

T.R.
GEBZE TECHNICAL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

**EXTENDING SHELF-LIFE OF MUSHROOM (*AGARICUS*
BISPORUS): MICROBIAL PARAMETER ESTIMATION AND
COMPUTER VISION SYSTEM DESIGN**

FATİH TARLAK
A THESIS SUBMITTED FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
DEPARTMENT OF CHEMICAL ENGINEERING

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THESIS SUPERVISOR
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MANTARIN (*AGARICUS BISPORUS*) RAF
ÖMRÜNÜN ARTTIRILMASI:
MİKROBİYAL PARAMETRE TAHMİNİ VE
BİLGİSAYARLI GÖRÜNTÜLEME SİSTEMİ
TASARIMI

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SUMMARY

Mushrooms were washed with sodium chlorite solution for 30, 60 and 120 seconds and were packed with a non-perforated PVC film. The changes in quality parameters such as colour, microbial load, firmness and weight loss were analysed throughout 12 days of storage. The quality parameters of the mushrooms washed with sodium chlorite solution for 60 seconds and packed with a non-perforated polyvinyl chloride (PVC) film were found close to those of fresh mushroom even at the end of the 12 days of storage. This showed that sodium chlorite solution could be used by the mushroom processing establishments to prolong the shelf-life of fresh mushroom. Experimental growth data of *Pseudomonas* spp. on sliced mushrooms were compared with the predicted growth data of *Pseudomonas* spp. found by microbial parameter estimation. High pseudo-determination coefficient and low mean square error values indicated that the models can be used to evaluate the growth behaviour of *Pseudomonas* spp. on sliced mushroom, set limits for the quantitative detection of the microbial spoilage and assess product shelf-life. Computer vision system was developed to measure the colour of foods as an alternative to conventional colorimeters. Data obtained by the computer vision system in the form of RGB colour units were transformed to conventional $L^*a^*b^*$ colour space. The quadratic model with the smallest error values was found to be the most appropriate model. The prediction capability of the quadratic model was tested and validated with the foods selected. High determination coefficient and low root mean square error values suggest that the computer vision system has the potential to be used as a simple, fast and inexpensive quality control tool by the food industry.

Key Words: Mushroom, Sodium Chlorite, Shelf-life, Predictive Modelling, Computer Vision System, Colour.

ÖZET

Kültür mantarları (*Agaricus bisporus*) sodyum klorit ile 30, 60 ve 120 saniye boyunca yıkandı ve deliksiz PVC film ile paketlenildi. Renk, mikrobiyal yük, sertlik ve ağırlık kaybı gibi kalite parametrelerindeki değişimler 12 gün boyunca analiz edildi. Sodyum klorit çözeltisi ile 60 saniye yıkanmış ve perforesiz polivinil klorür (PVC) film ile paketlenmiş mantarların kalite parametreleri 12 günlük depolamanın sonunda bile taze mantarlara çok yakın bulunmuştur. Sodyum klorit çözeltisinin mantar işleyen tesisler tarafından taze mantarların raf ömrünü uzatmak için kullanılabileceği görülmüştür. Dilimlenmiş mantarlar üzerindeki *Pseudomonas* spp.'nin deneysel mikrobiyal büyüme değerleri ile mikrobiyal parametre tahmini yöntemiyle bulunan *Pseudomonas* spp.'nin tahminsel büyüme değerleri karşılaştırıldı. Yüksek pseudo-determinasyon katsayısı ve düşük ortalama karesel hata değerleri modellerin dilimlenmiş mantar üzerindeki *Pseudomonas* spp.'nin büyüme davranışını değerlendirmek, mikrobiyolojik bozulmanın niceliksel tespiti için limit koymak ve ürün raf ömrünü değerlendirmek için kullanıldı. Geleneksel renk ölçüm cihazlarına alternatif olarak gıdaların rengini ölçmek için bilgisayarlı görüntüleme sistemi geliştirildi. Bilgisayarlı görüntüleme sistemi tarafından elde edilen RGB renk değerleri, doğrusal ve karesel modeller kullanılarak geleneksel $L^*a^*b^*$ renk değerlerine dönüştürüldü. Küçük hata değerlerine sahip olan karesel model en uygun model olarak bulundu. Karesel modelin tahmin başarısı seçilen gıdalarla test ve valide edildi. Yüksek determinasyon katsayısı ve düşük ortalama karesel hatanın karekökü değerleri, bilgisayarlı görüntüleme sisteminin gıda sanayinde basit, hızlı ve ucuz bir kalite kontrol yöntemi olarak kullanılma potansiyeline sahip olduğunu göstermektedir.

Anahtar Kelimeler: Mantar, Sodyum Klorit, Raf Ömrü, Tahminsel Modelleme, Bilgisayarlı Görüntüleme Sistemi, Renk.

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LIST of ABBREVIATIONS and ACRONYMS

<u>Abbreviations</u> <u>and Acronyms</u>	<u>Explanations</u>
Δe^*	: Total colour difference
L^*	: Lightness component or luminance from black (0) to white (100)
a^*	: Chromatic component from green (-120) to red (+120)
b^*	: Chromatic component from blue (-120) to yellow (+120)
σ	: Standard deviation
$ \bar{e} $	: Mean absolute error
x_0	: Initial number of microorganisms
$x_{\max}$	: Maximum number of microorganisms
λ	: Lag phase duration
$r_{\max}$	: Maximum specific growth rate
E_a	: Activation energy
pseudo- R^2	: Coefficient of determination for nonlinear regression
A_f	: Accuracy factor
B_f	: Bias factor
BI	: Browning Index
CFU	: Colony-Forming Unit
MAP	: Modified Atmosphere Packaging
MSE	: Mean Square Error
PVC	: Polyvinyl Chloride
RGB	: Red-Green-Blue
RMSE	: Root Mean Squared Error
s	: Second
SC	: Sodium Chlorite

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1. INTRODUCTION

Mushrooms have been consumed as a source of food and medicine for centuries because of their high amounts of proteins, minerals and bioactive compounds [Wani et al., 2010]. Mushrooms are also one of the best sources of vitamins, especially vitamin B. More than hundreds of edible mushroom species exist in nature, but a few of those are cultivated commercially on industrial scale. Among these species, the button mushroom (*Agaricus bisporus*) is the most common edible mushroom all over the world.

The button mushroom is a saprophyte, and traditionally commercial production of button mushroom requires a specific organic medium that is made from agricultural materials such as hay, straw, horse manure and poultry litter. The process of preparation of this organic medium is called composting, and the final product of composting process is known as compost. The compost provides large amounts of carbon and nitrogenous compounds that are easily available for the button mushroom.

In the composting process, although pasteurisation is done to the compost, not all the microbial load is inactivated. Therefore, the button mushroom that takes all necessary nutrients from compost is very susceptible to contamination with the spoilage microorganisms such as *Pseudomonas* spp. [Simón et al., 2005], [Venturini et al., 2011] and *Bacillus subtilis* [Amner et al., 1988]. This contamination even may be with pathogen microorganisms such as *Listeria monocytogenes* [Heisick et al., 1989], [Samadpour et al., 2006], *Salmonella* spp. [Samadpour et al., 2006], *enterohemorrhagic Escherichia coli* [Samadpour et al., 2006], *Campylobacter jejuni* [Doyle and Schoeni, 1986] and *Staphylococcus aureus* [González-Fandos et al., 2000].

There have been no reported outbreaks associated with consumption of the button mushroom. However, there have been several studies that some pathogens can be isolated from the button mushroom. Some of these pathogens are *Campylobacter jejuni*, *Listeria* spp., *Salmonella* spp. and *enterohemorrhagic Escherichia coli*. Doyle and Schoeni isolated *Campylobacter jejuni* in 3 out of 200 (1.5%) retail mushroom packages obtained from several Midwestern U.S. grocery stores [Doyle and Schoeni, 1986]. The authors reported that contamination might

have occurred through contact with inadequately pasteurized compost, casing material or by hand contact. Heisick et al. reported that *Listeria* spp. were found in 11 out of 92 (12.0%) mushroom samples [Heisick et al., 1989], and in another work Viswanath et al. found that *Listeria* spp. were found in 29 out of 184 (15.8%) mushroom samples [Viswanath et al., 2013]. Samadpour et al. reported that *Salmonella* spp. were found in 5 out of 100 mushrooms while *enterohemorrhagic Escherichia coli* were isolated from 4 out of 100 mushroom samples [Samadpour et al., 2006].

Pseudomonas spp. were highly isolated from the button mushrooms. The initial number of *Pseudomonas* spp. on mushrooms was found to be 6.9 log₁₀ CFU/g [González-Fandos et al., 2006], 7.8 log₁₀ CFU/g [Simón et al., 2005] and 8.1 log₁₀ CFU/g [Venturini et al., 2011]. These results show that *Pseudomonas* spp. are the most abundant bacterial genus at harvest time and are highly isolated from the button mushrooms [Soler-Rivas et al., 1999], [Wong and Preece, 1982].

The button mushrooms have no cuticle to protect them from physical damage, microbial attack and water loss. They have a very high respiration rate and high water content, making them prone to microbial spoilage and enzymatic browning [Brennan et al., 2000]. Therefore, the shelf-life of mushrooms is very limited, as compared to most vegetables especially at room temperature and they lose their commercial value within a few days. This is a big problem for the distribution and marketing of the fresh mushrooms. Therefore, prolonging the shelf-life of mushrooms without any adverse effect on their quality would be important for the mushroom industry as well as the consumers. There are extensive research going on finding an appropriate technology and method to extend the shelf-life of mushrooms. However, the shelf-life of the button mushrooms is still limited.

Predictive models are considered as important tools to assess product shelf-life and food safety, to perform hazard analysis and set critical control points, and to develop risk assessment plans. The use of predictive models is a very quick, efficient and cost effective way of assessing the potential for growth or predicting the growth of microorganisms under specific conditions without needing long, expensive and time consuming analysis techniques and specialized people. Microbial load can be determined with traditional microbial enumeration techniques, but these techniques used to determine microbial load in foods are time consuming and expensive, and they require specialized people. In addition, these techniques give information only

for conditions that the analysis was conducted, and the use of traditional enumeration techniques is not practical to determine the growth of microorganisms under variable environmental conditions. Predictive models, on the other hand, are useful in predicting the growth of microorganisms in foods during processing and storage in real time situations [Bovill et al., 2001]. Predictive models were used to describe the growth behaviour of spoilage microorganisms in which the time to reach a specified target level under variable temperature conditions is the most important parameter. The modelling of the growth behaviour of *Pseudomonas* spp. being one of the most abundant microorganisms widely isolated from various foods especially fresh button mushrooms is very important to determine optimum storage conditions for the mushrooms and to predict their shelf-life easily.

Colour analysis is an important issue for the food industry during harvesting, processing and preservation of foods. In industry, the colour of foods has generally been measured using $L^*a^*b^*$ or CIELab colour space, which is an international standard. In the CIELab colour space, L^* is the lightness component or luminance between 0 and 100, a^* is a chromatic component between -120 and +120 from green to red and b^* is a chromatic component between -120 and +120 from blue to yellow. In food industry, colour is analysed using commercial colour meters such as Minolta Chroma Meter and Hunterlab Color Difference Meter [Gras et al., 1990], [Holman et al., 2015]. However, colorimetric results usually vary due to the fact that measurements taken by a colorimeter are based on a number of point measurements, and the average of these measurements does not represent the whole colour of the object. This approach does not reflect the colour variation throughout the object, and the variation in colour is more dramatic when especially the surface to be measured is quite non-uniform. Therefore, it is hard to obtain reproducible results from the colorimetric measurements even if they are taken from the same point [Baardseth et al., 1988]. Alternatively, colour of foods can rapidly and accurately be analysed by using computerised image analysis techniques, known as “computer vision systems” [Mendoza et al., 2006]. Computer vision systems can be used to measure attributes of total appearance together with the measurement of uneven colouration [Brosnan and Sun, 2004], [Du and Sun, 2004]. Such systems provide an automated, cost-effective and non-destructive alternative to the conventional colour meter systems [Brosnan and Sun, 2004], [Yam and Papadakis, 2004].

1.1. Hypothesis

Shelf-life of mushrooms can be extended with antimicrobial agents that do not adversely affect the quality parameters of the button mushroom “*Agaricus bisporus*”. If the growth behaviour of the microorganisms on the button mushrooms is simulated with time under different conditions, optimum storage conditions can be determined to extend the shelf-life of the button mushrooms and mushroom shelf-life can be predicted easily using predictive models. Because colour analysis is the most important key response to determine the shelf-life of a food particularly the button mushrooms, if an alternative colour analysing method is developed, the colour analyses of the mushrooms can be performed easily, quickly and inexpensively.

1.2. Objectives

The objectives of this study are to:

- i) determine the effects of the washing with sodium chlorite solution and packaging with a non-perforated PVC film on the quality parameters of the button mushroom.
- ii) develop the primary and secondary models to describe the growth behaviour of *Pseudomonas* spp. on the button mushrooms and to determine the shelf-life of the mushrooms under different conditions by considering their microbial count.
- iii) develop a colour vision system to rapidly and accurately measure and analyse the colour of foods and to implement the colour vision system as a methodology in colour analysis of real foods.

2. METHODOLOGY

Details of the materials used and methods followed to:

- i) determine the effects of the washing with sodium chlorite solution and packaging with a non-perforated PVC film on the quality parameters of the button mushroom are explained in the “Materials and Methods for Extending Shelf-life of Mushroom”.
- ii) evaluate the growth behaviour of *Pseudomonas* spp. on sliced mushroom, set limits for the quantitative detection of the microbial spoilage and assess product shelf-life are explained in the “Materials and Methods for Microbial Parameter Estimation”.
- iii) develop a colour vision system to rapidly and accurately measure and analyse the colour of foods and to implement the colour vision system as a methodology in colour analysis of real foods are explained in the “Materials and Methods for Computer Vision System”.

3. EFFECTS of DIFFERENT WASHING TREATMENTS WITH SODIUM CHLORITE on QUALITY of BUTTON MUSHROOM (*AGARICUS BISPORUS*)

In recent years, there has been a rapid increase in the trade of fresh mushrooms. However, the shelf-life of mushrooms is very limited. This is an obstacle for the distribution and marketing of the fresh mushrooms. There are several methods to extend the shelf-life of mushrooms. The most preferred preservation methods are modified atmosphere packaging (MAP) and washing treatments to control respiration rate, enzymatic and microbiological activity of the mushrooms. Examples from the important studies related to the application of MAP and washing treatments in mushrooms are given below:

MAP is a technique used for prolonging the shelf-life of fresh or minimally processed foods. MAP consists of altering the composition of air to provide an atmosphere for decreasing the product's respiration rate, preserving quality and increasing shelf-life. This can be achieved by using active or passive modification. Active modification occurs by the displacement of gases in the package in which a desired mixture of gases is flushed into the package. On the other hand, passive modification occurs when the product is packed using a selected film type, and a desired atmosphere develops naturally as a consequence of product respiration and diffusion of gases through the film.

The respiration rate of button mushrooms is very high; therefore, MAP technique is commonly used for decreasing respiration rate and increasing shelf-life of button mushrooms by the mushroom industry. The storage temperature and the surrounding gas composition showed a major effect on the respiration rates of whole and sliced button mushrooms [Oliveira et al., 2012]. Results showed that both O₂ consumption and CO₂ production rates increased significantly as the storage temperature increased, and the gas levels (low O₂/high CO₂) reduced the respiration rates of whole and sliced mushrooms.

Simón et al. investigated the effect of passive MAP method using two different packaging films on the quality of sliced button mushrooms. For this purpose, the sliced mushrooms were packed and stored at 4 °C, and carbon dioxide and oxygen

content inside the packages were determined. The authors suggested a modified atmosphere containing 2.5% CO₂ and 10-20% O₂ because this gas mixture reduced the microbial counts and improved the appearance of button mushrooms as compared to the air atmosphere [Simón et al., 2005].

In the study implemented by Simón et al. the effect of washing the mushrooms with citric acid before slicing and the effect of MAP on the sensorial and microbiological quality of sliced mushrooms stored at 5 °C for up to 17 days was evaluated. The atmosphere generated with the perforated PVC film was similar to that of air atmosphere. CO₂ concentrations inside the packages having the non-perforated PVC films ranged from 3.1% to 6.9% while O₂ concentrations ranged from 2% to 6% depending on the storage time. Washing with citric acid reduced initial microbial counts by 2.5 log₁₀ CFU/g, and MAP also reduced the microbial counts by 0.8 log₁₀ CFU/g throughout the storage. The authors recommended washing with citric acid combined with packaging under modified atmosphere to improve quality parameters and extend the shelf-life of button mushrooms [Simón et al., 2010].

Lagnika et al. evaluated the effect of high argon pressure combined with active MAP processing (a gas mixture consisting of 5% O₂ and 10% CO₂) on the physicochemical and microbiological characteristics of button mushrooms stored at 4 °C for 15 days. Results showed that button mushrooms treated with MAP had the lowest polyphenol oxidase (PPO) activity and high phenolic contents. High argon pressure treatment reduced weight loss, and increased protein and flavonoid contents. Combining high argon pressure with MAP showed to be an effective treatment in reducing respiration rate, colour change and microbial deteriorations [Lagnika et al., 2011].

Jiang dipped button mushrooms in different concentrations (1%, 2% and 3%) of alginate for 2 minutes, then placed them in jars and ventilated the button mushrooms continuously with 100% O₂ for up to 16 days at 4 °C. Mushroom respiration rate, weight loss, texture, colour, percent open caps, chemical properties and activities of PPO, and peroxidase (POD) were measured. Results indicated that treatment with 2% alginate followed by ventilation with 100% O₂ maintained a high level of firmness, and delayed browning and cap opening [Jiang, 2012].

Microbial counts of *Agaricus bisporus* can be reduced by washing the mushrooms with solutions containing antimicrobial agents and/or antibrowning

compounds that do not adversely affect organoleptic quality [Simón et al., 2010]. In the last decade, mushroom washing has gained commercial popularity as a means of improving appearance by removing casing soil particles.

Brennan et al. soaked whole fresh mushrooms in solutions of citric acid and hydrogen peroxide for 10 minutes and then sliced, packed and stored at 4 °C for up to 19 days. Citric acid treatments reduced the counts of *Pseudomonas* spp. almost 2.5 log₁₀ CFU/g while hydrogen peroxide treatments reduced *Pseudomonas* spp. counts lower than 2.0 log₁₀ CFU/g. Yellowing and shrinkage of the cap surface were observed, and organoleptic properties were adversely affected by the treatments [Brennan et al., 2000].

Simón and González-Fandos investigated the effect of washing with a solution of 10 g/L citric acid and 100 mg/L acidified sodium hypochlorite solution (pH 6.5) on the visual and microbiological quality of whole mushrooms stored at 3 °C for up to 13 days. Washing with citric acid reduced *Pseudomonas* spp. counts almost 2 log₁₀ CFU/g throughout storage at 3 °C, and avoided bacterial blotch during 13 days of storage, but colour change was observed. Washing with sodium hypochlorite hardly reduced *Pseudomonas* spp. and caused mushroom browning [Simón and González-Fandos, 2010].

Singla et al. evaluated the use of malic acid on the quality and shelf-life of *Agaricus bisporus* during post-harvest storage at 4 and 15 °C. For this purpose, mushroom slices were artificially inoculated with five foodborne pathogens including *Escherichia coli* O157:H7, *Cronobacter sakazakii*, *Salmonella typhimurium*, *Listeria monocytogenes* and *Campylobacter jejuni* [Singla et al., 2012]. They reported that malic acid treatments inactivated some foodborne pathogens, but shelf-life is still limited with 4-5 days.

Sodium chlorite (SC) is an effective antimicrobial and antibrowning agent. The use of SC solution (0.5-1.2 g/L) has been approved by the US Food and Drug Administration (FDA) for sanitizing food products, including processed fruits and vegetables. Lu et al. used SC solution with the concentration ranging from 0.1 to 1 g/L to inhibit enzymatic browning in apple slices and found that the SC solution of 1 g/L was the most effective treatment in reducing browning in sliced apples [Lu et al., 2007]. Allende et al. used SC solution (1 g/L) to reduce total aerobic mesophilic bacteria and *E. coli* O157:H7 on the fresh-cut cilantro in which total aerobic mesophilic bacteria and *E. coli* O157:H7 were reduced by 3 log₁₀ CFU/g [Allende et

al., 2009]. Wang et al. used SC solution up to 1 g/L concentration to decrease the counts of inoculated *Salmonella typhimurium* and *Listeria monocytogenes* in chicken breast, and found that the SC solution of 1 g/L resulted in 1.1 and 1.9 log₁₀ CFU/g reductions in *Salmonella typhimurium* and *Listeria monocytogenes*, respectively [Wang et al., 2014]. Ban et al. dipped honey pomelo fruit in SC solution (0.6 g/L) for 5 minutes, and the reductions of mesophilic and psychrotrophic microorganisms were found to be 0.48 and 1.67 log₁₀ CFU/g, respectively [Ban et al., 2015]. These studies revealed that SC solution can be used as an antimicrobial and antibrowning agent to inhibit the growth of microorganisms and browning on button mushrooms.

3.1. Materials and Methods for Extending Shelf-life of Mushroom

Button mushrooms (*Agaricus bisporus*) were obtained from MUPA Agriculture and Industry Inc. (Izmit, Kocaeli, Turkey). The mushrooms were harvested at the closed cap stage with the cap diameter of 3.5-4.5 cm. After harvesting, the mushrooms were immediately shipped to the laboratory under chilled conditions. Stipes were trimmed at 1 cm, and the whole mushrooms were washed with 1 g/L SC (Merck KGaA, Darmstadt, Germany) solution for 30, 60 and 120 s. The whole mushrooms treated with SC solution for 30, 60 and 120 s were placed in polystyrene trays (125 g/tray) with the dimension of 22.5 × 13.5 × 3 cm. Some of the trays were overwrapped with a non-perforated PVC film with a thickness of 9 µm and O₂ permeability of 33000 mL m⁻² day⁻¹ atm⁻¹ under passive MAP conditions, and the others were not overwrapped with any kind of packaging film. The rest of the mushrooms were not subjected to any treatment and not overwrapped with any wrapping materials. All the mushrooms were stored at 4 °C ± 0.2 °C for 12 days in climatic cabinets. Mushrooms used in the experiments were categorized as follows:

- i) Control: Mushrooms put into trays without any treatment and stored without any overwrapping material,
- ii) Control-P: Mushrooms put into trays without any treatment and stored with a non-perforated PVC overwrapping material,

- iii) SC-30: Mushrooms washed with sodium chlorite solution for 30 s without any overwrapping material,
- iv) SCP-30: Mushrooms washed with sodium chlorite solution for 30 s and stored with a non-perforated PVC overwrapping material,
- v) SC-60: Mushrooms washed with sodium chlorite solution for 60 s without any overwrapping material,
- vi) SCP-60: Mushrooms washed with sodium chlorite solution for 60 s and stored with a non-perforated PVC overwrapping material,
- vii) SC-120: Mushrooms washed with sodium chlorite solution for 120 s without any overwrapping material,
- viii) SCP-120: Mushrooms washed with sodium chlorite solution for 120 s and stored with a non-perforated PVC overwrapping material.

3.1.1. Colour Analysis

The cap colour of seven mushrooms for each treatment and control samples were measured in CIELab colour space where L^* is lightness component or luminance from black (0) to white (100), a^* is a chromatic component from green (−120) to red (+120) and b^* is a chromatic component from blue (−120) to yellow (+120) using a chroma meter (Konica Minolta, CR-400, Tokyo, Japan) equipped with a D_{65} illuminant source. Prior to colour measurements, the chroma meter was calibrated with its white calibration tile ($Y = 86.6$, $x = 0.3188$ and $y = 0.3364$). Browning index (BI) and total colour difference (Δe^*) values are calculated using Equation (3.1) and Equation (3.2), respectively, [Borchert et al., 2014]:

$$BI = \frac{100(x - 0.31)}{0.17} \quad (3.1)$$

$$\Delta e^* = \sqrt{(L_i^* - L^*)^2 + (a_i^* - a^*)^2 + (b_i^* - b^*)^2} \quad (3.2)$$

where L_i^* , a_i^* and b_i^* are the initial colour values, L^* , a^* and b^* are the final colour values and $x = (a^* + 1.75L^*)/(5.645L^*) + (a^* - (3.012b^*))$.

3.1.2. Microbiological Analysis

Twenty-five grams of mushrooms were aseptically weighed and homogenized using a stomacher (Interscience, Bag Mixer 400VW, Woburn, MA, USA) at high speed for 2 minutes with 225 ml of sterile peptone (0.1%, w/v) water (Oxoid, Basingstoke, UK). Serial dilutions (10^{-2} - 10^{-10}) were made in serial dilution tubes by taking 1 ml sample with 9 ml of 0.1% (w/v) sterile peptone water. *Pseudomonas* spp. were determined in King's B medium [King et al., 1954], following incubation at 25 °C for 48 hours. Three different trays were used at each treatment and each sampling point. Results were expressed as the average of three measurements in terms of $\log_{10}$ CFU/g.

3.1.3. Texture Measurements and Weight Loss Analysis

The firmness of the mushrooms was measured using a texture analyser (Lloyd TA1, Lloyd Instruments Ltd., West Sussex, UK) equipped with a load cell of 250 N. Cylindrical probe with a 12.5 mm diameter was used for the whole mushrooms. The probe speed was set to a constant speed of 2 mm/s, and the contact force was 0.5 N. Samples were penetrated to a depth of 5 mm. Force and time data were recorded with Llyod software, and the maximum force was expressed as the firmness (N) of the mushrooms. Results were reported as the average of seven measurements.

The weight loss of the mushrooms was determined using a digital balance (Mettler Toledo, PB03-S, Columbus, OH, USA) with an accuracy of ± 0.001 g. Triplicate measurements were done to determine the weight loss. The weight loss (%) is given by Equation (3.3):

$$w_l(\%) = \frac{w_i - w}{w_i} \times 100 \quad (3.3)$$

where w_l is the weight loss (%), w_i is the initial weight (g) and w is the final weight (g) of the mushrooms.

3.1.4. Gas Composition Analysis and Measurement of pH

Oxygen and carbon dioxide levels inside the packages were measured using a gas analyser (Check Mate-II, PBI Dansensor, Ringsted, Denmark). Samples were taken with a syringe inserted through a gas tight septum placed on the exterior of the film. Measurements were performed in triplicate.

Mushrooms were homogenized using a kitchen type blender (Tefal, MB450, Cedex, France), and homogenised mushrooms were filtered through a muslin cloth. The pH of the filtrate was measured using a digital pH meter (SevenEasy S20-K, Mettler Toledo, Columbus, OH, USA). Measurements were done in triplicate.

3.1.5. Statistical Analysis

Experiments were performed using a completely randomized design. Data were subjected to one-way analysis of variance (ANOVA) using Matlab 7.12.0 (R2011a) software (MathWorks Inc., Natick, MA, USA). Statistical differences between the treatment means were determined by post hoc analysis using Tukey's multiple range test. The differences between the means are regarded as statistically significant if the p-value ≤ 0.05 .

3.2. Results and Discussion for Extending Shelf-life of Mushroom

3.2.1. Effect of Washing with Sodium Chlorite and Packaging on Mushroom Colour

Colour change in mushrooms is a crucial factor which directly affects consumer preference. The L^* value of the all the mushrooms decreased while BI and ΔE^* values increased during storage (Table 3.1). The L^* value of the fresh mushrooms was 90.14. Previously conducted studies on *Agaricus bisporus* showed that L^* value could be used as a criterion to determine the mushroom quality and shelf-life [Aguirre et al., 2008], [Gormley, 1975], [Taghizadeh et al., 2010]. Mushrooms with L^* value of less than 80 would not be acceptable at wholesale level [Gao et al., 2014], [Gormley, 1975], [Lagnika et al., 2011], [Lagnika et al., 2014],

[Lopez-Briones et al., 1993]. This grading criterion is the most widely used indicator to determine the mushroom shelf-life both in the industry and research [Aguirre et al., 2008].

Washing the mushrooms with the SC solution and packaging with a non-perforated PVC film slowed down the colour change of the mushrooms (Table 3.1). At the 4 days of storage, the L^* value of control was statistically different ($p \leq 0.05$) from the L^* value of the fresh mushrooms while there was no statistical difference ($p > 0.05$) in the L^* value of SCP-120 as compared with the L^* value of the fresh mushrooms at the 8 days of storage. The L^* value of SCP-120 as compared with the L^* value of the fresh mushrooms started to change at the 12 days of storage. This means that washing of mushrooms with the SC solution and packaging with a non-perforated PVC film retard browning of the mushrooms and retain the original colour of the mushrooms up to 12 days of storage. BI values followed an opposite trend as compared with the L^* values for each sampling day, and an increase in BI means that mushrooms become darker and the colour goes from white to brownish colour. The change in the colour of mushrooms is considered to occur due to the reactions between phenols and polyphenol oxidases [Cliffe-Byrnes and O'Beirne, 2010], [Simón and González-Fandos, 2010], [Xu et al., 2016].

The smallest Δe^* values were found for the SCP-60 and SCP-120 at each sampling day. Statistical analysis showed that there was no significant difference ($p > 0.05$) between the L^* , BI and Δe^* values of mushrooms denoted by SCP-60 and SCP-120 at each sampling day. The final BI and Δe^* values of SCP-60 were found to be slightly lower than the final BI and Δe^* values of SCP-120. This shows that subjecting mushrooms to SC solution longer than 60 s do not have a significant ($p \leq 0.05$) effect on colour. Therefore, SCP-60 was found to be the most appropriate treatment to minimize the colour change in *Agaricus bisporus*. The change in colour of mushrooms during 12 days of storage taken in the form of digital images by a computer vision system designed in this study is presented from Figures 3.1 to 3.4.

Table 3.1: Effect of different washing treatments on the colour of mushrooms stored at 4 °C for 12 days.

Colour	Treatments	Storage time (day)			
		0	4	8	12
L *	Control		84.35 ± 2.43 ^{Bb}	75.65 ± 4.36 ^{Cf}	66.01 ± 1.73 ^{Dc}
	Control-P		86.36 ± 3.49 ^{Aab}	79.11 ± 5.11 ^{Bef}	74.85 ± 7.06 ^{Bb}
	SC-30		85.75 ± 2.24 ^{Bab}	81.34 ± 1.79 ^{Ced}	74.35 ± 3.71 ^{Dabc}
	SCP-30	90.14 ± 1.12 ^A	87.31 ± 0.76 ^{Aab}	84.03 ± 2.34 ^{Bbcd}	79.46 ± 1.87 ^{Cab}
	SC-60		86.17 ± 2.58 ^{ABab}	82.47 ± 3.27 ^{Bcde}	76.78 ± 2.67 ^{Cab}
	SCP-60		87.81 ± 1.77 ^{ABa}	86.48 ± 1.53 ^{Babc}	82.04 ± 1.43 ^{Cab}
	SC-120		88.36 ± 1.35 ^{ABa}	87.66 ± 1.44 ^{Bab}	80.22 ± 2.58 ^{Cab}
	SCP-120		89.37 ± 2.39 ^{Aa}	89.11 ± 0.97 ^{Aa}	82.65 ± 5.30 ^{Ba}
BI	Control		28.93 ± 3.77 ^{Ba}	48.76 ± 7.56 ^{Ca}	57.05 ± 2.55 ^{Da}
	Control-P		24.63 ± 4.79 ^{Aab}	40.92 ± 8.00 ^{Bb}	49.83 ± 10.75 ^{Bab}
	SC-30		26.17 ± 2.57 ^{Aab}	35.54 ± 7.15 ^{Bbc}	45.73 ± 11.24 ^{Babc}
	SCP-30	17.37 ± 2.26 ^A	21.62 ± 2.98 ^{Abc}	27.41 ± 4.00 ^{Bde}	34.37 ± 3.69 ^{Cbc}
	SC-60		24.39 ± 3.98 ^{Aabc}	33.79 ± 4.38 ^{Bbcd}	41.82 ± 7.86 ^{Bbc}
	SCP-60		23.52 ± 2.90 ^{Bbc}	25.87 ± 3.80 ^{Bde}	33.96 ± 3.52 ^{Cc}
	SC-120		24.57 ± 2.58 ^{Bab}	27.74 ± 2.81 ^{Bcde}	42.27 ± 7.06 ^{Cabc}
	SCP-120		18.88 ± 4.36 ^{Ac}	20.68 ± 2.09 ^{Ae}	37.61 ± 6.45 ^{Bbc}
Δe*	Control		7.92 ± 2.66 ^a	19.41 ± 4.13 ^a	27.00 ± 1.60 ^a
	Control-P		5.56 ± 3.20 ^{ab}	15.32 ± 4.96 ^{ab}	20.31 ± 6.18 ^{ab}
	SC-30		6.06 ± 2.20 ^{ab}	12.06 ± 3.35 ^b	18.97 ± 5.13 ^{abc}
	SCP-30	-	3.34 ± 1.39 ^b	7.45 ± 2.73 ^c	12.75 ± 1.87 ^{bc}
	SC-60		5.11 ± 2.62 ^{ab}	10.78 ± 3.24 ^{bc}	16.52 ± 3.87 ^{bc}
	SCP-60		3.83 ± 2.10 ^b	5.50 ± 2.37 ^{cd}	11.13 ± 1.93 ^c
	SC-120		5.10 ± 1.48 ^{ab}	6.81 ± 1.62 ^c	15.45 ± 2.97 ^{bc}
	SCP-120		2.51 ± 2.16 ^b	2.53 ± 1.00 ^d	12.15 ± 6.94 ^c

Note: Control: unwashed and unpacked mushrooms. Control-P: unwashed mushrooms packed with a non-perforated PVC film. SC-30, SC-60 and SC-120 are the mushrooms washed with SC for 30, 60 and 120 s, respectively, and unpacked. SCP-30, SCP-60 and SCP-120 are the mushrooms washed with SC for 30, 60 and 120 s, respectively, and packed with a non-perforated PVC film.

^{A-D} values within the same raw followed by different letters are significantly different ($p \leq 0.05$).

^{a-f} values followed by different letters within the same group are significantly different ($p \leq 0.05$).

Data are presented as the mean ± standard deviation (n = 7).



Figure 3.1: Colour of mushrooms at the beginning of storage.

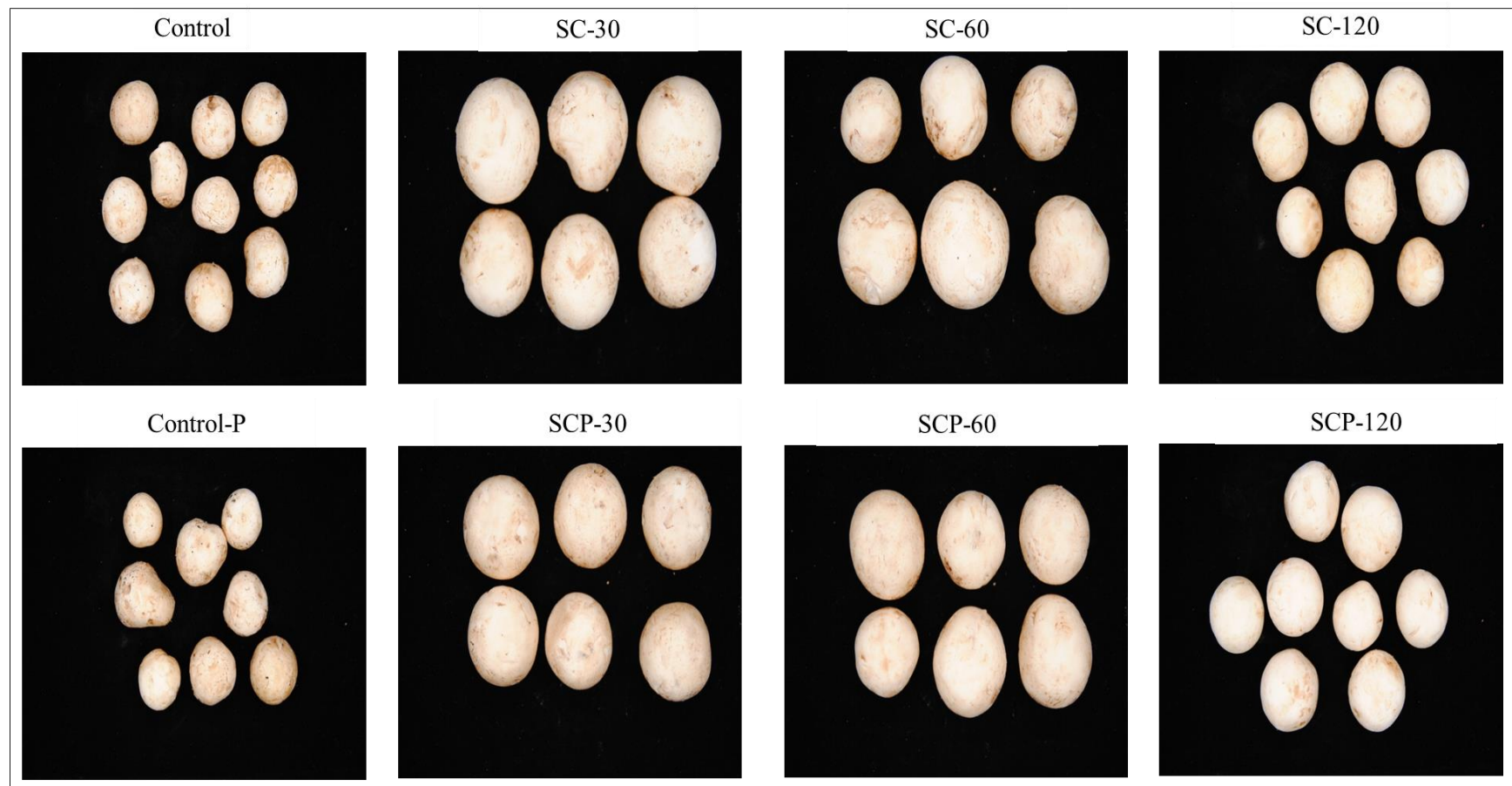


Figure 3.2: Colour of mushrooms on the fourth day of storage.



Figure 3.3: Colour of mushrooms on the eighth day of storage.

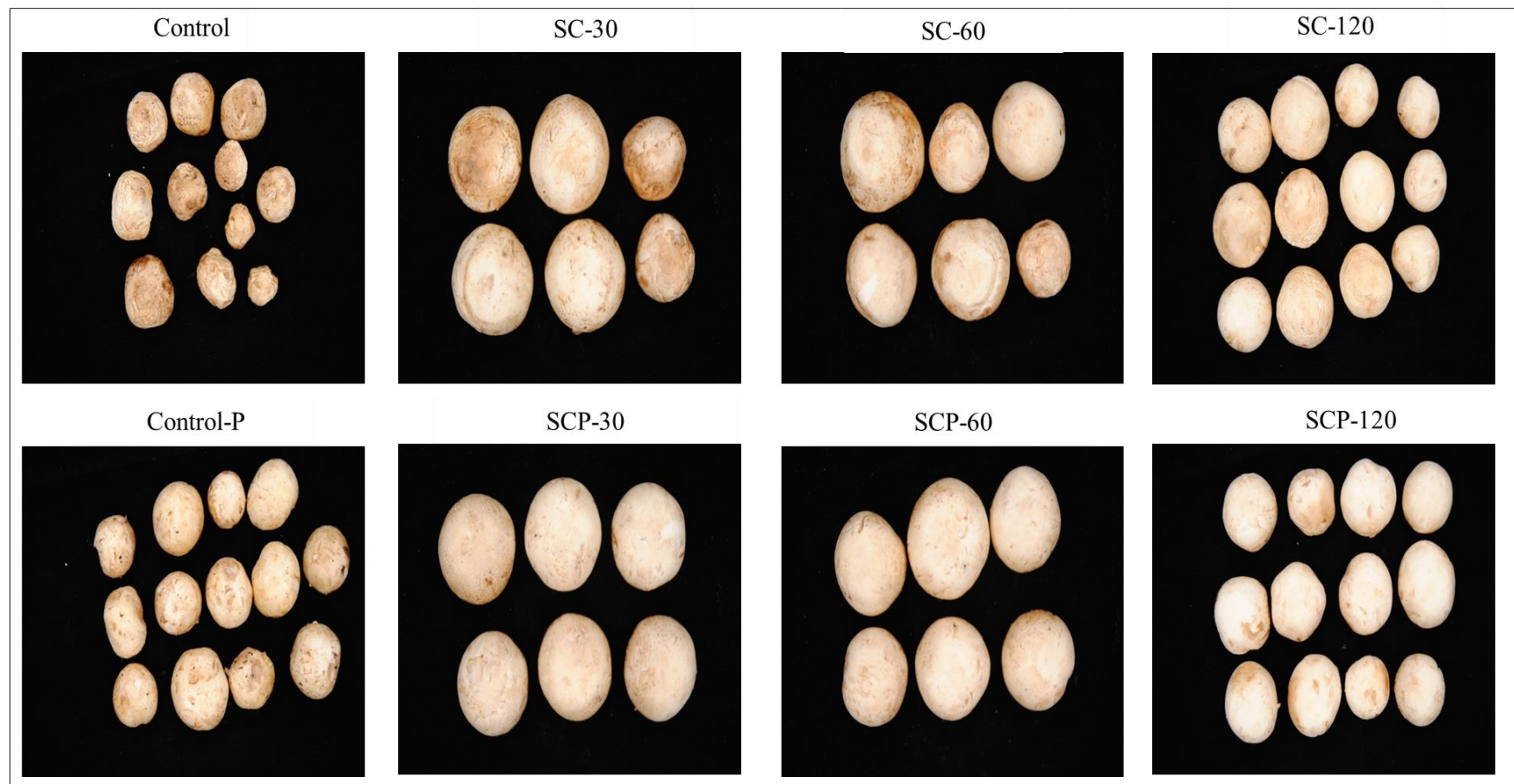


Figure 3.4: Colour of mushrooms on the twelve day of storage.

3.2.2. Effect of Washing with Sodium Chlorite and Packaging on the Number of *Pseudomonas* spp. on Mushroom

Pseudomonas spp. counts and the factors affecting the growth of *Pseudomonas* spp. play a key role in the post-harvest quality of the mushrooms. The initial count of *Pseudomonas* spp. on *Agaricus bisporus* in this study was found to be 7.59 log₁₀ CFU/g which is in good agreement with the *Pseudomonas* spp. counts on *Agaricus bisporus* which ranged from 6.90 to 8.10 log₁₀ CFU/g at harvest time [Simón et al., 2005], [Venturini et al., 2011]. Washing the mushrooms with SC solution and packaging with a non-perforated PVC film were found to be effective on the *Pseudomonas* spp. counts on mushrooms (Table 3.2).

Pseudomonas spp. counts increased with the storage time for mushrooms that are unwashed and unpacked, and mushrooms that are unwashed and packed with a non-perforated PVC film. On the other hand, washing the mushrooms with SC solution reduced the number of *Pseudomonas* spp. on mushrooms. Regardless of packaging, the number of *Pseudomonas* spp. on mushrooms washed with SC solution was lower than the initial number of *Pseudomonas* spp. on fresh mushrooms during 8 days of storage. This indicates that SC is an effective antimicrobial agent and subjecting the mushrooms to SC solution even for short times have a high lethal effect on the *Pseudomonas* spp. on mushrooms.

The lowest *Pseudomonas* spp. counts on mushrooms was found for SCP-120 at each sampling day. Statistical analysis showed that there was no significant difference ($p > 0.05$) between the numbers of *Pseudomonas* spp. for mushrooms subjected to SC solution for 60 s and 120 s at any sampling day. This result indicates that washing the mushrooms with the SC solution for 60 s or 120 s and packaging with a non-perforated PVC film have equal effect on the population of *Pseudomonas* spp. on mushrooms throughout the storage period.

Table 3.2: Effect of different treatments on the number of *Pseudomonas* spp. on mushrooms stored at 4 °C for 12 days.

Treatments	Storage time (day)		
	4	8	12
Control	8.23 ± 0.46 ^a	9.34 ± 0.52 ^a	10.30 ± 0.59 ^a
Control-P	8.14 ± 0.45 ^a	8.43 ± 0.47 ^a	10.02 ± 0.58 ^{ab}
SC-30	5.49 ± 0.32 ^b	7.26 ± 0.41 ^b	9.08 ± 0.53 ^{ab}
SCP-30	5.32 ± 0.32 ^b	7.09 ± 0.40 ^b	8.76 ± 0.52 ^{bc}
SC-60	5.11 ± 0.31 ^b	5.92 ± 0.35 ^c	7.66 ± 0.46 ^{cd}
SCP-60	4.86 ± 0.30 ^b	5.58 ± 0.33 ^c	7.40 ± 0.44 ^{cd}
SC-120	4.81 ± 0.29 ^b	5.29 ± 0.31 ^c	6.89 ± 0.42 ^d
SCP-120	4.56 ± 0.28 ^b	5.28 ± 0.32 ^c	6.62 ± 0.41 ^d

Note: Control: unwashed and unpacked mushrooms. Control-P: unwashed mushrooms packed with a non-perforated PVC film. SC-30, SC-60 and SC-120 are the mushrooms washed with SC for 30, 60 and 120 s, respectively, and unpacked. SCP-30, SCP-60 and SCP-120 are the mushrooms washed with SC for 30, 60 and 120 s, respectively, and packed with a non-perforated PVC film.

^{a-f} values followed by different letters within the same column are significantly different ($p \leq 0.05$). Data are presented as the mean ± standard deviation ($n = 3$).

3.2.3. Effect of Washing with Sodium Chlorite and Packaging on Mushroom Texture and Weight Loss

The firmness of the mushrooms is often one of the most important quality parameters considered by the consumers, and the firmness is related to metabolic changes and change in weight loss [Gao et al., 2014]. The initial firmness of the fresh mushrooms was found to be 40 N, and the firmness decreased with the storage time (Figure 3.5). In other words, all the mushroom samples softened during the 12 days of storage. The reason of the softening is the degradation of cell walls in mushrooms which is caused by bacterial enzymes and microorganisms especially *Pseudomonas* spp. that breaks down the intracellular matrix [Gao et al., 2014]. The firmness of the mushrooms washed with the SC solution was higher than the

firmness of the control. This means that bacteria-induced softening was slowed down with the aid of the SC washing treatments.

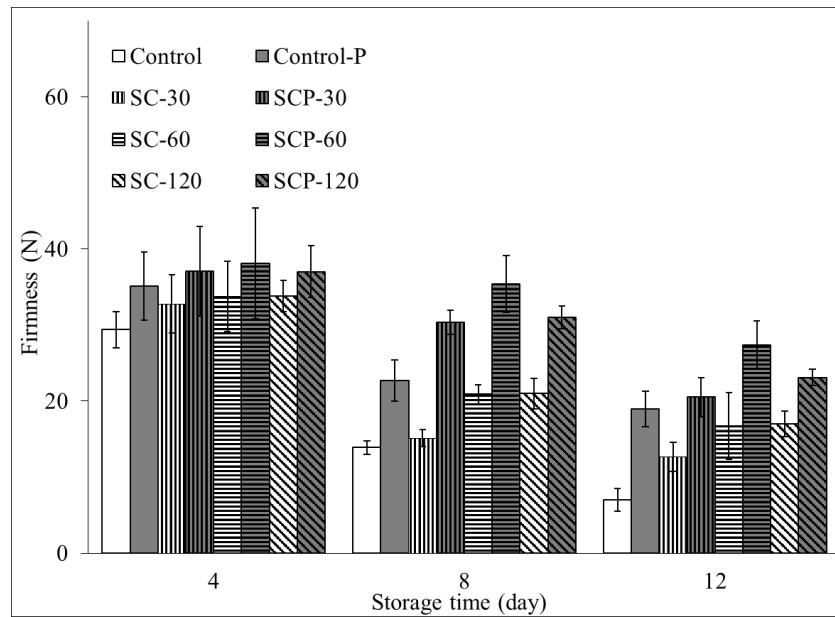


Figure 3.5: Change in firmness of mushrooms with storage time. Error bars represent the standard deviation of seven independent samples. Control: unwashed and unpacked mushrooms. Control-P: unwashed mushrooms packed with a non-perforated PVC film. SC-30, SC-60 and SC-120 are the mushrooms washed with SC for 30, 60 and 120 s, respectively, and unpacked. SCP-30, SCP-60 and SCP-120 are the mushrooms washed with SC for 30, 60 and 120 s, respectively, and packed with a non-perforated PVC film.

The firmness of all the mushroom samples packed with a non-perforated PVC film were significantly ($p \leq 0.05$) higher than the firmness of the controls at each sampling day (Figure 3.5). Similar results regarding the effect of packaging material on the firmness of mushrooms were also reported by González-Fandos et al. who found that the change in the firmness values of the mushrooms was delayed by packaging the mushrooms with a non-perforated PVC film that increases the CO_2 level in the package and decreases the respiration rate [González-Fandos et al., 2000]. At the end of 12 days of storage, the firmness value of control became 7 N while the firmness values of SCP-60 and SCP-120 were 27.4 N and 23.1 N, respectively. This indicated that subjecting the mushrooms to the SC solution longer than 60 s has an adverse effect on the texture of the mushrooms. Therefore, SCP-60 was found to be the most appropriate treatment to minimize the change in the firmness of *Agaricus bisporus*.

Dehydration is important and directly affects the mushroom quality because mushrooms lose water from their epidermal layer during the post-harvest storage [Khan et al., 2014]. Weight loss increased with the storage time for all the mushroom samples (Figure 3.6). The maximum weight loss occurred in the control and reached to almost 40% at the end of 12 days of storage. There was no statistical difference ($p > 0.05$) in the weight loss of control and the SC-30, SC-60 and SC-120 although washing the mushrooms with the SC solution slightly delayed the weight loss. When the mushrooms were packed with a non-perforated PVC film, weight loss was significantly ($p \leq 0.05$) reduced as compared with the unpacked mushrooms treated with the SC solution over the entire storage period. At the end of 12 days of storage, the weight loss for the mushroom samples packed with a non-perforated PVC film was less than 10%. This reveals that PVC film acts as a barrier against water loss from the mushrooms.

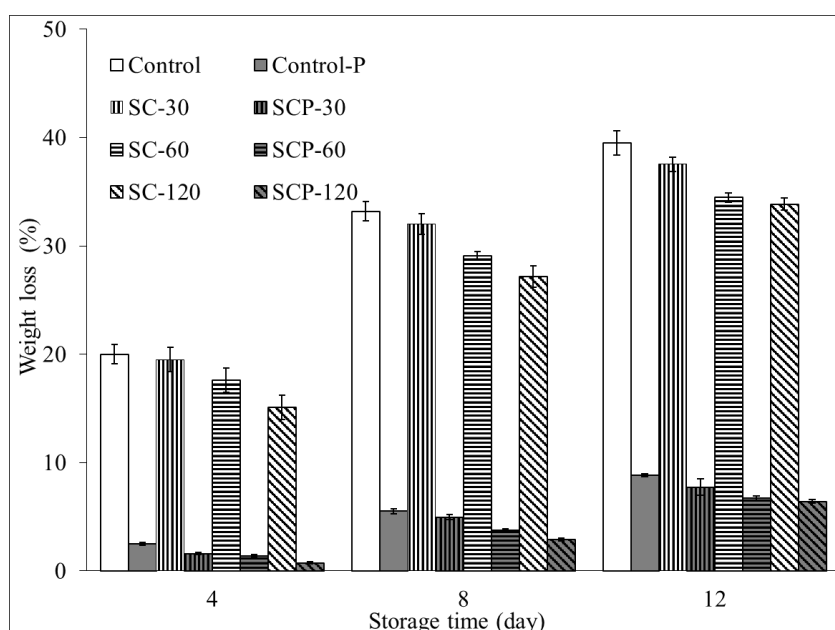


Figure 3.6: Change in weight loss of mushrooms with storage time. Error bars represent the standard deviation of three independent samples. Control: unwashed and unpacked mushrooms. Control-P: unwashed mushrooms packed with a non-perforated PVC film. SC-30, SC-60 and SC-120 are the mushrooms washed with SC for 30, 60 and 120 s, respectively, and unpacked. SCP-30, SCP-60 and SCP-120 are the mushrooms washed with SC for 30, 60 and 120 s, respectively, and packed with a non-perforated PVC film.

3.2.4. Effect of Washing with Sodium Chlorite and Packaging on Gas Composition and pH

The change in O₂ and CO₂ concentrations inside the packages was analysed since the O₂ and CO₂ levels inside the package directly affects the respiration rate. The concentrations of O₂ and CO₂ inside the packages equilibrated around 15% and 5%, respectively, at the end of 12 days of storage (Figure 3.7). The rate of change in O₂ and CO₂ levels inside the packages was high during the first 4 days of storage, and then the rate of change O₂ and CO₂ was gradual and levelled off. This means that the respiration rate of the mushrooms in the first 4 days was high and then the respiration rate decreased due to an increase in the CO₂ levels inside the packages. Similar results were also reported by Lagnika et al. who observed that an increase in CO₂ levels inside the packages caused an inhibitory effect on the respiration rate of mushrooms stored under MAP at 4 °C [Lagnika et al., 2011].

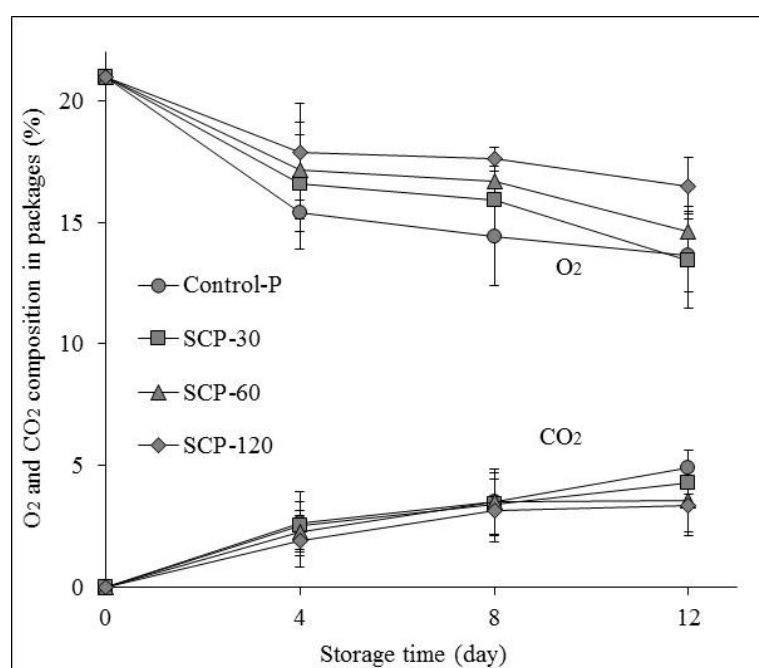


Figure 3.7: Change in gas composition inside packages with storage time. Error bars represent the standard deviation of three independent samples. Control-P: unwashed mushrooms packed with a non-perforated PVC film. SCP-30, SCP-60 and SCP-120 are the mushrooms washed with SC for 30, 60 and 120 s, respectively, and packed with a non-perforated PVC film.

pH is important during storage because it affects stability and shelf life of foods. The initial pH value of the fresh button mushroom *Agaricus bisporus* was

found to be 6.65 which is in good agreement with the pH values (6.50-6.92) reported for fresh *Agaricus bisporus* [Aday, 2016], [Masson et al., 2002]. The pH values of the mushroom samples changed between 6.58 and 6.85 during the 12 days of storage (Figure 3.8). There was no significant difference ($p > 0.05$) in pH values of the SC and SCP mushroom samples. This could be attributed to slight metabolic changes in SC and SCP mushroom samples as compared with the control.

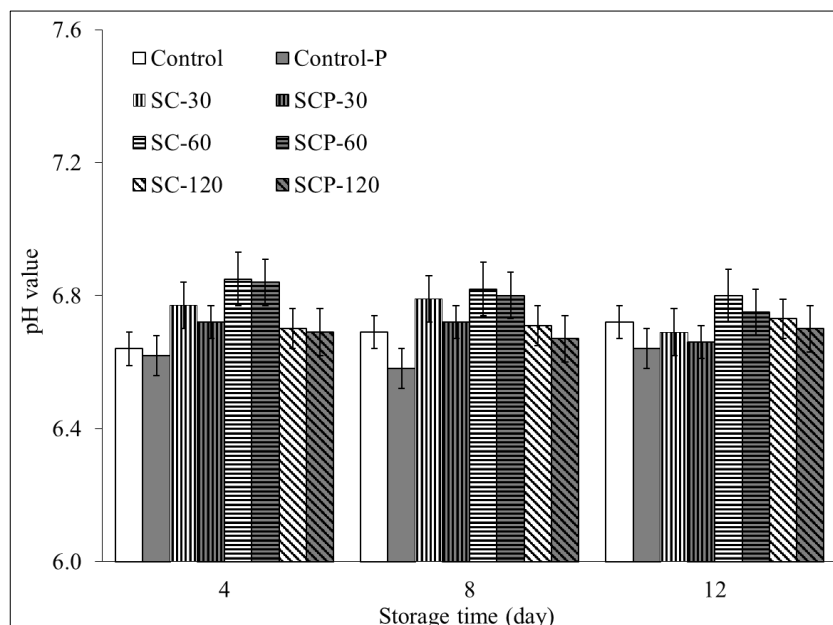


Figure 3.8: Change in pH value of mushrooms with storage time. Error bars represent the standard deviation of three independent samples. Control: unwashed and unpacked mushrooms. Control-P: unwashed mushrooms packed with a non-perforated PVC film. SC-30, SC-60 and SC-120 are the mushrooms washed with SC for 30, 60 and 120 s, respectively, and unpacked. SCP-30, SCP-60 and SCP-120 are the mushrooms washed with SC for 30, 60 and 120 s, respectively, and packed with a non-perforated PVC film.

4. MATHEMATICAL MODELLING of the GROWTH BEHAVIOUR of *PSEUDOMONAS* SPP. on *AGARICUS BISPORUS*

Mushrooms have been consumed as a source of food and medicine for centuries, because of their high amounts of proteins, minerals and bioactive compounds [Wani et al., 2010]. The cultivated button mushroom (*Agaricus bisporus*) is the most common edible mushroom in the world. *Agaricus bisporus* has a very short shelf-life because it has no cuticle to protect it from physical deterioration or microbial attack [Brennan et al., 2000]. Although no outbreak of pathogenic microorganisms such as *E. coli* O157:H7 and *L. monocytogenes* has been reported [Guan et al., 2012] for *Agaricus bisporus*, it is very susceptible to contamination with *Pseudomonas* spp. which are abundant in nature [González-Fandos et al., 2006], [Simón et al., 2005]. The *Pseudomonas* spp. are responsible for causing spoilage, and the initial count of *Pseudomonas* spp. on cultivated mushrooms is quite high, ranging from 6.90 to 8.1 log₁₀ CFU/g [Simón et al., 2005], [Venturini et al., 2011].

Predictive food microbiology aims to estimate the microbial growth using mathematical models under different conditions. These mathematical models are generally classified into three main categories known as primary, secondary and tertiary models [Whiting, 1995]. Primary models describe the growth data as a function of time under a constant environmental condition. Sigmoidal type models such as the modified Gompertz, logistic and Baranyi models are widely used as primary models for fitting microbial growth data. Secondary models describe the effects of environmental factors, such as temperature, pH and water activity (a_w) on the parameters of the primary models, including maximum specific growth rate and lag phase duration. One of the most important environmental factors from the food safety point of view is temperature. The most widely used secondary model to determine the relationship between temperature and maximum specific growth rate is known as the Ratkowsky or square root model [Ratkowsky et al., 1982]. Tertiary models combine both the primary and secondary models with user-friendly application software or expert systems to assess microbial behaviour under specific conditions [Wang et al., 2013], [Whiting, 1995].

Predictive models are considered as important tools to assess product shelf-life and food safety, to perform hazard analysis and set critical control points, and to develop risk assessment plans. Predictive models are a very quick, efficient and cost effective way of assessing the potential for growth or predicting the growth of microorganisms under specific conditions without needing long, expensive and time consuming analysis techniques and specialized people. Traditional microbial enumeration techniques give information only for conditions that the analysis was conducted, and they are not practical to determine the growth of microorganisms under variable environmental conditions. Predictive models, on the other hand, are useful in predicting the growth of microorganisms in foods during processing and storage in real time situations [Bovill et al., 2001]. Predictive models were used to describe the growth behaviour of spoilage microorganisms in which the time to reach a specified target level under variable temperature conditions is the most important parameter. The growth behaviour of *Pseudomonas* spp. being one of the most abundant microorganisms widely isolated from various foods has been studied and modelled using some predictive models. Koutsoumanis used the logistic model to describe the growth behaviour of *Pseudomonas* spp. in fish stored at temperatures ranging from 0 to 15 °C [Koutsoumanis, 2001]. Gospavic et al. used the modified Gompertz and the Baranyi models to implement a growth model for estimating the growth of *Pseudomonas* spp. in poultry under variable temperature conditions [Gospavic et al., 2008]. Zhang et al. used the Baranyi model to fit the number of *Pseudomonas* spp. in beef stored between 0 and 20 °C [Zhang et al., 2011]. Bruckner et al. used the modified Gompertz model to define growth behaviour of *Pseudomonas* spp. in pork and poultry meat stored between 2 and 15 °C [Bruckner et al., 2013]. Dabadé et al. used the Baranyi and the modified Gompertz models to describe the growth behaviour of *Pseudomonas* spp. in tropical fresh shrimp stored at temperatures ranging from 0 to 28 °C [Dabadé et al., 2015]. Lytou et al. used the Baranyi model to describe the growth of total viable bacteria including *Pseudomonas* spp. in marinated and unmarinated chicken breast fillets stored at 4, 10 and 15 °C, and correlated these data with shelf-life [Lytou et al., 2016]. Psychrotropic bacteria, mainly *Pseudomonas* spp., have been isolated as the most ubiquitous microorganisms responsible for the spoilage of mushrooms [Simón et al., 2005]. Temperature is the most important factor affecting the growth rate. The relationship between the growth rate of *Pseudomonas* spp. and the storage

temperature of sliced mushrooms was not investigated. Therefore, it is important to investigate and model the microbial growth of *Pseudomonas* spp. on sliced mushrooms.

The main objective of this part of the thesis was to develop primary and secondary models to describe the growth of *Pseudomonas* spp. on sliced mushrooms. First, several representative primary models such as the modified Gompertz, logistic and Baranyi models were used to describe the microbial growth data of *Pseudomonas* spp. on sliced mushrooms at different storage temperatures (4, 12, 20 and 28 °C). The goodness of fit of these three models were compared by considering the mean squared error (MSE) and the coefficient of determination for nonlinear regression (pseudo- R^2). Then, the maximum specific growth rate and lag phase duration derived from the primary model which has the best goodness of fit were correlated with the temperature using the Ratkowsky and Arrhenius models. Observed growth data from the independent experiments were used to validate the models. The bias factor (B_f) and accuracy factor (A_f) were employed to validate the models used. Finally, mushroom spoilage and mushroom shelf-life were correlated with the temperature using the models.

4.1. Materials and Methods for Microbial Parameter Estimation

4.1.1. Sample Preparation and Microbiological Analysis

Cultivated button mushrooms (*Agaricus bisporus*) were obtained from MUPA Agriculture and Industry Inc. (Izmit, Kocaeli, Turkey). The mushrooms were harvested at the closed cap stage with the cap diameter of 3.5–4.5 cm. After harvesting, mushrooms were immediately shipped to the laboratory under chilled conditions. Stipes were trimmed at 1 cm, and mushrooms were sliced carefully (5 mm wide) using a pre-sterilized stainless steel knife. The slices were placed in polystyrene trays (200 g/tray) with the dimension of 22.5 × 13.5 × 3 cm. The trays were not overwrapped with any packaging material and placed in an incubator at 4, 12, 20 and 28 °C. The temperature in the incubator did not vary more than ± 0.2 °C.

Twenty-five grams of mushrooms were aseptically weighed and homogenized using a stomacher (Interscience, Bag Mixer 400VW, USA) at high speed for 2 min

with 225 ml of sterile peptone (0.1%, w/v) water (Oxoid, Basingstoke, UK) for each temperature with the corresponding sampling frequency. Dilutions (10^{-2} - 10^{-10}) were made in serial dilution tubes by taking 1 ml of sample with 9 ml of 0.1% (w/v) sterile peptone water. *Pseudomonas* spp. were determined in King's B medium [King et al., 1954], following incubation at 25 °C for 48 h. Three different trays were used at each temperature and each sampling point. Results were expressed as the average of three measurements in terms of $\log_{10}$ CFU/g. Gormley reported that the mushrooms with L^* value lower than 69 were regarded as unacceptable by the consumers [Gormley, 1975]. Therefore, prior to microbiological analyses, the L^* value of the sliced mushrooms was measured using a chroma meter (Konica Minolta, CR-400, Tokyo, Japan) equipped with a D_{65} illuminant source, and microbiological analyses were carried out until the L^* value of the sliced mushrooms was lower than 69. Sampling frequency was based on the storage temperature to determine the growth behaviour of *Pseudomonas* spp. on sliced mushrooms.

4.2. Modelling

4.2.1. Primary Models

The modified Gompertz, logistic and Baranyi models were commonly used in literature as primary models which are sigmoidal functions that describe growth behaviour of microorganisms as a function of time at constant environmental conditions. The modified Gompertz and logistic models are defined by Equation (4.1) and Equation (4.2), respectively [Zwietering et al., 1990]:

$$x(t) = x_0 + (x_{\max} - x_0) \exp \left\{ -\exp \left[\frac{r_{\max} \cdot e}{(x_{\max} - x_0)} (\lambda - t) + 1 \right] \right\} \quad (4.1)$$

$$x(t) = x_0 + \frac{(x_{\max} - x_0)}{\left\{ 1 + \exp \left[\frac{4r_{\max}}{(x_{\max} - x_0)} (\lambda - t) + 2 \right] \right\}} \quad (4.2)$$

where t is the time (h), $x(t)$ is the number of microorganisms ($\log_{10}$ CFU/g) at time t , x_0 is the initial number of microorganisms ($\log_{10}$ CFU/g), $x_{\max}$ is the maximum

number of microorganisms ($\log_{10}$ CFU/g), $r_{\max}$ is the maximum specific growth rate ($\log_{10}$ CFU/h) and λ is the lag phase duration (h).

The Baranyi model at constant environmental conditions is given by:

$$x(t) = x_0 + r_{\max} \cdot A(t) - \frac{1}{\ln(10)} \cdot \ln \left(1 + \frac{e^{r_{\max} \cdot \ln(10) \cdot A(t)} - 1}{10^{(x_{\max} - x_0)}} \right) \quad (4.3)$$

where $A(t)$ is the adjustment function described by Baranyi and Roberts (1994), t is the time (h), $x(t)$ is the number of microorganisms ($\log_{10}$ CFU/g) at time t , x_0 is the initial number of microorganisms ($\log_{10}$ CFU/g), $x_{\max}$ is the maximum number of microorganisms ($\log_{10}$ CFU/g), $r_{\max}$ is the maximum specific growth rate ($\log_{10}$ CFU/h).

The growth data of *Pseudomonas* spp. obtained at isothermal conditions were fitted to three different primary models (the modified Gompertz, logistic and Baranyi models) and all parameters were calculated using ORIGIN 9.0 software (OriginLab Corporation, Northampton, MA, USA) which uses Levenberg-Marquardt algorithm.

4.2.2. Secondary Models

Secondary models are used to describe the effects of different environmental factors such as temperature, pH, a_w , oxygen availability, additives etc. on the parameters of the primary models [Juneja et al., 2007]. After the growth data were fitted to each primary model, secondary models were used. The Ratkowsky model is used to describe the relationship between maximum specific growth rate and temperature [Ratkowsky et al., 1982]. The Arrhenius model is widely used to describe the temperature dependence of chemical reactions, and is often adopted to define the effect of storage temperature on microbial growth in foods [Taoukis et al., 1999]. Therefore, the Ratkowsky (Equation 4.4) and Arrhenius (Equation 4.5) models were used to describe the effect of temperature on the maximum specific growth rate and lag phase duration obtained from the primary model that has the best goodness of fit:

$$\sqrt{r_{\max}} \text{ or } \sqrt{1/\lambda} = b_1(T - T_0) \quad (4.4)$$

$$r_{\max} \text{ or } 1/\lambda = b_2 \left[\exp \left(\frac{-E_a}{R\theta} \right) \right] \quad (4.5)$$

where T is the temperature ($^{\circ}\text{C}$), T_0 is the theoretical minimum temperature ($^{\circ}\text{C}$) for microbial growth, $r_{\max}$ is the maximum specific growth rate ($\log_{10}$ CFU/h) obtained from the primary model, λ is the lag phase duration (h) obtained from the primary model, b_1 and b_2 are the regression coefficients, E_a is the activation energy (J/mol), R is the universal gas constant (8.314 J/mol K) and θ is the absolute temperature (K). Similar to the model fitting for the primary models, the temperature data were fitted to $r_{\max}$ and λ using the ORIGIN 9.0 software for the secondary models

4.2.3. Comparison of the Goodness of Fit of the Primary and Secondary Models

The goodness of fit of the primary and secondary models used was evaluated by considering MSE and pseudo- R^2 values using Equation (4.6) and Equation (4.7), respectively:

$$\text{MSE} = \sum_{i=1}^n \frac{(\text{observed}_i - \text{fitted}_i)^2}{n - s} \quad (4.6)$$

$$\text{pseudo-}R^2 = 1 - \left(\frac{\text{SSR}}{\text{SST}_{\text{Corrected}}} \right) \quad (4.7)$$

where observed_i is the observed value, fitted_i is the fitted value, n is the number of observations, s is the number of parameters of the model, SSR is the sum of squared of residuals and $\text{SST}_{\text{Corrected}}$ is the corrected total sum of squares.

Temperature data are fitted to $\sqrt{r_{\max}}$ or $\sqrt{1/\lambda}$ for the Ratkowsky model, while temperature data are fitted to $r_{\max}$ or $1/\lambda$ for the Arrhenius model. Therefore, the MSE values obtained for the secondary models were calculated based on $r_{\max}$ and $1/\lambda$ values. The model with the lowest MSE and the highest pseudo- R^2 was selected as the best model to describe the growth behaviour of *Pseudomonas* spp. on sliced mushrooms.

4.2.4. Shelf-life Prediction

The number of *Pseudomonas* spp. on sliced mushrooms stored between 4 and 28 °C can be predicted as a function of time and storage temperature using Equation (4.8) proposed by Buchanan et al. (1997):

$$\begin{aligned}x(t) &= x_0, \text{ for } t \leq \lambda \\x(t) &= x_0 + r_{\max}(t - \lambda), \text{ for } \lambda < t < t_{\max} \\x(t) &= x_{\max}, \text{ for } t \geq t_{\max}\end{aligned}\tag{4.8}$$

where t is the time (h), $x(t)$ is the number of *Pseudomonas* spp. ($\log_{10}$ CFU/g) at time t , x_0 is the initial number of *Pseudomonas* spp. ($\log_{10}$ CFU/g), $t_{\max}$ is the time (h) when the maximum number of *Pseudomonas* spp. is reached, λ is the lag phase duration (h) at the storage temperature T (°C), and $r_{\max}$ is the fitted maximum specific growth rate ($\log_{10}$ CFU/h) at the storage temperature T (°C) obtained from the secondary model that has the best goodness of fit. The Equation (4.8) can also be used to assess the microbial spoilage and predict the product shelf-life by setting limits for the number of *Pseudomonas* spp. on sliced mushroom.

4.2.5. Validation of the Models

The data obtained from the independent experiments done in this work for different storage temperatures (15, 21 and 27 °C), and experimental growth data for *Pseudomonas* spp. on sliced mushrooms stored at 4 and 8 °C obtained from literature [Simón et al. 2005], [Cliffe-Byrnes and O’Beirne, 2010], respectively, were used to validate the models. Predicted number of microorganisms ($\log_{10}$ CFU/g) obtained from the models used for *Pseudomonas* spp. on sliced mushrooms at different temperatures were compared with the observed number of *Pseudomonas* spp. Model validation was assessed by considering the bias (B_f) and accuracy (A_f) factors using Equation (4.9) and Equation (4.10), respectively:

$$B_f = 10^{\frac{\sum_{i=1}^n \log(x_{\text{predicted}}/x_{\text{observed}})}{n}}\tag{4.9}$$

$$A_f = 10^{\frac{\sum_{i=1}^n |\log(x_{\text{predicted}}/x_{\text{observed}})|}{n}} \quad (4.10)$$

where $x_{\text{predicted}}$ is the predicted number of microorganisms ($\log_{10}$ CFU/g) from Equation (4.8), x_{observed} is the observed number of microorganisms ($\log_{10}$ CFU/g) and n is the number of observations. The B_f is a measure of average variation between the observed and predicted values. The A_f measures the average difference between the observed and predicted values by disregarding whether the difference is positive or negative. A value of 1 for B_f and A_f indicates that there is a perfect agreement between all the observed and predicted values.

4.3. Results and Discussion for Microbial Parameter Estimation

The microbiological analyses were carried out until the L^* value of the sliced mushrooms was lower than 69 as reported in literature [Gormley, 1975]. Colour analyses indicated that the required sampling times for the microbiological analyses were 240, 192, 120 and 96 hours for 4, 12, 20 and 28 °C, respectively (Figure 4.1).

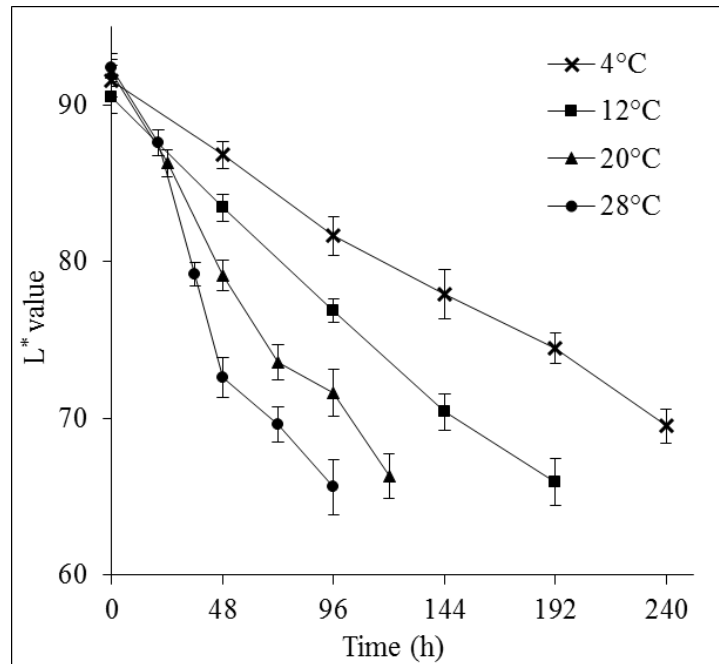


Figure 4.1: Effect of temperature on the L^* values of sliced mushrooms. Error bars represent the standard deviation of three independent samples.

The initial count of *Pseudomonas* spp. on sliced mushrooms obtained at different times was found to be $7.3 \pm 0.2 \log_{10}$ CFU/g. This result is in good agreement with the published data for sliced mushroom. The initial count of *Pseudomonas* spp. on sliced mushrooms was reported as 6.9 and 8.1 $\log_{10}$ CFU/g [Simón et al., 2005], [Venturini et al., 2011]. The growth data of *Pseudomonas* spp. on sliced mushrooms were obtained at storage temperatures of 4, 12, 20 and 28 °C (Figure 4.2). The final concentration of *Pseudomonas* spp. on sliced mushrooms at 4 °C was about 10 $\log_{10}$ CFU/g. Similar *Pseudomonas* counts in mushrooms stored at refrigeration temperatures were also reported [González-Fandos et al., 2006], [Simón et al., 2005]. In our study, the final population of *Pseudomonas* spp. reached up to 12.0 $\log_{10}$ CFU/g as the storage temperature was increased. One of the main factors which caused an increase in the population of *Pseudomonas* spp. is the temperature. Higher *Pseudomonas* populations near 11 $\log_{10}$ CFU/g were also obtained for the storage of mushroom above the refrigeration temperature. In most of the studies reported in literature, mushrooms are washed with water, citric acid, acidified hypochlorite, hydrogen peroxide and similar chemicals which reduce the initial load of the *Pseudomonas* spp., thus resulting in lower final concentrations. In these studies, mushrooms are packed and wrapped with some packaging films, thus also reducing the final *Pseudomonas* population during storage. In our study, mushrooms were not treated with water and any chemical agent, and they were not wrapped with a packaging film. It is also reported that slicing increases the respiration rate and provides larger area for the microbiological growth, thus resulting in higher microbiological counts [Brennan et al., 2000], [González-Fandos et al., 2006], [Simón et al., 2005]. All of these factors mentioned above are believed to contribute to attainment of higher final *Pseudomonas* populations in our study when compared with the *Pseudomonas* counts in mushrooms in the other studies.

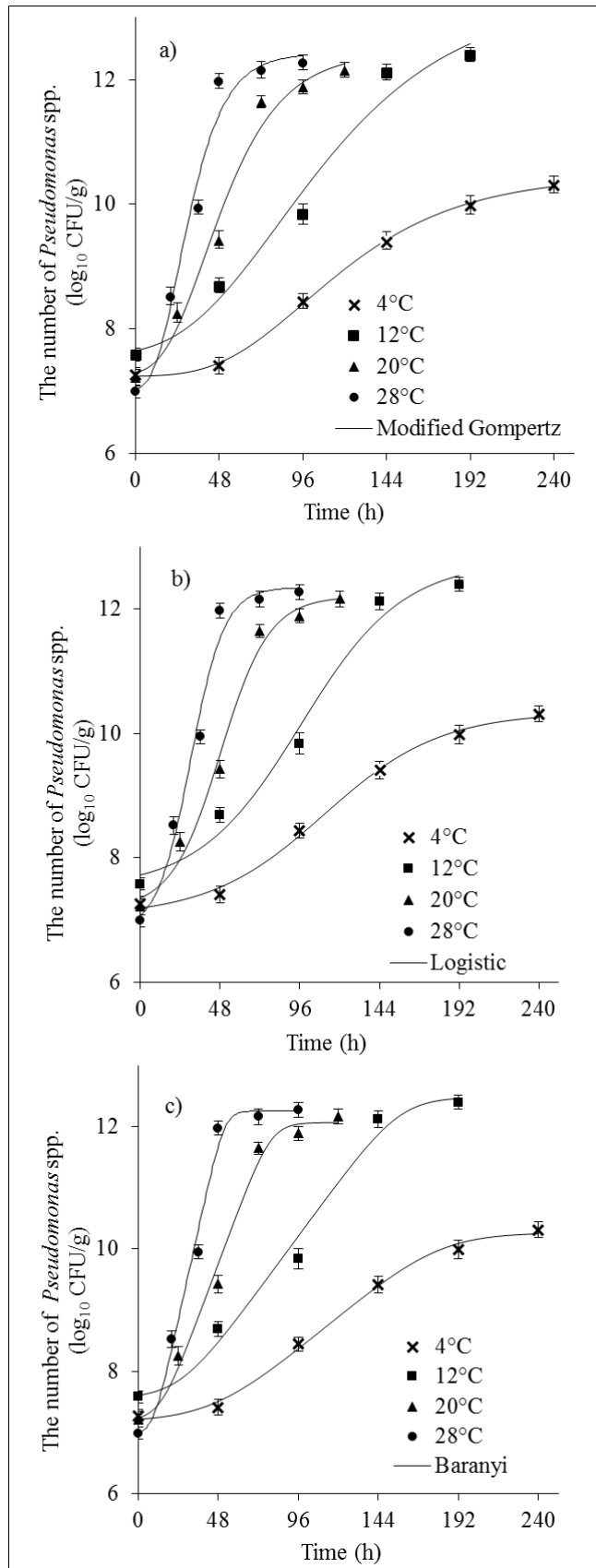


Figure 4.2: Growth rate data obtained at 4, 12, 20 and 28 °C fitted to the a) modified Gompertz model, b) logistic model and c) Baranyi model. Error bars represent the standard deviation of three independent samples.

The growth data were fitted to three primary models (modified Gompertz, logistic and Baranyi) for all storage temperatures (Figure. 4.2). The goodness of fit of the three primary models were analysed by comparing the corresponding MSE and pseudo-R² values for each model (Table 4.1). At 4 °C, each primary model (the modified Gompertz, logistic and Baranyi models) has the best goodness of fit to describe the growth of *Pseudomonas* spp. on sliced mushrooms. All the primary models gave MSE values lower than 0.0164 and pseudo-R² values higher than 0.996 for 4 °C. Although all the primary models have a high goodness of fit for the number of *Pseudomonas* spp. on sliced mushrooms stored at 4 °C, the goodness of fit of the Baranyi model for 12, 20 and 28 °C are better than the goodness of fit of the modified Gompertz and logistic models. The MSE and pseudo-R² for the modified Gompertz model ranged from 0.00335 to 0.386 and from 0.977 to 0.999, respectively. The MSE values for the logistic model were between 0.0163 and 0.277, and pseudo-R² values were between 0.982 and 0.996. The MSE and pseudo-R² for the Baranyi model ranged from 0.0164 to 0.247 and from 0.986 to 0.996, respectively. These results indicated that the Baranyi model yielded the lowest MSE and the highest pseudo-R² except for 4 °C. Therefore, the Baranyi model was selected as the best primary model to fit the number of *Pseudomonas* spp. on sliced mushrooms in the temperature range of 4 and 28 °C (Table 4.1).

Table 4.1: Estimated parameters for primary models.

Temperature	Primary models	x_0 (log ₁₀ CFU/g)	$x_{\max}$ (log ₁₀ CFU/g)	$r_{\max}$ (log ₁₀ CFU/h)	λ (h)	MSE	pseudo-R ²
4 °C	Modified Gompertz	7.24	10.5	0.0240	47.5	0.00335	0.999
	Logistic	7.06	10.3	0.0235	40.9	0.0163	0.996
	Baranyi	7.22	10.3	0.0231	47.2	0.0164	0.996
12 °C	Modified Gompertz	7.58	13.3	0.0379	26.4	0.386	0.978
	Logistic	7.51	12.8	0.0427	34.9	0.277	0.984
	Baranyi	7.61	12.5	0.0372	26.0	0.247	0.986
20 °C	Modified Gompertz	7.27	12.4	0.0786	15.5	0.192	0.983
	Logistic	7.18	12.2	0.0859	18.9	0.123	0.989
	Baranyi	7.24	12.1	0.0734	13.6	0.118	0.989
28 °C	Modified Gompertz	7.01	12.4	0.139	10.4	0.281	0.977
	Logistic	6.82	12.3	0.138	10.1	0.217	0.982
	Baranyi	7.00	12.3	0.124	9.22	0.131	0.989

Gospavic et al. used the Baranyi model to fit the number of *Pseudomonas* spp. on poultry meat stored between 2 and 20 °C, and they found that pseudo-R² values ranged from 0.978 to 0.993, and MSE values ranged from 0.0366 to 0.148 [Gospavic et al., 2008]. Zhang et al. also used the Baranyi model to fit the number of *Pseudomonas* spp. in chilled beef stored between 0 and 20 °C, and MSE values were found between 0.0309 and 0.277 [Zhang et al., 2011]. These results showed that the goodness of fit of the Baranyi model to fit the number of *Pseudomonas* spp. on sliced mushrooms is better than the goodness of fit of the Baranyi model used to fit *Pseudomonas* spp. counts on poultry and beef.

In exponential phase in which the growth rate reaches maximum value ($r_{\max}$) the changes in organoleptic properties of foods such as colour, odour and texture also reach to maxima. These changes are adversely affected due to high metabolic activity and growth of microorganisms. In lag phase, organoleptic properties do not change because the number of microorganisms remains relatively constant. Therefore, lower $r_{\max}$ and higher λ are desired in order to increase shelf-life of foods [Kreyenschmidt and Ibal, 2012]. Temperature is one of the most important factors affecting $r_{\max}$ and λ [Huang, 2008]. The $r_{\max}$ and λ derived from the Baranyi model were correlated with the temperature using the Ratkowsky (Figure 4.3a) and Arrhenius models (Figure 4.3b) for $r_{\max}$, and the Ratkowsky (Figure 4.3c) and Arrhenius models (Figure 4.3d) for λ . Similar to the primary models, MSE and pseudo-R² values were calculated for the Ratkowsky and Arrhenius models (Table 4.2). The MSE values of the Ratkowsky model for $r_{\max}$ and λ were 4.71×10^{-5} and 8.09×10^{-6} , respectively, while the pseudo-R² values were 0.980 and 0.995, respectively. The MSE values of the Arrhenius model for $r_{\max}$ and λ were 6.66×10^{-6} and 2.18×10^{-5} , respectively, while the pseudo-R² values were 0.998 and 0.990, respectively. High pseudo-R² and low MSE values indicated that the Arrhenius model has a higher goodness of fit than the Ratkowsky model to determine the effect of temperature on the maximum specific growth rate for *Pseudomonas* spp. on sliced mushrooms. The maximum specific growth rate is more sensitive to small changes in temperature compared to the lag phase duration. In other words, the change in temperature drastically affects the $r_{\max}$ much more than the λ . Therefore, modelling the temperature dependence of maximum specific growth is more important than modelling the temperature dependence of lag phase duration. Accordingly, the Arrhenius model was selected as the best secondary model.

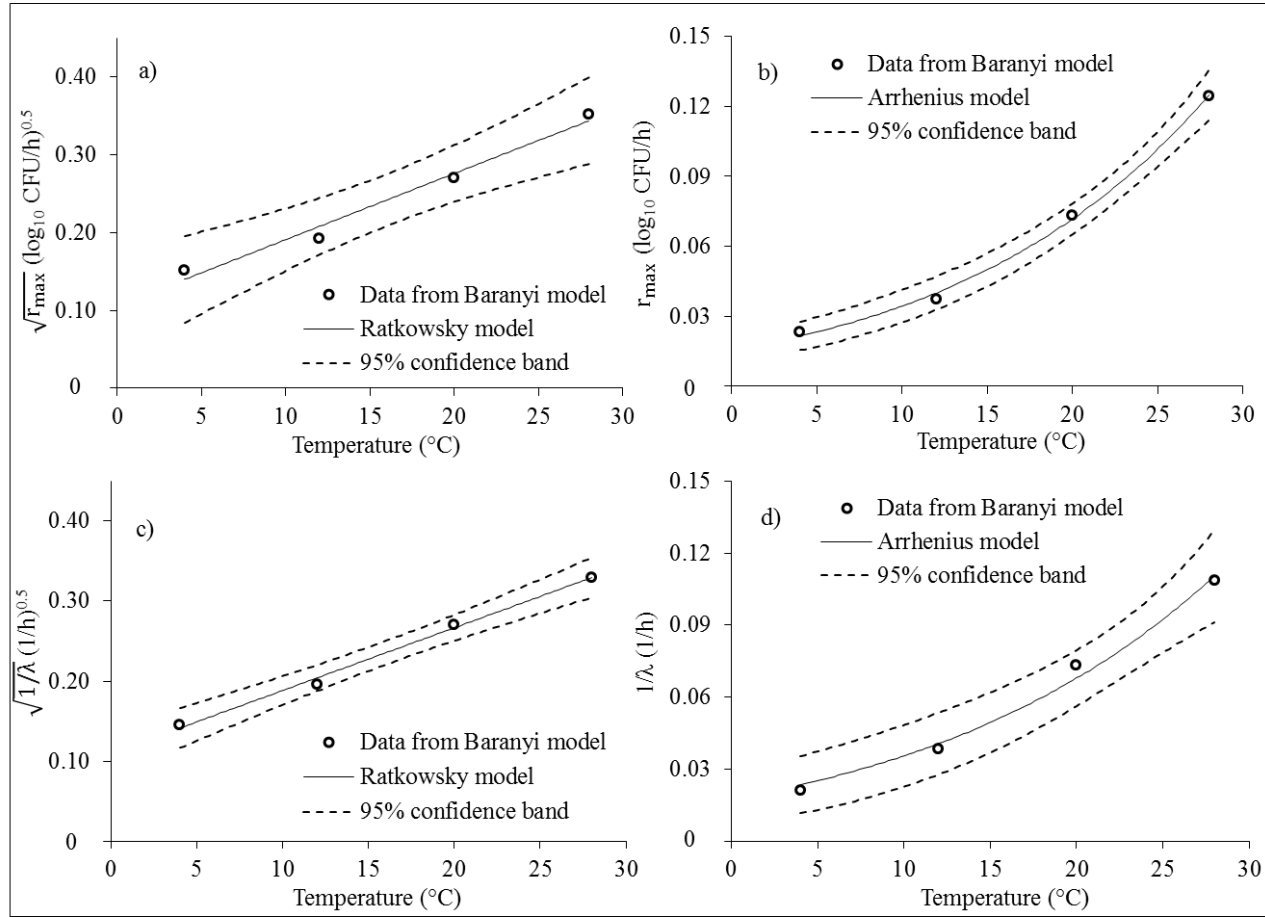


Figure 4.3: Maximum specific growth rates obtained from the Baranyi model fitted to the a) Ratkowsky and b) Arrhenius models, and lag phase durations obtained from the Baranyi model fitted to the c) Ratkowsky and d) Arrhenius models.

Table 4.2: Estimated parameters for secondary models.

Secondary models	Parameters	Estimate	Lower 95% confidence band	Upper 95% confidence band	MSE	pseudo-R ²
$\sqrt{r_{\max}} = b_1(T - T_0)$	b_1	0.00850	0.00478	0.0122	4.7×10^{-5}	0.980
	T_0 (°C)	-12.5	-25.6	-0.601		
$\sqrt{1/\lambda} = b_1(T - T_0)$	b_1	0.00782	0.00616	0.00949	8.09×10^{-6}	0.995
	T_0 (°C)	-14.1	-20.8	-7.42		
$r_{\max} = b_2 \left[\exp\left(\frac{-E_a}{R\theta}\right) \right]$	b_2	8.02×10^7	-2.18×10^8	3.78×10^8	6.66×10^{-6}	0.998
	E_a (kJ/mol)	50.8	41.6	59.9		
$1/\lambda = b_2 \left[\exp\left(\frac{-E_a}{R\theta}\right) \right]$	b_2	5.90×10^6	-3.40×10^7	4.58×10^7	2.18×10^{-5}	0.990
	E_a (kJ/mol)	44.5	27.8	61.2		

^a calculated based on $r_{\max}$ and $1/\lambda$.

The E_a for r_{max} of *Pseudomonas* spp. on sliced mushrooms was found to be 50.8 kJ/mol. Koutsoumanis et al. also used the Arrhenius model to determine the E_a of the growth of *Pseudomonas* spp. in minced pork packed aerobically with a high permeability film. The E_a for r_{max} of *Pseudomonas* spp. in minced pork was found to be 68.4 kJ/mol [Koutsoumanis et al., 2008]. Steeper slope obtained from the Arrhenius model for the minced pork means that minced pork is more sensitive to small changes in temperature compared to the sliced mushrooms. In other words, the change in temperature drastically affects the growth of *Pseudomonas* spp. in minced pork compared to the growth of *Pseudomonas* spp. on sliced mushrooms. For instance, when the temperature was increased from 5 to 15 °C, the fitted growth of *Pseudomonas* spp. in minced pork increased from 0.0478 to 0.140 log₁₀ CFU/h whereas the fitted growth of *Pseudomonas* spp. on sliced mushrooms increased from 0.0233 to 0.0500 log₁₀ CFU/h.

Some studies have been published pertaining to r_{max} and λ values of *Pseudomonas* spp. on poultry meat [Dominguez and Schaffner, 2007], [Gospavic et al., 2008] and chilled beef [Zhang et al., 2011]. The results collected from these studies together with our results obtained from this study were compared in Table 4.3 for r_{max} and λ values. At the same temperature, r_{max} values for *Pseudomonas* spp. on sliced mushroom are lower than the r_{max} values found for *Pseudomonas* spp. on poultry and beef. As far as λ values are compared, lag phase durations of *Pseudomonas* spp. on sliced mushroom are higher than the lag phase durations of *Pseudomonas* spp. on poultry and beef. The reason why lower r_{max} and higher λ were obtained for *Pseudomonas* spp. on sliced mushroom is that *Pseudomonas* spp. grow more easily in poultry meat and beef compared to the mushroom because poultry and beef are the mediums rich in nutrients required for the growth of *Pseudomonas* spp.

Table 4.3: Comparison of $r_{\max}$ and λ values for *Pseudomonas* spp. on poultry meat, beef and sliced mushroom.

Temperature (°C)	$r_{\max}$ (log ₁₀ CFU/h)				λ (h)		
	Poultry meat ^{a, b}		Beef ^c	Sliced mushroom ^d	Poultry meat ^a	Beef ^c	Sliced mushroom ^d
4	0.0375	–	0.0386	0.0215	27.8	26.4	42.3
10	0.0849	0.0886	0.0896	0.0344	12.3	16.6	28.1
12	–	0.112	–	0.0400	–	–	24.6
15	0.167	0.154	0.126	0.0500	6.24	6.13	20.2
20	0.264	0.236	0.180	0.0717	3.96	2.74	14.7

$r_{\max}$ and λ values ^a[Gospavic et al., 2008], ^b[Dominguez and Schaffner, 2007], ^c[Zhang et al., 2011] and ^dcalculated with the Arrhenius model in this work.

Validation based on an independent experiment is important to validate the reliability of the models used. The models must be validated with the data collected under conditions in which food products are usually stored and subjected to. Therefore, the data obtained from the independent experiments done in this work and experimental growth data collected for *Pseudomonas* spp. on sliced mushrooms were compared with the fitted number of *Pseudomonas* spp. (Figure 4.4) with the models used by considering the B_f and A_f values. The B_f and A_f values were found to be 0.974 and 1.036, respectively. A B_f factor of 1 indicates no structural deviation of the model. The B_f factor of 0.974 indicated that the model underestimates 2.6% whereas the A_f factor of 1.036 showed that on average the predicted value was 3.6% different (either smaller or larger) from the observed value. These results revealed that the models can be safely used because the error rates are relatively small.

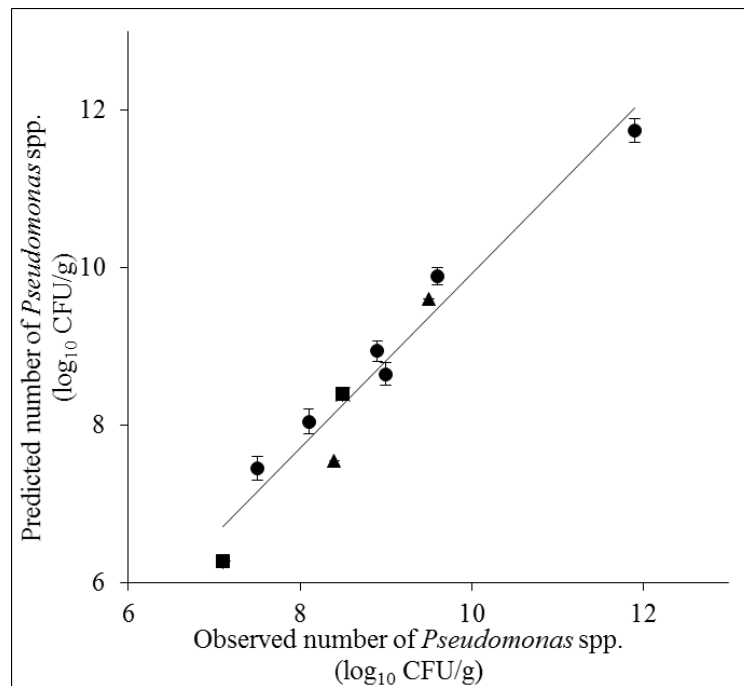


Figure 4.4: Observed and predicted number of *Pseudomonas* spp. on sliced mushrooms. (●) independent data from this work for 15, 21 and 28 °C, (■) data collected for 8 °C [Cliffe-Byrnes and O’Beirne, 2010] and (▲) data collected for 4 °C [Simón et al., 2005]. Error bars represent the standard deviation of three independent trays.

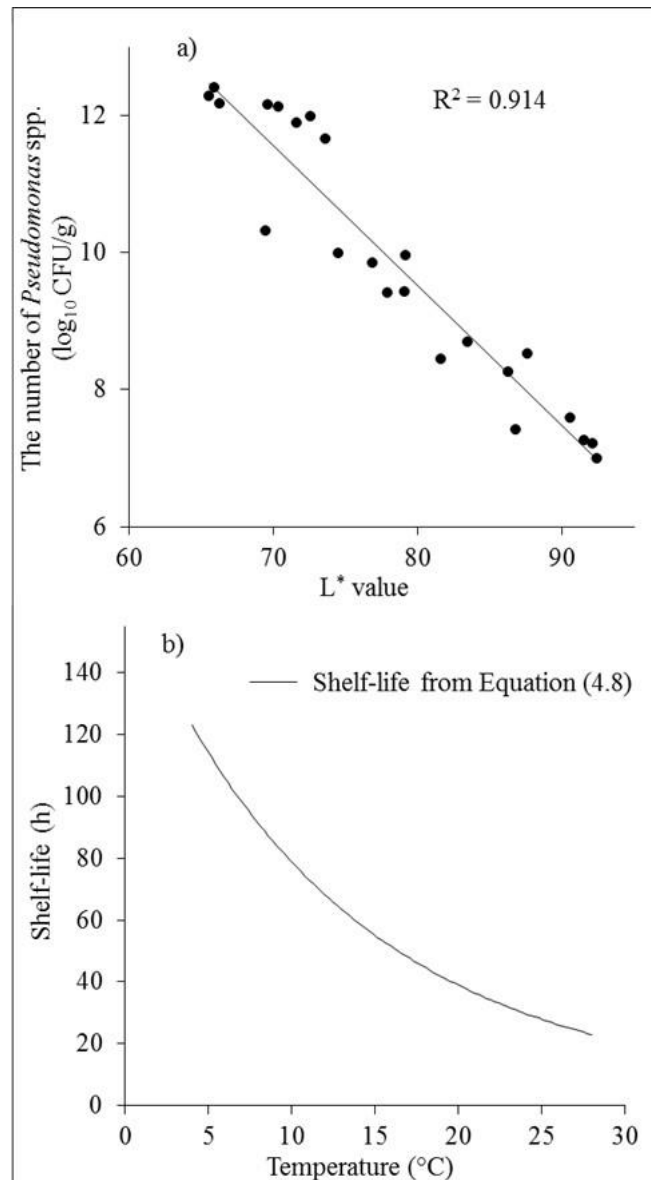


Figure 4.5. a) Relationship between the L^* values and the number of *Pseudomonas* spp. on sliced mushrooms and b) effect of temperature on the shelf-life of sliced mushroom. Shelf-life was predicted using $x_0 = 7.3 \log_{10}$ CFU/g and $x(t) = 9.0 \log_{10}$ CFU/g.

The L^* values of sliced mushrooms were correlated with the number of *Pseudomonas* spp. (Figure 4.5a). When the number of *Pseudomonas* spp. on sliced mushrooms reached a threshold value of $9 \log_{10}$ CFU/g which corresponds to a L^* value of approximately 80, off-odour formation and slime production were detectable. Therefore, $9 \log_{10}$ CFU/g was taken as the limiting microbial criterion for the sliced mushroom. The effect of temperature on microbial spoilage was correlated with the shelf-life of sliced mushrooms (Figure 4.5b). The temperature was found to be the critical factor affecting the mushroom shelf-life. As the temperature

increased, the shelf-life of sliced mushrooms decreased considerably. For instance, the shelf-life was 123 h at 4 °C, but it was only 23.0 h at 28 °C. The shelf-life is almost six times lower at 28 °C than at 4 °C. Therefore, sliced mushrooms should not be subjected to higher temperatures, and always be stored at chilled conditions, preferably less than 4 °C throughout their shelf-life. Because *Pseudomonas* spp. are good indicators of spoilage, the models can be used to evaluate the growth behaviour of *Pseudomonas* spp. on sliced mushroom, set limits for the quantitative detection of the microbial spoilage and assess product shelf-life.

5. DEVELOPMENT of ALTERNATIVE METHOD with COMPUTER VISION SYSTEM for COLOUR MEASUREMENTS of FOODS

Colour analysis is an important issue for the food industry during harvesting, processing and preservation of foods. For most people, it may sound like a simple thing, but in reality, colour analysis is a very complex issue. Colour is a cerebral perceptual response to the visible spectrum of light, which is reflected or emitted from an object; the response signal is linked up in the eye with the retina and transmitted by the optical nerve to the brain, as a result of this we can assign colours to this signal [MacDougall, 2002], [Wu and Sun, 2013]. Moreover, colour is considered as a psychophysical concept, which is related to the physiology of vision, the psychology of the observer and the spectral radiant energy of a source of light. All these factors make the colour analysis difficult to accurately analyse the colour of foods.

Colour of foods directly affects consumers' purchasing decision [Costa et al., 2011], [Meléndez-Martínez et al., 2005]. If the colour of the food is not what is expected, the consumers generally have a negative perception of quality [Francis, 1995], which makes it one of the most important selection criteria of how we purchase our food [Mendoza and Aguilera, 2004]. Therefore, colour of the foods must be analysed with utmost accuracy. The colour of an object can be determined either by human (visual) inspection or by using a colour measuring device because determination of colour is subjective and variable from observer to observer so it is generally recommended to determine the colour via a colour measuring instrument [Leon et al., 2006].

In industry, the colour of foods has generally been measured using $L^*a^*b^*$ or CIELab colour space, which is an international standard accepted by the Commission Internationale d'Eclairage (CIE) in 1976 [Leon et al., 2006]. In the CIELab colour space, L^* is the lightness component or luminance between 0 and 100, a^* is a chromatic component between -120 and +120 from green to red and b^* is a chromatic component between -120 and +120 from blue to yellow. In food industry, colour is analysed using commercial colour meters such as Minolta Chroma Meter and Hunterlab Color Difference Meter [Gras et al., 1990], [Holman et

al., 2015]. However, colorimetric results usually vary due to the fact that measurements taken by a colorimeter are based on a number of point measurements, and the average of these measurements does not represent the whole colour of the object. This approach does not reflect the colour variation throughout the object, and the variation in colour is more dramatic when especially the surface to be measured is quite non-uniform. Therefore, it is hard to obtain reproducible results from the colorimetric measurements even if they are taken from the same point [Baardseth et al., 1988].

Alternatively, colour of foods can rapidly and accurately be analysed by using computerised image analysis techniques, known as computer vision systems [Mendoza et al., 2006]. Computer vision systems can be used to measure attributes of total appearance together with the measurement of uneven colouration [Brosnan and Sun, 2004], [Du and Sun, 2004]. Such systems provide an automated, cost-effective and non-destructive alternative to the conventional colour meter systems [Brosnan and Sun, 2004], [Yam and Papadakis, 2004]. Computer vision system has some specific applications in the food industry. Mohebbi et al. used the computer vision system to predict moisture content of shrimp during drying [Mohebbi et al., 2009]. They found a high correlation coefficient between the colour and the change in moisture content of shrimp. Rodriguez-Pulido et al. also used the computer vision system and correlated the colour change of the grape berries with the chemical composition to determine the ripeness of grape berries [Rodriguez-Pulido et al., 2012]. Rodriguez-Pulido et al. developed an algorithm to determine the heterogeneity in some foods including cabbage, orange, apple and tomato by assessing a relationship between chemical properties and colour, appearance and composition using the computer vision system [Rodriguez-Pulido et al., 2013]. They have suggested that the proposed algorithm in conjunction with the computer vision system is the best way to measure the colour of food products, which have non-homogenous colour, and the system could be used for classification and prediction of chemical properties of foods. Recently, Yamamoto et al. have demonstrated that qualitative evaluation of the appearance of strawberries can be achieved based on a computer vision analysis technique [Yamamoto et al., 2015].

When using a digital camera, it is possible to register the colour of any pixel of the image, and the object can be registered using three colour sensors per pixel, which depend on the colour model being used [Forsyth and Ponce, 2012], [Pedreschi

et al., 2008]. In this field, Red-Green-Blue (RGB) image analysis techniques have gained an increasing interest in food colour analysis in the last 20 years [Ulrici et al., 2012]. The key point is the designation of proper automated methods to extract useful information from RGB images and employ it for calibration, classification and process monitoring [Foca et al., 2011]. RGB cannot be converted to $L^*a^*b^*$ directly, however, there are methodologies published in the literature to obtain accurate device independent $L^*a^*b^*$ colour units from device dependent RGB colour units captured by a digital camera [Leon et al., 2006]. Transformation of RGB colour units to $L^*a^*b^*$ colour space was achieved using limited amount of colour cards [Afshari-Jouybari and Farahnaky, 2011], [Leon et al., 2006]. The main drawback of their work was that they did not produce as many colour tones as possible to evaluate colour space.

The primary aim of this part of the thesis was to improve currently used methodology for the conversion of RGB colour units to $L^*a^*b^*$ colour space and implement this methodology in colour analysis of foods. For this purpose, two innovative ideas were proposed and tested. First, 120 colour tones were generated to cover as many points in all the colour space as possible as compared to the Leon et al. who used 32 colour tones [Leon et al., 2006]. Secondly, the colour space was calibrated separately, whereas in the literature, the colour space was calibrated simultaneously [Afshari-Jouybari and Farahnaky, 2011], [Leon et al., 2006]. Therefore, RGB colour units to $L^*a^*b^*$ colour space transformation approach proposed in this study is more logical and more accurate. In this study, 4 colours (red, yellow, green and black-white) were analysed separately because most of the foods are composed of these colours and their tones. Thirty tones of each colour were generated and imprinted on cards. The $L^*a^*b^*$ and RGB colour analysis of these cards were carried out with a chroma meter and the image acquisition system designed in this study, respectively. After the colour analysis, two polynomial models: linear and quadratic were generated for each colour. The predictive power of these models was analysed using advanced statistical analyses tools including % mean absolute error ($|\bar{e}|$), standard deviation of the % mean absolute error (σ), Euclidean distance (Δe^*), average root mean square error (RMSE) and correlation coefficient (R^2). Appropriate models for each colour were determined based on the results of the statistical analysis. The $L^*a^*b^*$ and RGB colour analysis of some selected foods were also carried out to verify the accuracy of these selected models

on real foods. Meat, salami, potato, cheddar cheese, broccoli, green bean, cauliflower and white cheese homogeneous and uniform in colour and shape were selected, and their colour analysis were done using the computer vision system developed. The developed computer vision system is specifically designed for foods that are homogeneous and uniform in colour and shape. As a next step, the system can be improved to be used for non-homogenous foods with non-homogenous colour.

5.1. Materials and Methods for Computer Vision System

The work was carried out in 5 separate phases: i) A colour map was generated for 4 different colours including red, yellow, green and black-white, ii) Digital images of the colour map were taken with an image acquisition system specifically designed in this work, iii) Background separation of the digital images was carried out, iv) RGB values obtained from the digital images were converted to $L^*a^*b^*$, and v) Validation process was performed by using colour cards for each colour. Schematic diagram of the experimental design is shown in Figure 5.1. Details of the phases in the experimental plan are explained in the following subsections.

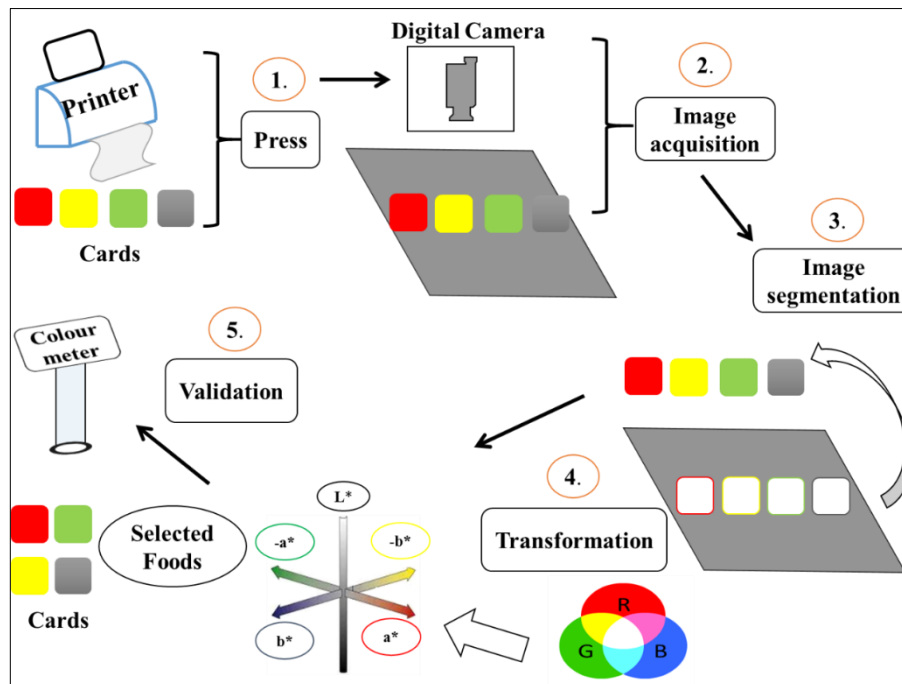


Figure 5.1: Schematic diagram of the experimental design.

5.1.1. Colour Map Design and Press

Thirty samples of lighter and darker tones of each colour (red, yellow, green and black-white) with a total of 120 colour cards (30 colour tones $\times$ 4 colours) in the form of $9 \times 9 \text{ cm}^2$ were systematically generated using the Adobe Photoshop (CS5.1) software (Adobe Systems Inc., San Jose, USA) in order to transform RGB values obtained from the image acquisition system to $L^*a^*b^*$ values. Thirty colour tones for each colour were generated, as shown in Figure. 5.2. Twenty four colour tones were used for calibration, and the remaining colour tones were used for validation. The generated colour map with a total of 120 colour cards was imprinted to a high quality printing paper (350 g/m^2), and the colour cards were stored in oxygen and water vapour proof sealed bags to protect them against environmental conditions and direct sunlight, which may change colour of cards.

5.1.2. Image Acquisition System

The digital image acquisition system (Figure 5.3) designed and used in this study consists of 4 main components: a black box, light source, digital camera and image processing software Details of these components are explained below:

Black box: The black box designed for the colour measurements was constructed from the medium-density fibreboard (MDF) wood, with dimensions of $1 \text{ m} \times 1 \text{ m} \times 0.6 \text{ m}$. All the interior surfaces of the box were covered with a matt black cloth to prevent any light reflection in the box and provide insulation for the ingress of any outside light [Sáenz et al., 2005].

Light source: Illumination was achieved with 4 fluorescent lamps (Philips, MASTER TL-D 90 Graphica), and each lamp has 60 cm long, 18 W power with a colour temperature of 6500 K and a colour-rendering index (Ra) larger than 95%. These 4 fluorescent lamps were placed forming a square vertically at a distance of 45 cm from the samples, so as to get uniform intensity of light over the samples. The fluorescent lamps were used together with their commercial luminaires (Philips, TCW060) with both electronic ballast (to prevent the stroboscopic effect) and plastic light diffuser (to filter the light) in order to provide constant and uniform illumination conditions.

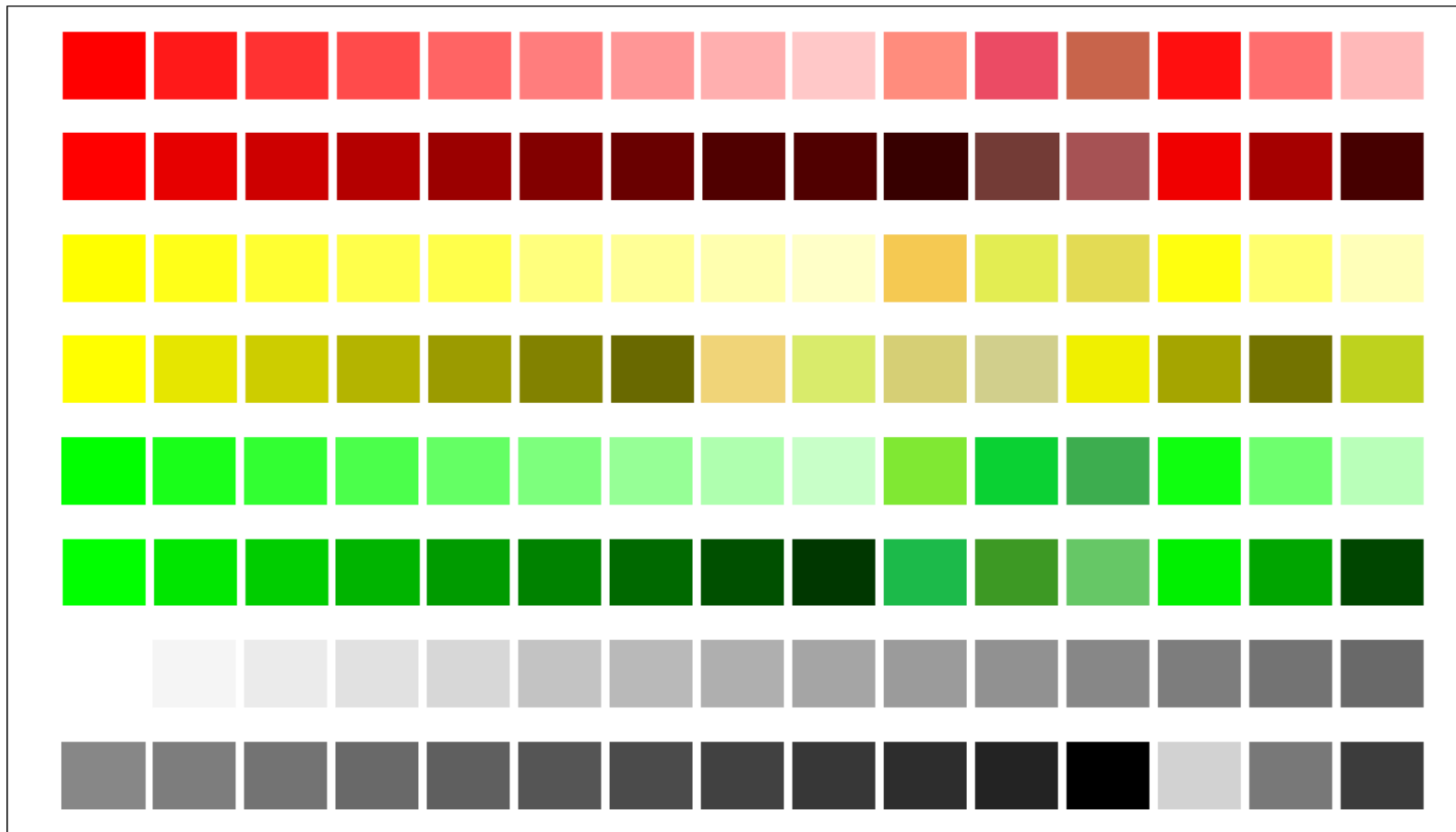


Figure 5.2: All generated colour tones for each colour.

Digital camera: Nikon D3000 with a 10.2 megapixel DX format DSLR Nikon F-mount camera (Nikon Corporation, Tokyo, Japan) was used to capture digital images. The digital camera was placed at distance of 20 cm from the samples using the elevation system constructed in the box shown in Figure 5.3. Before taking digital images, the white balance of the camera was calibrated using a $20 \times 25 \text{ cm}^2$ gray card with 18% reflectance (GC1890, Danes Picta Company, Praha 3, Czech Republic). The digital images were taken using the camera's manual mode. The settings of the digital camera used in the colour measurements are summarized in Table 5.1.

Table 5.1: Settings of the digital camera.

Variable	Value
Size of the image	3872×2592 pixels
Image file format	JPEG
Iso velocity	200
Aperture Av	f/4.0
Exposure Tv	1/40 s
Focal distance	18 mm
Zoom	Off
Flash	Off

Image processing software and hardware: The digital camera was connected to a personnel computer (Asus, IntelR, i7-2600) with a USB cable using remote control software specifically designed for the Nikon D3000 digital camera (SM Tether, v1.5). The angle between the axis of the lens and the sources of illumination was set to 45° [Leon et al., 2006]. In order to balance the illumination, the light source was switched on at least 30 minutes prior to the colour measurements [Valous et al., 2009]. Image processing, colour analysis and conversion of RGB colour units to $L^*a^*b^*$ colour space were carried out using the Matlab 7.12.0 (R2011a) software (MathWorks Inc., Natick, MA, USA).

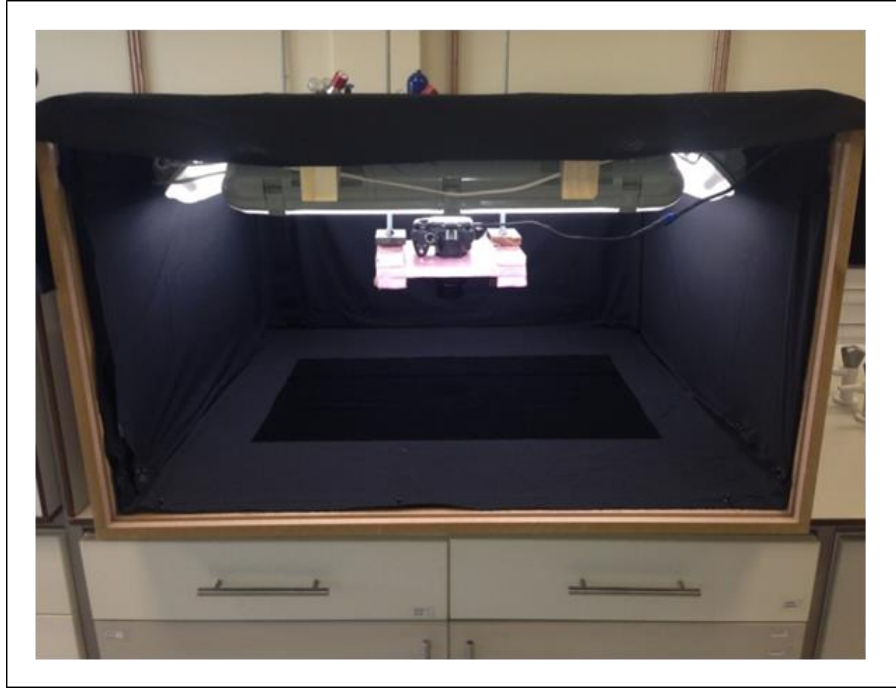


Figure 5.3: Digital image acquisition system.

5.1.3. Image Segmentation

All the interior surfaces of the image acquisition system were covered with a matt black cloth to assure that the background of the digital images was all black. However, before the conversion of the RGB colour system to $L^*a^*b^*$, the part of interest must be separated from the black background. This separation process is known as image segmentation. First, the digital images were read from the graphics file using the `imread` command of the Matlab software. Then, the digital images were filtered with a Gaussian low pass filter with a filter size $[3 \times 3]$ matrix and sigma 0.5, which allows pre-smoothing of noisy images [Mendoza and Aguilera, 2004], [Mendoza et al., 2006]. The filtered coloured images were converted to the grayscale intensity image using the `rgb2gray` command, and image segmentation was performed using the `graythresh` command of the Matlab software. Segmented images are in the form of binary images, so all pixels of these binary images are comprised of two values (0 and 1). Pixels with values of 0 and 1 represent black background and object in white colour, respectively.

5.1.4. Converting RGB Colour Units to $L^*a^*b^*$ Colour Space

Digital images of each card in red, green, yellow and black-white colour with 30 different colour tones for each colour were taken, and each colour card was divided into 5 zones as shown in Figure 5.4. The $L^*a^*b^*$ colour measurements were taken in each of the 5 zones by taking one colour measurement in each zone using a chroma meter (Konica Minolta, CR-400, Tokyo, Japan). Prior to colour measurements, the Chroma Meter was calibrated with its white calibration tile ($Y = 86.6$, $x = 0.3188$ and $y = 0.3364$). The average of these measurements representing the whole of the card analysed were coded as image 0-0. $L^*a^*b^*$ measurements taken from the first, second, third and fourth zones were coded as image 0-1, image 0-2, image 0-3 and image 0-4, respectively. The RGB values of the corresponding zones were calculated using the Matlab software, which computes mean of all pixels for each zone. Calculated RGB values for these five zones were coded with the same image codes as the $L^*a^*b^*$ measurements were taken.

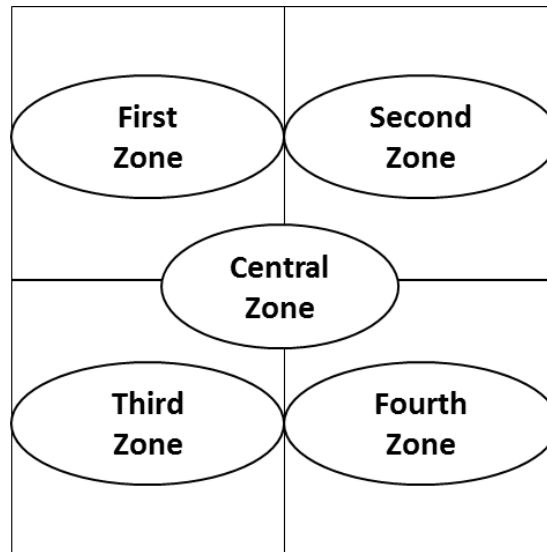


Figure 5.4: Zones of the colour cards.

Prior to the construction of linear and quadratic models, the colour cards were separated into two groups. For each colour, the first group of 24 cards ($24 \text{ cards} \times 5 \text{ zones} = 120 \text{ colour values}$) was used to calibrate and construct the models, and the remaining 6 cards ($6 \text{ cards} \times 5 \text{ zones} = 30 \text{ colour values}$) were used to validate the constructed models. In the calibration step, the linear and quadratic functions given

in Equation (5.1) and Equation (5.2) were used, respectively, to convert the RGB colour units to L* a* b* colour space.

$$\begin{bmatrix} \hat{L}^* \\ \hat{a}^* \\ \hat{b}^* \end{bmatrix} = \begin{bmatrix} P_{11} & P_{12} & P_{13} \\ P_{21} & P_{22} & P_{23} \\ P_{31} & P_{32} & P_{33} \end{bmatrix} \begin{bmatrix} R \\ G \\ B \end{bmatrix} \quad (5.1)$$

$$\begin{bmatrix} \hat{L}^* \\ \hat{a}^* \\ \hat{b}^* \end{bmatrix} = \begin{bmatrix} P_{11} & P_{12} & P_{13} & P_{14} & P_{15} & P_{16} & P_{17} & P_{18} & P_{19} \\ P_{21} & P_{22} & P_{23} & P_{24} & P_{25} & P_{26} & P_{27} & P_{28} & P_{29} \\ P_{31} & P_{32} & P_{33} & P_{34} & P_{35} & P_{36} & P_{37} & P_{38} & P_{39} \end{bmatrix} \begin{bmatrix} R \\ G \\ B \\ R^2 \\ G^2 \\ B^2 \\ RG \\ RB \\ GB \end{bmatrix} \quad (5.2)$$

In these equations, R, G and B are the digital colour variables of the samples; $\hat{L}^*$, $\hat{a}^*$ and $\hat{b}^*$ are the estimated L*, a* and b* colour variables, and P₁₁ - P₃₉ are the coefficients calculated using fminsearch command, which uses iteratively algorithm in the Matlab software.

5.1.5. Validation Process and Statistical Analysis

After the calibration, Equations (5.3) to (5.12) were used for verification. Mean normalized errors (e_L, e_a and e_b) for L*, a* and b* variables were calculated using Equation (5.3), Equation (5.4) and Equation (5.5), respectively.

$$e_L = \frac{1}{n} \sum_{i=1}^n \left| \frac{L_i^* - \hat{L}_i^*}{\Delta L} \right| \quad (5.3)$$

$$e_a = \frac{1}{n} \sum_{i=1}^n \left| \frac{a_i^* - \hat{a}_i^*}{\Delta a} \right| \quad (5.4)$$

$$e_b = \frac{1}{n} \sum_{i=1}^n \left| \frac{b_i^* - \hat{b}_i^*}{\Delta b} \right| \quad (5.5)$$

where $\hat{L}^*$, $\hat{a}^*$ and $\hat{b}^*$ are the colour values estimated with the model, and n is the number of measurements. L^* values range from 0 to 100, and a^* and b^* values are between -120 and +120 so $\Delta L = 100$ and $\Delta a = \Delta b = 240$.

Percent (%) mean absolute error ($|\bar{e}|$) was calculated by Equation (5.6), and the standard deviation (σ) of the % mean absolute error was determined by Equation (5.7):

$$|\bar{e}| = \frac{e_L + e_a + e_b}{3} 100 \quad (5.6)$$

$$\sigma = \sqrt{\frac{1}{n} \left(\sum_{i=1}^n |\bar{e}|_i - |\bar{e}| \right)^2} \quad (5.7)$$

Euclidean distance (Δe^*) between real and estimated L^* , a^* and b^* values was given by Equation (5.8):

$$\Delta e^* = \sum_{i=1}^n \sqrt{(L^*_i - \hat{L}^*_i)^2 + (a^*_i - \hat{a}^*_i)^2 + (b^*_i - \hat{b}^*_i)^2} \quad (5.8)$$

Root mean square errors ($RMSE_L$, RMS_a and $RMSE_b$) for L^* , a^* and b^* variables were calculated using Equations (5.9) to (5.11). Average root mean square error (RMSE) was obtained by Equation (5.12).

$$RMSE_L = \sqrt{\frac{\sum_{i=1}^n (L^*_i - \hat{L}^*_i)^2}{n}} \quad (5.9)$$

$$RMSE_a = \sqrt{\frac{\sum_{i=1}^n (a^*_i - \hat{a}^*_i)^2}{n}} \quad (5.10)$$

$$RMSE_b = \sqrt{\frac{\sum_{i=1}^n (b^*_i - \hat{b}^*_i)^2}{n}} \quad (5.11)$$

$$\text{RMSE} = \frac{\text{RMSE}_L + \text{RMSE}_a + \text{RMSE}_b}{3} \quad (5.12)$$

5.2. Results and Discussion for Computer Vision System

Calibration and validation processes for each colour (red, yellow, green and black-white) and each model (linear and quadratic) were carried out separately by calculating % mean absolute error ($|\bar{e}|$), standard deviation of the % mean absolute error (σ), Euclidean distance (Δe^*) and average root mean square error (RMSE) (Table 5.2). As can be seen from this table, the models that have the best prediction performance were the quadratic models for all the colours. The quadratic models gave $|\bar{e}|$, σ , Δe^* and RMSE values lower than 0.74, 1.30, 3.23 and 1.84, respectively, at the calibration. The $|\bar{e}|$, σ , Δe^* and RMSE values at the validation were lower than 0.58, 0.85, 2.39 and 1.94, respectively. This clearly showed that quadratic models provide reliable prediction performance for the transformation of RGB colour units to $L^*a^*b^*$ colour space for each colour.

Leon et al. used a quadratic model to transform RGB colour units to $L^*a^*b^*$ colour space, and they found $|\bar{e}|$ of 1.22% for calibration and 1.26% for validation [Leon et al., 2006]. Afshari-Jouybari and Farahnaky also used a quadratic model with a $|\bar{e}|$ value of 2.9% in which they did not improve the quadratic model [Leon et al. 2006] for the transformation of RGB colour units to $L^*a^*b^*$ colour space [Afshari-Jouybari and Farahnaky, 2011]. These researchers also used neural network models in which the neural network model used by Leon et al. had a $|\bar{e}|$ value of 0.93% while the neural network model used by Afshari-Jouybari and Farahnaky gave a $|\bar{e}|$ value of 1.3%. The $|\bar{e}|$ values of the quadratic models used in this study were much smaller than $|\bar{e}|$ values of the neural network models, thus, validating that the quadratic model is even better than the neural network based models.

Table 5.2: Results obtained from calibration and validation processes for the colour cards.

Colour	Model	Calibration Process				Validation Process			
		$ \bar{e} $	σ	Δe_{ab}^*	RMSE	$ \bar{e} $	σ	Δe_{ab}^*	RMSE
Red	Linear	2.18	4.02	10.65	5.71	1.77	3.38	8.72	4.69
	Quadratic	0.74	1.30	3.23	1.84	0.54	0.85	2.39	1.32
Yellow	Linear	1.53	2.74	7.48	3.88	0.80	1.14	3.66	1.92
	Quadratic	0.55	0.97	2.49	1.40	0.28	0.35	1.18	0.64
Green	Linear	0.91	2.07	5.25	2.74	1.49	2.74	7.38	3.94
	Quadratic	0.38	0.58	1.60	0.89	0.58	0.75	2.14	0.97
Black-white	Linear	1.20	1.75	4.45	2.53	0.61	0.70	2.07	1.18
	Quadratic	0.72	0.96	2.56	1.41	0.39	0.48	1.60	0.86

Leon et al. used the quadratic model to transform RGB colour units to $L^*a^*b^*$ colour space and found that σ is 1.42 for the calibration and 1.62 for the validation [Leon et al., 2006]. The same quadratic model as utilized by Leon et al. was also used in our work and the σ value was significantly improved both in calibration ($\sigma \leq 1.30$) and validation ($\sigma \leq 0.85$). This means that the model consistently predicts RGB colour units to $L^*a^*b^*$ colour space more precisely than the method [Leon et al., 2006].

Afshari-Jouybari and Farahnaky used the quadratic and neural network models to transform RGB colour units to $L^*a^*b^*$ colour space and found a RMSE value of 3.262 for the quadratic model and 1.973 for the neural network model [Afshari-Jouybari and Farahnaky, 2011]. In our work, RMSE values of the quadratic model were less than 1.84 for calibration and less than 1.32 for validation. These RMSE values are much smaller than that of Afshari-Jouybari and Farahnaky found for the quadratic model and even for the neural network model. This simply means that the prediction performance of our quadratic model is better than the neural network based models.

The Δe^* value between two different colours corresponds to colour difference perceived by the human eye [Leon et al., 2006]. If this value is high, the colour difference is easily perceived by the human eye. If this value is small, human eye has the difficulty in distinguishing the difference between two colours. Therefore, the Δe^* values were calculated for each colour to determine the error values between the predicted and measured $L^*a^*b^*$ values. Valous et al. used various polynomial models to transform RGB colour units to $L^*a^*b^*$ colour space [Valous et al., 2009]. In their work, Δe^* values ranged from 4 to 4.6 for the polynomial models composed of quadratic and cubic terms. In our work, all the Δe^* values for the quadratic models were lower than 3.23 for the calibration and 2.39 for the validation. Thus, even the quadratic model used in this work has smaller error than the polynomial models with quadratic and cubic terms used by Valous et al.

Measured $L^*a^*b^*$ values were plotted against estimated $L^*a^*b^*$ values, and R^2 values with root mean square errors (RMSE_L, RMSE_a and RMSE_b) for L^* , a^* and b^* variables were calculated to determine the existence of any relationship between measured and predicted values for the colour cards (Figure 5.5). Measured and predicted L^* , a^* and b^* values yielded R^2 higher than 0.9944 and RMSE smaller than 1.457. This indicated that the quadratic model has very high prediction performance

for L^* , a^* and b^* variables. For the quadratic model, R^2 values found by Leon et al. for L^* , a^* and b^* variables were 0.9843, 0.9851 and 0.9910, respectively [Leon et al., 2006], while Afshari-Jouybari and Farahnaky found R^2 values of 0.9841, 0.9663 and 0.9742 for L^* , a^* and b^* , respectively [Afshari-Jouybari and Farahnaky, 2011]. All the R^2 values determined in these two separate studies were less than the R^2 values found in this work for L^* , a^* and b^* variables. Higher R^2 and smaller RMSE values showed considerable improvement in the prediction performance of the quadratic models used in the transformation of RGB colour units to $L^*a^*b^*$ colour space compared to the prediction performance of the models [Leon et al., 2006], [Afshari-Jouybari and Farahnaky, 2011].

Barbin et al. used computer vision system for non-destructive determination of colour parameters of chicken and compared the measured $L^*a^*b^*$ values with the predicted $L^*a^*b^*$ values to assess chicken quality in a fast and rapid way [Barbin et al., 2016]. They worked with poultry meat which has non-flat and non-homogeneous surfaces causing high brightness and shadow effects. Therefore, their proposed approach was based on illumination normalisation step to reduce bright spot and shadow effects on poultry meat. In our work, the colour values of foods with surfaces that do not cause high brightness and shadow effects were measured. Therefore, an illumination normalisation step was not used in our work. In order to convert RGB colour units to $L^*a^*b^*$ colour space, Barbin et al. followed two step conversion model ($RGB \rightarrow XYZ \rightarrow L^*a^*b^*$) [Barbin et al., 2016]. They first converted RGB values to XYZ and then XYZ to $L^*a^*b^*$. Compared to the conversion method [Barbin et al., 2016], the method used in this work was based on a quadratic model that directly converts RGB colour units to $L^*a^*b^*$ colour space.

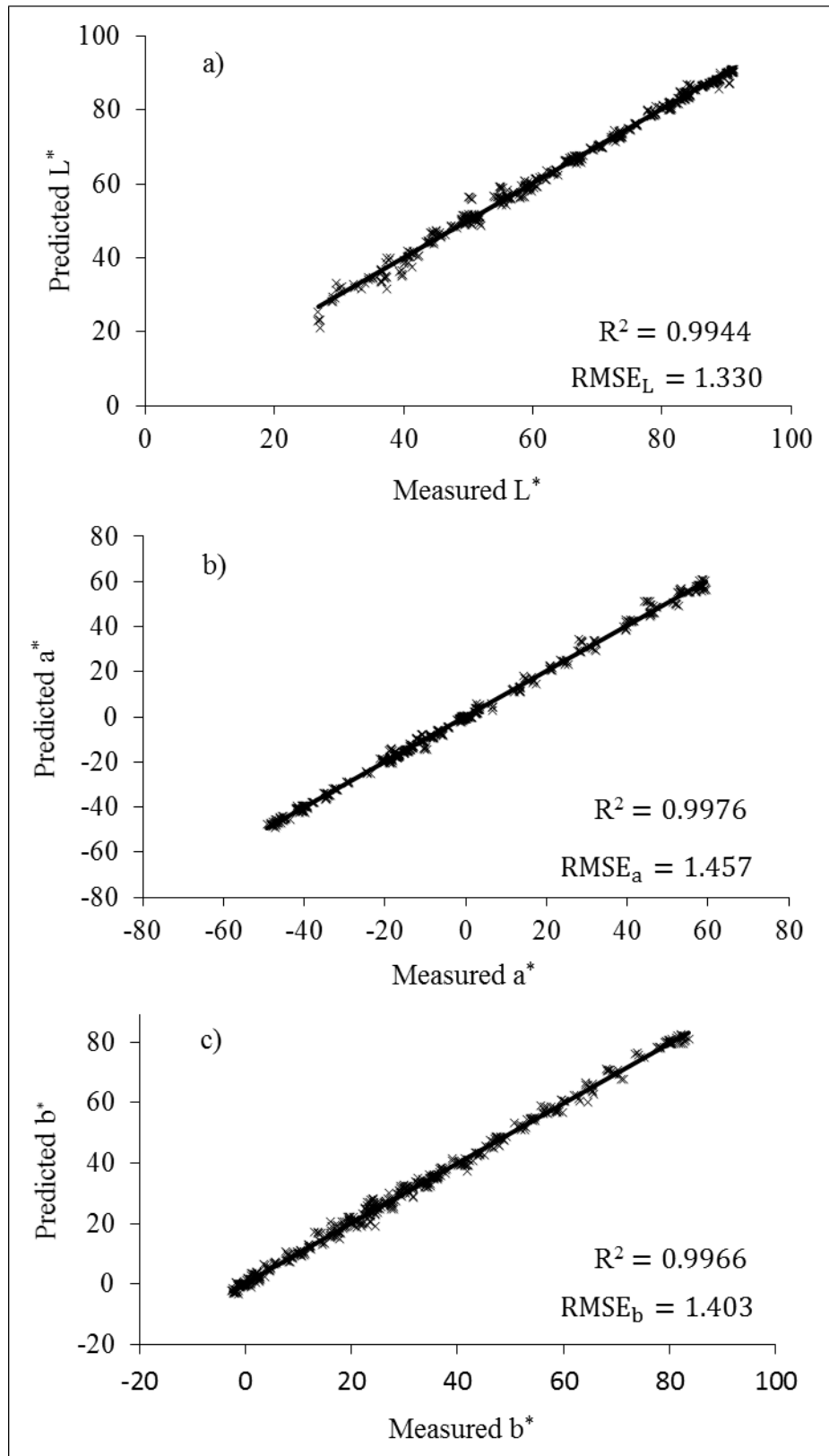


Figure 5.5: Relationship between measured and predicted a) L^* , b) a^* and c) b^* values for the colour cards, respectively.

The prediction performance of the quadratic model for each colour was tested on some real foods including raw meat, salami, sudjuk, crushed red pepper flakes, cheddar cheese, potato, biscuit, butter, green bean, broccoli, white cheese, cauliflower, black sesame and coconut flakes. The colour of these foods was measured by a chroma meter and the computer vision system used in this work (Figure 5.6).

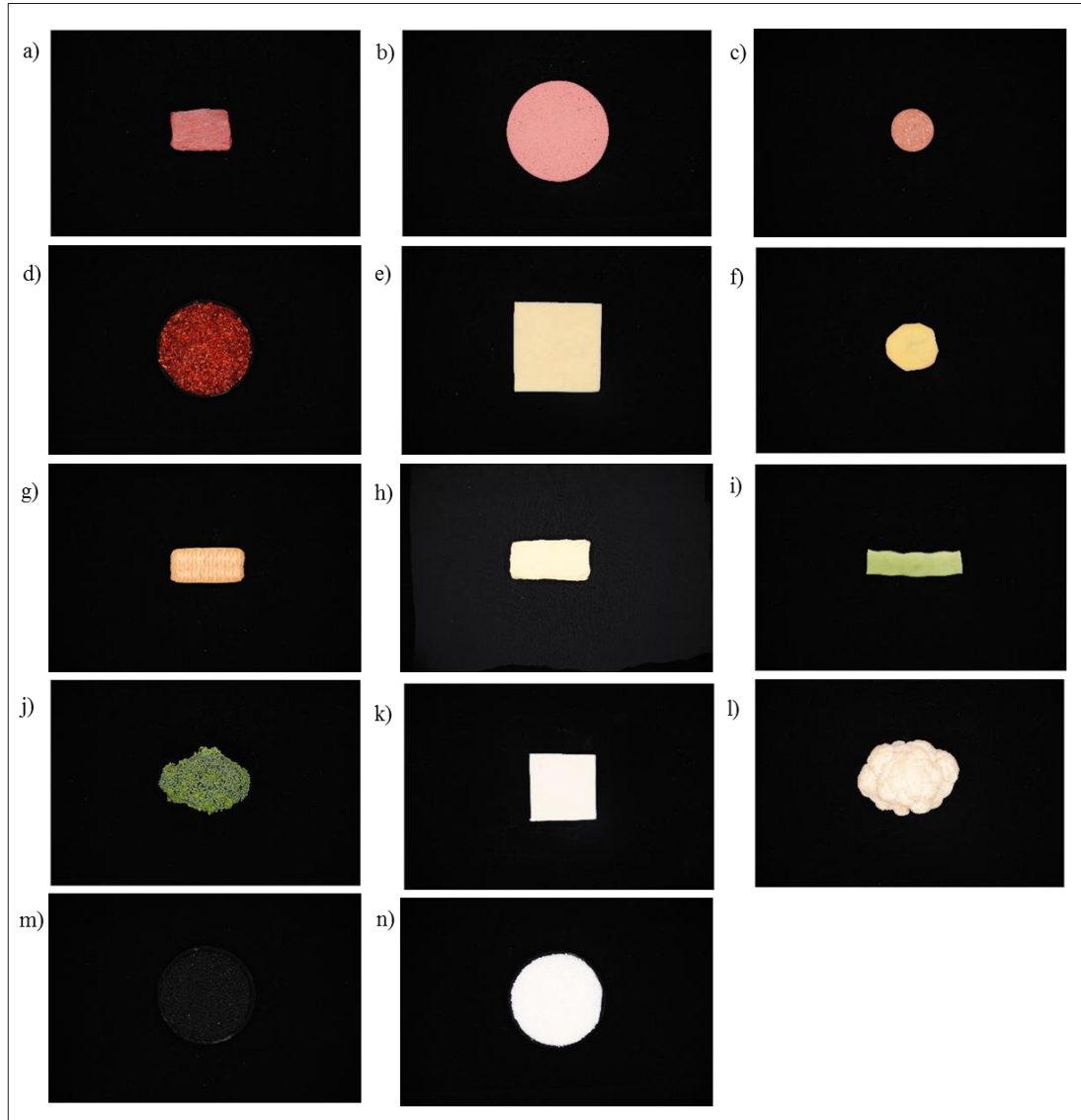


Figure 5.6: RGB images for a) raw meat, b) salami, c) sudjuk (Turkish style fermented sausage), d) crushed red pepper flakes, e) cheddar cheese, f) potato, g) biscuit, h) butter, i) green bean, j) broccoli, k) white cheese, l) cauliflower, m) black sesame and n) coconut flakes.

The quadratic model was used to convert RGB colour units to $L^*a^*b^*$ colour space for the data obtained from the computer vision system for the selected foods.

The prediction performance of the quadratic model for the real foods were analysed by calculating $|\bar{e}|$, Δe^* and RMSE values (Table 5.3). The $|\bar{e}|$, Δe^* and RMSE values for the selected foods ranged from 0.36 to 1.74, 0.94 to 5.93 and 0.48 to 2.69, respectively.

The coconut flakes have the smallest $|\bar{e}|$, Δe^* and RMSE values while the biscuit has the highest $|\bar{e}|$ and RMSE values and sudjuk has the highest Δe^* value. The reason why the smallest $|\bar{e}|$, Δe^* and RMSE values were obtained for the coconut flakes is that they have uniform colour and homogenous, resulting in obtaining more precise measurements by the chroma meter and the computer vision system. These results showed that homogeneity of the food colour was a critical factor affecting the measurement precision. If the colour contains some dark and light tones, this affects the colour measurement and causes some variation in repetitive colour measurements. This situation is observed in biscuit which has the dark and light tones of the yellow colour and sudjuk which contains fat having white tones.

Measured $L^*a^*b^*$ values were also plotted against predicted $L^*a^*b^*$ values, and R^2 values with root mean square errors (RMSE_L, RMSE_a and RMSE_b) for L^* , a^* and b^* variables were calculated to determine the existence of any relationship between the measured and predicted values for the selected foods (Figures 5.7-5.9). Measured and predicted L^* , a^* and b^* values yielded R^2 higher than 0.9735 and RMSE smaller than 1.7465. This indicated that the quadratic model has very high prediction performance for each L^* , a^* and b^* variable. Mendoza et al. used the computer vision system for the classification of ripening bananas and compared the measured $L^*a^*b^*$ values with the predicted $L^*a^*b^*$ values by two step conversion model from RGB to XYZ and XYZ to $L^*a^*b^*$ (RGB→XYZ→ $L^*a^*b^*$) [Mendoza et al., 2006]. They found R^2 values of 0.804, 0.972 and 0.609 for L^* , a^* and b^* , respectively. All the R^2 values were less than the R^2 values found in our work for L^* , a^* and b^* variables. Higher R^2 and smaller RMSE values showed considerable improvement in the prediction performance of the quadratic models used in the transformation of RGB colour units to $L^*a^*b^*$ colour space compared to the prediction performance of the models [Mendoza et al., 2006].

Table 5.3: Measured and predicted $L^*a^*b^*$ values with the quadratic model for some selected foods.

Food	Measured values ^a			Predicted values			$ \bar{e} $	Δe_{ab}^*	RMSE
	L^*	a^*	b^*	$\hat{L}^*$	$\hat{a}^*$	$\hat{b}^*$			
Meat	46.73 ± 1.01	21.94 ± 1.24	13.11 ± 1.00	46.73	24.21	12.31	1.15	3.25	1.75
Salami	60.38 ± 0.40	28.21 ± 0.19	14.13 ± 0.14	62.04	30.05	15.99	1.07	3.10	1.79
Potato	68.59 ± 1.02	-4.56 ± 0.23	36.73 ± 0.99	70.60	-5.67	39.31	1.18	3.45	1.90
Cheddar cheese	76.90 ± 0.45	-4.01 ± 0.12	27.67 ± 0.51	75.32	-4.30	27.75	0.58	1.61	0.65
Broccoli	39.71 ± 1.13	-9.27 ± 1.31	13.07 ± 2.38	36.01	-8.97	13.05	1.28	3.71	1.34
Green bean	63.06 ± 1.28	-17.16 ± 0.78	31.07 ± 1.24	60.63	-15.9	31.78	1.08	2.83	1.47
Cauliflower	82.18 ± 4.11	-0.51 ± 0.14	20.9 ± 1.26	81.95	-0.21	26.57	0.91	5.68	2.07
White cheese	89.59 ± 2.03	-2.08 ± 0.09	12.17 ± 0.47	88.36	-2.04	11.42	0.58	1.58	0.70

^aMeasured values are the average of twenty different measurements on each sample and $\pm$ values are the standard deviation.

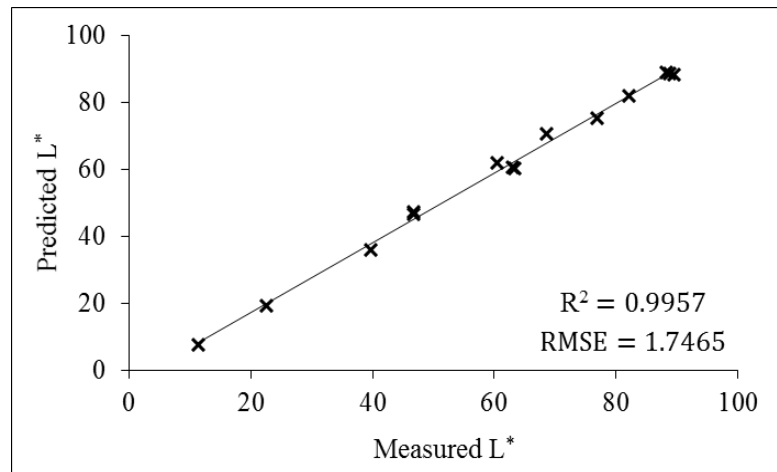


Figure 5.7: Relationship between measured and predicted L^* values for the selected foods.

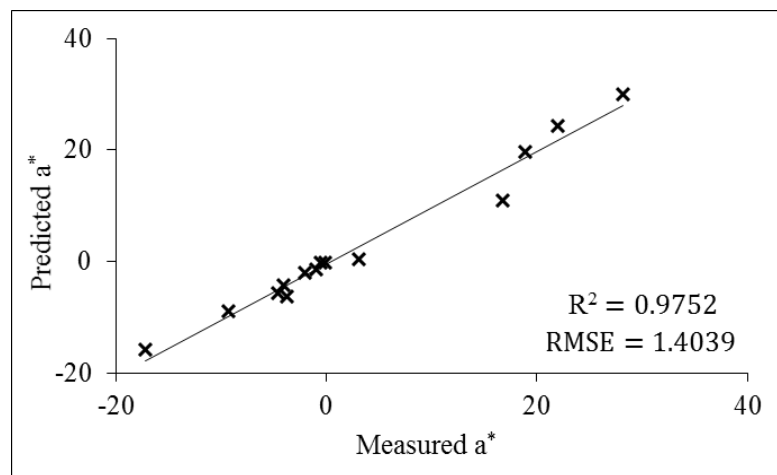


Figure 5.8: Relationship between measured and predicted a^* values for the selected foods.

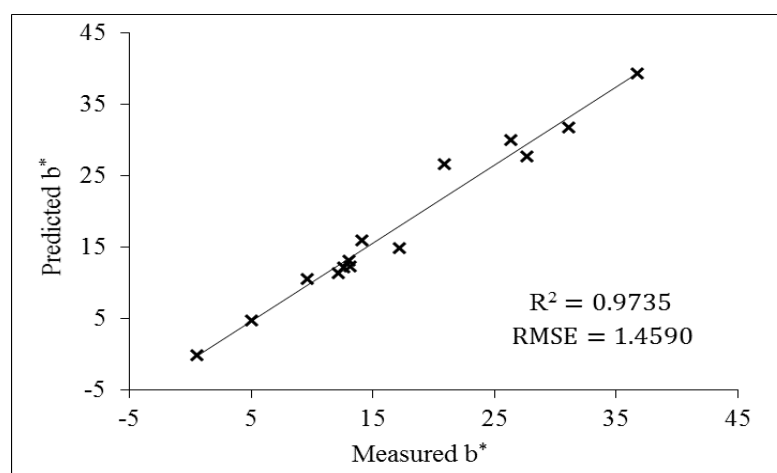


Figure 5.9: Relationship between measured and predicted b^* values for the selected foods.

Barbin et al. used the computer vision system for non-destructive determination of colour parameters of chicken and compared the measured $L^*a^*b^*$ values with the predicted $L^*a^*b^*$ values to assess chicken quality in a fast and rapid way [Barbin et al., 2016]. In order to convert RGB colour units to $L^*a^*b^*$ colour space, Barbin et al. followed two step conversion model ($RGB \rightarrow XYZ \rightarrow L^*a^*b^*$) [Barbin et al., 2016]. Compared to the conversion method [Barbin et al., 2016], the method used in this work was based on a quadratic model that directly converts RGB colour units to $L^*a^*b^*$ colour space. Barbin et al. reported that Δe^* value for the poultry meat was less than 5.2 [Barbin et al., 2016] while Δe^* values for all the foods used in this work changed between 0.94 and 5.93.

Trinderup et al. used multispectral imaging system to measure the colour values of some fresh and processed meat products and compared the measured $L^*a^*b^*$ values with the predicted $L^*a^*b^*$ values by their multispectral imaging system [Trinderup et al., 2015]. In their work, Δe^* values for the fresh meat ranged between 5 and 15 whereas for the processed meat Δe^* values ranged from 1.7 to 4.6. In our work, Δe^* value for the fresh meat was found to be 3.25 while the Δe^* value for the processed meat (salami) was 3.10. These results showed that the prediction power of the computer vision system used in this work is better than the multispectral imaging system [Trinderup et al., 2015].

Valous et al. used the computer vision system to evaluate the quality of pre-sliced hams [Valous et al., 2009]. In order to calibrate computer vision system, they used a colour checker with 24 colours and compared the prediction performance of two step conversion model ($RGB \rightarrow XYZ \rightarrow L^*a^*b^*$) with the prediction performance of some polynomial functions. In their work, Δe^* value was found to be 8.80 ± 4.2 for two step conversion model and greater than 4.00 for the polynomial models including quadratic and cubic terms. Nevertheless, they did not validate the performances of the models used with colour cards or real food samples. In other words, they did not measure the real $L^*a^*b^*$ values and did not compare them with the predicted $L^*a^*b^*$ values for the real foods.

Leon et al. used computer vision system to analyse the colour of potato chips and compared the measured $L^*a^*b^*$ values with the predicted $L^*a^*b^*$ values by neural network model [Leon et al., 2006]. They reported $|\bar{e}|$ value of 1.80% for the transformation of RGB colour units to $L^*a^*b^*$ colour space for potato chips. In our work, the quadratic model gave a $|\bar{e}|$ value of 1.18% for potato; thus, the quadratic

model had a smaller error than the neural network model [Leon et al., 2006]. Compared to the published works [Afshari-Jouybari and Farahnaky, 2011], [Leon et al., 2006] which both used neural network model with extremely complex algorithms, the approach used in this work is based on a quadratic model which does not need complex mathematical operations for converting RGB colour units to $L^*a^*b^*$ colour space as the neural network approach requires. The most important factor to obtain accurate prediction performance to convert RGB colour units to $L^*a^*b^*$ colour space is that as much colour tones as possible must be generated to cover as many points as possible in all the colour space. If this is done, there is no need to use complex algorithms such as the neural network model.

6. CONCLUSIONS

The quality parameters of the mushrooms washed with the SC solution for 60 s and packed with a non-perforated PVC film were very close to the fresh mushrooms even at the end of 12 days storage. In other words, the maximum benefit was achieved when the mushrooms were washed with the SC solution for 60 s and packed with a non-perforated PVC film. Treating mushrooms with the SC solution was found to be effective in reducing the *Pseudomonas* spp. on mushrooms, in controlling the change in colour of the mushrooms during storage. Therefore, SC solution could be used by the mushroom processing establishments to prolong the shelf-life of fresh mushroom.

Several representative primary models such as the modified Gompertz, logistic and Baranyi models were used to describe the growth behaviour of *Pseudomonas* spp. on sliced mushrooms at different isothermal storage temperatures (4, 12, 20 and 28 °C). The Baranyi model was found to be the best primary model with the lowest MSE and the highest pseudo- R^2 values to fit the number of *Pseudomonas* spp. on sliced mushrooms in the temperature range of 4 and 28 °C. The $r_{\max}$ and λ values obtained from the Baranyi model were correlated with the temperature using the Ratkowsky and Arrhenius models. High pseudo- R^2 and low MSE values showed that the Arrhenius model has a high goodness of fit to determine the effect of temperature on $r_{\max}$ and λ . Model validation with the B_f and A_f factors close to one indicated that the models used in this work have high capability to fit the number of *Pseudomonas* spp. on sliced mushroom. The shelf-life of the sliced mushrooms can be reliably fitted with the models used over the temperature range of 4 and 28 °C. The primary and secondary models used provide important information for mushroom spoilage and have high potential to be used as the predictive tools for the mushroom shelf-life. The information provided by the predictive models is valuable to perform quantitative detection of the *Pseudomonas* spp.

Computer vision system was developed to measure the colour of foods as an alternative to conventional colorimeters. Data obtained by the computer vision system in the form of RGB colour units was transformed to conventional $L^*a^*b^*$ colour space using linear and quadratic models. The quadratic model was found to be the appropriate model which gave small error values. The novel approach, in

which more colours were used and the colours were calibrated independently from each other, and contributed reduction in error of the quadratic model significantly. The prediction capability of the quadratic model was also tested and validated with the foods selected. The food industry could benefit from the computer vision system used in this study. With the aid of the computer vision system, foods that are homogenous in colour and shape could be classified with regard to their colours. This system could also be used to distinguish the defectives from the non-defectives. Quality parameters of meat and dairy products could be monitored without any physical contact, which causes contamination during sampling. The computer colour vision system has the potential to be used in the food industry as a quality control tool in a simple, fast and inexpensive way.

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BIOGRAPHY

Fatih TARLAK was born in 1987, Bursa. He received his B.Sc. degree from Department of Chemical Engineering, Gazi University in 2009. In 2010, he started his M.Sc. study at the Department of Chemical Engineering, Gebze Technical University under the supervision of Prof. Dr. Hasan Sadıkoğlu and Assoc. Prof. Dr. Tunahan Çakır. Between 2011 and 2013, he worked in a project entitled “Investigation of the cellular objective behind the metabolic behaviours of the cell by using bottom-up and top-down approaches of systems biology in an integrative manner: Applications to the metabolisms of microorganisms and human”. Being as a research assistant, he has been pursuing his Ph.D. study under the supervision of Prof. Dr. Murat Özdemir at the Department of Chemical Engineering, Institute of Natural and Applied Sciences, Gebze Technical University since 2013. During his Ph.D. study, he worked in two projects entitled “Modelling of microorganisms in mushroom (*Agaricus bisporus*) under different growth conditions” and “Reduction of microbial load and extension of shelf-life of button mushroom with application of acidic sodium chloride”. In the last six months of his Ph.D. study, he was a visiting researcher in the Department of Food Science and Human Nutrition, the Agricultural University of Athens, Athens, Greece under the supervision of Prof. Dr. George-John Nychas.