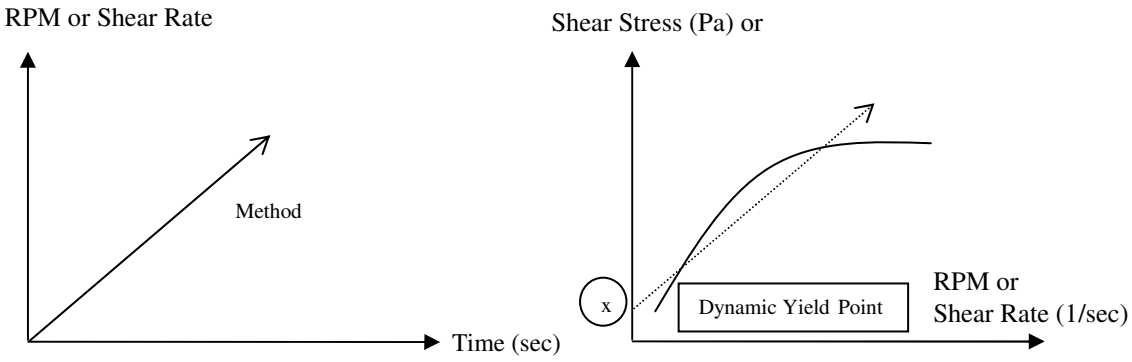


Figure 3.5. Dynamic yield test method.

The shear stress and shear rate were determined from the torque measurements, rotational speed and geometry of the system and calculated automatically by using the software attached to the system. Different rheological flow models (Newtonian (3.4), Power Law (3.5) and Herschel-Bulkley (3.6)) based on shear stress shear rate were tested (Rao, 1999). Fitted functions were obtained by using the least square fit method of commercial spread sheet (Excel, Microsoft Corp., 2003). The suitability of the fitted functions was evaluated by the coefficient of determination (r^2) as well as the standard error of estimate S_{est} (3.7):

$$\tau = \mu \gamma \tag{3.4}$$

$$\tau = K \gamma^n \tag{3.5}$$

$$\tau = \tau_0 + K \gamma^n \tag{3.6}$$

$$S_{est} = \tau_0 + K \gamma^n \tag{3.7}$$

where τ is the shear stress (Pa), τ_0 is the yield stress (Pa), γ is the shear rate (s^{-1}), μ is the Newtonian viscosity (Pa.s), K is the consistency index ($Pa.s^n$), n is the flow behavior index, In order to quantify the effect of temperature on the viscosity or consistency coefficient, an Arrhenius type equation (3.8) was applied:

$$\eta_a = \eta_0 e^{\frac{E_a}{RT}} \quad (3.8)$$

where η_a is the apparent viscosity (Pa.s), η_0 is a parameter considered as the viscosity at infinite temperature (Pa.s), E_a is the activation energy (J/mol), R is the molar gas constant (J/mol K), and T is the temperature (°K).

3.2.2. Rheological Properties of Model solutions (CMC)

Similar tests explained in Section 3.2.1, were applied on CMC model solutions, prepared at different concentrations (0.7, 1.25 and 3.5 % wt/v). Viscosity and density of CMC solutions were measured at different temperatures. Then, results were recorded, rheological behavior were determined similarly using (3.4), (3.5), (3.6), (3.7) and (3.8) and compared with that of LEPs.

3.2.3. Measurements of Physical Properties

3.2.3.1. Density of LEPs and CMC Solutions

Density and pH values of LEPs were measured by using densitometer (Kyoto Electronics DA, Japan) at different temperature gradient taking several data at different temperature ranges (4-55.6 °C for LEW, 4-60 °C for LEY and LWE). Fluid was filled in the measurement tube and density was recorded as a function of its instant temperature in g / cm^3.

3.2.3.2. pH of LEPs

pH of each LEPs before and after UV treatment in bench top and continuous system studies were measured using pH meter (Metrohm, Switzerland). Along with pH measurements, temperature changes were also recorded.

3.2.3.3. Total Solid Content of LEPs

Total dry solid content (by weight percent) of each product were also determined in terms of AOAC 925.30 method.–Total Solids in Eggs (Daniel Lebryk, 1999). A metal petri dish was previously dried at 98-100 °C and cooled in desiccator. Then weighted at room temperature. 5 g of sample was added in petri dish and final weight was recorded nearest to 0.0001 rapidly. Finally sample was dried at 98-100 °C in an incubator (Memmert GmbH Co. KF D-91126 Schwabach, Germany). Total weight was recorded in a very short period of time per hour.

3.2.4. Optical Properties

3.2.4.1. Absorbance of LEPs

Before each test, UV absorbance (A_e) (the fraction of UV intensity transmitted through 1 cm path length of the sample) was measured using a UV-VIS spectrophotometer (Cary 100 Bio, Varian Inc., CA, USA) set at a wavelength of 254 nm. LEPs were diluted at 1:50, 1:100, 1:250, 1:500 and 1:1000 dilution factors. Then UV absorbance was measured by 1 ml of cuvette for each dilution.

3.2.4.2 Turbidity of LEPs

Turbidity of LEPs were also determined both in bench top and continuous flow studies by using a turbidimeter (2100AN, HACH Company, CO, USA). 45-50 ml of samples were poured in a glass sample cell and turbidity measurements were done before and after UV inactivation of LEPs, for twice for each cell.

3.2.4.3. Color Measurement of LEPs

Color attributes of LEPs were determined by CR 400 chromometer (Konica Minolta, Japan) using Illuminant D_{65}. The instrument was calibrated using a standard white tile. A cylindrical glass cell (5.5 cm in diameter) filled with 90 ml of LEPs was placed on the top of the light source. CIE L*(Brightness), a*(redness-greenness) and b*

(yellowness–-blueness) values (CIE, 1976) of LEW, LEY and LWE were measured before and after UV exposure. Three readings were averaged for each depth and exposure time. The color change of the samples was also expressed as a single numerical value ΔE. This value defined the magnitude of the total color difference. The ΔE value was expressed by the following equation:

$$\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2}\ \Delta b^{*2}} \tag{3.9}$$

L* a* and b* values were determined for the samples treated with bench top collimated beam and continuous flow UV studies. ΔE values were determined for only the bench top collimated beam experiment. The data of the bench top collimated beam treatment was analyzed using Analysis of Variance and means were compared using Duncan Multiple Range Test.

3.3. Bench top Biodosimetry Studies

3.3.1. Target Microorganisms (Biological Dosimeter) and Growth Conditions

The non-pathogenic strain of *E.coli* (NRRL B-253), serotype *E.coli* O157:H7 (ATCC 700728) and *S.typhimurium* (CCM 5445) were used as biological dosimeters in the preliminary biodosimetric study. *E.coli* (NRRL B-253) was kindly provided from United States Department of Agriculture, Microbial Genomics and Bioprocessing Research Unit, Peoria, Illionis. serotype *E.coli* O157:H7 (ATCC 700728, Dr. Ali Aydın, Department of Food Hygiene and Technology, Faculty of Veterinary, İstanbul University, Turkey) and *S.typhimurium* (CCM 5445, Dr Ayse Handan Baysal, Department of Food Engineering, İzmir Institute of Technology, Turkey). During this study, they were maintained at -80°C. A broth subculture was prepared by inoculating loopful from stock culture into a test tube containing nutrient broth (NB, Merck, Darmstadt, Germany) and strains were incubated for overnight (24 h) at 37°C. Serial dilutions of the subculture were carried out to determine the inoculum concentration. Incubation of 24 h allowed the respective bacteria to approach stationary phase of growth at a concentration of ca. 10^8 CFU/ml.

The depth in 50 mm standard Petri-dishes was adjusted by adding a in different amount of volume to give sample depths of 0.153, 0.3 and 0.5 cm. Biodosimetric study of *E.coli* O157:H7 (ATCC 700728) and *S.typhimurium* (CCM 5445) were applied on 0.153 cm sample depth and 1.315 mW/cm^2 UV incident intensity level. Then, LEPs samples was directly inoculated with the subculture to provide a final inoculum of ~10^7-10^8 CFU/ml. For enumeration, appropriate dilutions were made with 0.1% peptone water and surface plated in duplicate on tryptic soy agar (TSA, Merck, Darmstadt, Germany). To determine the level of injured cells, serial dilutions of the UV treated samples were spread on Violet Red Bile Agar (VRBA, Fluka, Biochemica, Spain) and Eosin Methylene Blue (EMB, Fluka, Biochemica, Spain) plates. All the plates were incubated at 37 °C for 24h and counted. All studies were replicated twice.

3.3.2. UV Irradiation Equipment and Inactivation Treatments

In order to determine reduction rate of microorganisms in logarithmic scale under ultraviolet light, UV biodosimetry experiment was conducted with collimated beam apparatus described in Bolton 2003. Liquid egg white sample were UV exposured using closed bench top ultraviolet system, shown in Figure 3.6. Bench top system consists of two identical low pressure mercury vapor UV lamp at 254 nm wavelength (UVP XX-15, UVP Inc., CA, USA) (1), a platform on which petri dish and a vortex mixer was placed for UV exposure. It is easy raised or lowered by tray system (2 and 3), a hole, placed on top of the system with dimensions of one standard petri dish diameter (4), one passage system in front of the lamp to use blocking UV light before measurement and a shutter to block or allow passage of UV energy to a stage (5). Ultraviolet lamps were switched on about 30 minutes before the measurement to provide complete activation.

A cover was closed in front of the system during UV experiment to prevent contact of UV light to human skin directly. The whole system was constructed by cardboard with a flatblock point in order to minimize the loss of light. Samples were

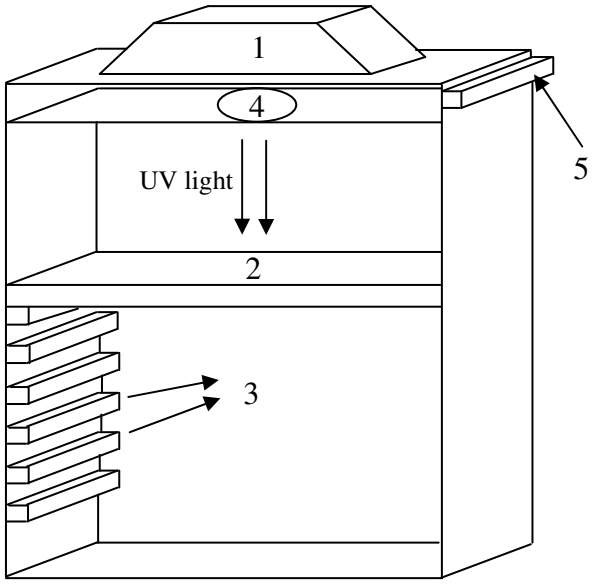


Figure 3.6. Closed bench top ultraviolet system.

placed in 50 mm diameter Petri dishes directly below the collimated UV beam and stirred continuously during the irradiation with a vortex mixer (IKA, Yellowline TTS 2, IKA® Werke GmbH & Co. KG, Germany) set at dial #5. The UV intensity at the surface of the sample (incident intensity (I_o) or irradiance at the surface) was measured using a radiometer with UVX-25 sensor (UVX, UVP Inc., CA, USA). The radiometer was placed at a similar distance from the UV lamp as the LEW samples. The UV lamp was switched on for about 30 min prior to UV treatment of LEW samples in order to minimize fluctuations in intensity.

Prior to usage, the collimated beam apparatus was cleaned and sanitized. For the UV inactivation treatments, inoculated plates were subjected to different doses of UVC light. The average UV intensity (average irradiance or fluence rate) in the stirred sample (I_{avg}) was calculated by an integration of Beer-Lambert law over the sample depth (Morowitz, 1950):

$$I_{avg} = I_0(1 - e^{-A_e L})/A_e L \tag{3.10}$$

where I_o is the incident intensity (mW/cm^2), A_e is the absorbance per centimeter and L is the path length (cm). UV dose can be calculated using these irradiance values from t = 0-20 min minutes using the following formula:

$$d = t * I \tag{3.11}$$

where "d" represents UV dose (mJ/cm^2), "t" is time (in minutes) and "I" refers to irradiance in mW / cm^2

Pasteurized products obtained from the egg product manufacturer (IPAY Izmir Pastörize San. And Tic. A.Ş., İzmir, Turkey) were selected for this study in order to make sure that samples did not contain significant levels of indigenous microflora. Inoculated LEW samples were exposed to UV radiation of known intensity levels (1.315 mW/cm^2, 0.709 mW/cm^2 and 0.383 mW/cm^2) for 5, 10 and 20 min excluding untreated control sample and incubated at 37 ^{o}C for 24 h. All studies were conducted within the UV Dose range of 0-100 mJ/cm^2 and replicated twice.

The total elapsed time between inoculation and UV treatment was controlled in order not to allow extra microbial growth. Enumeration of *E.coli* (NRRL B-253) after each treatment was made as described in previous section. The survival ratio of cells after each treatment was calculated from the following equation:

$$S = \frac{N}{N_0} \tag{3.12}$$

Where N is the microbial population after UV exposure, N_0 is the initial inoculation level. The inactivation of microorganism is generally reported in terms of log reduction per unit UV Dose. The inactivation rate of microorganism can be described by a linear first order model (Chick, 1908):

$$\log\left(\frac{N}{N_0}\right) = y - k * d \tag{3.13}$$

where d is the UV Dose (in mJ/cm^2), k is described as the inactivation rate constant (in cm^2/mJ) as the slope of the log N/N_0 vs. UV dose curve and estimated from the slope of log (N/N_o) versus UV Dose curve, y is the intercept. Experimental procedure was summarized in Figure 3.7.

3.3.3. Statistical Analysis

The effects of three factors, depth of liquid medium, UV intensity and time on the inactivation of UV resistant of *E.coli* (NRRL B-253) in LEPs were investigated by using of a general full factorial design. Average log reductions were used in statistical analysis. All treatments were replicated twice. Analysis of Variance (ANOVA) was performed using the general linear model procedure of MINITAB 14 (Minitab Inc., State College, PA, USA) in order to determine the significance of each factor. Regression analysis was performed for the kinetics of UV inactivation data by using commercial spread sheet (Excel, Microsoft Corp., 2003).

3.4. Continuous Flow UV Treatment of LEPs

Continuous flow UV reactor system not only differs from bench top collimated beam apparatus as a basis of design, operation method and equipment characteristics, but fluid motion also integrates more fundamental factors about microbial inactivation and UV light penetration to the sample fluid, than that of static system. Dramatically, it has not been possible until now to measure the slope of UV dose distribution in flow-through systems. Consequently, bench top biodosimetry studies were applied first in order to establish logarithmic reduction versus UV dose curve in LEPs. Then based on the logarithmic reduction obtained in flow-through system, applied UV-dose were found from this curve. Additionally, bench top studies pointed out that UV light radiation creates pronounced effect on LEW samples. UV inactivation of *E.coli* (NRRL B-253) in LEY and LWE samples were limited due to their high turbid nature. As a result of this conditions, continuous UV treatment was applied only to LEW samples.

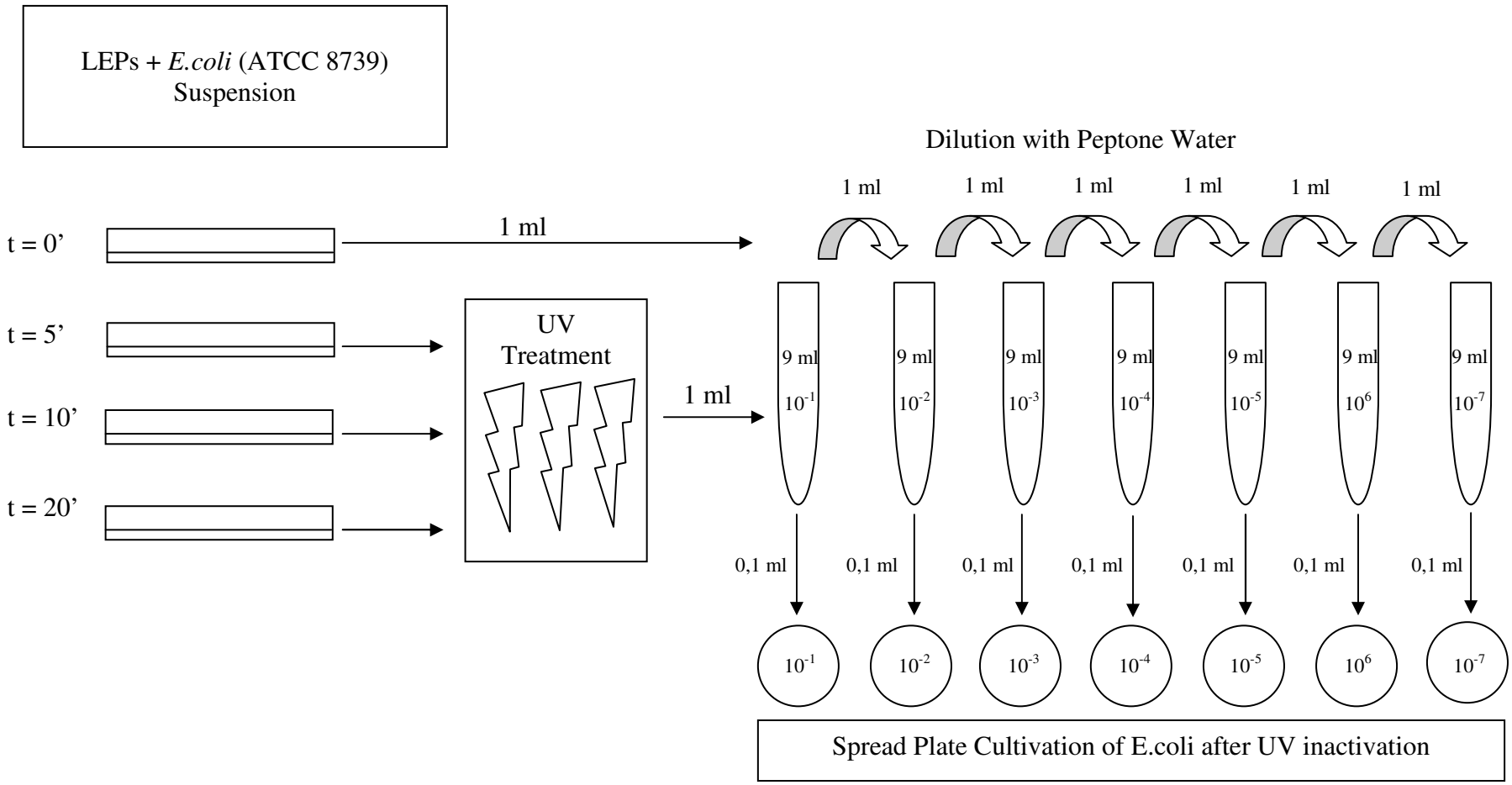


Figure 3.7. Experimental procedure of cultivation after bench top collimated beam UV inactivation of *E.coli* (NRRL B-25)

3.4.1. Processing Parameters for Continuous Flow UV Reactor

UV treatment system consists of following equipments:

- A vertical UV reactor (Wedeco-Durco AG Water Technology, Germany); equipped with 6 of 254 nm low mercury UV lamp and a quartz tube (See Table 3.1.).
- 150 lt of stainless steel tank,
- A 2M25 type stainless monopomp (Inokstek Inc, Turkey), having the capacity of 100-1000 lt per hour, with a frequency inventor.
- Stainless steel piping system and fitting equipments (manual valves, elbows, non-aseptic sample valves)
  - 3.5 m of stainles steel pipe (D=1")
  - 1.1 m of stainless steel pipe (D=2 ")
  - 1 m of quartz tube (D=2")

Continuous flow UV reactor system was illustrated in Figure 3.8. Since operating parameters plays an important role in the design of UV system, it is essential to determine UV residence time, total operation time per one cycle and volumetric flow rate for LEW samples in the system. Besides, great amount of LEPs was to be spent for the determination of parameters and cleaning of the whole system after operation was

Table 3.1. Specifications of Continuous Flow UV Reactor.

Reactor Type	E10
Reactor connections: male thread, stainless steel, 1.4571 (316 Ti)	R 2"
Width (mm)	240
Height (mm)	1090
Depth (mm)	245
Volume (lt.) app.	4.5
Weight (kg) app	31
Operating Pressure (bar)	10
Pressure Loss (bar) app.	0.01
Voltage (V/Hz)	230/50
Power Consumption (W / VA)	230/500
Protection Class	IP 54
Lamp Type	NLR1579W
Lamp Power (W)	30
UV-C Output 254 nm (W)	12.5
Quantity	6
Expected Lamp Life Time (h)	8000

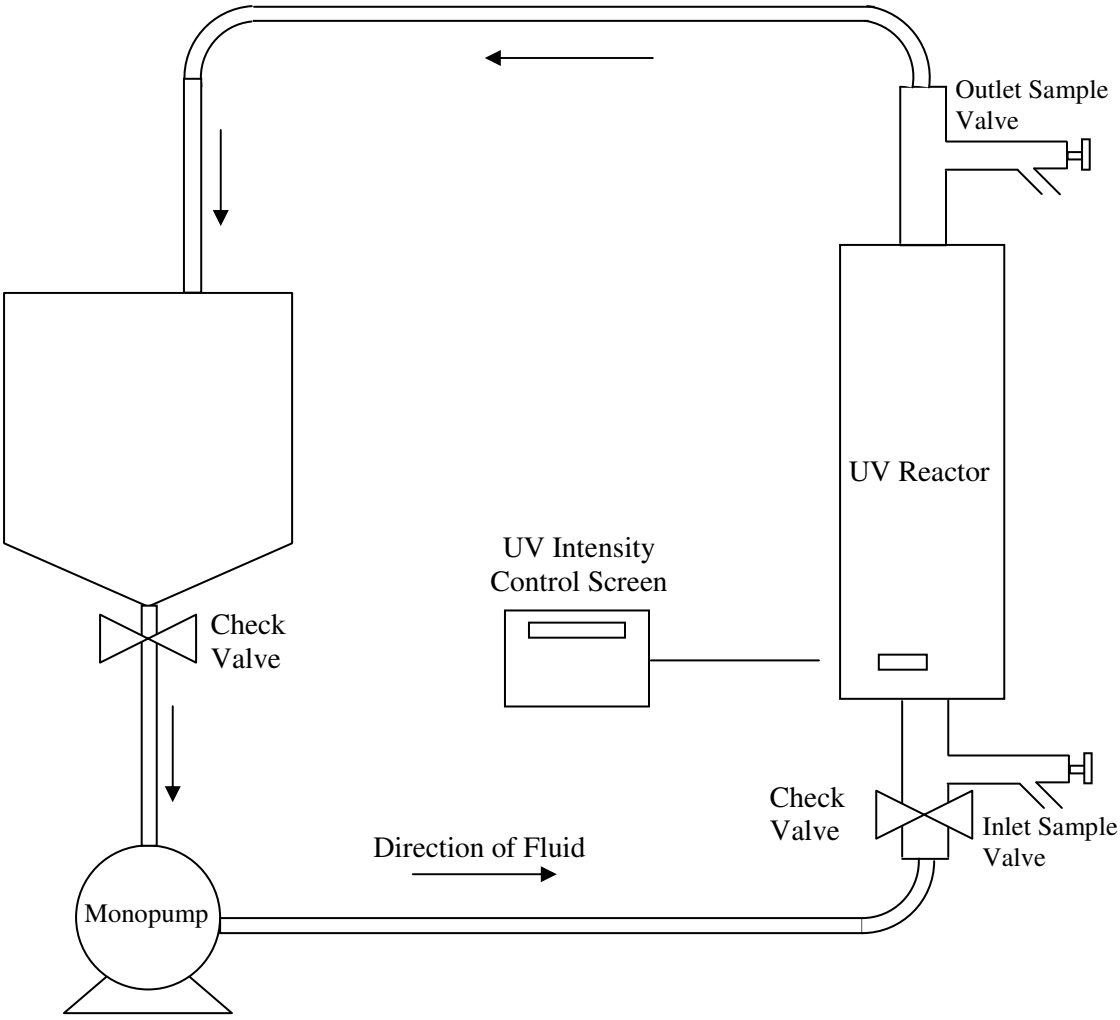


Figure 3.8. Continuous flow UV reactor system.

crucial. Consequently CMC model solutions, which mimic the rheological properties of LEW, were used initially to determine processing parameters such volumetric flow rate (m^3/s), Reynolds number, total cycling time and UV residence time in the continuous UV system. 0.7 % CMC solution was filled into the tank and pump was switched on. Meanwhile, chronometer was started and time was checked for UV residence time when passing from UV reactor and total cycle time until the first fluid dropped was detected at the outlet of the system. Moreover, volumetric flow rate for each solution was measured by collecting certain amount of fluid in a volumetric flask per unit time. Laminar flow is preferred in continuous flow system in order to prevent foaming in LEW caused by mixing and turbulence. Hence, Reynolds numbers were estimated for Newtonian and Non-Newtonian fluids in laminar flow by (3.14) and (3.15) (Telis-Romero 2006).

$$\mathrm{Re}_g = \frac{D^n v^{(2-n)} \rho}{v^{(n-1)} K} \left(\frac{4n}{1+3n} \right)^n \quad \text{(Non-Newtonian)} \tag{3.14}$$

$$\mathrm{Re} = \frac{Dv\rho}{\eta} \quad \text{(Newtonian)} \tag{3.15}$$

where Re is reynolds number and Re_g is generalized reynolds number, D is diameter (m), υ refers to average flow velocity (m/s), ρ is density (kg/m^3), n is flow behavior index, K is consistency index (Pa.s^n) and η is viscosity (Pa.s). Results of Reynolds number of both Newtonian and Non-Newtonian behaviour was summarized in Table 3.2.

Table 3.2. Flow Parameters of 0.7 % CMC in Continuous Flow UV Reactor.

Flow Rate Level	Volumetric Flow Rate (ml/min)	Time of one cycle (sec)	UV residence time (sec)	UV Q (m3/s)	UV v (m/s)	Re (Newtonian)	Reg (Non-Newtonian)
1	360	850	426	1,056E-05	0.0054	18.473	16.685
2	710	397	233	1.931E-05	0.0099	33.776	30.984
3	1080	292	156	2.885E-05	0.0147	50.447	46.760
4	1560	205	107	4.206E-05	0.0215	73.549	68.840
5	1840	183	91	4.945E-05	0.0252	86.481	81.283
6	2190	155	77	5.844E-05	0.0298	102.205	96.476

3.4.2. Inactivation Study

As similarly in UV biodosimetry studies in bench top collimated beam apparatus, explained in Section 3.3.1., the non-pathogenic strain of *E.coli* (NRRL B-253) was again used in this continuous system study, A broth subculture was prepared by inoculating loopful from stock culture into a test tube containing 10 ml of nutrient broth (NB, Merck, Darmstadt, Germany) and strains were incubated for overnight (18-24 h) at 37°C. Incubation of 24 h allowed for the respective bacteria to approach stationary phase of growth at a concentration of ca. 10^8 CFU/ml.

Logarithmic growth rate of *E.coli* (NRRL B-253) was determined by measuring optical densities. In this method, after an overnight incubation, 100 µl of *E coli* suspension from 10 ml of culturewas transferred into 4 of 500 ml erlenmayer filled with

250 ml of nutrient broth and left for incubation in an orbital shaker (Thermo Electron Corp., Ohio, USA) at 300 RPM. Optic density was measured by UV spectrophotometer at the wavelength of 600 nm and microbial count was determined by spread plate count method on TSA, per hour.

According to the activation curve (Figure 3.9.), it could be said that, *E.coli* (NRRL B-253) reached stationary phase after 5 hours of incubation by orbital shaking. Total microbial count was observed as 10^9 CFU / ml. Logarithmic growth rate curve as a basis of optical density values was plotted and shown in Figure 3.10.

It was decided to inoculate 10^6 CFU/ml in 10 lt of sample, 4 x 250 ml of *E.coli* (NRRL B-253) culture was added to 9 lt of sample. Figure 3.11. shows optical density procedure. 10 lt of LEW were prepared at 4 °C and stock culture was inoculated at the rate of 10^6 CFU/ml. Before the UV inactivation study, storage tank was filled with LEW and pump was started at a given flow rate. It was checked that LEW was passed at least one cycle in the system, completely before turning on the UV reactor. Sample valves were sterilized with 70 % ethanol by flaming.

50 ml of sample was taken before inoculation of stock culture for the enumeration of total aerobic count and stored in a dark container in the refrigerator until the microbiological examination. Enumeration of total aerobic count was done by spreading on Tryptic Soy Agar (TSA, Fluka, Biochemica, Spain) and Violet Red Bile Agar (VRBA, Fluka, Biochemica, Spain), also pouring by Plate Count Agar (PCA, Fluka, Biochemica, Spain).

After one cycle was ended, pump was swiched off and LEW was inoculated with 1 lt of stock culture. In order to distribute microbial population homogeneously, sample was mixed with a sterile rod or spoon very well without forming any foam structure.

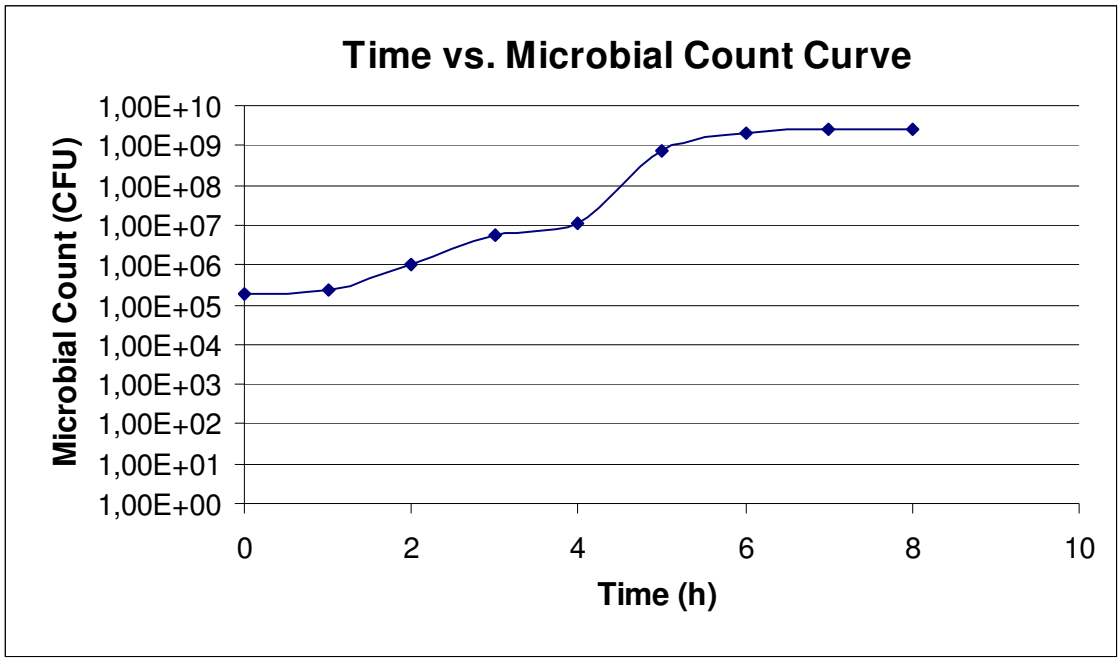


Figure 3.9. Time versus microbial count curve of *E.coli* (NRRL B-253).

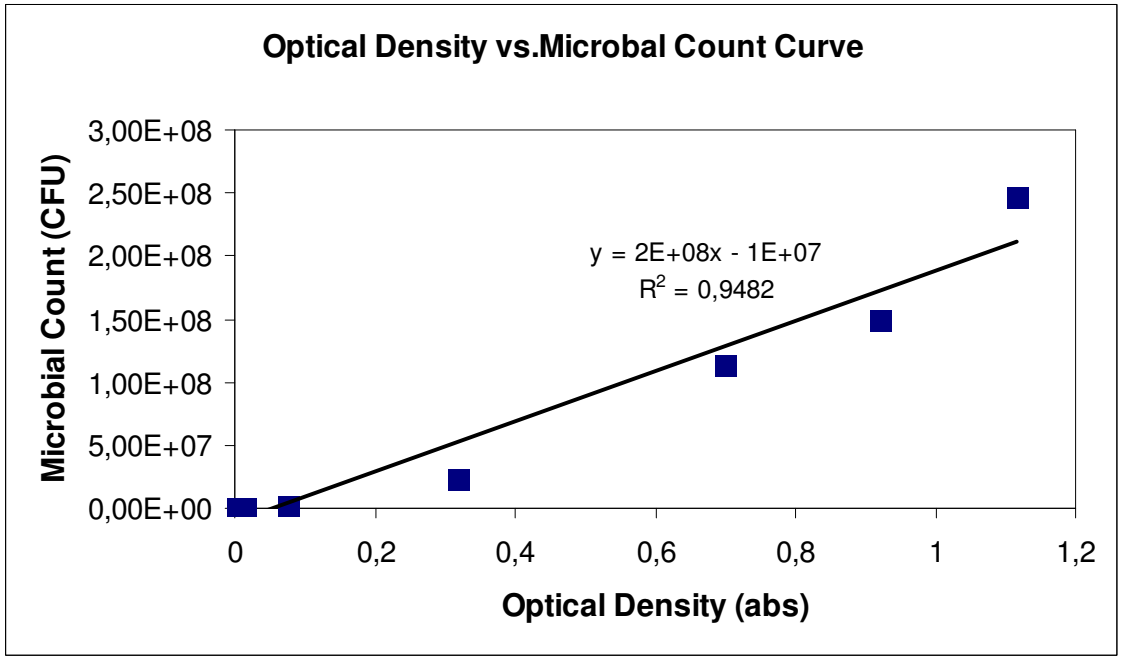


Figure 3.10. Optical density versus microbial count curve of *E.coli* (NRRL B-253).

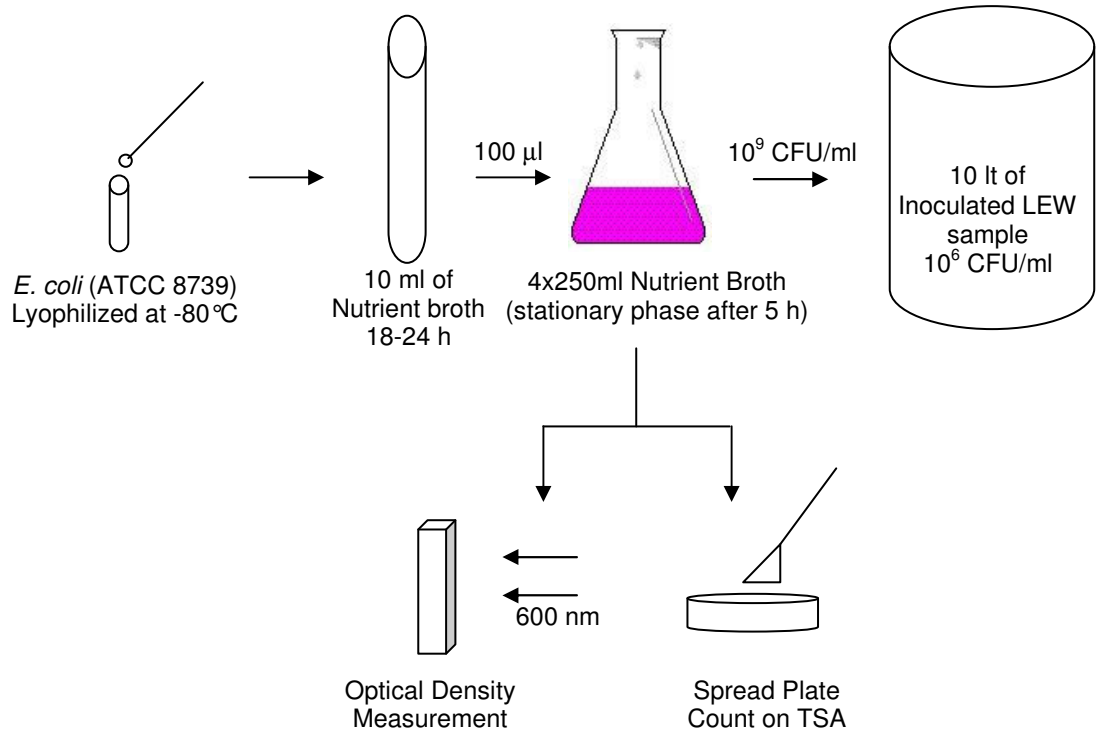


Figure 3.11. Determination of inoculum rate by optical density study.

Initially, 50 ml of sample was taken from the inlet sample valve to determine the initial microbial count in the whole system. UV lamps and UV intensity sensor was switched on, meanwhile pump was started at a given flow rate (1080 ml/min). A stop-watch was started in order to record the total cycle time in the whole system ($t=t_0$). Time was recorded when the liquid was reached to the inlet of UV reactor ($t=t_1$). 50 ml of sample was taken to a sterile erlenmeyer flask from the inlet of the UV system for the *E.coli* (NRRL B-253) enumeration as the initial count (UV inlet). During the passage, UV intensity in the UV reactor was recorded by the UV sensor.

At the end of first cycle, all the liquids in the system were allowed to collect in the storage tank. In order to determine the waiting time for collecting the whole fluid in the pipes, total cycling time levels of 0.7% CMC for each flow rates were used.

Figure 3.12. demonstrates that 300 sec (5 min) waiting time was necessary to complete one cycle in order to discard the whole fluid in the pipes for choosen volumetric flow rate (1080 ml/min).

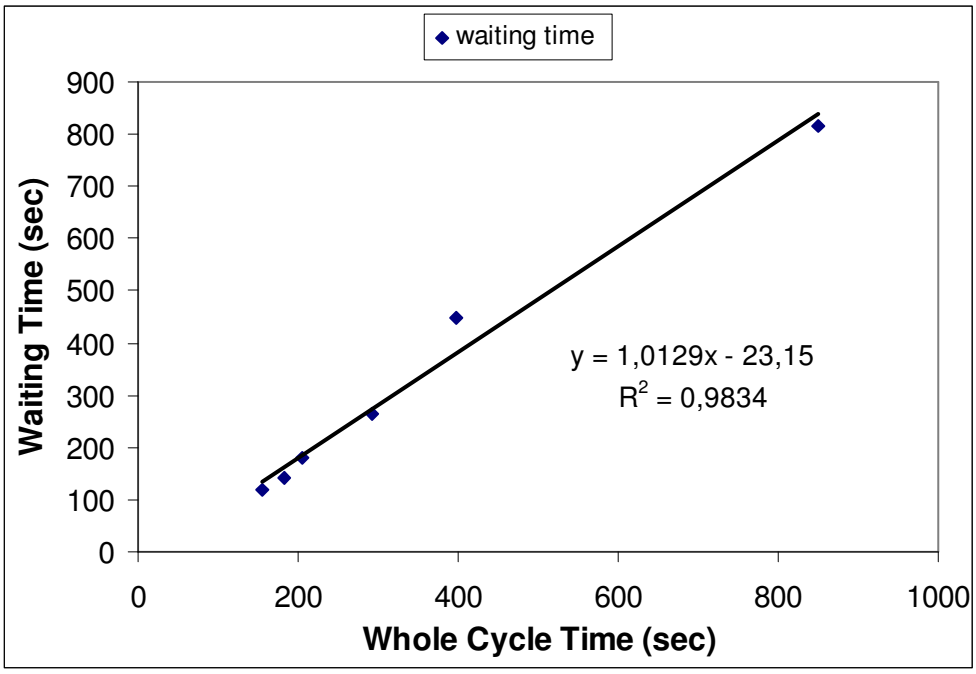


Figure 3.12. Waiting time versus whole cycle time curve for liquid egg white (LEW).

In addition to completing one cycle in UV reactor, UV residence time of one pass for the given LEW was recorded ($t=t_2$). Then 50 ml of sample was taken from the outlet sample valve for the *E.coli* (NRRL B-253) enumeration for the reduction after one pass (UV outlet). After the passage of liquid was completed in UV reactor, time was continued to record and stop-watch was stopped when one cycle was completed within turning LEW to storage tank ($t=t_3$). The total cycle time was found by the summation of t_1, t_2 and t_3, as 294 sec. Samples were refrigerated in aseptic storage conditions in dark container for further microbiological analysis.

3.4.3. Cleaning of the Whole System

3.4.3.1. Discharging and Washing by Disinfectants

In order to clean all the residual protein and microbial structure of LEW from piping and quartz tube of UV reactor, a disinfection agent was needed. To remove entire egg residue, 15 lt of 0.5 M NaOH solution was prepared by dissolving 300 g of NaOH (Panreac E-08110, Montcada I Reixac, Barcelona, Spain) in 15 lt of distilled water. Then, tank was filled with 0.5 M NaOH solution Three complete cycles were operated

to remove the whole LEW. Finally NaOH solution was discharged and system was rinsed with distilled water for three cycles.

To remove microbial population from the system, 15 lt of 2 % (or at least 200 ppm) chlorine solution was prepared by dissolving 300 ml of pure chlorine in 15 lt of distilled water, completely. As done with 0.5 M NaOH solution, 2 % chlorine solution was passed from the system for 3 cycles. At the end of 3rd cycle, chlorine solution was discharged and system was re-washed with 15-20 lt of hot distilled water. During rinsing, UV reactor was switched on to remove residual microbial population. From the outlet of the UV reactor, 50 ml of sample was taken, aseptically for enumeration of total microbial count by spreading on TSA-VRBA and pouring by PCA. 50-75 ml of water was taken to analyze the residual chlorine. At the end of the cleaning operation, all the connections were separated and allowed to dry. Connections were attached again to make the system ready for the next experiment.

All the physical (pH, T and Cl_2 residue), mirobiological and optical (color, absorbance and turbidity) measurements carried out during the continuous flow studies in UV reactor were summarized in Table 3.3.

3.4.3.2 Determination of Residual Chlorine After Cleaning Step

Residual chlorine in final-rinsed water was analyzed by 4500-CI B Iodometric Method I (American Public Health Association 2005). Since chlorine will disengage free iodine from potassium iodine (KI) solutions at pH 8 or less, the disengaged iodine was applied to titremetric method with a standard solution of sodium thiosulphate ($Na_2S_2O_3$) with starch as the indicator (Fluka Chemie, GmbH, CH-9471 Buchs, Switzerland). 0.025 N sodium thiosulphate (Merck KgaA, 64271, Darmstadt, Germany) was prepared by dissolving 6.2 g in 1 lt of distilled water, then standardized by potassium di-chromate (Carlo-Elba, 7778-50-9, Spain) after at least 2 weeks storage. 1 g of KI (Sigma-Aldrich Laborchemikalien GmbH.D-30926, Seelze) was dissolved in 5 ml of acetic acid to reduce pH between 3.0-4.0, since reaction is not stoichiometric at neutral pH due to partial oxidation of thiosulfate to sulfate. Mixture was poured to 50 ml of water-sample. Titration was done away from direct sunlight, 0.025 N of $Na_2S_2O_3$ was dropped to water sample until yellow color of the disengaged iodine was almost discharged. 1 ml of starch indicator solution was added and dark blue color was

appeared. Titration was continued until dark brown color was turned to colorless. For the determination of total available residual chlorine in the water sample, following correlation was applied

Table 3.3. Summary of Continuous Flow UV Reactor Studies.

Sampling Points	Microbiological Examination			Optical Measurements			Physical Measurements		
	TSA	VRBA	PCA	Color	Abs.	Turbidity	pH	T	Cl_2 residue
Pasteurized egg white (control, untreated)		√	√	√	√	√	√	√	
Cycle 1 inlet-outlet	√			√	√	√	√	√	
Cycle 2 inlet-outlet	√			√	√	√	√	√	
Cycle 3 inlet-outlet	√			√	√	√	√	√	
Cycle 4 inlet-outlet	√			√	√	√	√	√	
Cycle 5 inlet-outlet	√			√	√	√	√	√	
After cleaning	√	√	√						√

$$mg\ Cl\ as\ Cl_2 / ml = \frac{A\ x\ N\ x\ 35.45}{ml\ sample} \tag{3.16}$$

where A was ml titration for sample and N is normality of $Na_2S_2O_3$.

CHAPTER 4

RESULT AND DISCUSSION

The results of our study were expalined in four main sections. The results pertainning to rheological characterization, physical and optical measurements of the fresh and pasteurized LEPs were given in the section 4.1 and 4.2. Rheological properties are important for determination of processing parameters and selection of model solutions that simulates the LEPs during UV treatment in continuous flow reactor. In the section 4.3, the results of the biodosimetric study based on the UV treatment of LEPs by using collimated beam apparatus were presented. UV dose in the continuous flow UV reactor cannot be directly measured. Therefore, biodosimetry studies which are carried out by using collimated beam apparatus are essential in determination of the UV dose applied in continuous flow UV reactor studies. In the last section (4.4), the results of continuous flow UV inactivation studies were evaluated and presented.

4.1. Rheological, Physical and Optical Measurements of Liquid Egg Products (LEPs) and CMC Model Solutions

4.1.1. Rheological Measurements

4.1.1.1. Time Dependency

A change in the LEPs´ viscosity over time indicates time-dependent behavior; a decrease signifies thixotropy. This decrease is an important consideration in process design. As the sample is sheared during its passage through the process system, the linkages between particles or molecules in the food are broken, resulting in reduction in the size of the structural units and offer lower resistance to flow (Rao 1999).

In this study, thixotropic behavior of LEPs was examined by measuring hysteresis loop at different temperatures. Results were summarized in Figures 4.1.,4.2.and 4.3.

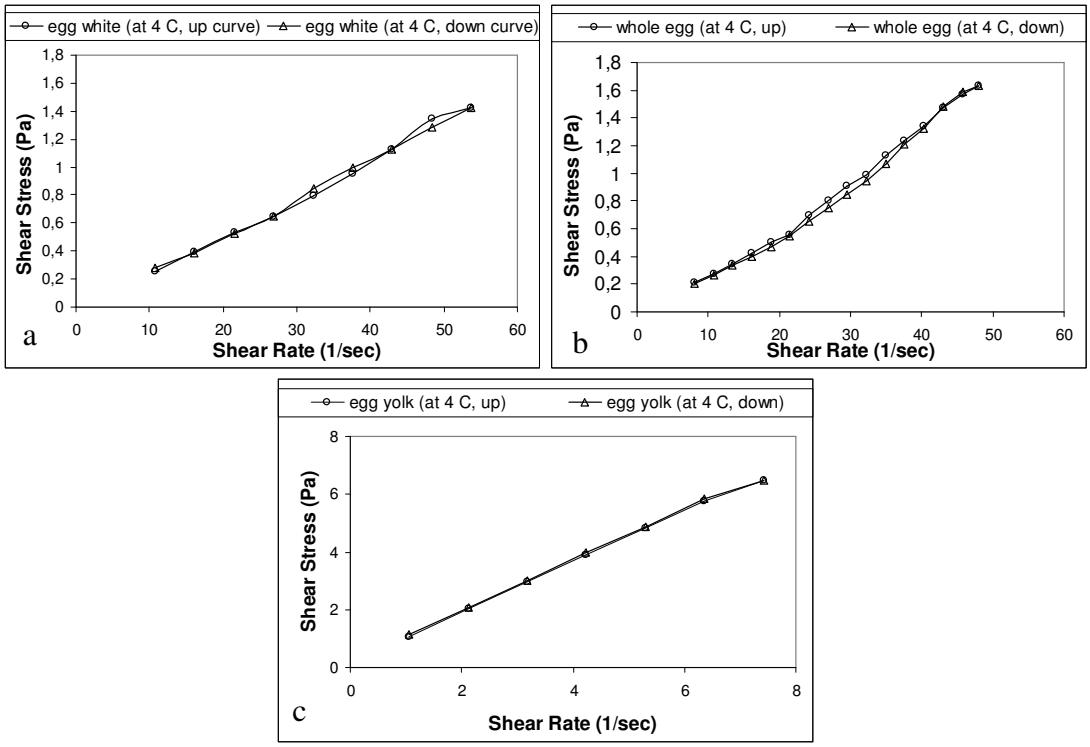


Figure 4.1. Thixotropy of liquid egg products at 4 °C (a) Egg white (b) Whole egg (c) Egg yolk.

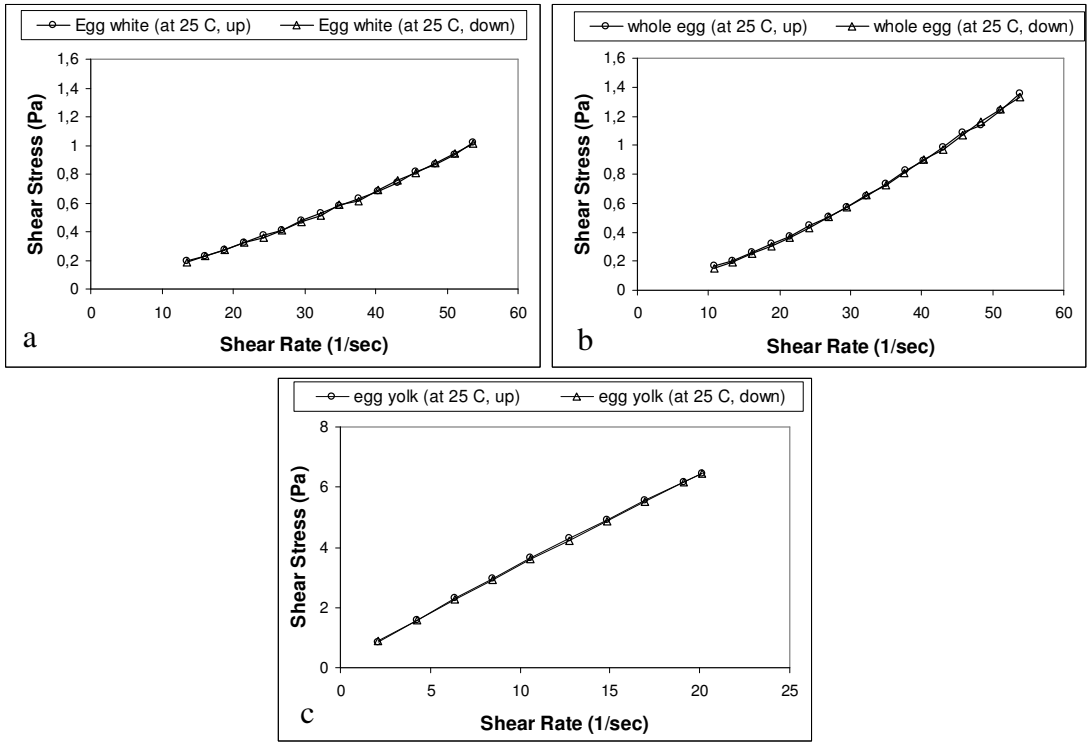


Figure 4.2. Thixotropy of liquid egg products at 25 °C (a) Egg white (b) Whole egg (c) Egg yolk.

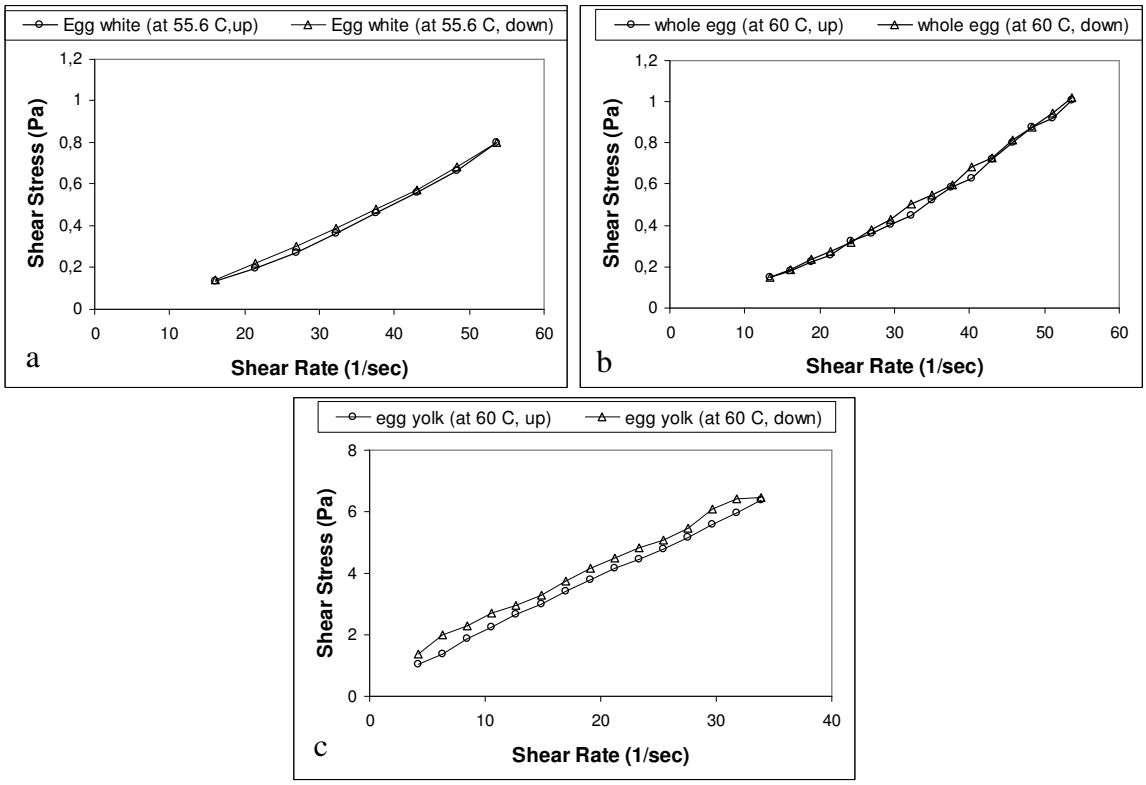


Figure 4.3. Thixotropy of liquid egg products at pasteurization temperatures (a) Egg white (at 55.6 °C) (b) Whole egg (at 60°C) (c) Egg yolk (at 60 °C).

Although LEW and LWE showed evidence of hysteresis at pasteurization temperature of 4 °C, LEY samples exhibited higher degree of hysteresis at 60 °C. It is obvious from the figures that none of the products exhibited thixotropy at low shear rates and temperatures of 4 and 25 °C.

This was also confirmed by recording apparent viscosity at regular time intervals (s 4.4.). Apparent viscosity of these products did not show any time-dependency at low shear rates and 25 °C.

As a result, LEW and LWE were determined to be time-independent in the temperature ranges from 4 °C to 60 °C. But, LEY having higher protein content was especially affected at 60 °C, indicating that the chemical structure was broken primarily because of a susceptibility to heat (coagulation of proteins). Therefore, LEY could be also considered to be time-independent in the temperature ranges from 4 °C to 25 °C but time-dependent at pasteurization temperature.

4.1.1.2. Rheological Parameters

Different rheological models (Newtonian, Herschel- Bulkley and Power law) have been tested to ascertain the correct flow behavior of liquid egg products. Rheological parameters of unpasteurized LEPs were reported in Table 4.1. The coefficient of determination (r^2), for all cases, were higher than 0.99 ($p<0.05$) and standard error of estimates (S_{est}) were lower than 0.16 which confirms that selected models were adequately suitable for describing the flow behavior of LEPs within the range studied (see in Appendix A).

Yield stress was observed in the cases of LWE and LEW (Table 4.2.) and shear stress and shear rate data fitted Herschel-Bulkley model (3.6.) well in the range of temperatures from 4 to 60 °C. The flow behavior index (n) of LWE and LEW obtained from this model showed values varying from 0.9290 to 0.9878 indicating that, in this range of temperature, the liquid whole egg and liquid egg white are slightly pseudoplastic in nature. Because the magnitudes of n were higher than 0.9, one could assume that these products have Newtonian like behavior and can be considered as Newtonian fluids in engineering calculations (Scalzo et al. 1970).

The consistency index (K) of both products varied from 0.021 to 0.038 $Pa.s^n$ and decreased with increasing temperature. Newtonian model (3.4.) was also applied to shear stress shear rate data of LWE and LEW. Newtonian viscosity values were found to be in the range of 0.017 and 0.037 Pa.s and, in the same way as the consistency coefficient (K), decreased when increasing temperature.

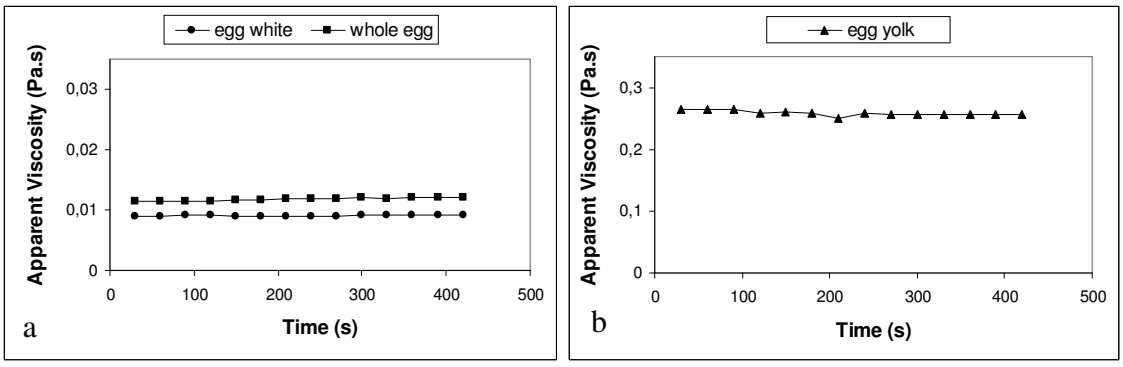


Figure 4.4. (a) Time dependency of LEW and LWE at shear rate of 16.12 s^{-1} and 25 °C. (b) Time dependency of egg yolk at 8.48 $s^{-1}$and 25 °C.

Table 4.1. Flow Parameters of Fresh LEPs at Selected Temperatures.

Herschel-Bulkley Model					
Product	Temperature (°C)	Consistency index, K (Pa s^n)	Flow behavior index, n	r^2	S_{est}
Whole egg	4	0.038	0.9878	0.991	0.0325
	25	0.030	0.9720	0.992	0.0138
	60	0.028	0.9290	0.980	0.0252
Egg white	4	0.032	0.9607	0.995	0.1421
	25	0.024	0.9545	0.992	0.1609
	55.6	0.021	0.9436	0.986	0.0219
Newtonian Model					
	Temperature (°C)	Viscosity (Pa.s)		r^2	S_{est}
Whole egg	4	0.037		0.993	0.0402
	25	0.028		0.991	0.0365
	60	0.021		0.986	0.0343
Egg white	4	0.028		0.994	0.0336
	25	0.020		0.994	0.0213
	55.6	0.017		0.989	0.0254
Power Law Model					
	Temperature (°C)	Consistency index, K (Pa s^n)	Flow behavior index, n	r^2	S_{est}
Egg yolk	4	1.013	0.9324	0.999	0.0045
	25	0.438	0.8970	1	0.0016
	60	0.284	0.8777	0.999	0.0073

Table 4.2. Yield Stress at Selected Temperatures.

Product	Temperature (°C)	Yield Stress (Pa)
Whole Egg	4	0.1654
	25	0.2049
	60	0.1947
Egg White	4	0.0704
	25	0.1157
	55.6	0.1799

Those findings were in agreement with the results of Scalzo et al., Hamid Samimi et al. and Hamid-Samimi and Swartzel (Scalzo et al. 1970, Hamid Samimi et al. 1984, Hamid-Samimi and Swartzel 1985). They reported that whole egg behaves as a Newtonian fluid below 60 °C and pseudoplastic at temperatures above 60 °C. Tung et al. also observed that egg white is slightly pseudoplastic at its respective pasteurization temperature (Tung et al. 1971).

In Figures 4.1., 4.2. and 4.3, it was evident that the shear stress-shear rate relationship at all temperatures were non-linear for LEY. Experimental data of LEY were fitted to power law model in the range of temperatures from 4 to 60 °C. The value of consistency coefficient (K) ranged from 0.28 to 1.013 $Pa.s^n$ and the flow behavior index (n) ranged between 0.87 and 0.93 indicating pseudoplastic behavior.

The egg yolk exhibited non-Newtonian and shear-thinning (pseudoplastic) behavior, which could be attributed to its chemical structure. The lipids of the eggs are all contained in the yolk. Nearly all of the yolk lipids are present as lipoproteins, which are classified as high density, low density and very low density lipoproteins (Lee 2002).

These types of high molecular weight polymers increase the viscosity even at very low concentrations (Damodaran 1996). Those findings were in agreement with those previously reported (Kaufman et al. 1968, Ibarz and Sintes 1989, Punidadas and McKellar 1999, Telis-Romero et al. 2006).

4.1.1.3. Effect of Temperature

For each liquid egg product, the effect of temperature on the viscosity at different shear rates is illustrated in Figure 4.5. It was observed that apparent viscosities of LEPs decrease with increasing temperature. An Arrhenius-type equation (3.9) was used to quantify the effect of temperature on the rheological behavior of these products. Linear form of (3.9) was fitted to experimental data to find the values of the flow activation energies for different products tested. Flow actication energy results were listed in Table 4.3.

It can be seen that egg yolk has high activation energy (E_a) value which means that this product is more sensitive to temperature than other samples. It needs to have a great care if typical thermal pasteurization process will be applied to this product.

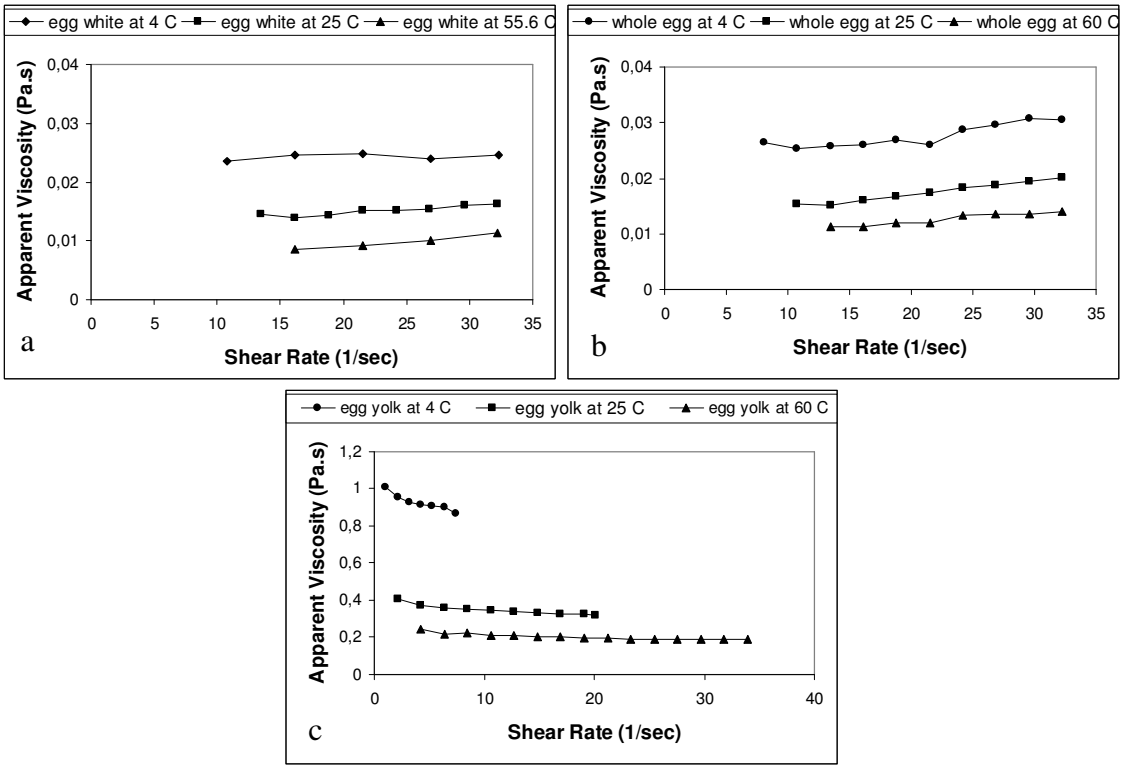


Figure 4.5. Effect of temperature on the viscosity as a function of shear rate (s^{-1}) for (a) egg white (b) whole egg and (c) egg yolk.

Table 4.3. Flow Activation Energies for Fresh LEPs.

Product	E_a (J/mol)	r^2
Whole Egg (LWE)	10,523.86[a]	0.9728
Egg White (LEW)	15,495.63[a]	0.9912
Egg Yolk (LEY)	20,095.76[b]	0.9813

[a]calculated from data measured at 16.12 s^{-1}
[b]calculated from data measured at 8.48 s^{-1}

As done in fresh LEPs, Herschel Bulkley, Power Law and Newtonian models were applied on pasteurized LEPs. Table 4.4. represents the rheological parameters of pasteurized LEPs at appropriate models. Rheological property curves as a basis of shear stress and shear rate for Herschel Bulkey, Power Law and Newtonian Models were represented in Figures 4.6, 4.7 and 4.8.

Table 4.4. Results of Rheological Properties for Pasteurized LEPs.

	Herschel-Bulkley Model			Power Law Model	
Product (pasteurized)	Temperature (^{0}C)	Consistency index, K (Pa s^n)	Flow behavior index, n	Consistency index, K (Pa s^n)	Flow behavior index, n
Whole egg	25	0.020	1.0616	0.0724	0.7499
Egg white	25	0.015	0.9729	-	-
		Newtonian Model			
	Temperature (^{0}C)	Viscosity (Pa.s)		r^2	S_{est}
Whole egg	25	0.0247		-	-
Egg white	25	0.014		-	-
Egg yolk	25	0.153		-	-
		Power Law Model			
	Temperature (^{0}C)	Consistency index, K (Pa.s^n)	Flow behavior index, n	r^2	S_{est}
Egg yolk	25	0.1936	0.9422		

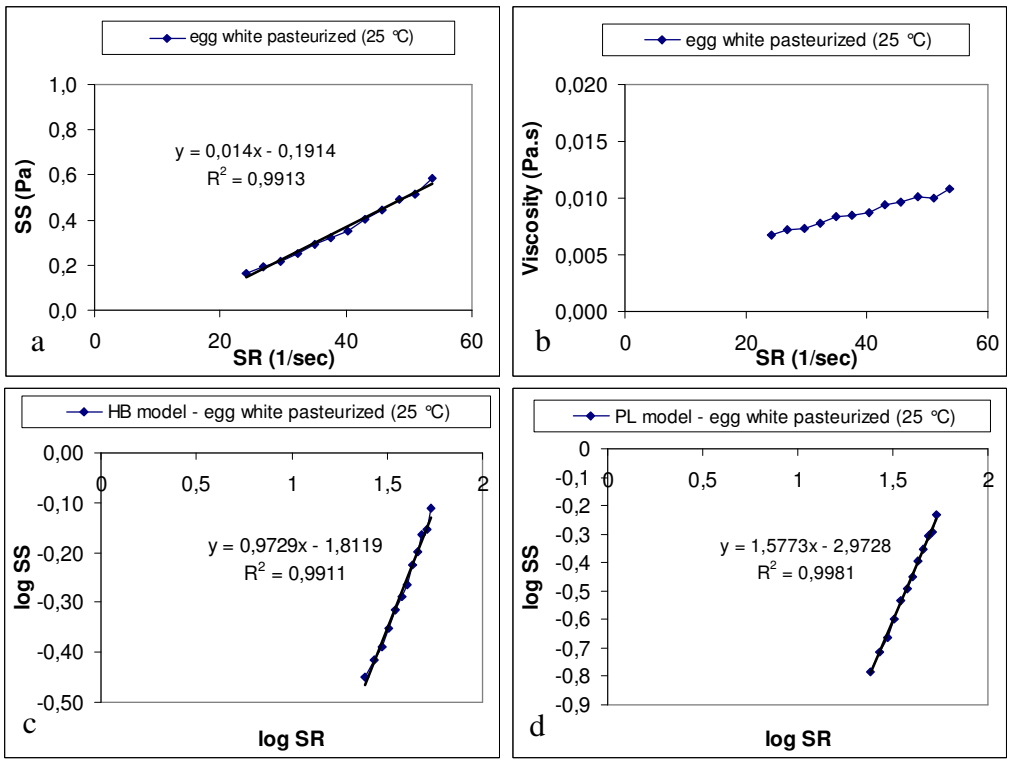


Figure 4.6. (a) Shear stress (SS)-shear rate (SR) (b) viscosity-shear rate curves (c) Log SS-Log SR curve for Herschel-Bulkley Model (d) Log SS-Log SR curve for Power Law Model for pasteurized liquid egg white (LEW).

appeared. Titration was continued until dark brown color was turned to colorless. For the determination of total available residual chlorine in the water sample, following correlation was applied.

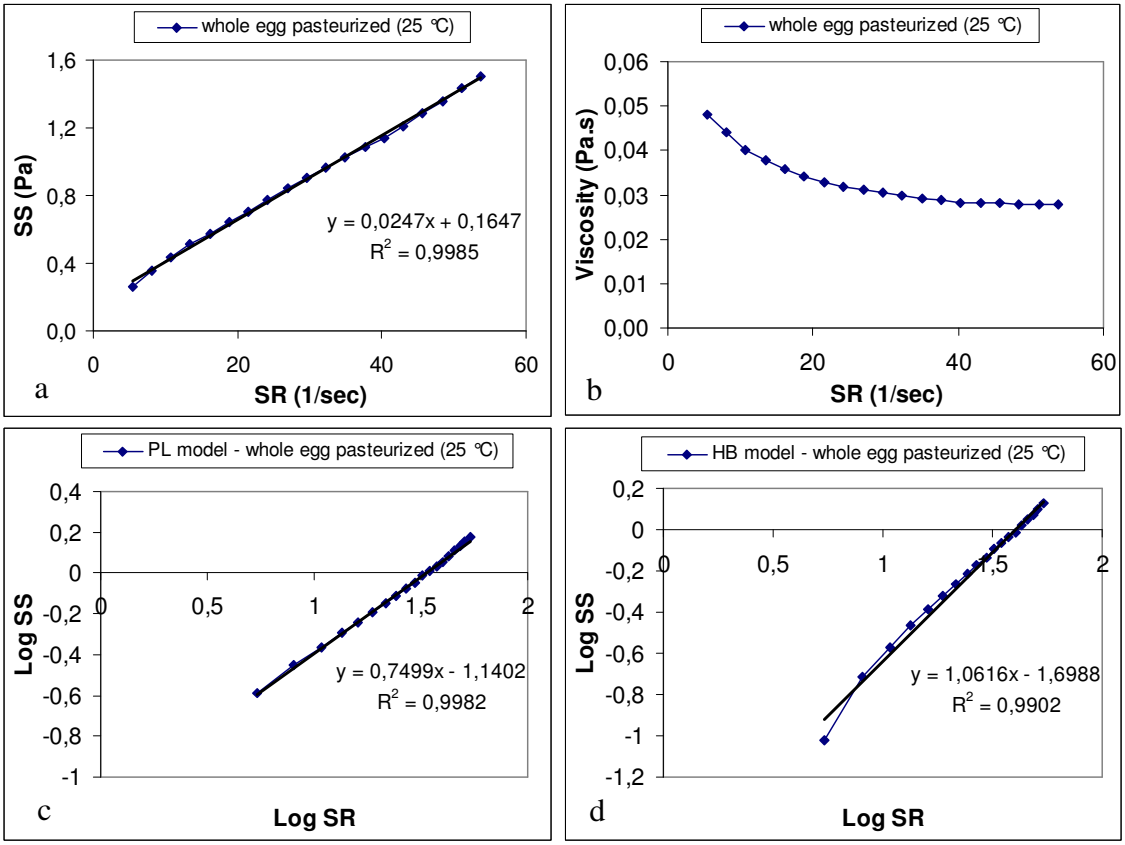


Figure 4.7. (a) Shear stress (SS)-shear rate (SR) (b) viscosity-shear rate curves (c) Log SS-Log SR curve for Power Law Model (d) Log SS-Log SR curve for Herschel-Bulkley Model for pasteurized liquid whole egg (LWE).

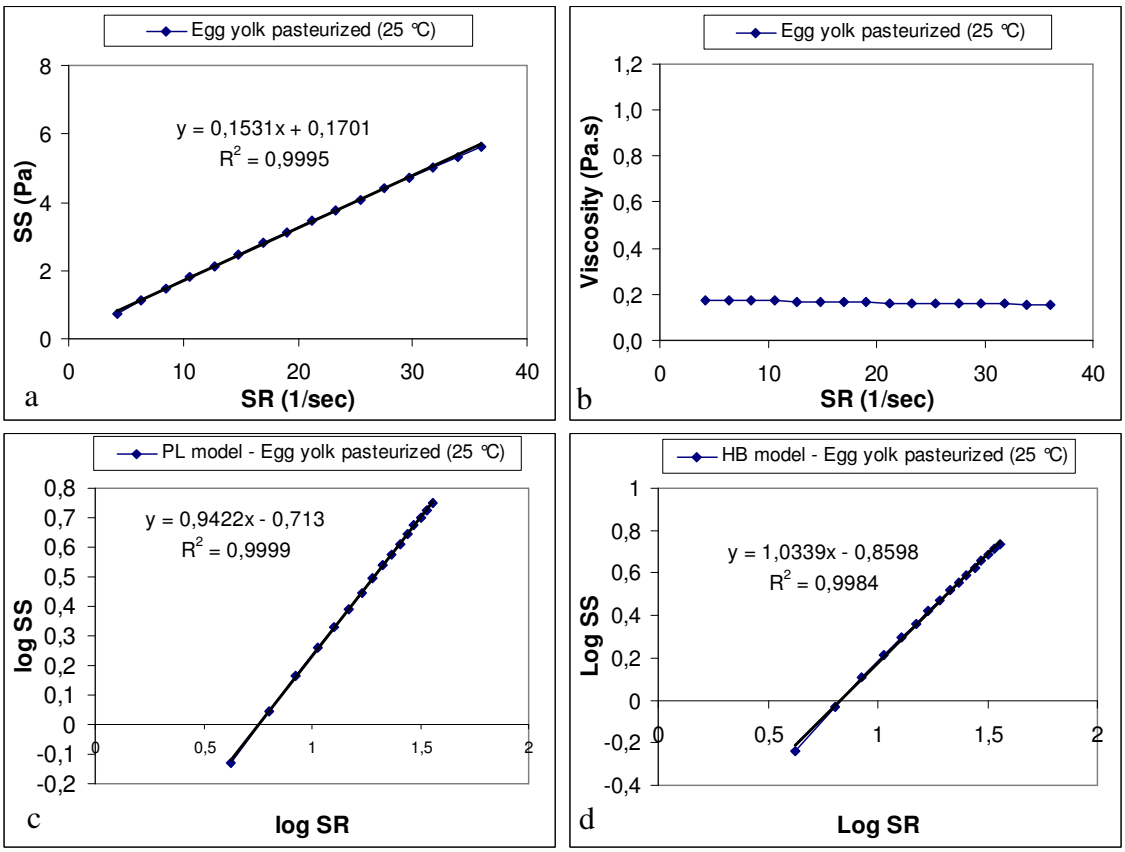


Figure 4.8. (a) Shear stress (SS)-shear rate (SR) (b) viscosity-shear rate curves (c) Log SS-Log SR curve for Power Law Model (d) Log SS-Log SR curve for Herschel-Bulkley Model for pasteurized liquid egg yolk (LEY).

The flow behavior index (n) of pasteurized LWE and LEW obtained from Herschel-Bulkley model indicates that, the liquid whole egg and liquid egg white are slightly pseudoplastic at ambient temperature (Figure 4.6., 4.7.). On the other hand, it was seen that most appropriate rheological model for LEY was determined as Power Law, which was the result of non-linear behavior of shear stress vs. shear rate (Figure 4.8.).

It was seen that, shear stress – shear rate relationship at ambient temperature could be observed as non-linear for pasteurized LEY. Consequently, experimental data of LEY were applied on Power Law model. The value of consistency coefficient (K) was detected as 0.1936 $Pa.s^n$ and the flow behavior index (n) was determined as 0.9422 indicating pseudoplastic behavior.

As a result of the comparison of rheological properties between fresh and pasteurized egg products (Table 4.1 and 4.4), it could be concluded that, consistency index (K) and flow behavior index (n) parameters of both LEW samples are not extremely different by applying

Herschel-Bulkley Model at ambient temperature. For the same model, difference of flow behavior index between fresh (1.0616) and pasteurized (0.9720) LWE samples may be explained by the thermal treatment effect at high temperature. But in contrast, Newtonian Model shows that pasteurization effect may be negligible since viscosity levels are slightly identical. According to Power Law Model, it may be defined that pasteurization effect seems to be great for LEY by the difference between K and n indexes, due to its high percent of denaturable protein content than that of LWE.

4.1.2. Physical Measurements

The density of all three products decreased with increasing temperature (Figure 4.9.). Density (ρ) data were correlated by polynomial models (Table 4.5.). All fitted functions presented r^2 values higher than 0.99 ($p<0.05$) and S_{est} lower than 1.1845. The results were in good agreement with those reported by Punidadas and McKellar (Punidadas and McKellar 1999).

In present study, the pH and total dry solid content (% weight) were determined before the rheological and density measurements in order to obtain the information about the quality attributes of liquid egg products. pH data are listed in Table 4.6. pH values of all the egg products were found to be decreased by increasing temperature.

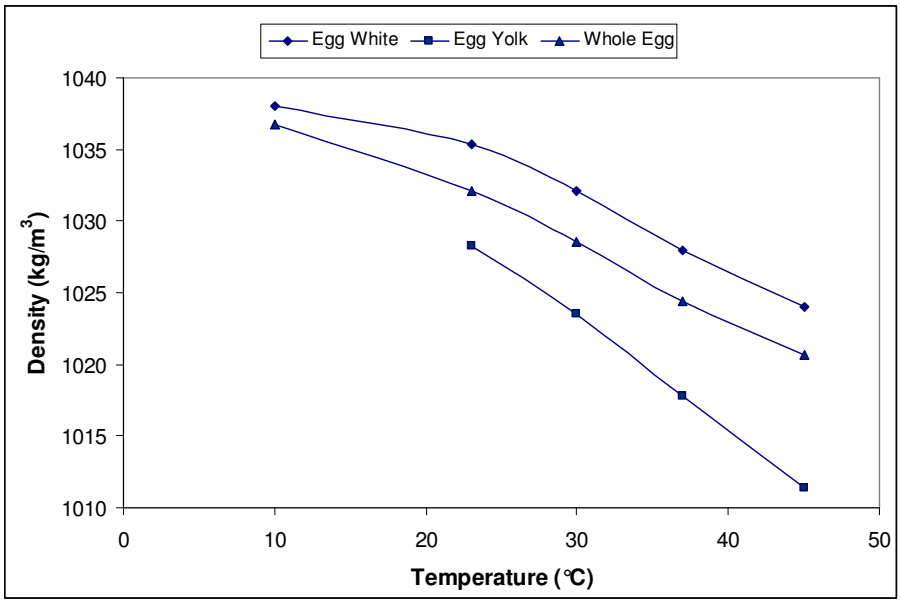


Figure 4.9. Density of different fresh egg products as a function of temperature.

Table 4.5. Temperature Dependency of Density of Fresh LEPs.

Product	Temperature Range (°C)	Equation[1]	r^2	S_{est}
Egg White (LEW)	4-55.6	$\rho = -0.0074T^2 - 0.0039T + 1038.9$	0.9943	1.1845
Whole Egg (LWE)	4-60	$\rho = -0.0036T^2 - 0.272T + 1039.9$	0.9968	1.6488
Egg Yolk (LEY)	4-60	$\rho = -0.0037T^2 - 0.52T + 1042.3$	0.9997	0.1756

[1]T is the temperature (°C)

The observed standard deviation values of total solid content (% weight) were 0.307, 0.060, 0.079 for liquid egg yolk (LEY), liquid egg white (LEW) and liquid whole egg (LWE), respectively. Data are listed in Table 4.7.

Table 4.6. pH of Fresh and Pasteurized LEPs at Different Temperatures.

		pH	
Product	Temperature (°C)	Fresh LEPs	Pasteurized LEPs
Egg White	4	9.43	6.72
	25	9.14	
	55.6	8.61	
Egg Yolk	4	6.25	6.42
	25	6.13	
	60	5.92	
Whole Egg	4	8.00	7.46
	25	7.96	
	60	7.74	

Table 4.7. Total Dry Solid Content of Fresh and Pasteurized LEPs.

	Total Solid (%)	
Product	Fresh Egg Products	Pasteurized Egg Products
Egg White	12.60	11.50
Egg Yolk	48.87	41.0
Whole Egg	25.10	23.50

Density of pasteurized LEPs were also measured as in Section 3.2.3.1. Density vs. temperature curve was represented in Figure 4.10. Besides, Table 4.8. shows the density equations as a function of temperature. As in unpasteurized LEPs samples, all fitted functions presented r^2 values higher than 0.99 ($p<0.05$) and S_{est} lower than 5.1555. Estimated standard deviation of pasteurized LEW is lower than that of unpasteurized LEW. But in contrast, pasteurized LEY and LWE give higher standard deviation in density results due to the effect of thermal pasteurization.

Table 4.8. Temperature Dependency of Density of Pasteurized LEPs.

Product	Temperature Range (°C)	Equation[1]	r^2	S_{est}
Egg White (LEW)	4-55.6	$\rho = -0.0002T^3 - 0.0264T^2 + 0.5379T + 1037.1$	0.9985	.0319
Whole Egg (LWE)	4-60	$\rho = -0.0046T^2 - 0.1499T + 1039.4$	0.9933	5.1555
Egg Yolk (LEY)	4-60	$\rho = -0.0002T^3 - 0.0169T^2 + 0.0115T + 1036.2$	0.999	3.2856

[1]T is the temperature (°C)

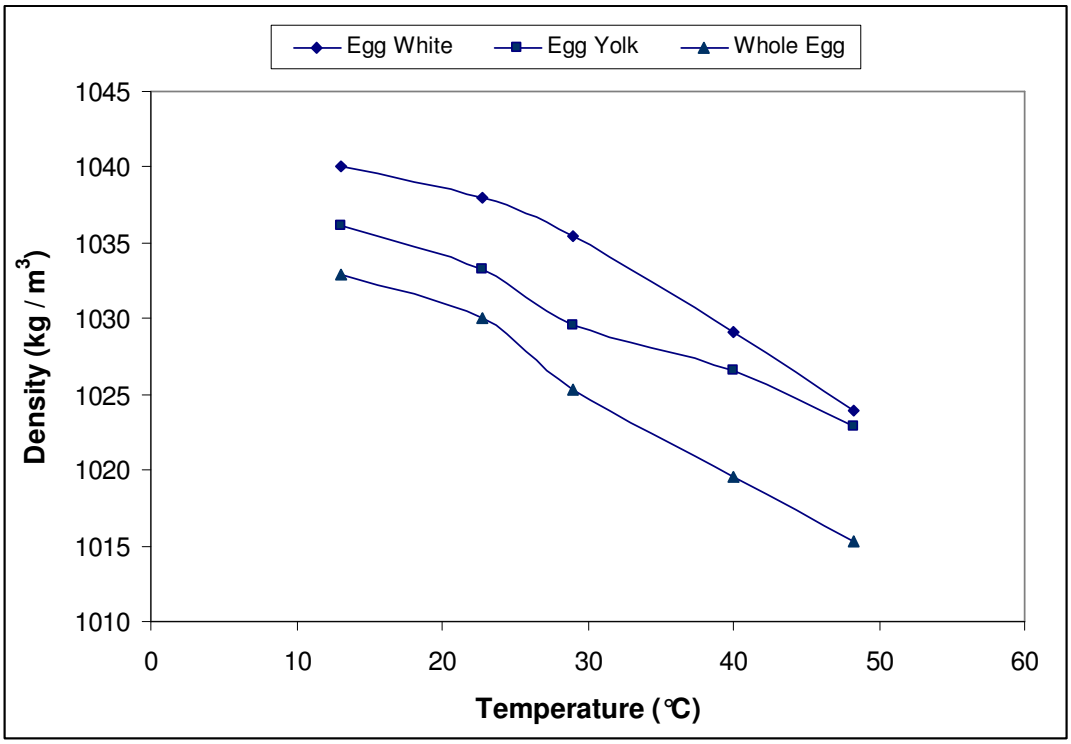


Figure 4.10. Density of different pasteurized egg products as a function of temperature.

4.1.3. Optical Measurements

Absorbance values of fresh LEPs were measured at 254 nm wavelength by applying different dilution factors (1:50, 1:100, 1:250, 1:500 and 1:1000). Then, absorbance (A_{254}) and absorbance coefficients (a_{254}) were estimated. These parameters were calculated from the following correlation:

$$A_{254} = abs * df \tag{4.1}$$

Where L refers to the measurement length of spectrometer cuvette that is 10 mm for each measurement. Results were given in Table 4.9.

Absorbance for pasteurized LEPs at 254 nm were collected for most appropriate diluton factor of "500" for all the samples. As done for unpasteurized LEPs, absorbance coefficients were estimated with (4.1) and shown in Table 4.9. It was seen that absorbance coefficients differs from unpasteurized LEPs samples. This may be occurred by the heat effect of thermal treatment for pasteurization samples.

UV light penetration is much better in egg white than others on the basis of absorbance values. Other than absorbance, turbidity and color has found also to be important in the UV light penetration. Results of turbidity for fresh and pasteurized LEPs and color measurement of fresh LEPs were shown in Table 4.10. and Table 4.11. Turbidity levels of fresh and pasteurized LEW samples are not close, whereas LEY and LWE keeps their high protein content behavior by high turbidity levels, which are slightly close for both.

Table 4.9. Absorbance Results of Fresh and Pasteurized LEPs.

	A_{254}	
Sample	Fresh Egg Products	Pasteurized Egg Products
Egg White	873	104.65 ± 6.22
Egg Yolk	345,7	630.75 ± 6.64
Whole Egg	336,2	807.5 ± 1.62

Table 4.10. Turbidity Results of Fresh and Pasteurized LEPs.

	Turbidity (NTU)	
Sample	Fresh Egg Products	Pasteurized Egg Products
Egg White	62,86	398 ± 8.86
Egg Yolk	7147,625	8114.25 ± 33.50
Whole Egg	8807,62	8369.83 ± 91.01

Table 4.11. Color Measurements of Fresh and Pasteurized LEPs.

	Fresh Egg Products			Pasteurized Egg Products		
Sample	L* [1]	a* [2]	b* [3]	L*	a*	b*
Egg White	21,07	-0,67	2,99	29,08	-0,15	-0,41
Egg Yolk	52,00	6,72	51,42	56,75	2,95	47,15
Whole Egg	62,34	3,62	40,55	58,73	2,71	29,44

[1] Brightness
[2] Redness-greenness
[3] Yellowness-blueness

Egg yolk and whole egg have higher turbidity values than egg white. This can be attributed to the high protein content of these products. Moreover, color measurement results showed that, brightness property of LWE is greater than that of LEW and LEY and yellowness index of LEY is the highest. But in contrast, LEW has greatest opaque characters that may allow more UV permeability than the other LEPs.

4.1.4. Rheological and Physical Measurements of CMC Model Solutions

Rheological measurements of model solutions (0.7, 1.25 and 3.5 % CMC) at ambient temperature were analyzed by different rheological models (Newtonian, Herschel- Bulkley and Power law) to detect the correct flow behavior of the model solutions. Figure 4.11. represents rheological behavior of 0.7 % CMC model solution. Rheological parameters of model solutions at ambient temperature are shown in Table 4.12.

Table 4.12. Results of Rheological Parameters for Model Solutions.

	Herschel-Bulkley Model			Power law Model	
Product (pasteurized)	Temperature (^{0}C)	Consistency index, K (Pa s^n)	Flow behavior index, n	Consistency index, K (Pa s^n)	Flow behavior index, n
1.0 CMC	25	0.002	0.9687		
0.7 CMC	25	0.016	0.9742		
1.25 CMC	25	0.0246	0.9257	0.02157	0.9554
2.5 CMC	25	0.0460	1.0899	0.0985	0.8558
3.0 CMC	25	0.0906	0.9814	0.1302	0.8912
3.5 CMC	25	0.1185	1.0364	0.2070	0.8909
4.0 CMC	25	0.172	1.1062	0.412	0.8234

Newtonian Model

	Temperature (^{0}C)	Viscosity (Pa.s)
1.0 CMC	25	0.017
0.7 CMC	25	0.0146
1.25 CMC	25	0.019
2.5 CMC	25	0.0585
3.0 CMC	25	0.0853
3.5 CMC	25	0.1332
4.0 CMC	25	0.2245

Power Law Model

	Temperature (^{0}C)	Consistency index, K (Pa.s^n)	Flow behavior index, n	r^2
4.0 CMC	25	0.4121	0.8234	1
2.5 CMC	25	0.0985	0.8558	0.9999
3.0 CMC	25	0.1302	0.8912	0.9973
3.5 CMC	25	0.2070	0.8909	0.9997
1.25 CMC	25	0.0215	0.9554	0.9865

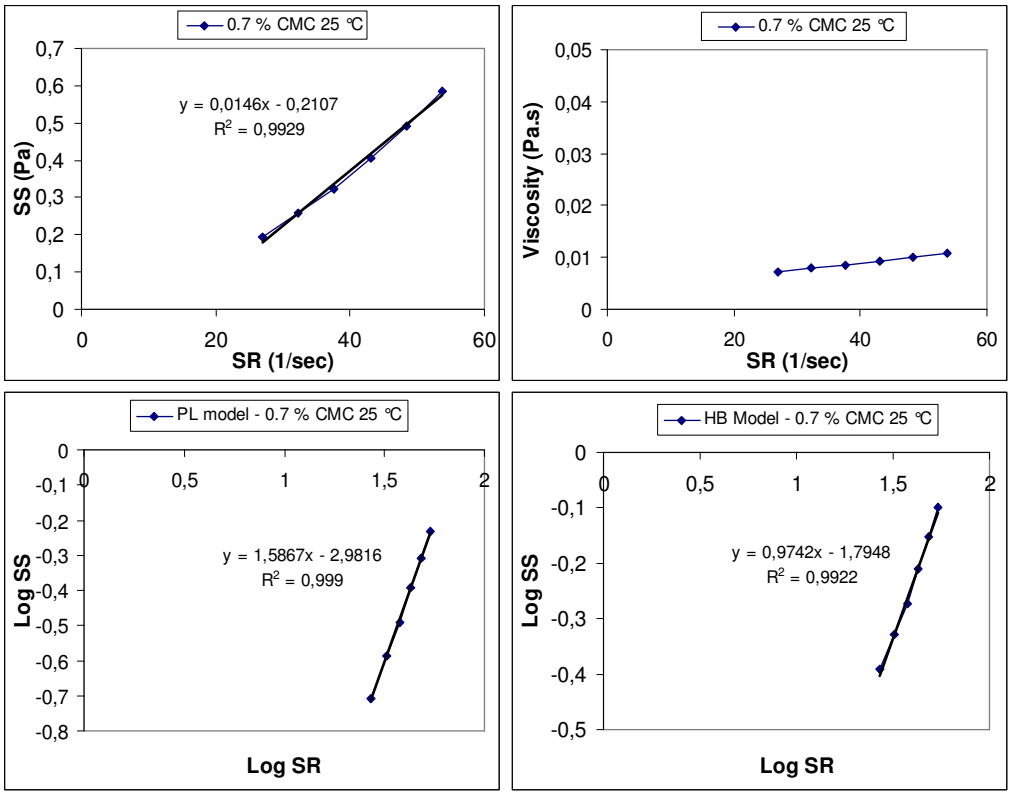


Figure 4.11. Rheological Properties of 0.7 % CMC.

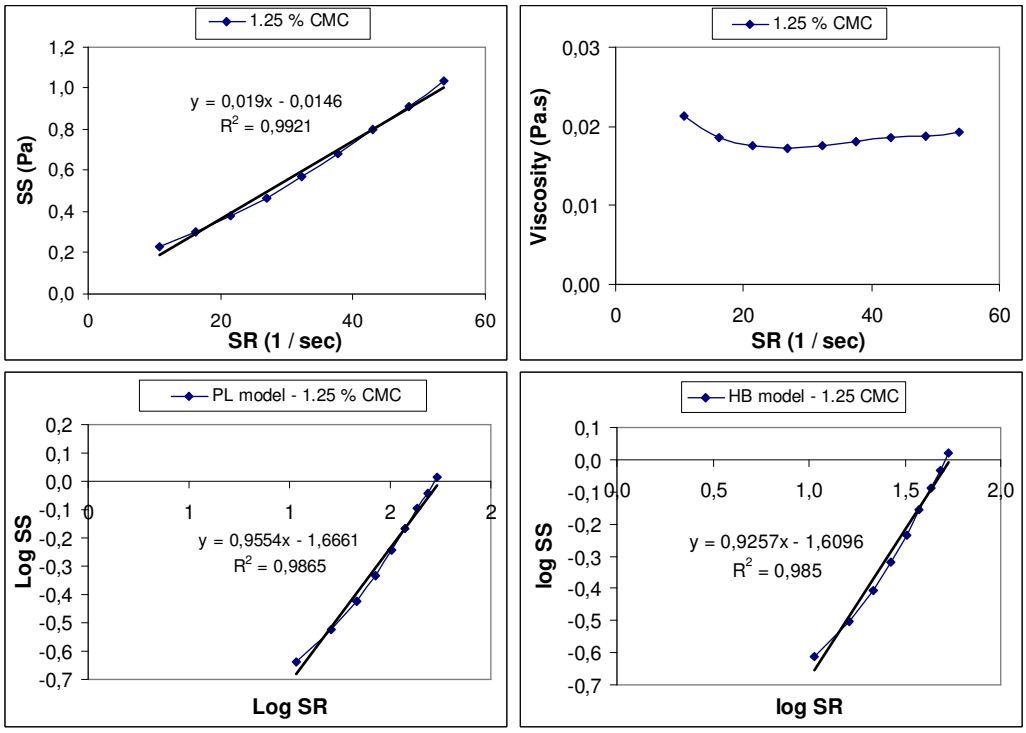


Figure 4.12. Rheological properties of 1.25 % CMC.

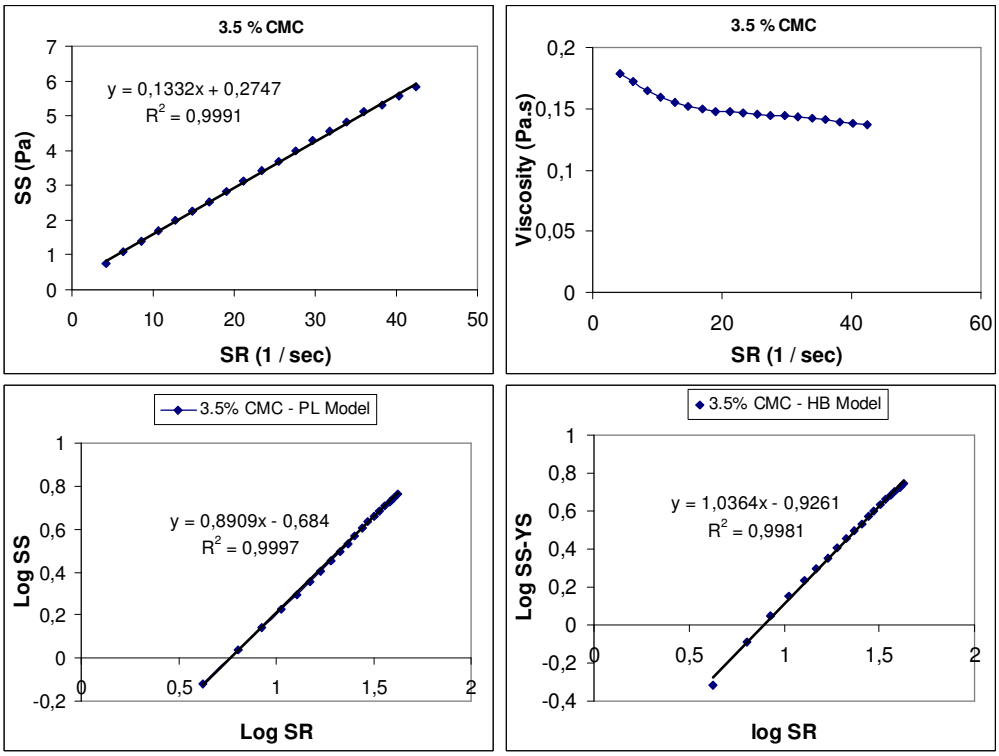


Figure 4.13. Rheological Properties of 3.5 % CMC.

Flow behavior index (n) differs from 0.9257 to 1.1062 for 0.7-4.0 % CMC model solutions, having the consistency index range of 0.002-0.172 Pa s^n according to Herschel-Bulkley model. Flow behavior and consistency index of 1.25 % CMC solution were nearest to that of LWE as a result of this model. Moreover, Newtonian model indicated that 0.7 % CMC solution might be a proper simulator of LEW. As approached to LEY, rheological parameters of 3.5 % CMC solution were simulated by Power Law model and found to be similar flow behavior index and consistency factor.

4.2. Microbiological Results of Bench Top Biodosimetry Studies

The standard plate count for LEW samples was found to be 3-4 log units CFU/ml and *E.coli* was not detected in any of the tested LEPs samples prior to inoculation. Additionally, the number of surviving microorganism on VRBA and EMB plates were not different than those enumerated on TSA plates indicating that *E.coli* (NRRL B-253) cells were mostly inactivated by UV-C radiation rather than just injured.

Influence of UV radiation of *E.coli* (NRRL B-253) inactivation in liquid egg product samples at different fluid medium depth and UV intensity (I_0) levels are depicted in Figure 4.14., 4.15 and 4.16. The inactivation curve was constructed by plotting the log reduction (log (N/N_0)) versus UV Dose (mJ/cm^2).

4.2.1. Liquid Egg White

The best reduction (2.2-log CFU/ml) of *E.coli* (NRRL B-253) was achieved in liquid egg white samples (LEW) subjected to the highest UV intensity (1.314 mW/cm^2), and the lowest medium depth (0.153 cm) after 20 min UV exposure (UV dose of 98 mJ/cm^2) (Figure 4.14). Ngadi, Smith and Cayouette applied 300 mJ/cm^2 (5 mW.min/cm^2) UV dose at 0.315 mW/cm^2 incident UV intensity for 16 min in order to achieve 3.8-log (CFU/ml) reduction of *E.coli* O157:H7 (ATCC 35150) in LEW samples of 0.1 cm in depth (Ngadi, Smith and Cayouette 2003). In their study, they calculated average UV intensity from Bouguer-Lambert law, which resulted in higher UV dose values than Beer-Lambert law, used in this work. The variation in the log reduction values might be a result of differences in *E.coli* strains, background flora and absorbance of LEW samples used in these studies.

Ngadi et al. reported higher UV sensitivity of *E.coli* O157:H7 compared to non-pathogenic *E.coli* strain used in this study (Ngadi et al. 2003). Although, no *E.coli* cells were detected in LEW before treatment, initial population of aerobic mesophilic bacteria in LEW ranged from 10^3 to 10^4 CFU/ml. Ngadi et al. has not reported any background flora in their work. Additionally, LEW samples used in our study had absorbance of 104.65±6.22 (<0.01 % UV transmittance) at 254 nm.

Ngadi et al. measured UV transmittance of LEW as 0.02 %. Decrease in UV transmittance reduces the UV dose by diminishing UV intensity and results in lower inactivation rate (Sommer et al., 1997). As a result, it was expected to have higher UV dose (98 mJ/cm^2) and lower inactivation rate (2.2 log) for *E.coli* (NRRL B-253) in LEW than those reported by Ngadi et al. (Ngadi et al. 2003)

Besides, 3.2 log CFU/ml reduction of *E.coli* O157:H7 (ATCC 700728) and 2.6 log CFU/ml reduction of *S.typhimurium* (CCM 5445) were achieved at the highest UV intensity (1.314 mW/cm^2), and the lowest medium depth (0.153 cm) after 20 min UV exposure (See in Appendix C).

4.2.2. Liquid Egg Yolk

In LEY samples, maximum inactivation (0.675-log CFU/ml) was observed at the highest UV intensity (1.314 mW/cm^2) and the lowest medium depth (0.153 cm) after being exposed to treatment time of 20 min (UV dose of 13.25 mJ/cm^2, Fig 4.15.). The amount of light that penetrates through a liquid decreases with increasing liquid medium absorbance (Murakami et al. 2006).

Since absorbance of LEY was higher than LEW, the lower inactivation of *E.coli* was expected in these samples. At the highest UV intensity (1.314 mW/cm^2), and the lowest medium depth (0.153 cm) after 20 min UV exposure, *E.coli* O157:H7 (ATCC 700728) was inactivated maximum 1.25 log CFU/ml and reduction of *S.typhimurium* (CCM 5445) was estimated as 0.62 log CFU/ml (See in Appendix C).

4.2.3. Liquid Whole Egg

LWE samples showed different inactivation response (Figure 4.16) where the maximum reduction (0.316-log CFU/ml) was achieved at the medium UV intensity (0.709 mW/cm^2) and the lowest depth (0.153 cm) after 20 min treatment time (UV dose of 16.55 mJ/cm^2). On the other hand, 0.4 log CFU/ml reduction of *E.coli* O157:H7 (ATCC 700728) and 0.34 log CFU/ml reduction of *S.typhimurium* (CCM 5445) were found at 1.314 mW/cm^2 UV intensity and 0.153 cm medium depth after 20 min UV exposure.

This can be due to the fact that LWE samples have the highest absorbance and turbidity (suspended material) (see Table 4.15.). Suspended materials influence the inactivation of bacteria by protecting them during irradiation (Quintero-Ramos 2004, Murakami 2006). Therefore it was thought that the mixing was not homogeneous and microorganisms were shadowed by suspended material, which led to different inactivation pattern in LWE samples.

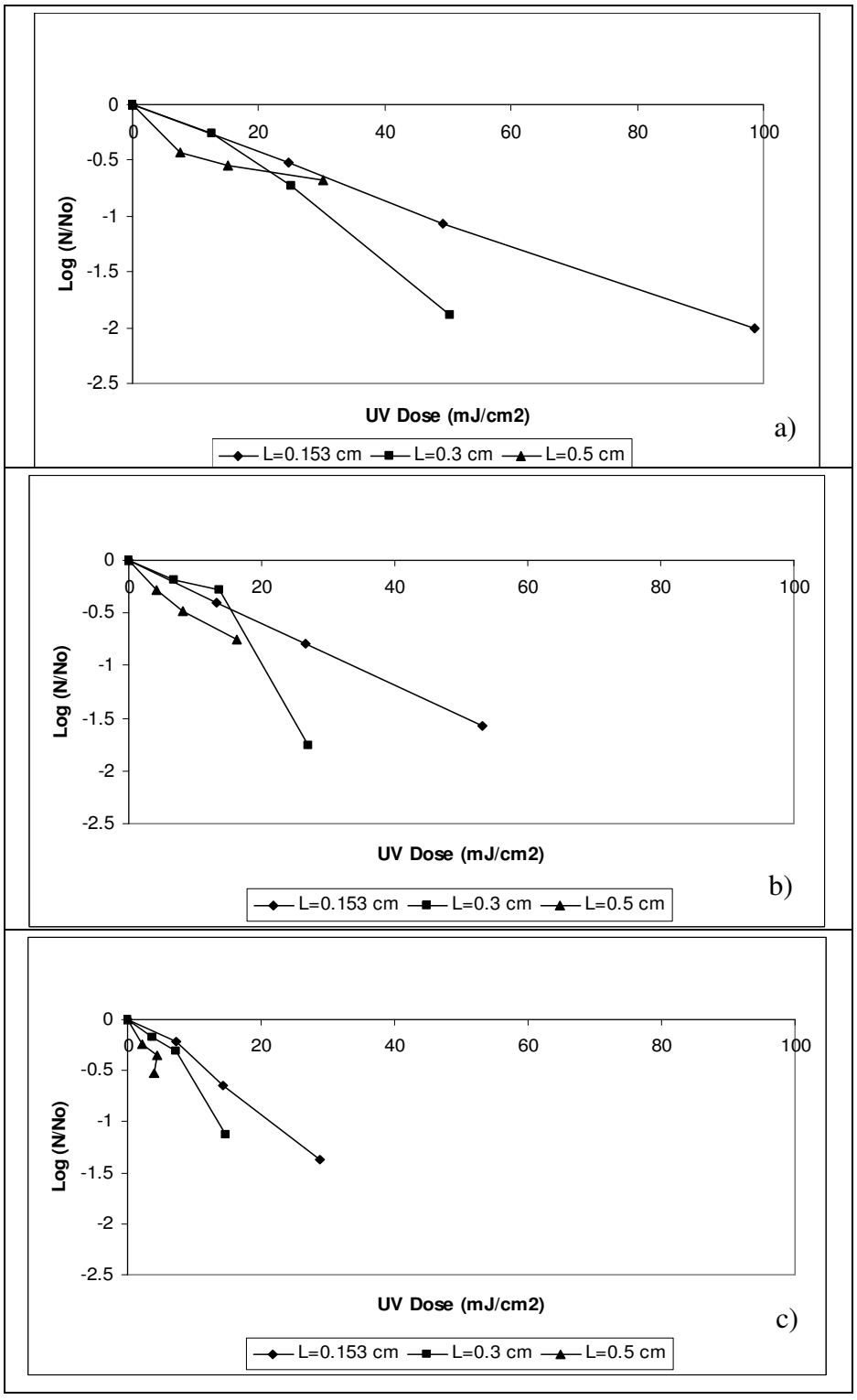


Figure 4.14. Influence of UV-C radiation on *E.coli* (NRRL B-253) inactivation in liquid egg white (LEW) at different fluid medium depth and UV intensity levels (I) (a) I= 1.314 mW/cm^2, (b) I= 0.709 mW/cm^2, (c) I= 0.383 mW/cm^2.

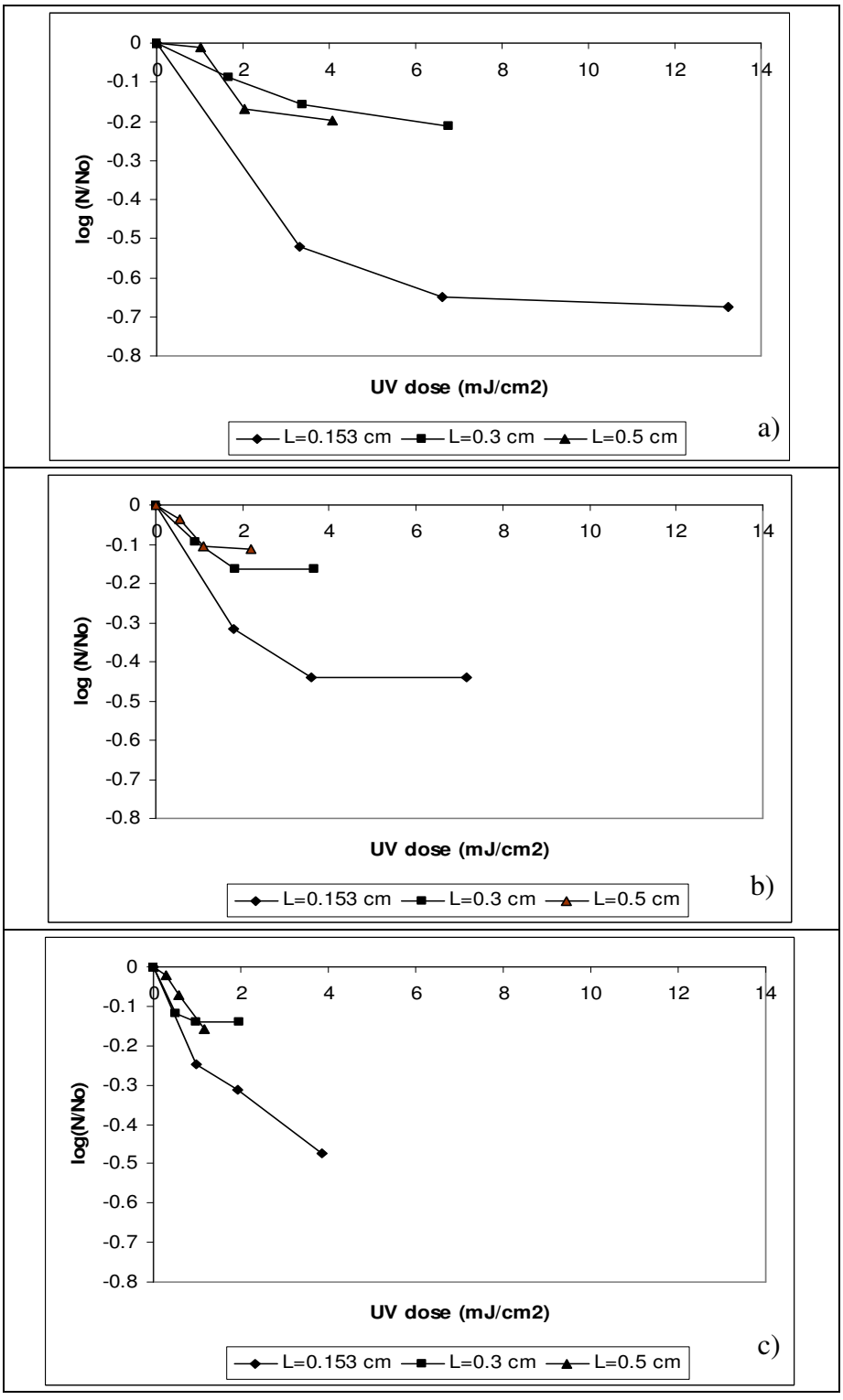


Figure 4.15. Influence of UV-C radiation on *E.coli* (NRRL B-253) inactivation in liquid egg yolk (LEY) at different fluid medium depth and UV intensity levels (I) (a) I= 1.314 mW/cm^2, (b) I= 0.709 mW/cm^2, (c) I= 0.383 mW/cm^2.

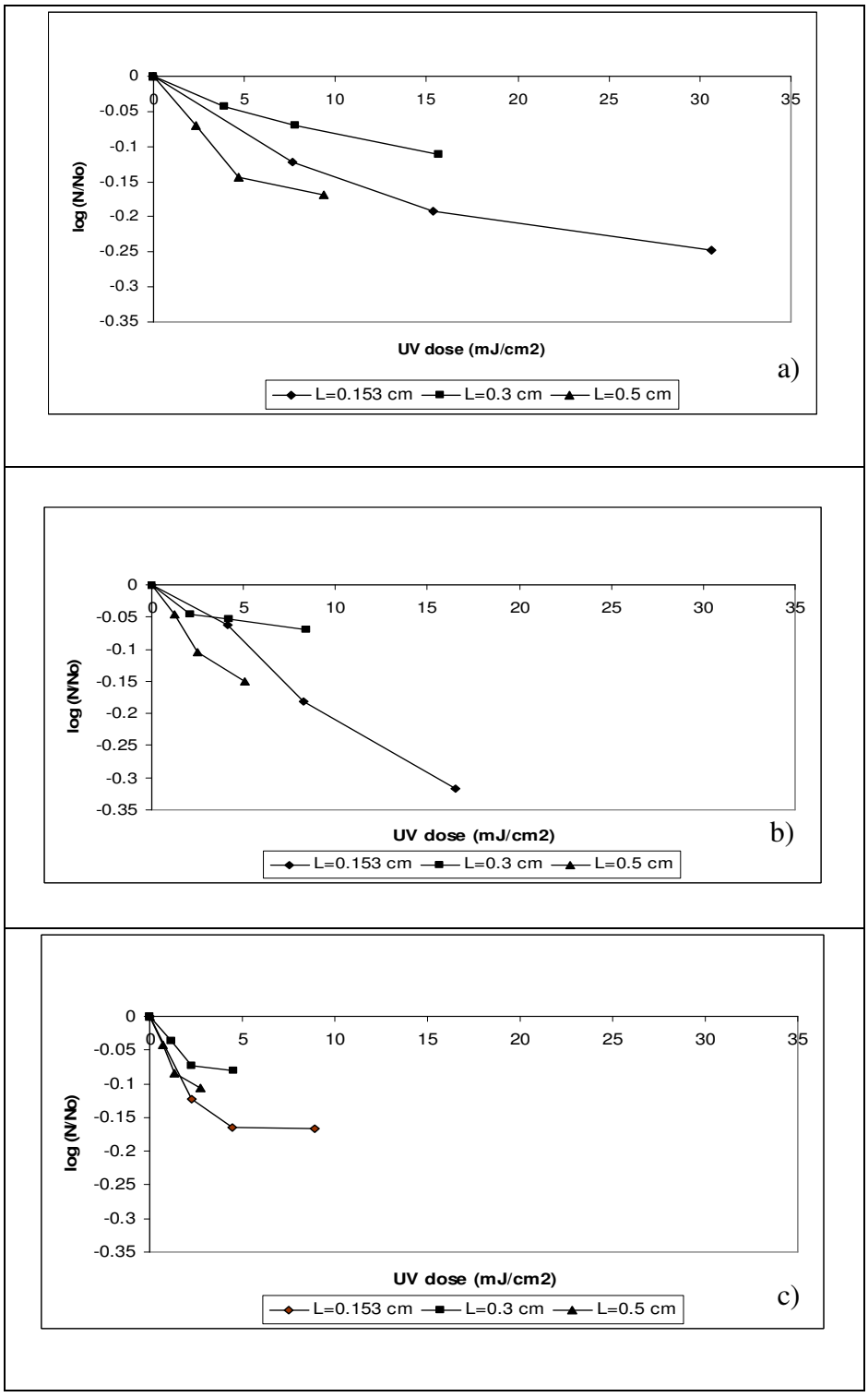


Figure 4.16. Influence of UV-C radiation on *E.coli* (NRRL B-253) inactivation in liquid whole egg (LWE) at different fluid medium depth and UV intensity levels (I) (a) I= 1.314 mW/cm^2, (b) I= 0.709 mW/cm^2, (c) I= 0.383 mW/cm^2.

4.2.4. Statistical Analysis

Statistical analysis of full factorial design, which considers the factors (liquid depth, UV intensity and exposure time) and their interactions on the reduction of *E.coli* (NRRL B-253) in LEPs (presented in Table 4.13.). Small p-values ($p < 0.05$) show that the parameter is significant on the log reduction at the 5% level of significance. The intensity and its interaction with depth and time were found to be insignificant ($p >> 0.05$) in LWE samples, which support our observation (See in Appendix B). To best of our knowledge there is no available literature on the UV-C inactivation of LEY and LWE. Hence, it is not possible to make comparison with any of the literature data. Overall, it can be concluded that increasing the liquid depth resulted in decrease of the inactivating effect of UV-C radiation on *E.coli* (NRRL B-253) in LEPs ($p << 0.05$).

Many researchers (Wright et al. 2000; Ngadi et al. 2003; Yaun et al. 2003; Quintero-Ramos et al., 2004; Matak et al. 2005 and Murakami et al. 2006) found UV dose (time x UV incident intensity) as the major factor for inactivation of *E.coli* . Additionally, the relationship between time and UV incident intensity was reported to be important for the design of UV equipment (Murakami et al. 2006; Sommer et al. 1998).

The influence of exposure time on the inactivation of *E coli* (NRRL B-253) was examined in Figure 4.17. With exposure time between 0 and 20 min, killing rate of *E.coli* was linear in LEW samples. On the other hand, the log reduction of *E.coli* was parabolic in LEY. and LWE samples suggesting that exposure time was also significant ($p << 0.05$) for UV-C radiation process.

E.coli was linear in LEW samples. On the other hand, the log reduction of *E.coli* was parabolic in LEY. and LWE samples suggesting that exposure time was also significant ($p << 0.05$) for UV-C radiation process. The evaluation of interaction data between time and incident intensity revealed the existence of time-incident intensity reciprocity for LEW whereas such a linear trend was not observed in LWE and LEY samples. This means that the same level of microbial reduction can be achieved by applying higher incident intensity for a shorter period of time for the same UV dose level. Murakami et al. also reported the same interdependence of time and incident intensity for UV inactivation of *E.coli* K12 in apple juice (Murakami et al. 2006).

Table 4.13. Results of Full Factorial Design: Effect of Factors and Interactions on the Log_{10} Reduction (N/N_0) of *E.coli* (NRRL B-253) in LEPs.

Response: log_{10} reduction (N/N_0)	*p*-value
Factor: time	
LEW	$<10^{-3}$
LWE	$<10^{-3}$
LEY	$<10^{-3}$
Factor: incident intensity	
LEW	$<10^{-3}$
LWE	>>0.05, insignificant
LEY	$<10^{-3}$
Factor: depth	
LEW	$<10^{-3}$
LWE	$<10^{-3}$
LEY	$<10^{-3}$
Interaction: time x incident intensity	
LEW	$<10^{-3}$
LWE	>>0.05, insignificant
LEY	>>0.05, insignificant
Interaction: time x depth	
LEW	$<10^{-3}$
LWE	>>0.05, insignificant
LEY	$<10^{-3}$
Interaction: incident intensity x depth	
LEW	>>0.05, insignificant
LWE	>>0.05, insignificant
LEY	0.014

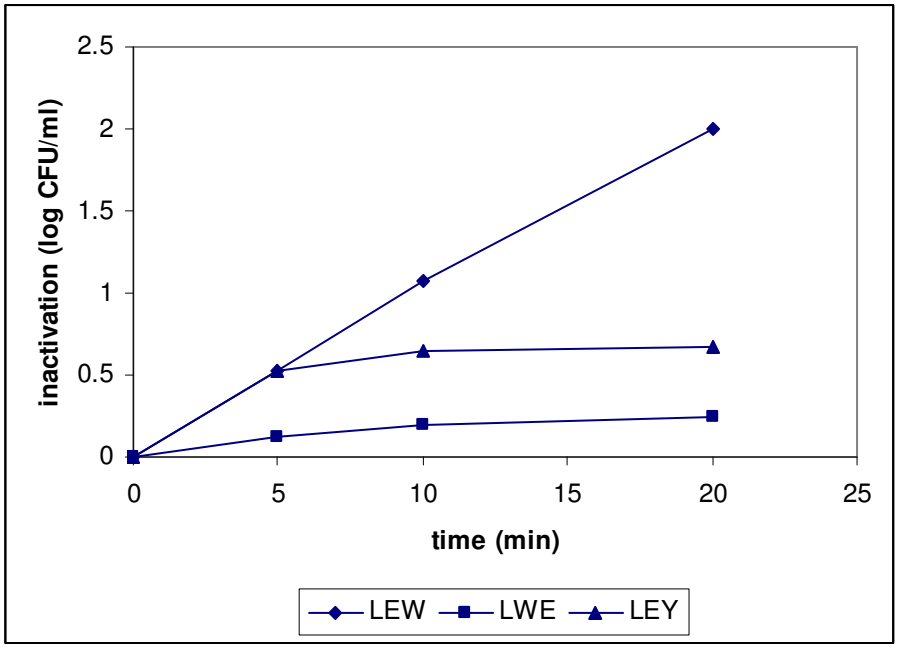


Figure 4.17. Inactivation of *E.coli* NRRL B-253 as a function of treatment time at $I_0 = 1.314$ mW/cm^2.

4.2.5. The UV Inactivation Kinetics of LEPs

The kinetics of UV inactivation of *E.coli* (NRRL B-253) in LEPs was studied. The inactivation rate constant (k) calculated from (3.12) for the different fluid medium depths and UV intensities with regression coefficients are shown in Table 4.14. inactivation rate constant values calculated in different depths at a fixed UV intensity showed variations. UV light penetration was expected to decrease with increasing fluid depth. Even though UV intensity was kept high (I=1.314 mW/cm^2), increasing the fluid depth did not show any decreasing trend in k values as expected. In some cases, regression coefficients were also found to be lower. This is attributed to the nonlinear relationship between logarithm of the survival fractions and UV dose. The inactivation curves were in sigmoidal shape when the fluid depth increased to 0.3 cm and 0.5 cm (Figures 4.1, 4.2 and 4.3.). Sigmoidal curve has an initial lag in the slope called "shoulder "and tailing at the higher UV doses. While the fluid depth was 0.3 cm, shoulder curve was dominant for LEW and tailing curve was the main in LEY and LWE. When the fluid depth was 0.5 cm, tailing was more pronounced regardless of UV intensity. Sigmoidal shape is a common survival curve observed in the UV inactivation of microorganisms and explained with single-hit and multiple-hit phenomena (Fenner and Komvuschara, 2005).

Table 4.14. Kinetic Parameters for UV Inactivation of *E.coli* (NRRL B-253) in LEPs.

Intensity (mW/cm^2)	Depth (cm)	LEW		LWE		LEY	
		k (cm^2/mJ)	R^2	k (cm^2/mJ)	R^2	k (cm^2/m)	R^2
1.314	0.153	0.0204	0.9987	0.0076	0.8791	0.0445	0.6421
	0.3	0.0386	0.9792	0.0068	0.9661	0.0305	0.9244
	0.5	0.0203	0.7986	0.0176	0.8650	0.0543	0.8222
0.709	0.153	0.0296	0.9999	0.0196	0.9866	0.0551	0.6581
	0.3	0.0657	0.8787	0.0074	0.7952	0.0427	0.7378
	0.5	0.0453	0.9681	0.0299	0.9569	0.0529	0.8252
0.383	0.153	0.0491	0.9907	0.0166	0.6530	0.1134	0.9031
	0.3	0.0776	0.9432	0.0174	0.8206	0.0613	0.5865
	0.5	0.0558	0.9298	0.0381	0.8938	0.1382	0.9836

The initial lag (shoulder) in the survival curve might be due to initiation of cell injury, as exposure to UV radiation continues, maximum cell damage occurs and minimal additional treatment becomes lethal. Tailing can result from suspended material in the medium showing high turbidity that protects bacteria during irradiation, aggregates of cells caused by inhomogeneous distribution of microorganisms in the liquid medium and in-homogeneity in radiation. Thus, (3.13) (Chick´s model of disinfection) can only be used to predict inactivation parameter k from the linear inactivation curve. In order to model the UV death kinetics more precisely, a nonlinear model needs to be developed to take into account variations in the UV intensity and microorganism sensitivity in liquid egg products.

Nevertheless, comparison of inactivation rate constants of *E.coli* O157:H7 (ATCC 700728) and *S.typhimurium* (CCM 5445) with that of *E.coli* (NRRL B-253) (See Table A.9.), indicates that, *E.coli* (NRRL B-253) strain is more resistant to UV exposure having lower inactivation rate constant and needs more UV light. This commend was also supported by comparing inactivation rates of these strains in LEPs. (See in Appendix C)

4.2.6. The Effect of UV on the Optical Properties

Statistical analysis of color parameters L*(Brightness), a*(redness-greenness) and b* (yellowness-blueness) for LEPs before and after UV treatment were carried out by applying Two-Way Analysis of Variance (ANOVA) within the factors of liquid sample depth (1.53, 3 and 5 mm), UV biodosimetry time levels (0, 5, 10 and 20 minutes) and interaction of three factors. After comparing of P-values of factors and interaction with alpha ($\alpha = 0.05$), it could be concluded that color parameters seem to be liquid depth dependent by lower level of P values than 0.0001. As liquid depth increases, increment of L*, a* and b* values occur for each LEPs. Besides, there is no significant effect of time-depth interaction on each parameter (Table 4.16.).

During the measurements of the color parameters for liquid depth dependency, it was seen that brightness of LEW remains between the same tolerance range for 3 and 5 mm of sample depth after 10 min. UV exposure. Moreover, brightness of LEY and LWE have distinct intervals for each depth. But in contrast, similar brightness could be differed after 20 min. of UV application. Redness of LEY have dissimilar characteristics

after 5 mm UV exposure, whereas greenness of LEW represents the same range in the increase of sample depth. Yellowness of LWE and LEY not only have no change as a function of UV exposure time, but also have dissimilarities in depth level dependency. Nonetheless UV exposure time have a significant effect on yellowness of LEW.

It is obvious that liquid egg products are UV-opaque wherein penetration of UV-C light is lower than clear liquids. Numeric results of the statistical analysis for color parameters before and after UV exposure were shown in Appendix D, Table A.10.

After the analysis of ΔE parameters for LEPs as a basis of UV biodosimetry results, significancy of time in LEW and sample depth in LEY was indicated in two replicates. Time-depth interaction was insignificant for ΔE after UV exposure. Numerical results of statistical analysis for ΔE values of LEPs were shown in Appendix D.

Table 4.15. Statistical Analysis of ΔE for LEPs Before and After UV Biodosimetric Study (at I_0=1.314 mW/cm^2).

Response: Color Parameter	*p*-value	
ΔE	Replicate-1	Replicate-2
Factor: time		
LEW	<0.05	>>0.05, insignificant
LWE	>>0.05, insignificant	>>0.05, insignificant
LEY	>>0.05, insignificant	>>0.05, insignificant
Factor: depth		
LEW	>>0.05, insignificant	>>0.05, insignificant
LWE	>>0.05, insignificant	>>0.05, insignificant
LEY	>>0.05, insignificant	<0.05
Interaction: time x depth		
LEW	>>0.05, insignificant	>>0.05, insignificant
LWE	>>0.05, insignificant	>>0.05, insignificant
LEY	>>0.05, insignificant	>>0.05, insignificant

Table 4.16. Color Parameters of LEPs After UV Biodosimetric Study (at I_0=1.314 mW/cm^2)

Response: Color Parameter	*p*-value
L*-Brightness	
Factor: time	
LEW	>>0.05, insignificant
LWE	>>0.05, insignificant
LEY	>>0.05, insignificant
Factor: depth	
LEW	<0.05
LWE	<0.05
LEY	<0.05
Interaction: time x depth	
LEW	>>0.05, insignificant
LWE	>>0.05, insignificant
LEY	>>0.05, insignificant
a*-Redness-greenness	
Factor: time	
LEW	>>0.05, insignificant
LWE	<0.05
LEY	>>0.05, insignificant
Factor: depth	
LEW	<0.05
LWE	<0.05
LEY	<0.05
Interaction: time x depth	
LEW	>>0.05, insignificant
LWE	>>0.05, insignificant
LEY	>>0.05, insignificant
b*-Yellowness-blueness	
Factor: time	
LEW	<0.05
LWE	>>0.05, insignificant
LEY	>>0.05, insignificant
Factor: depth	
LEW	<<0.05
LWE	<0.05
LEY	<0.05
Interaction: time x depth	
LEW	>>0.05, insignificant
LWE	>>0.05, insignificant
LEY	>>0.05, insignificant

4.3. Results of Continuous Flow UV Treatment of Liquid Egg White (LEW)

4.3.1. Results of Inactivation Study

Inactivation study in continuous flow UV reactor was applied on liquid egg white (LEW) samples based on the results derived from biodosimetric study. Since, USDA listed five approved continuous pasteurization process for egg white which are carried out at two different pH (pH 7.0 and 9.0). In Lineweaver-Cunningham method pasteurization was applied at pH 7.0 adjusted with lactic acid or aluminum sulfate. In simple heat treatment method, pH of liquid egg white (LEW) was kept at its natural value of 9.0. Therefore, the effect of pH was also investigated in this part of work.

In the first study, UV treatment of liquid egg white (LEW) was carried out pH 7.0. Unlike in the Lineweaver-Cunningham method, pH was adjusted by using potassium bitartrate (cream of tartar) in the local production company. liquid egg white (LEW) was inoculated from the stationary phase culture to give an approximately 10^6 CFU/ml of microbial population. 10 lt of inoculated liquid egg white (LEW) was passed through the UV reactor for five consecutive passes (5 cycles) at a flow rate of 1080 ml/min. At this flow rate, the lamps generated approximately 2.93 mJ/cm^2 UV dose at an approximate exposure time of 257 sec. Before and after each cycle color, turbidity, absorbance, pH and temperature were measured. Serial dilutions were also spread plated on TSA to check the reduction in microbial counts. Additionally, initial background flora of liquid egg white (LEW) was assessed for each new product before processing.

Maximum 0.176 logarithmic reduction was achieved (Figure 4.18). There was an increase in microbial population during the intermediate cycle of UV treatment. One of the reason of this may be due to an increase in temperature during each cycle. Measured temperature and pH at the inlet and outlet of UV reactor was shown in Table 4.17. Passage of liquid egg white (LEW) through the UV reactor resulted in a temperature increase of 18 ^{o}C. This may have caused slight unfolding of egg proteins (e.g. ovalbumin) and change in the lipid structure resulted in an increase in the suspended material. Eventually, suspended materials influence the inactivation of bacteria by protecting them during irradiation. This findings were supported by application of color,

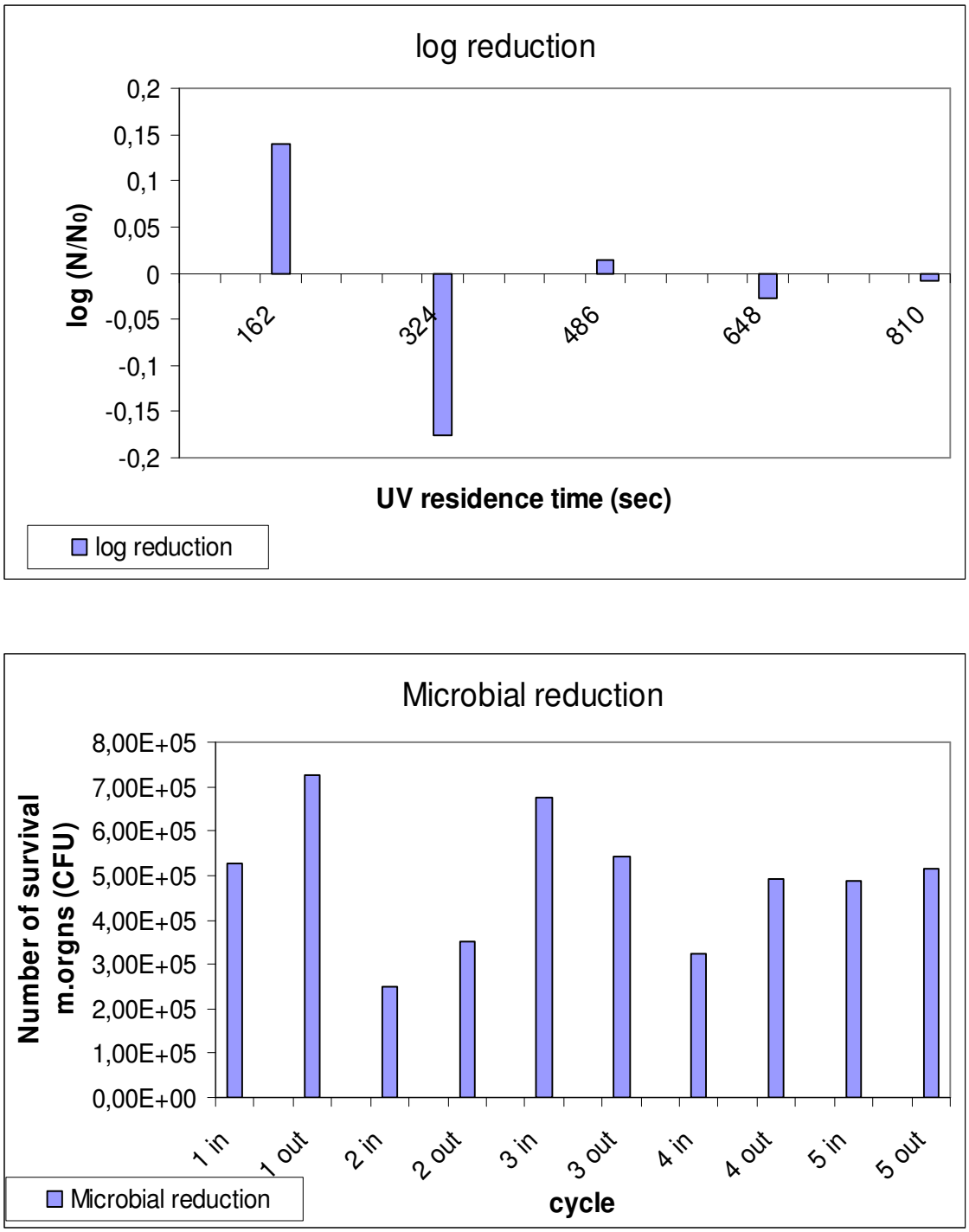
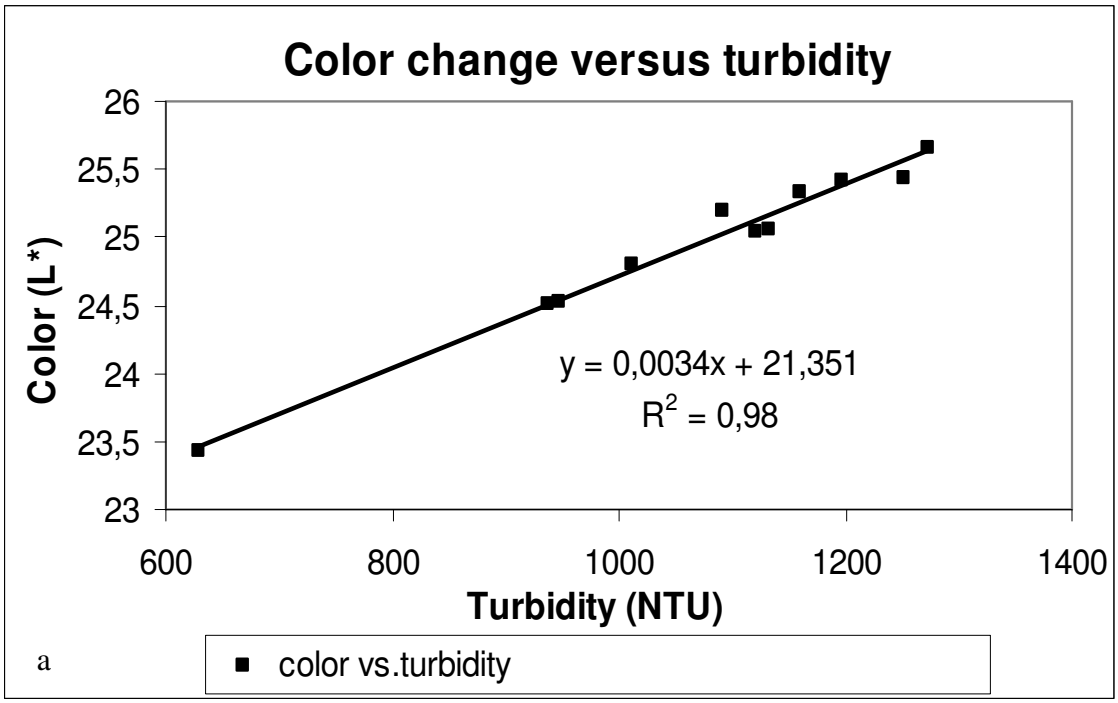
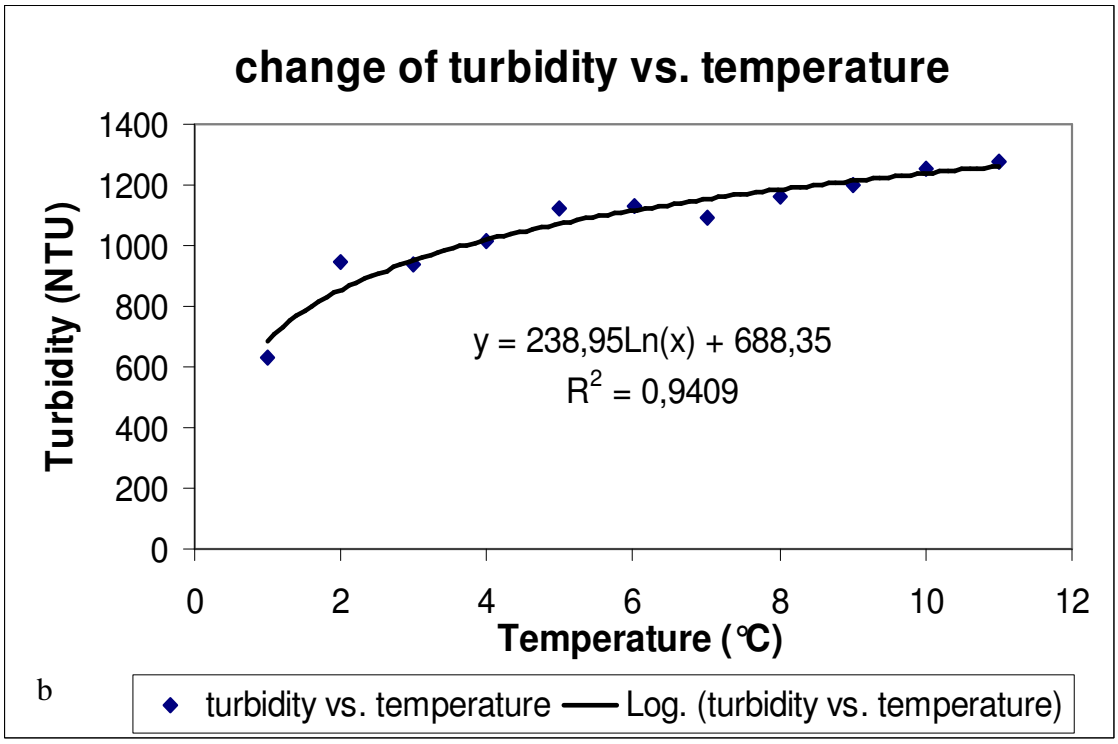


Figure 4.18. Microbial inactivation results of 1st continuous flow UV reactor experiment carried out at pH 7.0 and flow rate of 1080 ml/min.



a



b

Figure 4.19. (a) Color-turbidity and (b) turbidity-temperature effect after 1st continuous flow UV reactor experiment carried out at pH 7.0 and 1080 ml/min.

turbidity and absorbance analysis for each samples of these, absorbance coefficient was slightly drawn from different cycle of continuous flow UV treatment. It was realized that the increase in temperature directly affected the turbidity level and color of liquid egg white LEW (Figure 4.19.a). Color change versus turbidity curve (Figure 4.19.b) showed that color parameters were automatically affected by turbidity change after each cycle, causing a decrease in the UV penetration depth. Second reason may be due to the fact that the bacteria was more resistant at pH 7.0 than the alkaline conditions (pH 9.0) to UV irradiation. Robertson and Muriana (2004) were also reported significant difference between thermal pasteurization methods applied to egg white at different pH values. They observed lower microbial reduction with egg white of pH 8.2 compared with pH 9.0.

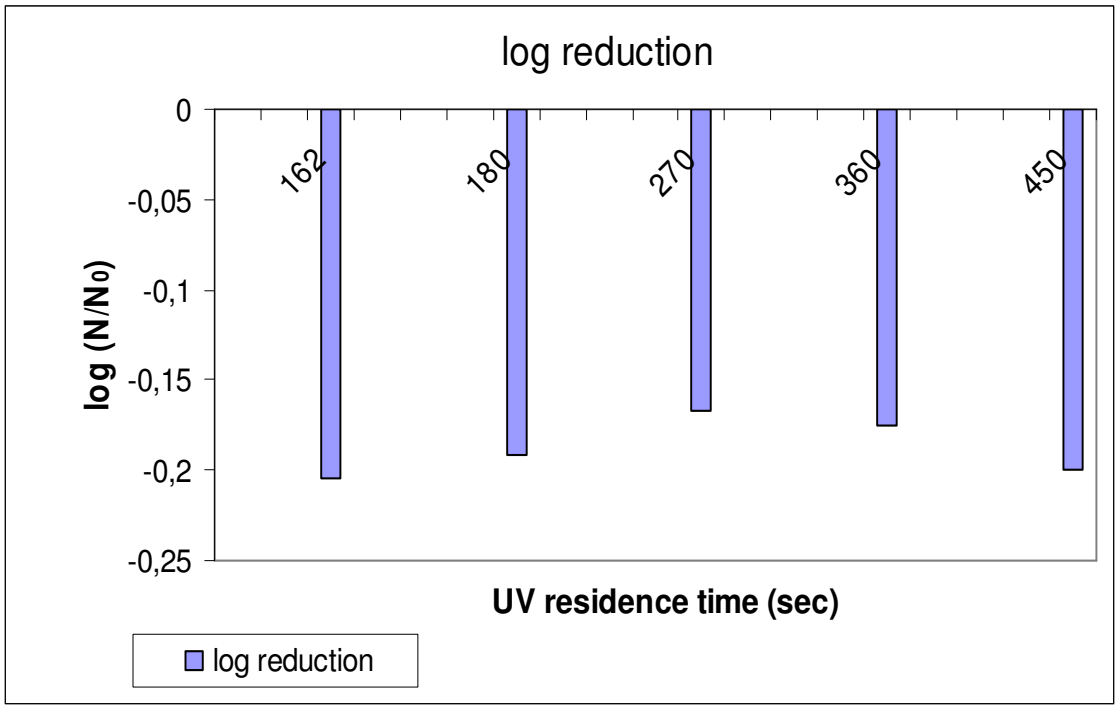
Table 4.17. Temperature and pH Change of Liquid Egg White (LEW) Sample in Each Cycle (first experiment).

	Temp (°C)	pH
Initial Sample	10.2	6,78
C_{1in}	19.9	6.79
C_{1out}	19.8	6.77
C_{2in}	21.0	6.71
C_{2out}	22.1	6.72
C_{3in}	22.6	6.79
C_{3out}	24.2	6.77
C_{4in}	24.8	6.77
C_{4out}	26.2	6.72
C_{5in}	26.6	6.73
C_{5out}	28.3	6.73

In the second study of continuous flow UV reactor, pH of liquid egg white (LEW) samples was kept at its natural pH value (approximately 9-9.2). In this case, the flow rate was adjusted to slightly higher value (1860 ml/min) compared to first experiment. At this flow rate, the lamps generated approximately 31.57 mJ/cm^2 UV dose at an approximate exposure time of 180 sec. The applied UV dose was much higher than the first experiment.

This is because, the liquid egg white (LEW) samples were free from any additives used to adjust its pH value. Therefore average absorbance coefficient was around 1.5 which increased the UV light penetration in the sample. However, similar results were obtained in microbial reduction as in the first experiment. Although maximum log reduction was

slightly higher (0.2 log) than the first experiment, an increase in temperature and microbial population during intermediate cycles were still the problem.



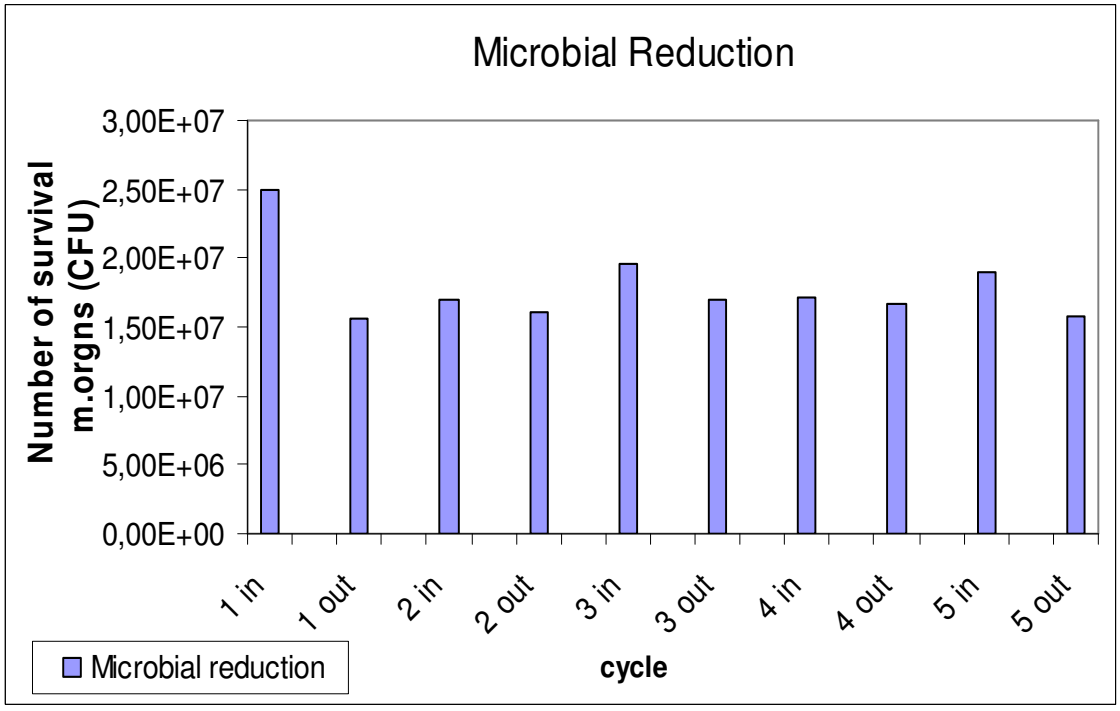
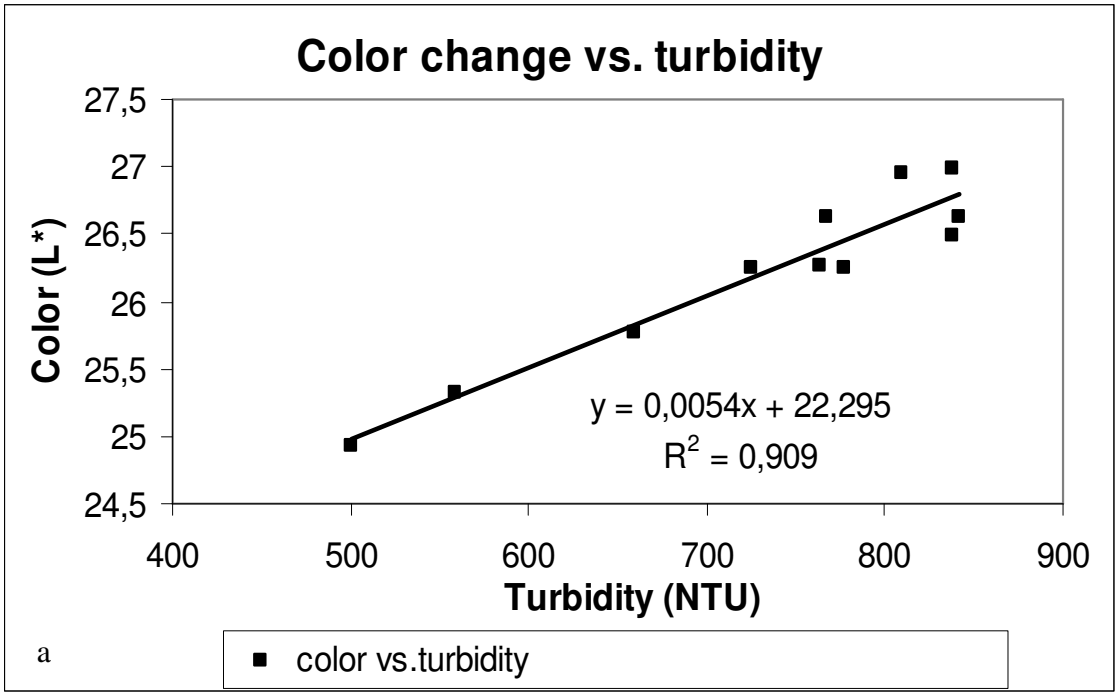
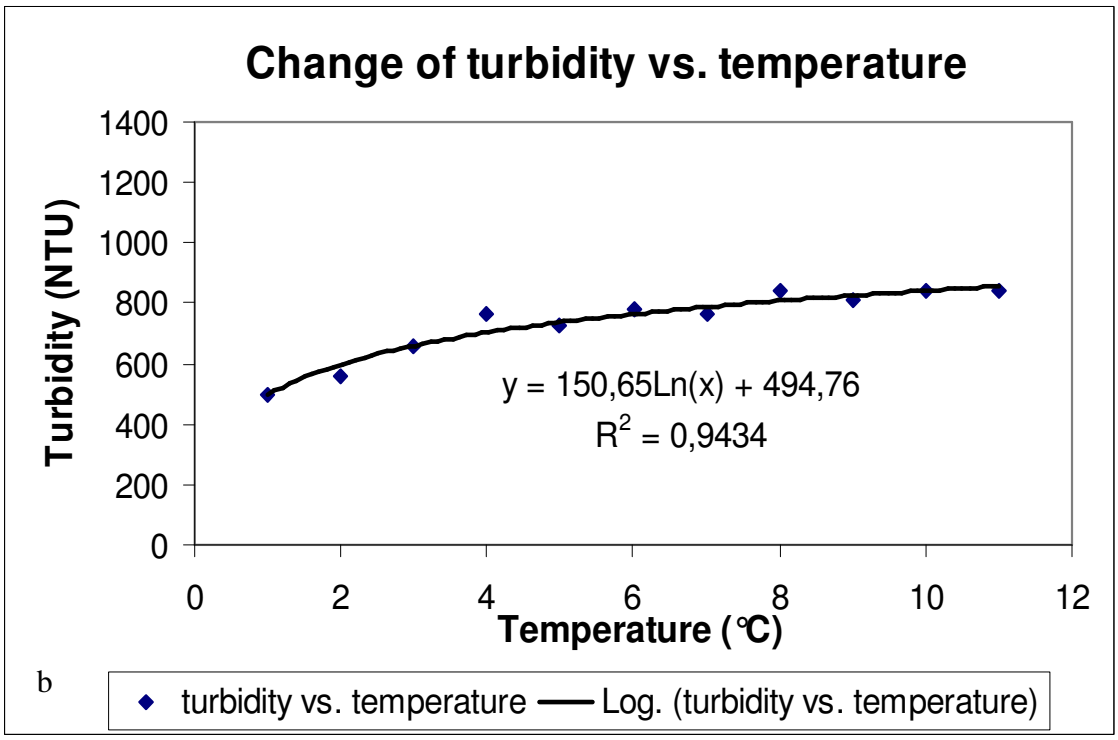


Figure 4.20. Microbial inactivation results of 2nd continuous flow UV Reactor experiment carried out at pH 9.0 and flow rate of 1860 ml/min.



a



b

Figure 4.21. Color-turbidity and turbidity-temperature effect after 2nd continuous flow UV reactor experiment carried out at pH 9.0 and flow rate of 1860 ml/min.

In summary, the UV light treatment of liquid egg white (LEW) samples resulted in inefficient microbial reduction (Table 4.18, Figure 4.20 and 4.21 a and b) at pH 7.0 and 9.0 for a given UV system. Based on our experimental observations we suggest the change of UV reactor design to thin film and addition of cooling jacket to the system in order to supply efficient reduction in liquid egg white (LEW) samples.

Table 4.18. Temperature and pH Change of Liquid Egg White (LEW) Sample in Each Cycle (second experiment)

	Temp (°C)	pH
Initial Sample	10,1	9,17
C_{1in}	17,8	9,11
C_{1out}	20,8	9,08
C_{2in}	22,6	9,04
C_{2out}	23,3	9,0
C_{3in}	23,6	9,0
C_{3out}	25	8,97
C_{4in}	24,5	8,98
C_{4out}	26	8,94
C_{5in}	25,9	8,95
C_{5out}	27,6	8,91

4.3.2. Results of Residual Chlorine Determination After Cleaning Step

After application of continuous flow UV reactor studies, cleaning procedure was done as explained in Section 3.4.3.2. system was firstly washed with 15 lt of 0.5 M NaOH, and then cleaned with 15 lt of 2 % sodium hypochloride disinfectant. Between each solution, system was cleaned by distilled water to remove the residuals, which had the potential reactivity risk of themselves. System was finally cleaned by hot distilled water to completely remove the residual chlorine and UV light was switched on to disinfect the residual microbial population in water. This operation was ended after 3-4 cycle and 100 ml of water sample was taken from outlet of the reactor. After application of titrametric chlorine test, explained in Section 3.4.3.2., it was seen that residual chlorine level was non detectable in the pipe wall and quartz sleeve as a result of thiosulphate titration.

CHAPTER 5

CONCLUSION

The results of this work are concluded under three basic headlines including rheological, biodosimetric and continuous flow UV inactivation studies of liquid egg products.

Rheological and selected physical properties of liquid egg products (LWE, LEW, and LEY) were studied as a function of temperature. Density data were well correlated by polynomial models and decreased by increasing temperature. Different rheological flow models (Newtonian, Power law and Herschel-Bulkley) based on shear stress and shear rate were applied to fit the experimental data. The good agreement between values predicted by these models and experimental values confirmed appropriateness of the equations proposed for describing the selected physical and rheological properties of liquid egg products (LEPs).

Experimental data of LEW and LWE were successfully fitted to Herschel-Bulkley model, LEY data was well suited to power law model. It was found that liquid egg white (LEW), liquid whole egg (LWE) and liquid egg yolk (LEY) exhibited mildly shear thinning (pseudoplastic) behavior at 4, 25 and 55.6/60 °C. LEW and LWE showed thixotropy and time-dependency at their pasteurization temperatures (55.6 °C for LEW, 60 °C for LWE). As a result, LEW and LWE were determined to be time-dependent at their pasteurization temperatures and time-independent below these temperatures.

On the other hand, liquid egg yolk (LEY) exhibited time-dependent behavior at 4 °C and 60 °C. But its rheological behavior showed no thixotropy and time-dependency at 25 °C. An Arrhenius-type equation was found to be useful to quantify the effect of temperature on the rheological behavior of these products. The activation energy of LEY samples was found to be highest indicating higher sensitivity to temperature than other samples.

Biodosimetric studies were conducted with using bench top collimated beam apparatus for LEPs. UV-C inactivation data from *E.coli* (NRRL B-253) provides a conservative estimate of inactivation rate in liquid egg products. The results of this study indicate that UV-C light inactivation could achieve a greater than 2 log reduction of UV resistant *E.coli* (NRRL B-253) in LEW emphasizing that UV-C light treatment

can be used as a pre-treatment process or alternative method when combined with mild heat treatment in order to reduce the adverse effects of thermal pasteurization of LEW. Due to very low UV-C light penetration, *E.coli* inactivation was inefficient in LEY and LWE samples. Therefore, the continuous flow UV inactivation studies were applied on LEW only.

The continuous flow UV inactivation studies revealed that maximum 0.2 logarithmic reduction could be obtained after 5 cycle of operation. Inefficiency of system might be due to undesired temperature increase caused by inappropriate UV reactor design. Based on our experimental observations, in order to supply efficient reduction in LEW samples we suggest the change of UV reactor design to thin film in order to increase the UV light penetration, addition of cooling jacket to the storage tank to keep the product temperature at 4-10 ^{0}C and installation a fan to the UV reactor to remove the heat generated by the lamps. Additionally, there should be an air temperature control at the place where the UV reactor is located.

Further research needs to be done on the modeling of UV irradiation kinetics of *E.coli* (NRRL B-253) and the phenomenon of photo-regeneration in UV-C treated liquid egg products. Future research should also asses the re-design of the UV reactor.

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APPENDIX A

ESTIMATION OF STANDARD DEVIATION FOR RHEOLOGY OF LEPs

Table A.1. Standard Deviations of Liquid Egg White (LEW) for Herchel Bulkey Model.

HB model for LEW at 4 °C					
X	Y	Y´=0.9607x-1.494	Y-Y´	$(Y-Y´)^2$	Sest
log SR	log (SS-YS)				
1,0315	-0,4879	-0,5030	0,0151	0,0002	**0.0198**
1,2076	-0,3307	-0,3338	0,0032	1,012E-05	
1,3325	-0,2166	-0,2139	-0,0027	7,314E-06	
1,4295	-0,1445	-0,1207	-0,0238	0,0006	
1,5086	-0,0637	-0,0446	-0,0190	0,0004	
HB model for LEW at 25 °C					
		Y´=0.9545x-1.622			
log SR	log SS-YS				
1,1283	-0,5075	-0,5451	0,0375	0,00141	**0,01609**
1,2074	-0,4667	-0,4695	0,0028	7,8E-06	
1,2746	-0,4146	-0,4054	-0,0092	8,5E-05	
1,3324	-0,3536	-0,3502	-0,0034	1,1E-05	
1,3836	-0,3129	-0,3013	-0,0116	0,00013	
1,4293	-0,2770	-0,2577	-0,0193	0,00037	
1,4709	-0,2294	-0,2180	-0,0113	0,00013	
1,5085	-0,1918	-0,1822	-0,0097	9,4E-05	
1,5432	-0,1603	-0,1490	-0,0113	0,00013	
1,5755	-0,1281	-0,1182	-0,0099	9,8E-05	
1,6054	-0,0982	-0,0897	-0,0085	7,2E-05	
1,6334	-0,0668	-0,0629	-0,0039	1,5E-05	
1,6598	-0,0293	-0,0377	0,0084	7,1E-05	
1,6847	-0,0081	-0,0139	0,0058	3,4E-05	
1,7080	0,0235	0,0083	0,0152	0,00023	
1,7303	0,0567	0,0296	0,0271	0,00074	
HB model for LEW at 55.6 °C					
		Y´=0.9436x-1.669			
log SR	log SS-YS				
1,2075	-0,4990	-0,5303	0,0313	0,0010	**0,0219**
1,3325	-0,4204	-0,4124	-0,0080	6,5E-05	
1,4294	-0,3461	-0,3209	-0,0251	0,0006	
1,5086	-0,2642	-0,2462	-0,0180	0,0003	
1,5755	-0,1931	-0,1831	-0,0101	0,0001	
1,6334	-0,1312	-0,1284	-0,0028	7,8E-06	
1,6845	-0,0745	-0,0802	0,0057	3,2E-05	
1,7303	-0,0097	-0,0370	0,0272	0,0007	

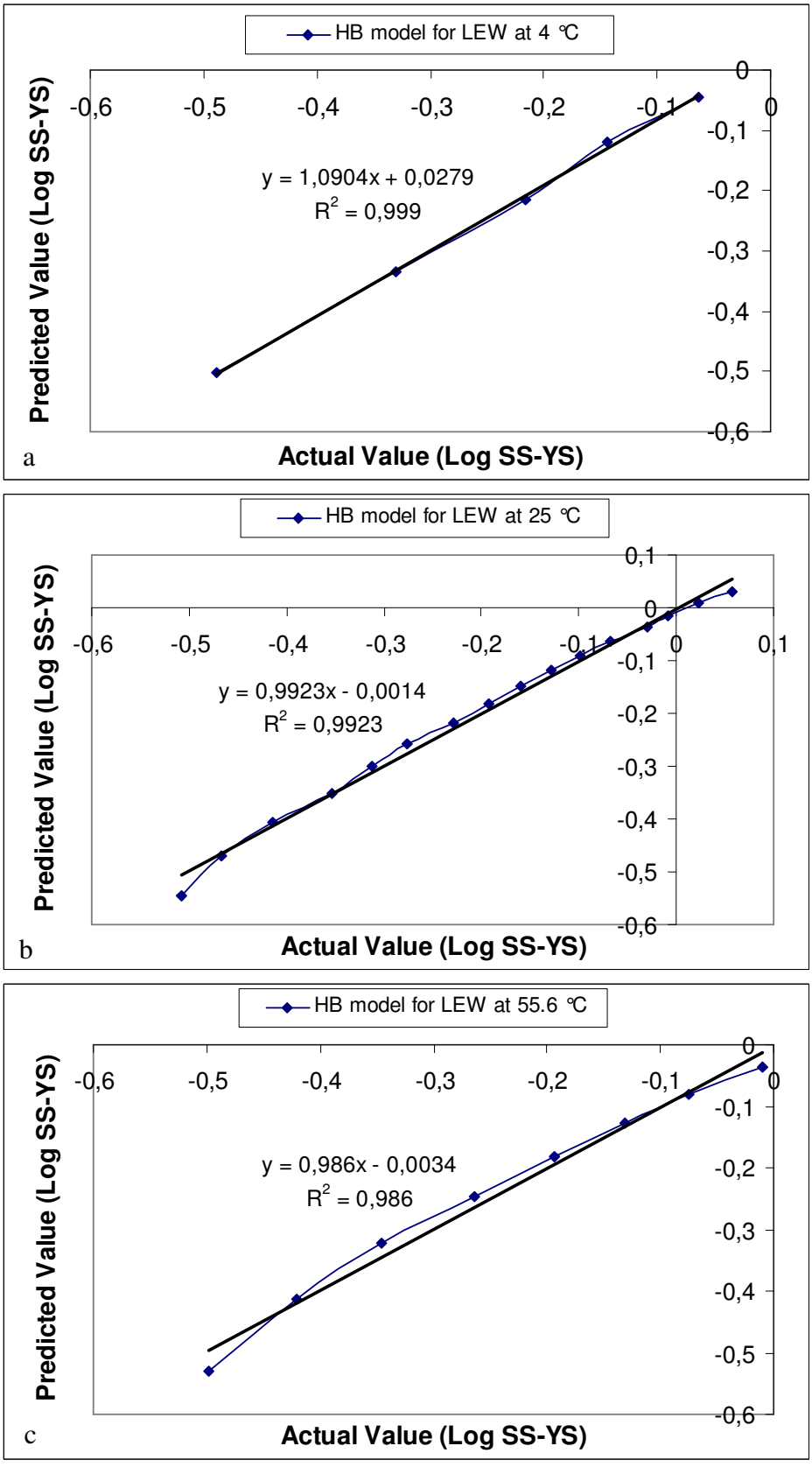

Figure A.1. Standart deviation curves of Herchel Bulkey model for the rheology of fresh liquid egg white (LEW) (a) 4 °C (b) 25 °C (c) 55.6 °C.

Table A.2. Standard Deviations of Liquid Egg White (LEW) for Newtonian Model.

Newtonian Model for LEW at 4 °C					
X	Y	Y´=0.0279x-0.0704	Y-Y´	$(Y-Y´)^2$	Sest
SS (Pa)	SR (sec^{-1})				
0,2547	10,7530	0,2296	0,0251	0,0006	0.0336
0,3966	16,1292	0,3796	0,0170	0,0003	
0,5369	21,5012	0,5295	0,0074	5,48E-05	
0,6465	26,8823	0,6796	-0,0331	0,0011	
0,7932	32,2585	0,8296	-0,0364	0,0013	
Newtonian Model for LEW at 25 °C					
		Y´=0.0203x-0.1157			
SS (Pa)	SR (sec^{-1})				
0,1951	13,4356	0,1570	0,0380	0,0014	0,0213
0,2257	16,1227	0,2116	0,0141	0,0002	
0,2692	18,8197	0,2663	0,0029	8,47E-06	
0,3273	21,4969	0,3207	0,0066	4,36E-05	
0,3708	24,1904	0,3754	-0,0045	2,07E-05	
0,4127	26,8711	0,4298	-0,0170	0,00029	
0,4740	29,5720	0,4846	-0,0106	0,0001	
0,5272	32,2453	0,5389	-0,0117	0,0001	
0,5756	34,9288	0,5934	-0,0178	0,0003	
0,6288	37,6231	0,6480	-0,0193	0,0004	
0,6820	40,3067	0,7025	-0,0205	0,0004	
0,7416	42,9938	0,7571	-0,0154	0,0002	
0,8190	45,6877	0,8118	0,0073	5,29E-05	
0,8658	48,3874	0,8666	-0,0008	6,02E-07	
0,9400	51,0520	0,9207	0,0193	0,0004	
1,0238	53,7422	0,9753	0,0485	0,0024	
Newtonian Model for LEW at 55.6 °C					
		Y´=0.8777x-0.5462			
SS (Pa)	SR (sec^{-1})				
0,1370	16,1261	0,1023	0,0347	0,0012	0,0255
0,1999	21,5015	0,1964	0,0035	1,26E-05	
0,2709	26,8768	0,2904	-0,0196	0,0004	
0,3644	32,2522	0,3845	-0,0201	0,0004	
0,4611	37,6276	0,4786	-0,0175	0,0003	
0,5593	42,9906	0,5724	-0,0131	0,0002	
0,6625	48,3666	0,6665	-0,0040	1,63E-05	
0,7979	53,7428	0,7606	0,0373	0,0014	

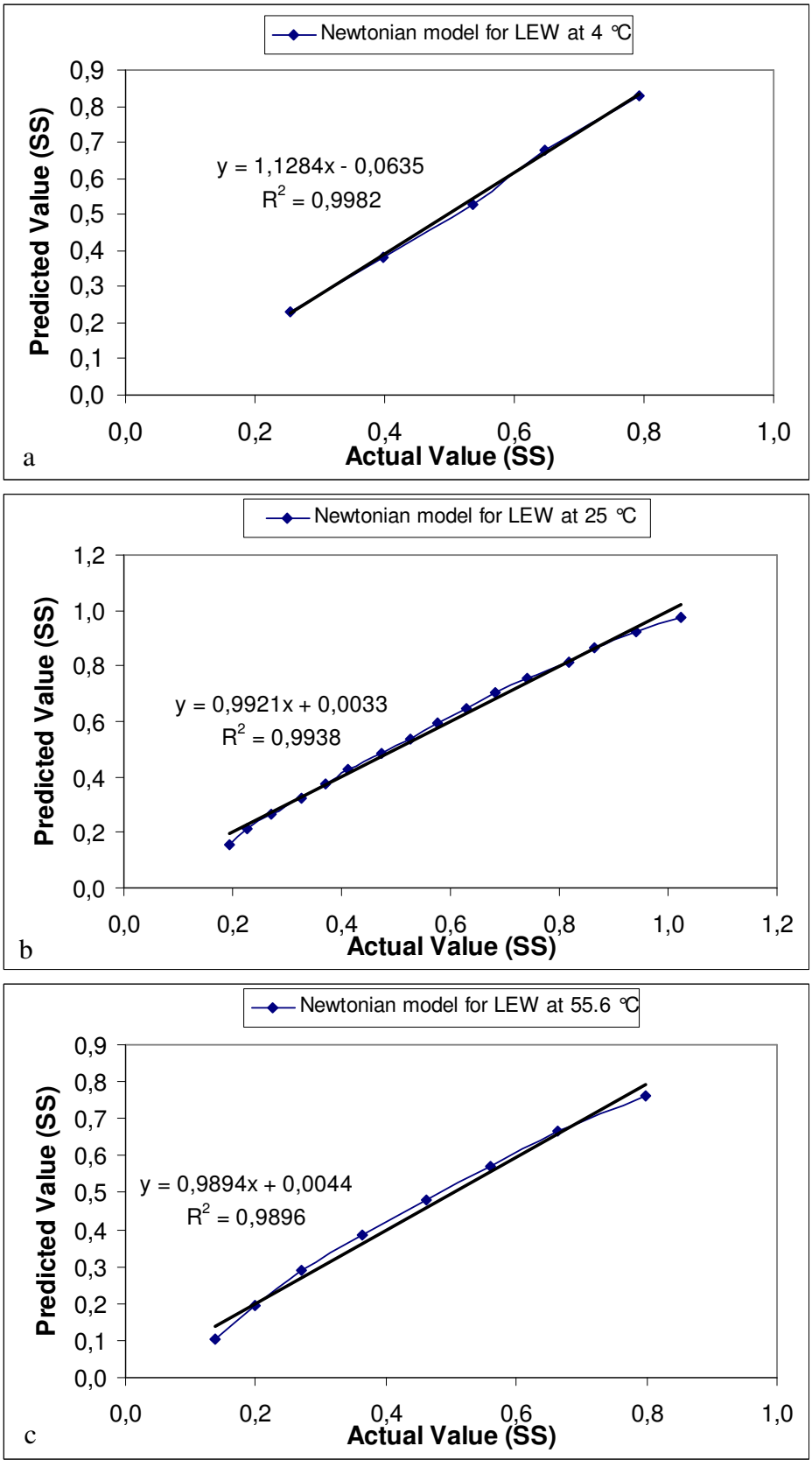

Figure A.2. Standart deviation curves of Newtonian model for the rheology of fresh liquid egg white (LEW) (a) 4 °C (b) 25 °C (c) 55.6 °C.

Table A.3. Standard Deviations of Liquid Egg Yolk (LEY) for Power Law Model.

Power Law for LEY at 4 °C					
X	Y	Y´=0.9324X+0.0058	Y-Y´	$(Y-Y´)^2$	Sest
log SS	log SR				
0,0253	0,0314	0,0294	0,0020	4,0532E-06	**0,0046**
0,3263	0,3071	0,3101	-0,0030	9,0456E-06	
0,5024	0,4709	0,4743	-0,0034	1,164E-05	
0,6274	0,5915	0,5908	0,0008	5,683E-07	
0,7243	0,6848	0,6811	0,0036	1,325E-05	
0,8035	0,7605	0,7549	0,0056	3,0852E-05	
0,8704	0,8115	0,8174	-0,0059	3,43E-05	
Power Law for LEY at 25 °C					
		Y´=0.897x-0.3583			
log SS	log SR				
-0,0630	0,3263	-0,0656	0,0026	6,7228E-06	**0,0016**
0,2014	0,6274	0,2044	-0,0031	9,303E-06	
0,3608	0,8035	0,3624	-0,0016	2,619E-06	
0,4745	0,9284	0,4745	3,66E-05	1,336E-09	
0,5616	1,0253	0,5614	0,0002	3,204E-08	
0,6333	1,1045	0,6324	0,0008	7,081E-07	
0,6927	1,1714	0,6925	0,0002	3,7826E-08	
0,7448	1,2294	0,7445	0,0003	1,1931E-07	
0,7915	1,2806	0,7904	0,0011	1,213E-06	
0,8107	1,3041	0,8114	-0,0007	5,453E-07	
Power Law for LEY at 60 °C					
		Y´= 0.929x-1.5599			
log SS	log SR				
0,0154	0,6274	0,0044	0,0109	0,0001	**0,0073**
0,1418	0,8035	0,1590	-0,0172	0,0003	
0,2762	0,9284	0,2687	0,0076	5,744E-05	
0,3535	1,0253	0,3537	-0,0002	3,2184E-08	
0,4236	1,1045	0,4232	0,0004	1,3336E-07	
0,4764	1,1714	0,4820	-0,0056	3,1033E-05	
0,5358	1,2294	0,5329	0,0029	8,604E-06	
0,5808	1,2806	0,5778	0,0030	9,286E-06	
0,6196	1,3263	0,6179	0,0017	2,8562E-06	
0,6485	1,3677	0,6543	-0,0058	3,361E-05	
0,6807	1,4055	0,6874	-0,0067	4,5217E-05	
0,7145	1,4403	0,7179	-0,0034	1,1804E-05	
0,7469	1,4725	0,7462	0,0007	4,707E-07	
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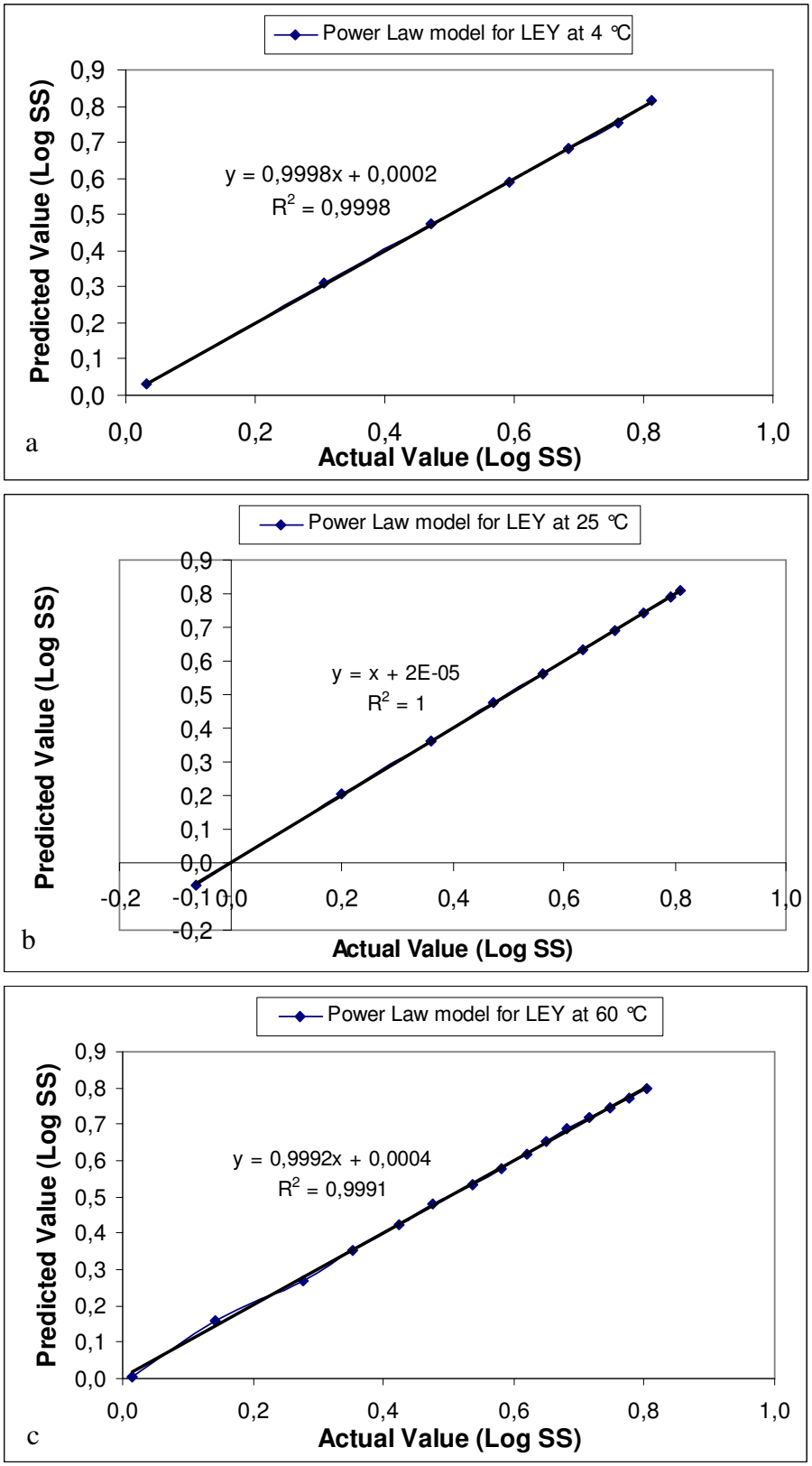

Figure A.3. Standart deviation curves of Power Law model for the rheology of fresh liquid egg yolk (LEY) (a) 4 °C (b) 25 °C (c) 60 °C.

Table A.4. Standard Deviations of Liquid Whole Egg (LWE) for Herschel Bulkey Model.

HB Model for LWE at 4 °C					
log SR	Observed Xm log SS-YS	Observed log SS	Predicted Xc eqn HB	HB model obs-pred	S_{est}
0,9066	-0,4204	-0,6687	-0,5202	0,0998	**0,0033**
1,0315	-0,3571	-0,5621	-0,3968	0,0397	
1,1284	-0,2893	-0,4581	-0,3010	0,0117	
1,2076	-0,2308	-0,3743	-0,2228	-0,0079	
1,2745	-0,1729	-0,2956	-0,1568	-0,0161	
1,3325	-0,1397	-0,2522	-0,0995	-0,0403	
1,3837	-0,0645	-0,1571	-0,0489	-0,0156	
1,4294	-0,0147	-0,0962	-0,0037	-0,0110	
1,4707	0,0326	-0,0397	0,0371	-0,0045	
1,5086	0,0621	-0,0051	0,0745	-0,0124	
1,5433	0,1125	0,0532	0,1088	0,0037	
1,5755	0,1458	0,0911	0,1406	0,0052	
1,6055	0,1771	0,1265	0,1702	0,0069	
1,6335	0,2137	0,1674	0,1979	0,0158	
1,6598	0,2403	0,1969	0,2239	0,0164	
HB Model for LWE at 25 °C					
X log SR	Y log SS-YS	Y´= 0.972x-1.5185	Y-Y´	$(Y-Y´)^2$	S_{est}
1,3325	-0,2033	-0,2233	0,0200	0,0004	**0,0139**
1,4293	-0,1419	-0,1292	-0,0126	0,0002	
1,5085	-0,0688	-0,0523	-0,0165	0,0003	
1,5756	0,0084	0,0130	-0,0046	2,08E-05	
1,6335	0,0692	0,0693	-6,6E-05	4,38E-09	
1,6846	0,1230	0,1190	0,0041	1,67E-05	
1,7303	0,1728	0,1634	0,0095	8,94E-05	
HB Model for LWE at 60 °C					
log SR	log SS-YS	Y´= 0.929x-1.5599			S_{est}
1,1283	-0,4606	-0,5118	0,0511	0,0026	**0,0253**
1,2074	-0,4238	-0,4382	0,0144	0,0002	
1,2744	-0,3763	-0,3760	-0,0003	1,036E-07	
1,3324	-0,3442	-0,3221	-0,0221	0,0005	
1,3836	-0,2864	-0,2745	-0,0119	0,0001	
1,4293	-0,2538	-0,2321	-0,0217	0,0005	
1,4707	-0,2223	-0,1936	-0,0287	0,0008	
1,5085	-0,1908	-0,1585	-0,0322	0,0010	
1,5435	-0,1454	-0,1260	-0,0194	0,0004	
1,5754	-0,1070	-0,0963	-0,0107	0,0001	
1,6054	-0,0860	-0,0685	-0,0175	0,0003	
1,6334	-0,0376	-0,0425	0,0048	2,336E-05	
1,6599	-0,0024	-0,0179	0,0154	0,0002	
1,6846	0,0301	0,0051	0,0250	0,0006	
1,7081	0,0474	0,0269	0,0205	0,0004	
1,7303	0,0800	0,0476	0,0325	0,0011	

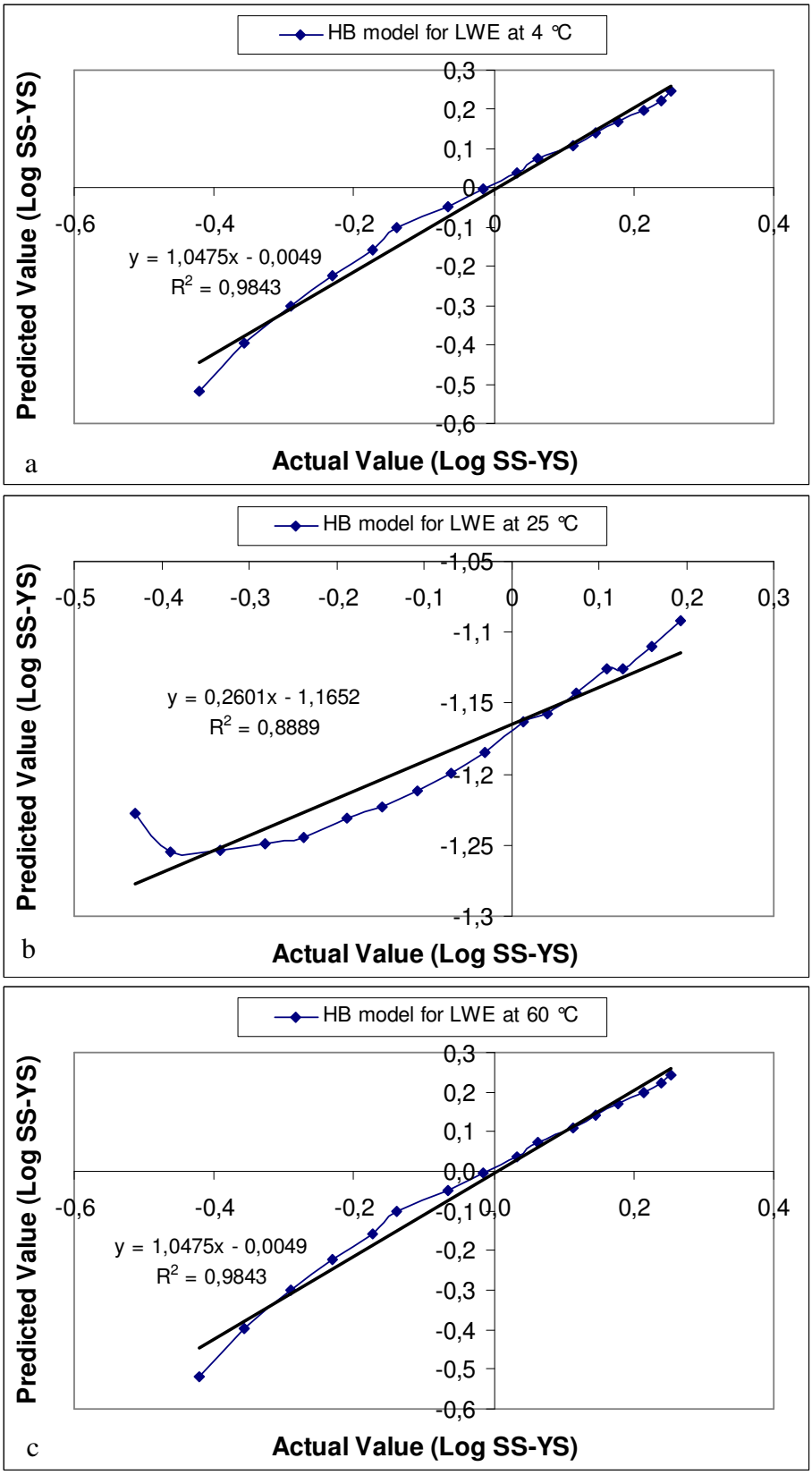

Figure A.4. Standart deviation curves of Herchel Bulkey model for the rheology of fresh liquid whole egg (LWE) (a) 4 °C (b) 25 °C (c) 60 °C.

Table A.5. Standard Deviations of Liquid Whole Egg (LWE) for Newtonian Model.

Newtonian Model for LWE at 4 °C					
X	Y	Y´=0.0279x-0.0704	Y-Y´	$(Y-Y´)^2$	Sest
SS (Pa)	SR (sec^{-1})				
0,2144	8,0644	0,1338	0,0806	0,0065	**0,04024**
0,2741	10,7527	0,2335	0,0406	0,0016	
0,3482	13,4407	0,3333	0,0150	0,0002	
0,4224	16,1288	0,4330	-0,0106	0,0001	
0,5063	18,8128	0,5326	-0,0263	0,0007	
0,5595	21,5010	0,6323	-0,0728	0,0053	
0,6965	24,1924	0,7321	-0,0356	0,0013	
0,8013	26,8801	0,8319	-0,0306	0,0009	
0,9125	29,5608	0,9313	-0,0188	0,0004	
0,9883	32,2559	1,0313	-0,0430	0,0018	
1,1302	34,9366	1,1307	-0,0005	3,01E-07	
1,2334	37,6261	1,2305	0,0029	8,15E-06	
1,3382	40,3188	1,3304	0,0078	6,01E-05	
1,4704	43,0064	1,4301	0,0403	0,0016	
1,5736	45,6903	1,5297	0,0439	0,0019	
1,6284	47,9926	1,6151	0,0133	0,0002	
Newtonian Model for LWE at 25 °C					
		Y´=0.0203x-0.1157			
SS (Pa)	SR (sec^{-1})				
0,1661	10,7484	0,0939	0,0722	0,0052	**0,0366**
0,2031	13,4356	0,1686	0,0345	0,0012	
0,2596	16,1227	0,2433	0,0163	0,0003	
0,3176	18,8161	0,3182	-0,0006	3,28E-07	
0,3740	21,4969	0,3927	-0,0187	0,0003	
0,4450	24,1840	0,4674	-0,0224	0,0005	
0,5063	26,8711	0,5421	-0,0359	0,0013	
0,5756	29,5624	0,6169	-0,0414	0,0017	
0,6481	32,2453	0,6915	-0,0434	0,0019	
0,7287	34,9350	0,7663	-0,0375	0,0014	
0,8287	37,6342	0,8413	-0,0126	0,0002	
0,8932	40,3067	0,9156	-0,0224	0,0005	
0,9835	43,0032	0,9906	-0,0071	5,05E-05	
1,0834	45,6956	1,0654	0,0180	0,0003	
1,1383	48,3749	1,1399	-0,0017	2,76E-06	
1,2398	51,0640	1,2147	0,0252	0,0006	
1,3575	53,7635	1,2897	0,0678	0,0046	
Newtonian Model for LWE at 60 °C					
		Y´=0.8777x-0.5462			
SS (Pa)	SR (sec^{-1})				
0,1516	13,4356	0,0942	0,0574	0,0033	**0,0344**
0,1822	16,1227	0,1519	0,0302	0,0009	
0,2257	18,8098	0,2097	0,0160	0,0003	
0,2580	21,4969	0,2675	-0,0095	9,06E-05	
0,3225	24,1901	0,3254	-0,0029	8,6E-06	

Table A.5. (Cont.)

Newtonian Model for LWE at 60 °C					
		Y´=0.8777x-0.5462			
SS (Pa)	SR (sec^{-1})				
0,3628	26,8711	0,3830	-0,0203	0,0004	0,3628
0,4047	29,5602	0,4408	-0,0362	0,0013	0,4047
0,4498	32,2453	0,4986	-0,0488	0,0024	0,4498
0,5208	34,9505	0,5567	-0,0360	0,0013	0,5208
0,5869	37,6196	0,6141	-0,0273	0,0007	0,5869
0,6256	40,3067	0,6719	-0,0463	0,0021	0,6256
0,7223	42,9938	0,7297	-0,0074	5,43E-05	0,7223
0,7997	45,6963	0,7878	0,0119	0,0001	0,7997
0,8771	48,3769	0,8454	0,0317	0,0010	0,8771
0,9206	51,0596	0,9031	0,0175	0,0003	0,9206
1,0077	53,7422	0,9608	0,0469	0,0022	1,0077

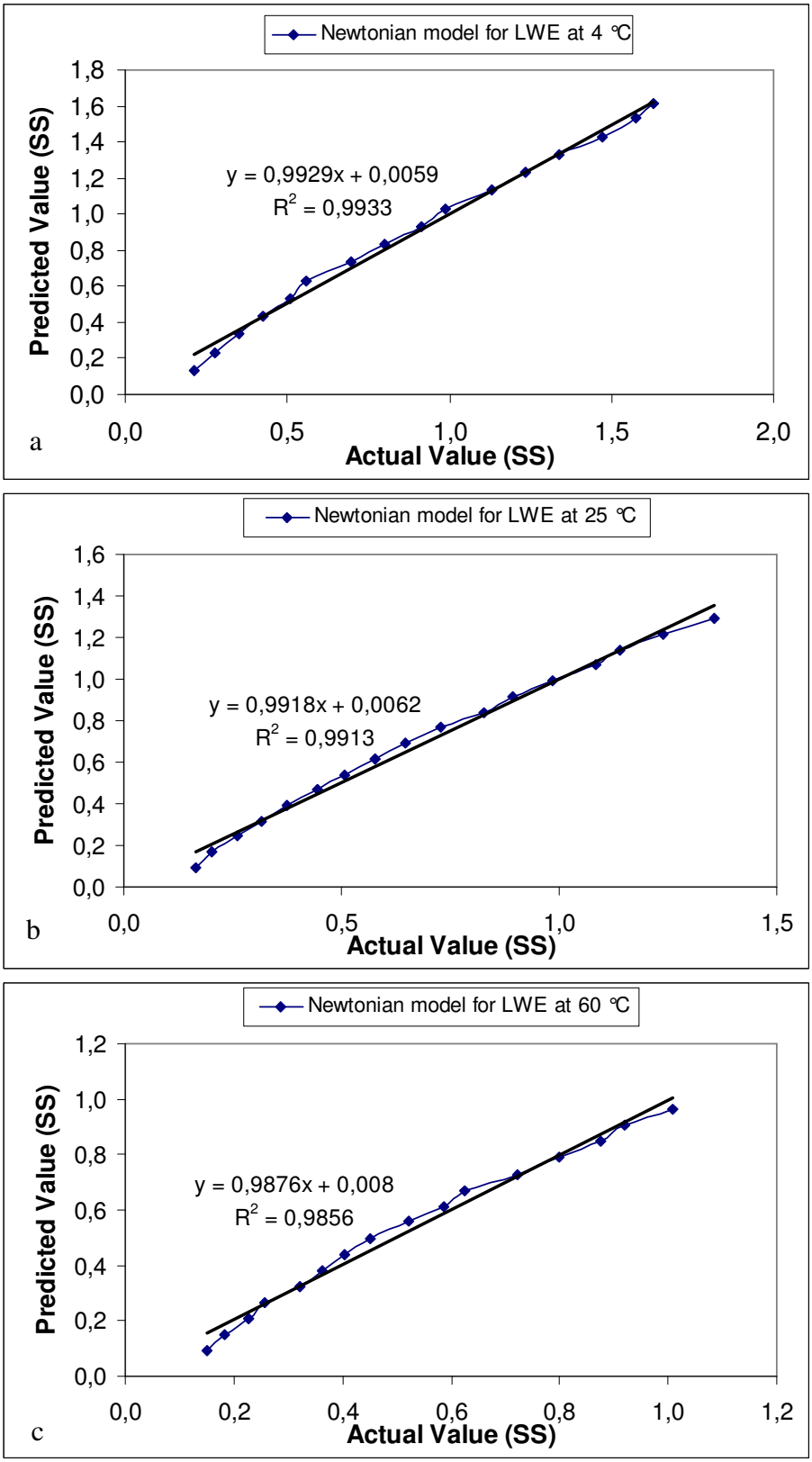

Figure A.5. Standart deviation curves of Newtonian model for the rheology of fresh liquid whole egg (LWE) (a) 4 °C (b) 25 °C (c) 60 °C.

APPENDIX B

STATISTICAL TABLES OF BIODOSIMETRY STUDIES FOR *E.coli* (NRRL B-253)

Table A.6. Summary of Statistical Analysis for Biodosimetry Study of *E.coli* (NRRL B-253) in Liquid Egg White (LEW).

General Linear Model: Log Reduction versus Time; Intensity; Depth						
Factor	**Type**	**Levels**	**Values**			
Time	fixed	4	0; 5; 10; 20			
Intensity	fixed	3	1.315; 0.709; 0.383[1]			
Depth	fixed	3	1.53; 3.00; 5.00[2]			
Analysis of Variance for log reduction, using adjusted SS for Tests						
Source	**DF**[3]	**Seq SS**[4]	**Adj SS**	**Adj MS**[5]	**F**	**P**
Time	3	20.99717	20.99717	6.99906	447.52	0.000
Intensity	2	0.89283	0.89283	0.44641	28.54	0.000
Depth	2	1.07845	1.07845	0.53923	34.48	0.000
Time* Intensity	6	1.13665	1.13665	0.18944	12.11	0.000
Time* Depth	6	1.51949	1.51949	0.25325	16.19	0.000
Intensity* Depth	4	0.12950	0.12950	0.03238	2.07	0.105
Time* Intensity* Depth	12	0.64565	0.64565	0.05380	3.44	0.002
Error	36	0.56302	0.56302	0.01564		
Total	71	26.96277				
S=0.125058	R-Sq=97.91%			R-Sq(adj)=95.88%		

[1] Unit in mW/cm^2
[2] Unit in mm
[3] DF: Degrees of Freedom
[4] SS: Sum of Square
[5] MS: Mean of Square

Table A.7. Summary of Statistical Analysis for Biodosimetry Study of *E.coli* (NRRL B-253) in Liquid Egg Yolk (LEY).

General Linear Model: Log Reduction versus Time; Intensity; Depth						
Factor	**Type**	**Levels**	**Values**			
Time	fixed	4	0; 5; 10; 20			
Intensity	fixed	3	1.315; 0.709; 0.383			
Depth	fixed	3	1.53; 3.00; 5.00			
Analysis of Variance for log reduction, using adjusted SS for Tests						
Source	**DF**	**Seq SS**	**Adj SS**	**Adj MS**	**F**	**P**
Time	3	0.848612	0.848612	0.282871	41.17	0.000
Intensity	2	0.12581	0.12581	0.051290	7.47	0.002
Depth	2	1.032962	1.032962	0.516481	75.17	0.000
Time* Intensity	6	0.055628	0.055628	0.009271	1.35	0.261
Time* Depth	6	0.366172	0.366172	0.061029	8.88	0.000
Intensity* Depth	4	0.098393	0.098393	0.024598	3.58	0.015
Time* Intensity* Depth	12	0.047625	0.047625	0.003973	0.58	0.845
Error	36	0.247346	0.247346	0.006871		
Total	71	2.766369				
S=0.0828898	R-Sq=91.16%			R-Sq(adj)=82.57%		

Table A.8. Summary of Statistical Analysis for Biodosimetry Study of *E.coli* (NRRL B-253) in Liquid Whole Egg (LWE).

General Linear Model: Log Reduction versus Time; Intensity; Depth						
Factor	**Type**	**Levels**	**Values**			
Time	fixed	4	0; 5; 10; 20			
Intensity	fixed	3	1.315; 0.709; 0.383[1]			
Depth	fixed	3	1.53; 3.00; 5.00[2]			
Analysis of Variance for log reduction, using adjusted SS for Tests						
Source	**DF**[3]	**Seq SS**[4]	**Adj SS**	**Adj MS**[5]	**F**	**P**
Time	3	0.249540	0.249540	0.083180	24.35	0.000
Intensity	2	0.005293	0.005293	0.002647	0.77	0.468
Depth	2	0.095266	0.095266	0.047633	13.94	0.000
Time* Intensity	6	0.013482	0.013482	0.02247	0.66	0.684
Time* Depth	6	0.043479	0.043479	0.007246	2.12	0.075
Intensity* Depth	4	0.003894	0.003894	0.000973	0.28	0.886
Time* Intensity* Depth	12	0.017253	0.017253	0.001438	0.42	0.945
Error	36	0.123001	0.123001	0.003417		
Total	71	0.551207				
S=0.0584525	R-Sq=77.69%			R-Sq(adj)=55.99%		

APPENDIX C

RESULTS OF BIODOSIMETRY STUDIES FOR *S.typhimurium* (CCM 5445) AND *E.coli* O157:H7 (ATCC 700728)

Table A.9. Inactivation Rate Constant Values (in cm^2/mj) for Target Microorganisms at 0.153 cm Sample Depth and 1.314 mW/cm^2 UV Intensity.

	Inactivation Rate Constant (cm^2/mj)		
M.organism	**LEW**	**LEY**	**LWE**
E.coli (ATCC 8739)	0.0204	0.0493	0.0229
E.coli O157:H7	0.0317	0.1922	0.0126
S.Typhimurium	0.0265	0.1071	0.027

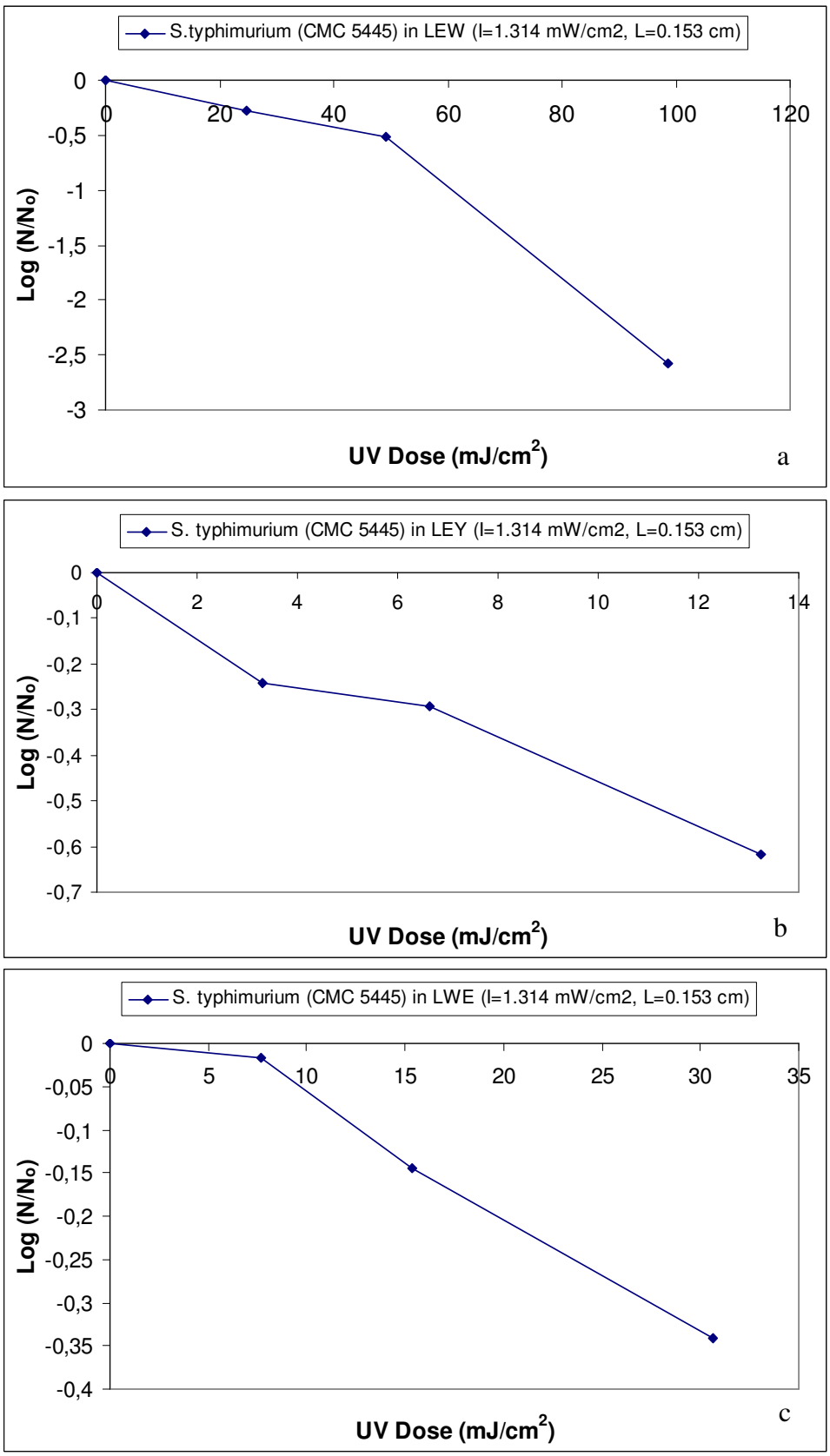

Figure A.6. Influence of UV-C radiation on *S.typhimurium* inactivation at 0.153 cm fluid medium depth and 1.314 mW/cm^2 UV intensity levels (I) (a) Liquid egg white (LEW) (b) liquid egg yolk (LEY) (c) liquid whole egg (LWE).

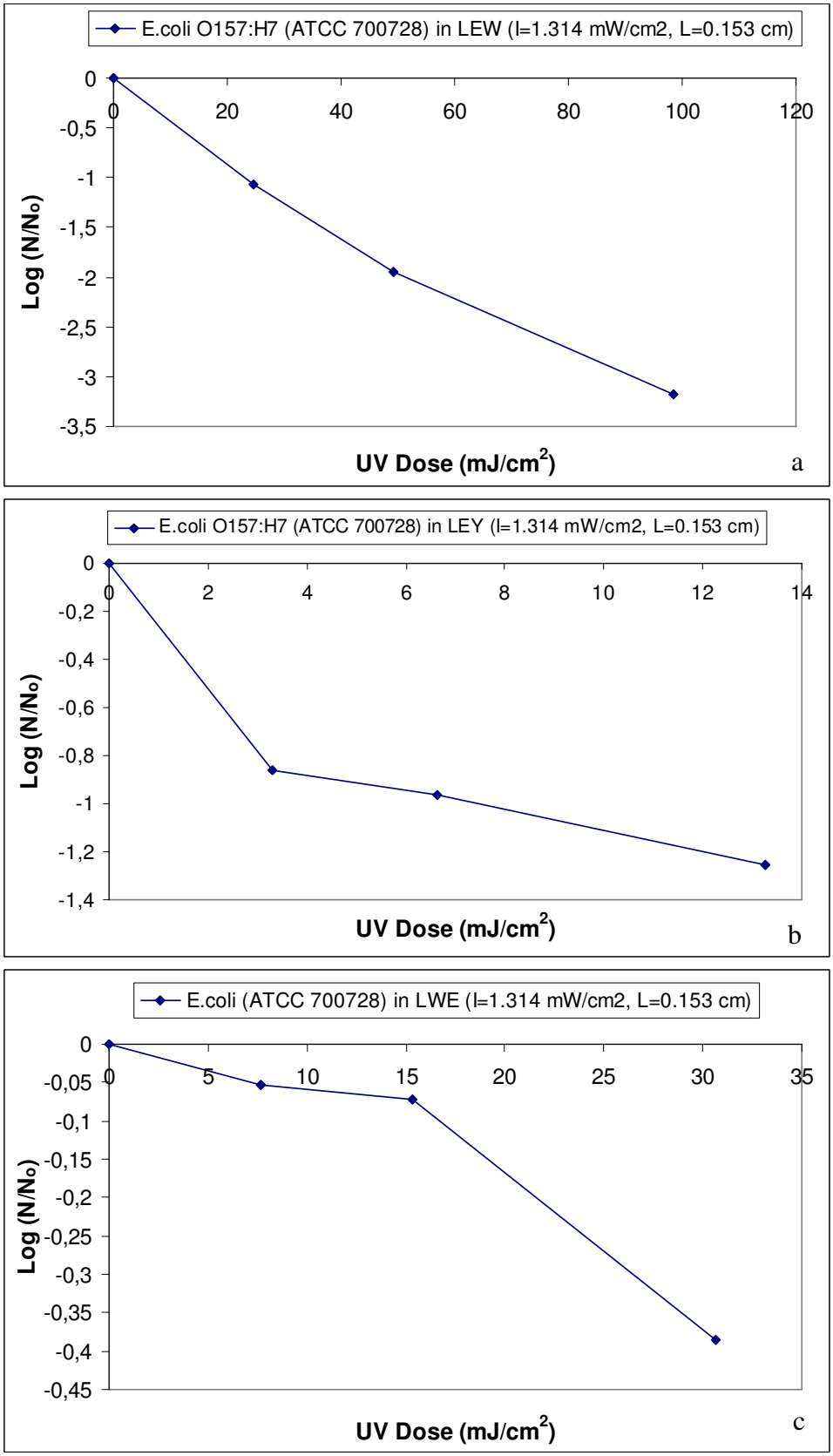

Figure A.7. Influence of UV-C radiation on *E.coli* inactivation at 0.153 cm fluid medium depth and 1.314 mW/cm^2 UV intensity levels (I) (a) Liquid egg white (LEW) (b) liquid egg yolk (LEY) (c) liquid whole egg (LWE).

APPENDIX D

RESULTS OF THE COLOR ANALYSIS OF LEPs

Table A.10. Statistical Analysis of Color Parameters of LEPs Before and After UV Treatment in Bench Top Collimated Beam UV Apparatus.

Parameter	Product	Depth (mm)	Time (min) 0	5	10	20
L*	LEW	1.53	28.51 ± 0.45^{c}	28.57 ± 0.24^{c}	28.56 ± 0.44^{b}	28.16 ± 0.64^{b}
		3	29.57 ± 0.25^{b}	29.25 ± 0.33^{b}	29.35 ± 0.32^{a}	29.42 ± 0.59^{a}
		5	30.16 ± 0.28^{a}	29.74 ± 0.19^{a}	29.80 ± 0.21^{a}	29.66 ± 0.22^{a}
	LEY	1.53	55.93 ± 0.64^{c}	55.23 ± 0.83^{c}	55.21 ± 0.95^{c}	54.70 ± 0.57^{c}
		3	57.31 ± 1.00^{b}	57.32 ± 0.74^{b}	57.27 ± 0.78^{b}	56.75 ± 0.50^{b}
		5	58.92 ± 0.32^{a}	58.90 ± 0.26^{a}	58.79 ± 0.44^{a}	58.83 ± 0.29^{a}
	LWE	1.53	57.23 ± 0.87^{c}	57.34 ± 0.88^{c}	57.31 ± 0.63^{c}	56.72 ± 0.79^{c}
		3	59.75 ± 1.09^{b}	59.66 ± 1.24^{b}	59.79 ± 0.96^{b}	59.58 ± 1.30^{b}
		5	61.56 ± 0.79^{a}	61.32 ± 0.86^{a}	61.14 ± 0.69^{a}	61.21 ± 0.91^{a}
a*	LEW	1.53	0.14 ± 0.20^{a}	0.18 ± 0.18^{a}	0.11 ± 0.20^{a}	0.11 ± 0.14^{a}
		3	-0.21 ± 0.10^{b}	-0.09 ± 0.17$^{a, b}$	-0.01 ± 0.12$^{a, b}$	-0.05 ± 0.23$^{a, b}$
		5	-0.36 ± 0.22^{b}	-0.33 ± 0.25^{b}	-0.32 ± 0.26^{b}	-0.30 ± 0.20^{b}
	LEY	1.53	1.05 ± 0.51^{c}	0.52 ± 0.64^{c}	0.46 ± 083^{c}	0.37 ± 0.27^{c}
		3	3.40 ± 0.64^{b}	3.27 ± 0.61^{b}	3.31 ± 0.60^{b}	2.93 ± 0.31^{b}
		5	6.02 ± 0.17$^{a, x}$	5.60 ± 0.14$^{a, y}$	5.66 ± 0.18$^{a, y}$	5.68 ± 0.22$^{a, y}$
	LWE	1.53	-0.34 ± 0.52^{c}	-0.13 ± 0.74^{c}	-0.22 ± 0.77c	-0.28 ± 0.56^{c}
		3	2.45 ± 0.35^{b}	2.22 ± 0.35^{b}	2.21 ± 0.30^{b}	2.19 ± 0.34^{b}
		5	4.86 ± 0.76^{a}	4.74 ± 0.77^{a}	4.62 ± 0.59^{a}	4.63 ± 0.66^{a}
b*	LEW	1.53	-0.65 ± 0.12$^{b, x}$	-0.58 ± 0.08$^{b, x}$	-0.63 ± 0.05$^{b, x}$	-0.53 ± 0.25$^{b, x}$
		3	-0.68 ± 0.19$^{b, y}$	-0.48 ± 0.09$^{b, xy}$	-0.52 ± 0.16$^{b, xy}$	-0.34 ± 0.18$^{b, x}$
		5	-0.16 ± 0.15$^{a, y}$	0.05 ± 0.11$^{a, xy}$	0.01 ± 0.18$^{a, xy}$	0.16 ± 0.13$^{a, x}$
	LEY	1.53	44.45 ± 0.72^{c}	43.39 ± 1.14^{c}	43.16 ± 1.96^{c}	43.64 ± 1.99^{c}
		3	47.45 ± 1.41^{b}	47.10 ± 0.90^{b}	46.96 ± 0.81^{b}	46.34 ± 1.01^{b}
		5	49.51 ± 0.90^{a}	49.60 ± 1.05^{a}	49.70 ± 0.75^{a}	49.33 ± 0.93^{a}
	LWE	1.53	25.74 ± 0.70^{c}	26.05 ± 0.98^{c}	26.02 ± 0.94^{c}	25.79 ± 0.68^{c}
		3	30.74 ± 0.92^{b}	30.94 ± 1.15^{b}	30.71 ± 1.11^{b}	30.51 ± 1.31^{b}
		5	33.31 ± 0.76^{a}	33.36 ± 1.09^{a}	33.25 ± 0.86^{a}	33.35 ± 0.83^{a}

$^{a, b, c}$ Column means having a different letter are significantly different ($p<0.05$, depth efffect)
$^{x, y}$ Row means having a different letter are significantly different ($p<0.05$, time efffect)

Table A.11. Results of Statistical Analysis of ΔE for LEPs Before and After UV Treatment in Bench Top Collimated Beam UV Apparatus.

Parameter	Depth (mm)	Time (min)	Replicate-1	Replicate-2
LEW	1.53	5	0.33 ± 0.18 [a b]	0.64 ± 0.41
		10	0.18 ± 0.14 [b]	0.45 ± 0.62
		20	0.69 ± 0.24 [b]	0.27 ± 0.12
	3	5	0.59 ± 0.33	0.36 ± 0.24
		10	0.33 ± 0.10	0.46 ± 0.13
		20	0.90 ± 0.33	0.40 ± 0.10
	5	5	0.57 ± 0.22	0.68 ± 0.44
		10	0.43 ± 0.18	0.70 ± 0.29
		20	0.60 ± 0.34	0.72 ± 0.32
LEY	1.53	5	1.60 ± 0.95	1.27 ± 0.93
		10	1.16 ± 0.82	2.13 ± 2.55
		20	1.17 ± 0.54	2.84 ± 1.17 [y]
	3	5	0.92 ± 0.64	0.52 ± 0.11
		10	1.34 ± 0.61	0.71 ± 0.23
		20	1.41 ± 0.86	1.79 ± 0.69 [xy]
	5	5	0.41 ± 0.23	0.85 ± 0.13
		10	0.75 ± 0.48	0.98 ± 0.39
		20	0.49 ± 0.09	0.53 ± 0.18 [x]
LWE	1.53	5	0.98 ± 0.61	0.48 ± 0.37
		10	1.47 ± 1.01	0.84 ± 0.61
		20	0.68 ± 0.19	0.79 ± 0.38
	3	5	0.64 ± 0.49	0.53 ± 0.36
		10	0.89 ± 0.42	0.63 ± 0.42
		20	1.34 ± 0.57	0.91 ± 0.64
	5	5	0.45 ± 0.27	0.50 ± 0.05
		10	0.60 ± 0.05	0.53 ± 0.17
		20	0.63 ± 0.09	0.41 ± 0.04

[a, b] Means having a different letter are significantly different ($p<0.05$, time efffect)
[x, y] Means having a different letter are significantly different ($p<0.05$, depth efffect)