Hungarian University of Agriculture and Life Sciences

Use of minimal processing technologies in extending shelf-life of egg products

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Budapest 2022

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Contents

LIST OF ABBREVIATIONS

n: flow behaviour index
1xPas: One-time Pasteurization, Pasteurized
2xPas: Two-time Pasteurization, Pasteurized
a*: Redness, Green
AcA: Acetic Acid
AMPs: Antimicrobial Peptides
AscA: Ascorbic Acid
b*: Yellowness
C*: Chroma
CA: Citric Acid
CaS: Calcium Sorbate
CFU: Colony Forming Unit
DSC: Differential Scanning Calorimetry
FA: Foam Ability
FE: Foam Expansion
FS: Foam Stability
h°: Hue°
HDL: High-density Lipoproteins (HDL)
HHP: High Hydrostatic Pressure
HPP: high-pressure processing
HT: Heat Treatment
IOR: ionizing irradiation
K: consistency coefficient
L*: Lightness
LA: Lactic Acid
LDL: Low-density Lipoproteins
LEP: Liquid Egg Products
LEW: Liquid Egg White
LEY: Liquid Egg Yolk
LWE: Liquid Whole Egg
Lys: Lysozyme
N: Nisin
Pas: Pasteurization, Pasteurized
PEF: pulsed electric filed
T_d: denaturation temperature
UHT: Ultrahigh Temperature
USDA: United States Department of Agriculture

UV: Ultraviolet radiation
UV-C: short wave ultraviolet
ΔE^*_{ab}: Color difference, Delta E
ΔH: enthalpy, Delta H
$\tau_{0:}$ yield stress
η: apparent viscosity

INTRODUCTION

Throughout ages, poultry and its products have been one of the important sources of proteins. Their uses and the conservation methods evolved by time in respect to its vulnerability as it easily perishable.

The sensitivity of poultry and its products comes from the high level and quality of proteins. Besides the protein value of the whole egg protein which is advised to be 100 and considering the high value of egg proteins, it is considered as standard for measuring nutritional quality of food proteins.

In fact, egg takes a huge part of human diet because of its rich chemical composition of minerals, vitamins, fats, and it boasts all the essential amino acids that human body needs. This richness not only enforces nutritional and sensory characteristics, but it emphasizes the functional properties too. These functional qualities remain the main core for food industries. Emulsifying, gelling, coloring, aromatic, and antioxidant properties are the main functional properties spotted in eggs and its products. However, various treatment was applied by food industries to ensure the microbiological safety of egg products although these treatments could be harmful to some properties, mainly the functional ones.

Liquid Whole Egg (LWE), Liquid Egg White (LEW) and Liquid Egg Yolk (LEY) are the predominant egg products in the world. They are obtained by cracking the egg, separating the white, yolk and whole egg and passing the products through homogenizer. Afterwards, treatment takes place to ensure the hygiene and the safety of the egg products. As with all foodstuffs, heat treatment (HT) was the first treatment choice for industries; although the sensitivity of egg proteins was one of the challenges that HT had. Thus, decreasing the temperature of HT is a necessity to minimize the damages and maintain the quality of egg products. Moreover, to provide the same microbiological safety produced by HT, it was combined with other preservation methods as chemical, essential, and active compounds or/and non-thermal methods. In fact, the food industry sector looks to the non-thermal methods with a favorable perspective.

Ultraviolet radiation (UV), ionizing irradiation (IOR), pulsed electric filed (PEF) and high-pressure processing (HPP) are some of the conventional and novel food preservation methods adopted by the food industry (Khan et al. 2017; Pou and Raghavan 2020). In fact, food preservation

techniques can be physical, chemical, and biological based (Pou and Raghavan 2020). It provides possibilities for eliminating the probable risks for contamination of foods with foodborne pathogens without drastically changing the natural characteristic of foodstuffs (Naderi, House, et al. 2017; Smelt 1998). Because of the diversity of food products, different pressure levels required to provide microbiologically safe products e.g., meat products are mainly pasteurized, thus they are generally treated in the range of 300 – 600 MPa, to inactivate vegetative cells form (Chung et al. 2005; Aymerich et al. 2008; Tóth et al. 2017).

As all the conventional food preservation techniques, High Hydrostatic Pressure (HHP) method had some impacts on the liquid egg products. Thus, some researchers started to combine HHP treatment with other methods as HT or additives to mitigate these impacts.

Additives are one of the non-thermal conventional food preservation methods that have been used for decades. A wide range of preservatives is available in the food market. They can be differentiated by utilities (preservatives, colorant, antioxidant…), by origin (natural or synthetic…). Nowadays, consumers are more and more aware about natural and healthy nutrition, thus they tend to use natural products to their diet. Currently, acids and active compounds are more popular additives for food factories to satisfy the needs of the consumers.

To emphasize the preservation of egg products characteristics and minimize the damages induced by treatments, hurdle concept is usually used. The hurdle concept (generally known as combined methods, combination preservation, combined processes, barrier technology or combination techniques) has become a promising technology that simultaneously reduces losses of nutritional and sensory quality and improves food safety (Rahman 2015; Khan et al. 2017).

Scrolling the research under egg and egg products topic, LWE had majority of experiment studies unlike for LEW and LEY where there is dearth of information about their changes during storage after treatments. Yet, more research is needed on egg products to improve shelf-life and reduce the impact of treatment on the main characteristics of the products.

Objectives

Egg products effortlessly deteriorate during refrigeration storage, making both consumers and producers face difficulties and health issues. According to the literature, many spoilages of food can occur such as physicochemical changes, growth of pathogenic microorganism, and alteration of organoleptic properties. These qualities are correlated with shelf life of egg products and their alteration indicate that the products are not consumable anymore and decrease the period of the shelf life.

The main objective of this study was to highlight the use of active compounds such as acids and bioactive peptides using the minimal processing technologies simultaneously to extend the shelf life and improving the functional properties of refrigerated liquid egg products.

In the first part of the research, heat treatment of egg products with a preliminary addition of acids has been carried out. As it has been mentioned by (Ponce et al. 1998) that the effect of nisin can be enhanced by its synergism with lysozyme, the second part of the work focused on the effect performed by nisin and lysozyme accompanied with high hydrostatic pressure as a minimal process.

The main interests of the study are:

- To investigate the effect of heat treatment on LEP with citric acid and calcium sorbate during storage time on physicochemical, microbiological, and sensory properties of liquid egg products.
- To investigate the effect of heat treatment on LWE with citric acid or lactic acid during storage time on rheological, physicochemical, microbiological, and sensory properties of liquid egg products.
- To illustrate the effects of HHP treatment on LEP with nisin and lysozyme during storage time on rheological, physicochemical, microbiological, and sensory properties of liquid egg products.

1. Literature overview

1.1. Egg and its products

Hen eggs are considered as a good source of nutrients highly bioavailable (Neira et al. 2017). It is consumed throughout the world without having any use restrictions (Miranda et al. 2015) and it is inexpensive source of proteins (Miranda et al. 2015; Muñoz et al. 2015).

According to many researchers (Abeyrathne et al. 2013; Natoli et al. 2007; Rêgo et al. 2014; Tolik et al. 2014), eggs are considered as a highly nutritious food providing fatty acids, lipids, 18 vitamins, minerals, and proteins that provide several essential amino acids of excellent biological value (histidine; isoleucine; leucine; lysine; methionine; phenylalanine; threonine; tryptophan; valine). For these reasons, eggs are consider as the most complete foods for human consumption (Rêgo et al. 2014).

They are prepared boiled, fried, or at times taken raw or as food supplement prepared in different forms depending on locality (Oladejo 2015). In addition to food uses, eggs also contain a range of bioactive components that could be used for improving human health and other non-food applications. Extraction and fractionation of bioactive egg components such as lysozyme, avidin, ovotransferrin, ovomucin, antibody (IgY), phospholipids and sialic acid, and development of bioactive peptides from egg proteins represent great opportunities in novel applications of egg components in the future (Wu 2014).

Due to the high nutriment quantity of the egg, it can be an excellent substrate for spoilage related microorganisms and food-borne pathogens so they are a highly perishable product even under refrigeration (De Souza et al. 2015). Actually, the aging process of egg begins soon as eggs are laid, altering their chemical, physical, microbial and functional properties (Mudannayaka et al. 2016). Usually, the desirable functional attributes for egg are foaming, emulsification, gelling, coloring, coagulation, and flavoring (Lechevalier et al. 2017; Yang et al. 1995).

1.1.1. Egg composition

Egg is considered as a perfect protein source and contains other high quality nutrients (Uysal et al. 2017); specially the nutritional profile of egg in egg yolk can be modified through diet leading to “designer eggs” such as “omega-3 eggs” and “vitamin-enriched eggs” with additional health attributes (Wu 2014; Zaheer 2015). The three main components constitute the hen egg are eggshell,

egg white and yolk, representing 7-9.5%, 60-63% and 33-27.5%, respectively (Wu 2014; Sunwoo and Gujral 2014).

1.1.1.1. Whole egg

Whole egg can be considered as a major source of high quality proteins and essential nutrients and provides many desirable functional attributes (Yang et al. 1995; Lechevalier et al. 2017). For the high protein value of whole egg, whole egg protein is considered to be 100 and used as standard for measuring nutritional quality of other food proteins (Sunwoo and Gujral 2014).

The main compound of egg is water, it constitutes approximately 75% of chemical composition; while proteins and lipids constitute only 12% of it (50% of proteins are located in egg white, 44% in egg yolk and the rest are based in the shell egg); only 1% for carbohydrates and minerals (Wu 2014).

The main components of whole egg are egg white (albumin); it represents 58% of the total egg weight approximately twice as much as egg yolk (31%). Although, the shell contributes about 11% to the total egg weight (Campbell et al. 2003).

The eggshell, represent 9-12% of the egg, is a complex compound composed of 95 % minerals, of which calcium carbonate is more than 98 %. Other inorganic components include phosphorus, magnesium, and trace amounts of iron and sulfur comprising less than 0.05% (Sunwoo and Gujral 2014; Zaheer 2015). Eggshells color is frequently white or brown but may vary to other color such as blue or even green, this variability is due to hen's genetics (Zaheer 2015). The shell is a calcified protein layer coating the egg and works as a physical barrier from external dangers but it may let the microorganisms pass through its pores (Baron and Jan 2011).

1.1.1.2. Egg white

Albumen or egg white comprised of 88-90% water, 10-12% protein, 0.2% of fat and 0.8% of ash (Campbell et *al*., 2003b; Zaheer, 2015). Some of the main egg white proteins are enumerated in Table 1. Usually, the egg white start to rigid or form a gel at 71 °C and increase at 83 °C, and its elasticity develops between 70 and 74 °C (Montejano et al. 1984; Alleoni 2006). The denaturation temperatures of egg white proteins range between 60 °C and 85 °C according to (Van der Plancken et al. 2006; Chalamaiah et al. 2017). The protein denaturation induces a modification in the egg white protein components such as the appearance of S-ovalbumin protein. Egg white plays a

similar role as an intercellular fluid and performs an important line of defense against invading bacteria because it does not represent a favorable environment (lack of nutriment, alkaline pH, and high viscosity), moreover it contains some antibacterial molecules such as lysozyme, ovo-transferrin, and some proteinase inhibitors (cystatin, ovomucoid, and ovoinhibitor) (Techer et al. 2013).

Table 1: Main egg white protein and their denaturation temperature according to the literature

Protein	% (dry mass basis)	T_d*
Ovalbumin	54	75-79 °C to 84-90 °C
Conalbumin or Ovo-transferrin	12	60-73 °C
Ovomucoid	11	80-100 °C**
Lysozyme	3.4	75-81.5 °C
Ovomucin	1.5-4	-

*T_d: denaturation temperature.

**: under certain conditions.

Ovalbumin

Ovalbumin is the most abundant protein in egg white, representing 54% of it (Huopalahti et al. 2007; Wu 2014; Renzetti et al. 2020). It was one of the first protein isolated from egg white (Abeyrathne et al. 2013). Ovalbumin plays a key role in the protein network formation, by its denaturation the four free sulfhydryl rapidly initiate polymerization through SH-SS exchange reactions thereby interconnecting different egg white proteins (Renzetti et al. 2020). According to the researchers, ovalbumin is the only albumen protein to contain four free sulfhydryl groups (SH) which are buried in the protein core (Wilderjans et al. 2010; Sunwoo and Gujral 2014; Wu 2014). The temperature denaturation of ovalbumin is located between 75-79 °C to 84-90 °C (Alleoni 2006; Wilderjans et al. 2010). Actually, with time and storage the ovalbumin denatures and transforms to S-ovalbumin protein, which is more heat-stable (denaturation at 92.5 °C) (Alleoni 2006; Sunwoo and Gujral 2014).

Ovo-transferrin or conalbumin

Ovo-transferrin or conalbumin is the second protein present in egg white protein reaching 12-13%, and it belongs to the transferrin family (Huopalahti et al. 2007; Takeuchi and Nagashima 2010; Abeyrathne et al. 2013; Wu 2014). As it is a member of the transferrin family, ovo-transferrin is able to bind iron (Corry 2007; Baron and Jan 2011; Wu 2014). For this reasons, it has a bacteriostatic effect through the creation of an iron-deficient environment (Huopalahti et al. 2007; Baron and Jan 2011). The antimicrobial activity mainly affects the outer membrane of Gram-negative bacteria (Valenti et al. 1986; Sunwoo and Gujral 2014).

Ovo-transferrin is the most abundant heat-sensitive egg white protein (Sunwoo and Gujral 2014). It has the lowest denaturation temperature range between 60 and 73 °C but conalbumin does not form aggregates at temperature below 57 °C, thus this temperature is generally used in the conventional pasteurization procedure (Németh et al. 2010; Wilderjans et al. 2010; Radványi et al. 2012; Sunwoo and Gujral 2014).

Lysozyme

Lysozyme is the most soluble and stable among the egg white proteins, and accounts to 3.5% of it (Abeyrathne et al. 2013; Wu 2014). It is widely used in the food industry due to its antibacterial properties (Huopalahti et al. 2007; Techer et al. 2013; Sunwoo and Gujral 2014).

Lysozyme is an enzyme that attacks cell-wall peptidoglycan in Gram-positive bacteria such as *Clostridium tyrobutyricum*, and *Clostridium thermosaccharolyticum* (Corry 2007; Wu 2014). The thermal denaturation temperature of lysozyme is around 75-81.5 °C but depends on pH and medium conditions (Sunwoo and Gujral 2014; Wu 2014).

Ovomucoid and ovomucin

While ovomucoid accounts for 11% of total egg white protein, ovomucin accounts only 1.5-4% of it (Alleoni 2006; Abeyrathne et al. 2013). Ovomucoid known as trypsin inhibitor and is considered as the principal cause of food allergy in egg white (Wu 2014; Miranda et al. 2015).

Ovomucin is present in chalaza; it is the component responsible for the gel-like properties of thick albumen and during the storage ovomucin denature thus the egg white thinning (Huopalahti et al. 2007; Wu 2014). There are two forms of ovomucin in egg white: soluble which is present in both thick and thin albumen; and insoluble ovomucin that is found only in thick albumen (Huopalahti

et al. 2007; Abeyrathne et al. 2013). Besides, it serves to prevent the spread of microorganisms and possess foaming and emulsifying abilities (Sunwoo and Gujral 2014). Indeed, the viscosity of hen egg albumen is principally attributed to ovomucin, a glycoprotein which plays a role in the decrease of the viscosity of the albumen if its structure denatures during storage time (Takeuchi and Nagashima 2010; Sunwoo and Gujral 2014).

Only ovomucoid and ovomucin are not coagulable by heat (Johnson and Zabik 1981; Alleoni 2006). Even though ovomucoid shows high thermo-stability (100 °C), it can be rapidly denature in the presence of lysozyme at 80 °C and pH 9.0 (Sunwoo and Gujral 2014).

1.1.1.3. Egg yolk

The yolk is the place where the cell division happen if the egg is fertile (Zaheer 2015); it compose up to 36% of the weight of the fresh whole hen egg (Huopalahti et al. 2007). It is composed mainly of 51% water, 31-35% lipids, 15-17% proteins, 1.7% minerals, and 0.6-1% carbohydrates (Huopalahti et al. 2007; Abeyrathne et al. 2013; Wu 2014). Major egg yolk elements were recited in Table 2. Egg yolk represents a natural oil-in-water emulsion made of lipid–protein particles in suspension in a clear yellow fluid (Mine and Yang 2010; Rayner et al. 2014). Generally, the egg yolk is fractionated into two main fraction: plasma and granules (Mine and Yang 2010; Anton 2013; Xu et al. 2019). And by controlling the hens diet, a “design egg” can be generated where the nutritional profile of egg yolk have additional health attributes such as omega-3 and vitamin (Wu 2014).

Egg yolk lipids

Lipids are exclusively present in egg yolk (Wu 2014). They have been found in the form of lipoproteins and made up of 62% triglycerides, 33% phospholipids, less than 5% cholesterol and carotenoids represent less than 1% of yolk lipids, and give its color (Huopalahti et al. 2007; Wu 2014).

- Fatty Acids

The standard composition of lipids in fatty acids is 30-35% saturated fatty acids (SFA), 40–46% of monounsaturated fatty acids (MUFA), and 20–25% of polyunsaturated fatty acids (PUFA) (Huopalahti et al. 2007; Sunwoo and Gujral 2014). With the presence of the three main PUFA of omega-3: alpha-linolenic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Sunwoo and Gujral 2014; Zaheer 2015).

Table 2: Main egg yolk components according to the literature

Egg yolk component	% (dry mass basis)	
Water	51	
Lipids	31-35	
	Fatty Acids	62
	Phospholipids	33
	Cholesterol	<5
	Fat-soluble Vitamins	<1
	Pigments	<1
Protein	15-17	
	Low-density Lipoproteins (LDL)	68
	High-density Lipoproteins (HDL)	16
	Phosvitin or phosphoprotein	4-7
	Livetin Fractions	10
Minerals	1.7	
Carbohydrates	0.6-1	

- Phospholipids

Phospholipids are amphiphilic molecules that are composed from two main heads: hydrophilic head group can be phosphoric acid + alcohol, amino acid or polyol, and one hydrophobic head group containing two fatty acids (Huopalahti et al. 2007). For the most part, they are present as a component of lipoprotein (Mine and Yang 2010). Phospholipids in egg yolk are extraordinarily rich with phatidylcholine (PC) range between 76-81%, phosphatidylethanolamine (PE) (12-22%), phosphatidylinositol (PI), phosphatidylserine (PS), sphingomyelin (SM), cardiolipins (CL),

lysoPC, and lysoPE, which are present at extremely low amounts (Sunwoo and Gujral 2014; Wu 2014).

- Fat-soluble Vitamins

A, D, E, B_{12} vitamins are exclusively found in egg yolk (Mine and Yang 2010). Other vitamins could be present in the egg such as K, thiamine B_1, riboflavin B_2 and niacin B_3 (Huopalahti et al. 2007; Baron and Jan 2011; Tolik et al. 2014; Zaheer 2015). It is considered as a source for the nine necessary vitamins for human nutrition (Sunwoo and Gujral 2014; De Souza et al. 2015). As with many component of eggs, vitamins content and quantities can vary with the variation of the composition of the hen's diet (Huopalahti et al. 2007).

- Pigments in Yolk

The yellow color of hen egg yolks comes from the presence of carotenoids which are a natural pigment. Their color can be range from very pale yellow to dark brilliant orange (Miranda et al. 2015). The different colors of egg yolk depend upon the laying hen's diet and does not have any connection with the nutritive value of an egg (Zaheer 2015). The carotenoids are mainly carotene and xanthophylls (lutein, cryptoxanthin, and zeaxanthin) (Huopalahti et al. 2007; Zaheer 2015). They represent usually less than 1% of egg yolk lipids (Abeyrathne et al. 2013; Zaheer 2015).

Egg yolk proteins

Egg yolk proteins are mainly composed by 68% low-density lipoproteins (LDL), 16% high-density lipoproteins (HDL), 4-7% phosvitin, and 10% livetin (Mine and Yang 2010; Xu et al. 2018). Results of differential scanning calorimetry (DSC) by (Cordobés et al. 2004) showed that the denaturation of egg yolk protein takes place above 60 °C.

- Low-density lipoproteins (LDL) and high-density lipoproteins (HDL) or lipovitellenin fraction

Lipovitellenin fraction is commonly used for low-density lipoprotein (LDL) and the high-density lipoproteins (HDL) consist of α- and β-lipovitellins (Mine and Yang 2010). LDL are spherical micelle with a neutral lipid core (triacylglycerols, cholesterol, and cholesteryl esters) surrounded by a layer of apolipoproteins and phospholipids (Huopalahti et al. 2007; Sunwoo and Gujral 2014; Anton 2013; Blume et al. 2015). Dissimilar to LDL, HDL does not have a spherical micelle

structure instead it have a pseudo-molecular structure close to that of globular proteins (Huopalahti et al. 2007; Mine and Yang 2010).

LDL has been extensively studied for its emulsifying, cryoprotective and antioxidative characteristics (Zhou et al. 2018). It is the main constituent of egg yolk plasma fraction (85%) and present only 12% of egg yolk granules fraction (Mine and Yang 2010; Xu et al. 2019). HDL compose 60-70% of egg yolk granules fraction (Mine and Yang 2010; Xu et al. 2019). They are linked together by phosphocalcic bridges forming the granular structure (Huopalahti et al. 2007; Mine and Yang 2010; Anton 2013). LDL confirmed their techno-functional properties by being the main contributors to emulsifying properties of egg yolk (Huopalahti et al. 2007).

The complete denaturation of LDL and HDL occurs at 76 °C and 84.3 °C respectively (Xu et al. 2019).

- Phosvitin or phosphoprotein

Phosvitin is a phosphoglycoprotein present in egg yolk representing 16% from egg yolk granule fraction (Miranda et al. 2015; Xu et al. 2019), it has also been reported to possess good emulsifying properties at various pH levels (Chalamaiah et al. 2017).

- Livetin Fractions

Livetin is the second component of egg yolk plasma and its fraction α, β, and γ-livetin (referred to as *immunoglobulin* Y (IgY)) are relatively heterogeneous and accounts for about 9.3% of hen egg yolk proteins, all of them are water-soluble (Mine and Yang 2010; Chalamaiah et al. 2017). The complete denaturation of the structure of α, β, and γ-livetin takes place at 76, 81 and 69 °C correspondingly (Xu et al. 2019).

1.1.2. Egg properties

1.1.2.1. Rheological properties

Usually, the rheological properties of egg are brought to the surface when egg liquids are involved. The knowledge of the rheological properties and viscosity behavior is essential for the product development, quality control, sensory evaluation and design (Kumbár, Strnková, et al. 2015).

Numerous papers (Jones 2007; Atılgan and Unluturk 2008; Toyosaki 2010; Takeuchi and Nagashima 2010; Alamprese et al. 2012; De Souza and Fernández 2013; Kumbár, Strnková, et al.

2015) reported that the rheological characteristics of egg yolk, whites, and liquid whole egg could be time-dependent non-Newtonian flow behavior.

Maintaining the structure and viscosity of egg white is largely ascribed to ovomucin, which is a glycoprotein , and it plays a role in decreasing the viscosity of thick white during storage (Robinson and Monsey 1972; Takeuchi and Nagashima 2010; Sunwoo and Gujral 2014).

1.1.2.2. Emulsifying properties

Food dispersions or food emulsions are of 3 types: oil-in-water, and water-in-oil emulsions, in which 1 liquid phase is dispersed in another liquid phase; foam, in which air (gas) bubbles are dispersed in an aqueous medium; and sol, which is small solid particles dispersed in a liquid medium (Damodaran 2005).

Contradictorily to egg white, egg yolk is particularly recommended for its emulsifying and thickening properties in mayonnaise, salad dressings, ice creams and bakery products, joined to its coloration effect (Rannou et al. 2015). All along the formation of an emulsion, oil droplets are dispersed into a continuous phase (Ghoush et al. 2008). As a matter of fact, egg yolk granules are labelled for what called “Pickering” stabilization effect of emulsion droplet for their particle-like structure (Gmach et al. 2019; Rayner et al. 2014).

1.1.2.3. Foaming properties

Foam is defined as two-phase systems composed of a discontinuous gas phase dispersed in a continuous liquid (Sun et al. 2022; Wouters et al. 2018). Foams and bubbles play a very important role in aerated food products (such as cakes, cookies, desserts shells, and chocolate mousses) in terms of their structure and texture (Duan et al. 2018). Unfortunately, foams are thermodynamically unstable, but can be stabilize by proteins (Damodaran 2005; Murray 2007; Wouters et al. 2018).

Owed to egg white protein globulins, ovalbumin, ovotransferrin, lysozyme, ovomucoid, and ovomucin, egg white have a high foaming property (Mine 1995; Alleoni 2006; Radványi et al. 2012; Campbell et al. 2003). Egg globular albumen proteins could improve foaming properties if they are partially unfolded before foaming to expose more hydrophobic (Liang and Kristinsson 2007). Indeed, the unique foaming abilities of egg white are due to the interaction between the various constituent proteins (Campbell et al. 2003).

Three main characteristics define a good foaming agent, which are: (1) able to adsorb rapidly at the air-water interface, (2) undergo rapid conformational change at the interface, and (3) form a cohesive viscoelastic film via intermolecular interactions (Mine 1995; Campbell et al. 2003).

1.1.2.4. Sensory properties

Egg and its products attributes to food products different sensory properties such as flavor, color, taste, and odor (Mine and Yang 2010; Sedoski et al. 2012; Miranda et al. 2015).

In a cake system, the denaturation of white egg protein occurs at a higher temperature than a pure white egg because of the high concentration of sucrose which can increase the denaturation temperature of white egg protein up to 13 °C so responding to that , their gelation will be delayed (Donovan 1977; Renzetti et al. 2020). The change in temperature denaturation of egg white proteins affects baking quality, because their gelation provides resistance from collapsing and does not affect the final cake volume, and crumb texture properties such as springiness and cohesiveness (Wilderjans et al. 2010; Deleu et al. 2015; Renzetti et al. 2020).

Egg yolk oil have a prominent level of bioactive compounds and an intensive yellow color that allows it to enrich food products with egg nutrients and flavor in a low dosage (Kovalcuks et al. 2016). In addition of an excellent emulsifying activity, egg yolk has an ability to form gels due to protein interactions and this plays a decisive role in determining the desire rheological, shape, and textural characteristics of bakery products, egg-based sauces, omelets, etc. (Blume et al. 2015).

1.1.2.5. Microbiological properties

Microorganisms, including pathogenic bacteria, can be attached to the eggshell surface and for some species they are even able to form biofilms (Neira et al. 2017). This is due to the moment of egg laying, where the egg passes through the cloaca of the hen into an environment contaminated with a variety of microorganisms coming from feces, dust, feed (Corry 2007). Some of the main strain can be found on the hen egg shell mentioned in Hester (2017) book are listed in Table 3. *Salmonella* species, mainly the serovars Enteritidis and Typhimurium are responsible for most foodborne illnesses associated with the consumption of eggs and egg products (Patrignani et al. 2013). In general, it has been known that the microbiological contamination of the food products depends essentially on the quality of the raw materials, and it influences the processes of transformation. The egg content may be contaminated at breaking by *Staphylococcus* sp. a pathogen microorganisms present on the eggshell (Neira et al. 2017).

Salmonella growth may occur at temperature between 4 and 10 °C in egg yolk, whole egg or white egg (Baron and Jan 2011; Gumudavelli et al. 2007). (Cwiková and Nedomová 2014) showed in their study that the highest total aerobic count and incidence rate of coliform bacteria are in egg yolk comparing to whole and egg white.

Table 3: Occurrence frequency of some bacteria strain found on the shell of poultry eggs according to Hester (2017) book

Strain of bacteria	Occurrence frequency
Staphylococcus	+
Pseudomonas	+
Escherichia	+
Aerobacter	+
Bacillus	+
Aerobacter	+
Micrococcus	++

+: occurs in small number in most cases

++: always present in large number

1.1.3. Egg products

Egg products are becoming increasingly popular in food service operations (Shahbaz et al. 2018). In recent years, the food industry prefers eggs broken and pasteurized for use. Liquid egg is obtained after breaking, filtration, add ingredients if needed, blending, standardizing, and pasteurizing prior to packaging in refrigeration room or further frozen or dried treatment (Rossi et al. 2010; Wu 2014; Uysal et al. 2017). The deteriorating and pathogenic microbes may contaminate the inner part of the egg during the breaking procedure (Németh et al. 2011).

By European laws, food industries can produce egg products with both grade A (fresh eggs) and B (second quality) eggs as they fit for human consumption (Smith 2004). The shells must be clean, dry, fully developed, and with no cracks. However, cracked eggs should be processed as soon as possible. Eggs must be broken in a manner that minimizes contamination, from the shells, thus contents may not be obtained by the centrifuging or crushing of eggs (Smith 2004).

Three main liquid egg products are distinguished (Wu 2014):

(i) Liquid whole egg is the fuse of egg white and egg yolk, its solid level should be standardized to 24.2% according to the USDA regulation generally achieved by the addition of egg yolk. The pH of liquid whole egg ranges from 7.0 to 7.6.

(ii) Liquid egg white has about 12 % of solid content with pH ranges from 7.6 to 9.3.

(iii) Liquid egg yolk, in which the solid level is standardized to 43-44% by adding egg white and the pH is around 6.0.

1.2. Preservation Methods

Many conservation methods are used to extend the shelf life of egg products and preserve their properties. The main methods were coating the eggs with petroleum jelly (Vaseline), immersions in limewater and water glass (Oladejo 2015). Coating of the eggshell takes a considerable duration of time to apply, but according to (Mudannayaka et al. 2016) coating with one of these materials (Beeswax, Gelatin and Aloe vera gel) can preserve the eggs for about 6 weeks of storage at 30 °C. In fact, treating them with limewater is likely to give the eggs a limy flavor (Oladejo 2015).

1.2.1. Heat treatment

In fact, heat-treatment of food products is often required to ensure microbial safety or to obtain desirable organoleptic attributes. On the other hand, major protein denaturation can happen with significant changes in the physicochemical properties demonstrated by the changes in the functional properties of the food such as gelling or foaming; all this is depending on the severity of the heat-treatment and intrinsic factors such as composition and pH (Van der Plancken et al. 2006).

Actually, in the egg product industry the extermination of microorganisms is mainly performed at temperatures around 65 to 68 °C for 5 to 6 min for both whole egg and egg yolk; and because of the thermo-labile protein egg white is treated with a milder temperature around 55 to 57 °C for 2 to 5 min (Baron and Jan 2011; Techer et al. 2013). Nevertheless, there is much standardization of the heat-treatment. The USDA requires liquid egg pasteurization (conventional processing) to be conducted on a critical temperature-time condition where egg protein coagulation may not occur. The minimum temperature and holding time requirements for the egg yolk is 60 °C and 6.2 min. For the egg white and whole egg, minimum temperature and holding time requirements are 55.6 °C and 6.2 min., 60 °C and 3.5 min, respectively (Atılgan and Unluturk 2008; Lechevalier et al. 2017). While in France, only microbiological results are determined by regulation. Classic

treatments use pasteurize liquid whole eggs from 65 to 68 °C for 2-5 min in order to ensure 5 to 6 decimal reduction of vegetative micro-organisms and especially *Salmonella* Enteridis and *Listeria monocytogenes* (Lechevalier et al. 2017).

Alteration of the physical and functional properties of eggs have been reported after an intensive heat treatment and induce formation of destruction of covalent bond, which promotes changes in egg quality due to severe thermal protein denaturation (TPD) (Dawson and Martinez-Dawson 1998; Llave et al. 2018).

1.2.2. High hydrostatic pressure

In 1899, Hite demonstrate that microbial contamination of milk could be postponed by applying high pressure (Smelt 1998). In time, high hydrostatic pressure (HHP) started to become a promising technique for food preservation and allows better retention of product flavor, texture, color and nutrient content than a thermal conventional treatment (Masschalck et al. 2000; Smelt 1998).

Regarding the laws for HHP treatment, until nowadays there is not a clear law for it. For European Union, most of the products undergo HHP treatment and are classified as Novel Food and subject to their regulation but it still need assessment on a case-by-case basis and it back to the food companies to verify whether or not a food or food ingredient falls under the Novel Food Regulation (Aganovic et al. 2021).

Other countries such as United States, Australia and New Zealand mention that food processors have to determine pressure-time condition of high pressure processing and validate that the treatment consistently achieves a minimum 5-log reduction of pertinent microorganisms for that type of a product; to validate that the process can eliminate the spores of *Clostridium botulinum* in low acid products; or effective post-packaged intervention method to control *L. monocytogenes*, which is considered the pertinent pathogen in food (Aganovic et al. 2021; Stewart et al. 2016). HHP technology uses isostatic pressures between 100 and 1000 MPa, with or without heat treatments, to eliminate different forms of spoilage and pathogenic microorganisms, viruses, molds, and yeasts to ensure the microbiological safety of final food products (Naderi, Doyen, et al. 2017).

Isostatic pressure is applied instantly with an equal pressure to the entire mass of food molecules (Aganovic et al. 2021; Patterson et al. 2006). This pressure could be transmitted by pressure transmitting fluids which are water, castor oil, silicone oil, ethanol, sodium benzoate, and glycol (Pou and Raghavan 2020). The working mechanism were simplified by a figure in the review article of Picart-Palmade et al. (2019) in Figure 1.

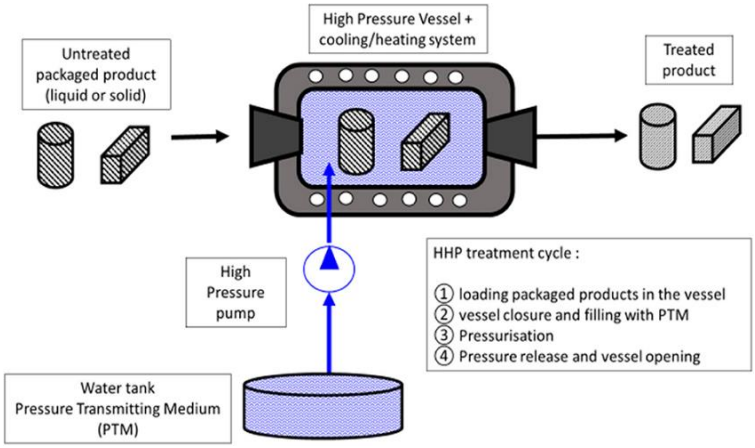


Figure 1:Schematic layout for a High Hydrostatic Pressure (HHP) treatment pilot adapted by Picart-Palmade et al. (2019)

Despite the advantage of the HHP treatment, it shows few limitations in food processing such as difficulties in elimination of bacterial spores, some enzymes, dissolved oxygen, and remaining enzymes action-induced oxidative and enzymatic activities, and most of the pressure-treated products require low temperature handling and storage to hold their organoleptic and dietary properties (Ginsau 2015; Pou and Raghavan 2020). To avoid increasing the pressure of HHP treatment, hurdle technology has must be implemented in combination with HHP at the selected treatment conditions (Monfort et al. 2012).

1.2.3. Hurdle technology

The hurdle concept, known also as combined methods, combination preservation, combined processes, barrier technology or combination techniques, has become a promising technology that simultaneously reduces loss of nutritional and sensory quality and minimizes the degradation of food qualities and improves food safety to enhance the shelf-life of food products (Khan et al. 2017; Rahman 2015).

Hurdle concept relies on combining moderate doses of inactivating and growth-retarding factors, instead of using a high dose of single inactivation factor such as heat or HHP (Hauben et al. 1996). The synergistic combination according Hurdle technologies of different moderate factors improve food safety, compensating for individual process limitations and minimizing the use of extreme levels of any one treatment (López-Pedemonte et al. 2003).

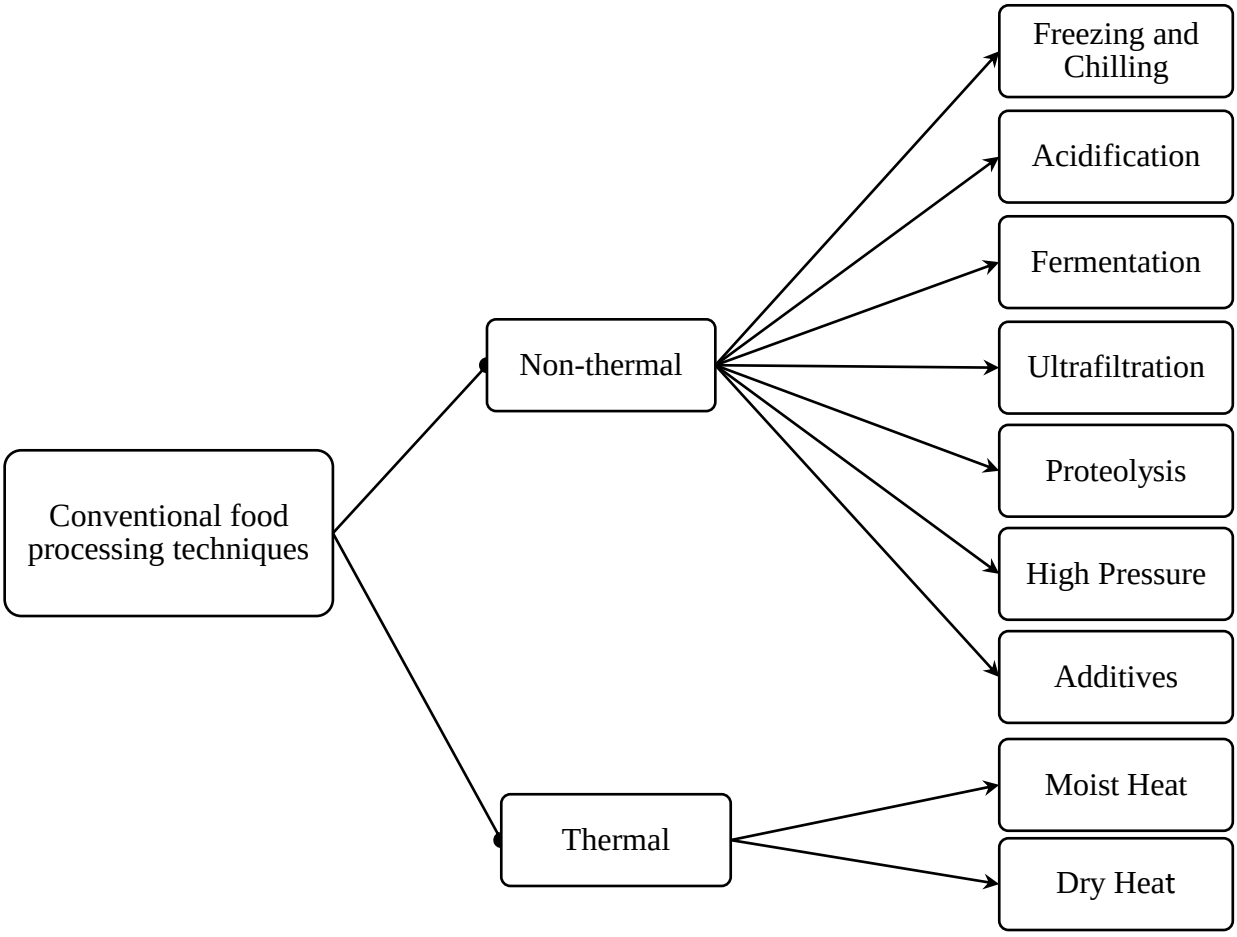


Figure 2: Conventional food conservation methods adopted by the food industry according to Khan et al. (2017) and Pou and Raghavan (2020)

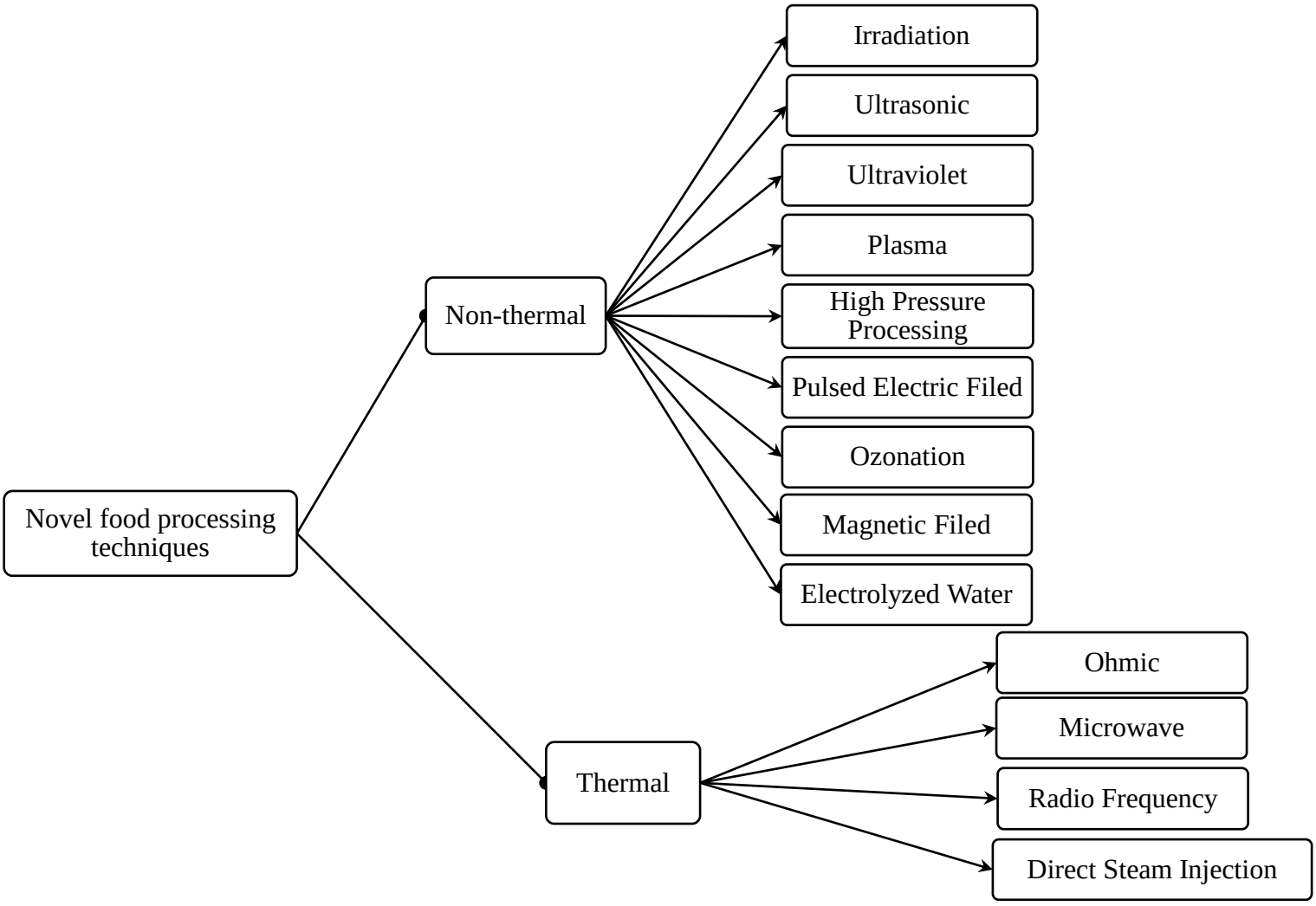


Figure 3: Novel food conservation methods adopted by the food industry according to Khan et al. (2017) and Pou and Raghavan (2020)

The main hurdles used in food preservation are temperature (high/low), water activity (a_w), acidity (pH), redox potential (Eh), preservatives (sorbate, nitrite…), and competitive microorganisms (lactic acid bacteria) (Leistner 2000). However, minimal processing technique is based on a hurdle concept involving the development of combined effects of different conventional and novel food preservation techniques (Leistner 2000; Naderi, House, et al. 2017). These techniques are summarized in Figures 2 and 3 by Khan et al. (2017) and Pou and Raghavan (2020).

1.2.4. Additives: Acids and Bioactive Compounds

The shelf-life of liquid egg ranges from a few days to several weeks depending on the initial bacterial load (Ponce et al. 1998), to prevent the easy contamination of liquid egg novel food processing methods are involved such as HHP and others.

Nevertheless, the selected high-pressure processing conditions were not severe enough to inactivate all kinds of the test microorganisms, and resistant microorganisms like *Listeria seeligeri* were not at all affected by such processing conditions (Lee et al. 2003). To overcome these problems researchers Leistner and Gorris (1995) and Masschalck et al. (2000) suggested the application of the hurdle technology which implicates the synergetic combination of moderate doses of inactivating and/or growth-retarding factors. Several publications: Hauben et al. (1996); Kalchayanand et al. (1998); Kalchayanand et al. (1994) and Masschalck et al. (2000) focus in the interesting synergetic inactivation exist between high pressure and a number of antimicrobial peptides, including nisin and lysozyme (Masschalck et al. 2000). The addition of lysozyme or other antimicrobials to food products before pressure treatment could reduce the required pressure levels, making the high pressure preservation more economical (Nakimbugwe et al. 2006).

1.2.4.1. Antimicrobial peptides

In 1922, Alexander Fleming discovered lysozyme and from that date, a modern innate immunity has seen the light; antibiotics and antimicrobial peptides (AMPs) come upon (Huan et al. 2020). Antimicrobial peptides have a broad spectrum of activity, including activity against bacteria, fungi, viruses, and even cancer cells (Kamysz et al. 2003). As the entire component, AMPs are classified according to different principals. First classification is based on their biological source. The distinguish sources are human, mammalian (such as cathelicidin and defensing), amphibians, fish, insects, and plants (Kościuczuk et al. 2012; Masso-Silva and Diamond 2014; Rima et al. 2021; Tam et al. 2015; Wu et al. 2018). AMPs category could also be based on biological functions such as antibacterial, antiviral, antifungal, antiparasitic peptides. The final category is generated according to their biochemical properties (amino acid sequence, composition, length, hydrophobicity, charge) (Huan et al. 2020; Rima et al. 2021).

The mode of action of AMPs have some factor that can modulate the activity and specificity of it for example size, charge, hydrophobicity (Rima et al. 2021). The ability of AMPs to kill bacteria depends on the interaction with cell membranes; in fact peptides which possess amphipathic

structures interact more accurately with the membrane of pathogens (Rima et al. 2021; Zhang and Gallo 2016). This is due to the membrane permeabilization action and/or act on certain intracellular functions of AMPs (Figure 4) by Lei et al. (2019) and Rima et al. (2021).

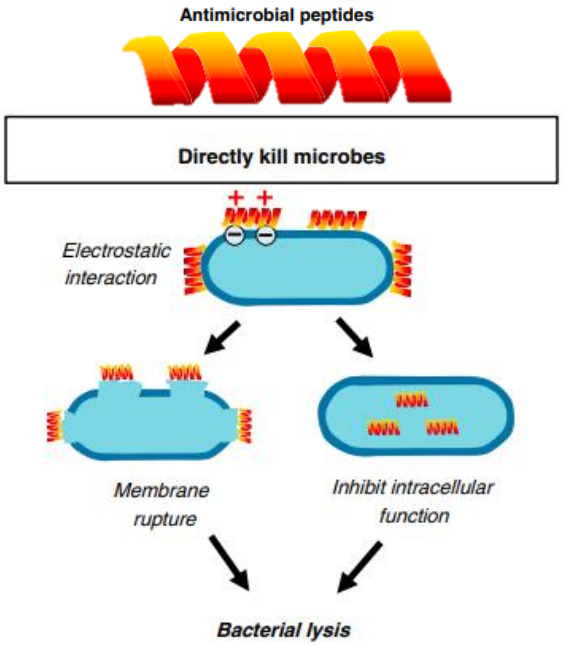


Figure 4: Membrane permeabilization action and/or act on certain intracellular functions of AMPs according to Lei et al. (2019) and Rima et al. (2021)

Nisin

The antimicrobial nisin is a peptide bacteriocin composed of 34 amino acids produced by certain strains of *Lactococcus lactis subsp. lactis* (Ponce et al. 1998; Calderón-Miranda et al. 1999; Lee et al. 2003; Ruiz et al. 2009; Ethiraj 2012; Hofstetter et al. 2013; Modugno et al. 2018).

Nisin is consider as an effective food preservative, not toxic to humans and it is rapidly inactivated in the intestine by digestive enzymes (Calderón-Miranda et al. 1999). According to (Huan et al. 2020; Modugno et al. 2018), nisin cecropins and defensins have shown good inhibition activity to Gram-positive bacteria and Gram-negative bacteria are usually resistant to nisin effect since their outer membrane blocks the access of nisin to cytoplasmic membrane. The mode of action of nisin is established on the pore formation in the cytoplasmic membrane of the target microorganisms

that leads to a loss of small intracellular molecules and a collapse of the proton motive force (Driessen et al. 1995; Lee et al. 2003).

In combination with moderate heat and pressure may be suitable to achieve minimal processing of foods and control of endospore outgrowth and viability (Hofstetter et al. 2013).

Lysozyme

Lysozyme is an antimicrobial enzyme produced by hen egg white which contains 129 amino acid residues (Fu et al. 2017; Sudagidan and Yemenicioğlu 2012).

Lysozyme exhibits a lytic action on the bacterial cell wall of Gram positive (Bi et al. 2020; Fu et al. 2017). In fact, lysozyme hydrolyze the β-1,4-linkage between N-acetylmuramic acid (NAM) and N-acetyl-glucosamine (NAG) of large polymers $(NAM\text{-}NAG)_n$ of the peptidoglycan component of the cell wall (Delves-Broughton 2012). The addition of lysozyme to food before pressure treatment may therefore reduce the pressure levels required for preservation (Nakimbugwe et al. 2006). However, lysozyme has a desirable property as a food preservative and is considered as a safe food ingredient (Huopalahti et al. 2007). It has the ability to control two major pathogens that cause problems in food industry, which are *Listeria monocytogens* and *Clostridium botulinum* (Abeyrathne et al. 2013).

1.2.4.2. Acids

Citric Acid

From food to non-food industries, citric acid is considered as one of the exceedingly popular additive used nowadays (Sweis and Cressey 2018). Usually, citric acid (*Acidum citricum*) a natural antioxidant component of living organism is mainly found in citrus fruits such as oranges and lemons (Drabik et al. 2021). It could also be manufactured and it is called manufactured citric acid (MCA); approximately 99% of MCA is product by the strain of the black mold *Aspergillus niger* (Kirimura et al. 2011; Sweis and Cressey 2018; Drabik et al. 2021). Citric acid takes many forms and its salt form (citrate) is used in many industrial field; for a long time it has been used as an acidulant, a flavoring, a preservative, and to provide pH control in the manufacture of beverages and food, as an aid to the setting of jams (Kirimura et al. 2011; Sweis and Cressey 2018). In general, it is used in the confectionery industry because it is recognized as safe, with pleasant acid taste, and high water solubility (Kirimura et al. 2011)

According to (Marušić Radovčić et al. 2021), citric acid can prevent the green discoloration of egg products and lower pH. It was proven by (Góngora-Nieto et al. 2003; Elez-Martínez et al. 2007) that the use of 0.15% and 0.5% of citric acid to stabilize liquid whole egg can avoid the color darkening during the shelf life storage at 4 °C; and increase the effectiveness of the pulsed electric field treatment and storage between 7-31 days.

Lactic Acid

Lactic acid, acetic acid, and their salts (individually or in various combinations) have been included among organic acids. They also demonstrated the potential to be used for preservation (Fialová et al. 2008; Necidová et al. 2019). In fact, lactic acid is produced by lactic acid bacteria (LAB) such as *Lactococcus, Lactobacillus, Enterococcus, and Streptococcus spp*. and constitute one of the key fermentation products of them (Ameen and Caruso 2017; Ayivi et al. 2020; Wee et al. 2006).

Lactic acid can be produced by microbial fermentation or chemical synthesis (Ameen and Caruso 2017; Wee et al. 2006). It is considerd as one of the most useful used chemical additive in food industry as preservative, acidulant, and flavoring (Ameen and Caruso 2017; Wee et al. 2006).

Ascorbic Acid

Vitamin C or ascorbic acid is used in wide ways in food products and its evident potential to be involved in Maillard reaction and free radical cycles (Farahnaky et al. 2003; Mohammadi Nafchi et al. 2013). It is used in the production and transformation phases of different food products such as gelatins, sweets and confectionery, fruit juices, beer and wine, fishing and it is essential for the production activities of the ground meat and cold cuts (Varnam et al. 1995; Varvara et al. 2016).

The name of ascorbic acid comes from its ability to cure and prevent scurvy (Johnston et al. 2013; Varvara et al. 2016; Doseděl et al. 2021). Even though the main sources of ascorbic acid are fruits and vegetables, two fermentation processes could produce it industrially: the Reichstein –Grussner process or fermentation starting with D-glucose or L-sorbitol (Johnston et al. 2013; Doseděl et al. 2021).

Acetic Acid

Acetic acid is known also by other names ethanoic acid, ethylic acid, and vinegar acid. In fact, vinegar is mainly an aqueous solution of acetic acid and other components which is consumed worldwide as a food condiment and preservative (Gomes et al. 2018). It is obtained by the anerobic

fermentation of sugars to ethanol by yeast than the aerobic oxidation of ethanol to acetic acid by bacteria which are acetic acid bacteria (Gullo et al. 2014; Santos et al. 2019). Besides that, it is the fundamental element of vinegar, acetic acid is recognized as an efficient antimicrobial compound that prevents pathogenic contamination in fermented foods although it can cause some beverages to spoil such as wine (Gullo et al. 2014).

2. MATERIALS AND METHODS

2.1. Preparation of raw liquid egg

For all the thesis experiments, the raw liquid egg was supplied from production line of Capriovus Ltd (Szigetcsép, Hungary). To produce different liquid egg, shell whole egg from caged laying hens were disinfected then passed by the breaker-separator egg machine. Three products were generated: raw liquid egg white (LEW), raw liquid egg yolk (LEY), and raw liquid whole egg (LWE). The egg products were directly sent to homogenization in a piston-gap homogenizer at 100 bars. Afterward, the raw liquid products were stored and transported at 4 °C to the laboratories of department of livestock products and food preservation technology (Hungarian University of Agriculture and Life Sciences).

2.2. Capriovus egg products used in the experiment

Final liquid egg products coming from Capriovus Ltd were pasteurized by a tubular pasteurizer specialized for liquid egg with a capacity of 2000 kg/h and 600 kg/h for liquid egg yolk. According to the nature of the product, the heat temperature parameter was adjusted. It was regulated to 70 °C, 56 °C, and 67 °C for whole, white and yolk liquid egg respectively with a holding time of 190 seconds (3 minutes approximately). Before pasteurization, citric acid (CA) 0.5% and calcium sorbate (CaS) 0.3% were added to the various products as additives. The samples were stored at 4 °C ± 2 °C in the refrigerator room. LWE was pasteurized at 65 °C for 10 min for one-time pasteurization (1xPas) and for 20 min for two-time pasteurization (2xPas). The products were then cooled down and transported at 4°C to the department. The samples were stored at 4°C for 21 days.

2.3. Preparation of liquid egg products with acid for heat treatment

Arriving to the laboratory, the various liquid egg products were stored at the refrigeration chamber at 4 °C. Then, with batch system, one liquid egg product was treated each time.

Liquid egg was poured in a big 1000 ml beaker, pH was measured then according to the pH target value, different volume of acidic solution was added. Citric acid (CA), lactic acid (LA), acetic acid (AcA), and ascorbic acids (AscA) were used with 20% concentration each. The target pH values were 5.0, 5.5, 6.0, 6.5 and 7.0. After reaching the appropriate pH, the samples were packed in polyethylene bags. During the hot sealing of the bags, the air was eliminated as much as possible.

2.4. Preparation of liquid egg with nisin and lysozyme for HHP treatment

Nisin from *Lactococcus lactis* 2.5% (N5764-5G) and lysozyme from hen egg white (62971-10G-F) were purchased from Sigma Aldrich. The liquid egg products were divided to batches, and for each batch an exact weighted amount of nisin (N) and lysozyme (Lys) were added. The additives were weighted on glass dishes using a precision balance. The quantity of additives and HHP pressure treatment are defined by a central composite design (Table 4). After adding the nisin and lysozyme to liquid egg, the sample was mixed with a stainless-steel wood until it was completely dissolved, and no debris remained in the bottom of beakers. Subsequently, the batch of liquid egg with nisin and lysozyme was divided into small quantities by pouring out into polyethylene bags. During the hot sealing of the bags, the air was eliminated as much as possible.

Table 4: Quantity of additives and HHP pressure treatment are defined by a central composite design

Sample	HHP (MPa)	nisin (mg)	lysozyme (mg)
1	226	3	1
2	435	3	1
3	350	0	1
4	350	6.35	1
5	350	3	0.16
6	350	3	1.84
7	300	1	0.5
8	400	1	0.5
9	300	5	0.5
10	400	5	0.5
11	300	1	1.5
12	400	1	1.5
13	300	5	1.5
14	400	5	1.5
15	350	3	1
16	350	3	1
17	350	3	1

2.5. Heat treatment

After the addition of acid to LWE, and sealing the bags, the water bath was preheated at 70 °C. The samples were submerged in the water with a control. One bag served to monitor the temperature (when the coldest point of the sample reaches the desired temperature). The heat treatment lasted for 3 minutes approximately. At the end of the treatment, the samples were directly placed in a sink full of ice to stop any further heat treatment. The moment the samples were cooled down, they were transferred to the refrigeration room to be stored for 15 days at 4 ± 0.5 °C. Three parallel measurements were performed.

2.6. HHP treatment

Before the HHP treatment, the prepared liquid egg samples were packed in polyethylene bags of 100 ml. The pressure treatment of samples was processed by the semi-industrial machine RESATO EPU 100-2000 HHP unit (Resato International B.V., the Netherlands), where glycol-oil mixture is used as a pressure-transmitting medium. The pressure build-up rate was 100 MPa/ min, build-up and decompression times were not included in the treatment time. The HHP treatment was fulfilled at room temperature, with a change in temperature in the samples in the build-up and decompression times. The machine is showed in Figure 5. At the end of the treatment, samples were stored at 4 ± 0.5 °C for 21 days. Three parallel measurements were performed.

Figure 5: HHP machine used during the experiment [Internet1]

2.7. Measurements

The applied measurements for each product are the following which they were summarized in Table in annex 2.

2.7.1. pH

The pH value was determined by emptying the bag of sample into 50 ml beakers before measurement by a pre-calibrated pH electrode meter (Testo 206; Testo-AG, Germany). Three parallel measurements were performed per sample.

2.7.2. Color

Color measurements were performed by using a Minolta Chroma Meter CR-400 tristimulus color analyzer (Konica Minolta Sensing Inc., Japan) for measuring reflected-light color. The measurements were taking from five different random points of the liquid egg bag, and then were analyzed and the average value was calculated for all samples. Color-difference (ΔE^*_{ab}) was calculated using CIELAB system where L* is lightness (black point L*=0, white point: L*=100), a* is characteristic to red-green color (+a* red, -a* green), and b* is the blue-yellow color (+b* yellow, -b* blue). Whereas saturation or color intensity is expressed by chroma (C^*_{ab}). Calibration was performed with calibration tile before starting the measurements.

$$\Delta E^*_{ab} = \sqrt{(\Delta a^*)^2 + (\Delta b^*)^2 + (\Delta L^*)^2}$$

Δa^*: Difference in redness or greenness of the sample and the control,

Δb^*: Difference in yellowness or blueness of the sample and the control,

ΔL^* : Difference in the lightness of the sample and the control.

ΔE^*_{ab}: can be ranges by which the numerical value of the resulting color stimulus can be assigned to the level of human perception explained in Table 5.

Chroma (C^*_{ab}) and hue° angle were calculated from the respective a^* and b^*:

$$C^*_{ab} = \sqrt{(a^*)^2 + (b^*)^2}$$

$$Hue^\circ = \tan^{-1}\left(\frac{b^*}{a^*}\right)$$

Table 5: Values of perceptible color differences

ΔE^*_{ab}	Perceptible color difference
0.0 – 0.5	Not noticeable by the human eye
0.5 – 1.5	Perceptible through close observation
1.5 – 3	Clearly noticeable
3 – 6	Noticeable by the human eye
6 – ≥ 12	High difference

2.7.3. Determination of the calorimetric properties

The thermal denaturation of liquid egg samples was examined by a Micro DSC III type (SETARAM, France) differential scanning calorimeter in dynamic measurement mode. Samples were conditioned in a tared stainless steel cylinder sample holder, weighed 210 ± 5 mg of liquid egg and the bi-distilled water was used as reference material (210 mg). The measurement program started by thermos stating at 20 °C for 2 minutes, then heating the samples to 95 °C at a heating rate of 1.5 °C/min. At 95 °C, the samples were re-cooled to 20 °C with a cooling rate of 3 °C/min. Heat flow curves were recorded. The measurement program was controlled by SetSoft 2000 software. The evaluation was conducted on the heat flow curves of the heating phase as a function of temperature with Callisto Processing version 1.076. A linear baseline was set to the heat flow curves and the area under the curve was calculated, which gives the denaturation enthalpy (ΔH, [J/g]). Peak temperatures were also recorded. Three parallel measurements were performed per sample.

2.7.4. Viscosity

The rheological behaviour of liquid egg samples was investigated by the MCR 92 rotational rheometer (Anton Paar, Les Ulis, France). Properties of the probe were the following: cup diameter 28.920 mm, bob diameter 26.651 mm, bob length 40.003 mm, active length 120.2 mm, positioning length 72.5 mm. The device was operated using Anton Paar RheoCompassTM software. The flow curves of the samples were recorded at an increasing shear rate of 10 to 1000 1/sec at 20 °C. Three parallel measurements were performed per sample.

The Herschel-Bulkley model (Equation) was used to analyse the flow curves. This model was used to describe the rheological properties of liquid egg products at 4 °C (Atılgan and Unluturk 2008). All R^2 values of the fitted model were higher than 0.99.

$$\tau = \tau_0 + K\,\gamma$$

where:

τ – shear stress (Pa),

τ_0 – yield stress (Pa),

γ – shear rate (s^{-1}),

K – consistency coefficient (Pa s^n),

n – flow behaviour index (dimensionless).

2.7.5. Sensory analysis

To highlight the effect of citric acid and lactic acid on the functional properties of liquid whole egg and the perception of customers, a panel was formed consisting of 10 different nonprofessional judges (researchers, teachers, and students of MATE) who were familiar with egg consumption such as omelet muffins.

The assessment was conducted using a 10 points hedonic scale: 2: Hate; 4: Do not like; 6: Do not mind; 8: Like; 10: Love. Four sensory attributes were involved: color, smell, taste, and texture. These attributes were used to draw the sensory profile in radar chart. The samples were coded randomly with a 3-digits code. In addition, the omelet muffins were offered to the panelist in a random order at room temperature (25 °C).

One person conducted the sample preparation. Liquid egg samples were filled in baking cups (d = 70 mm, h = 1/3 of the baking cup). The samples were labelled with their names before baking. Then, the samples were baked at 180 °C for 15 min. Later, the samples were cooled down to the room temperature before serving.

2.7.6. Emulsion stability

Emulsion stability towards creaming was determined as follows.

2.7.6.1. Emulsion preparation: Mayonnaise

The mayonnaise emulsion was prepared according Ghoush et al. (2008) and Huang et al. (2016) with modification. The formulation of mayonnaise is 0.67 g salt, 4 ml vinegar, 40 ml vegetable oil and 10 g of egg yolk. The description of the procedure of making mayonnaise was as follows: salt

and egg yolk were mix first at high speed for 3-4 minutes with a mixer. Simultaneously, the oil was added dropwise during the blending. The last step was adding the vinegar and blending it for another minute.

2.7.6.2. Heat stability of mayonnaise

Heat stability examination of mayonnaise was inspired by Huang et al. (2016). In their method, only 2 ml of emulsion preparation is used. In this experiment, 50 ml of mayonnaise were poured into 100 ml beaker, and then the beaker was put in a different water bath temperature (20, 40, and 60 °C). The time at which the oil separated out from mayonnaise was recorded within the 60 min observation time. Mayonnaise exudes more oil unless it is stable. If emulsion did not break within the 60 min, then no time is recorded. Three parallel measurements were performed per sample.

2.7.7. Foamability and foam stability

The foaming property can be determined by whipping test described below using the method of Li, Wang, et al. (2018) with modification. The foam was prepared using liquid egg white samples. 50 ml of liquid white egg was whipped for 10 min with a standard kitchen mix beater, with two stainless steel beaters. Then, the foam was transferred into a 500 ml plastic graduated measuring cylinder by gently scooping it out using a rubber spatula. Foaming properties were obtained by observing the change in foam volume and the volume of liquid exude from it. The foamability represents the volume of air entrapped by a solution. The samples were heled for 30 min to evaluate foam expansion (FE) and foam stability (FS). Three parallel measurements were performed per sample. Foam stability is expressed as percent of drained foam in relation to the initial liquid volume after a holding time of 30 min at the room temperature. Foam ability, foam expansion and foam stability were calculated as follows:

$$Foaming\ ability\ \% = \frac{V_T}{V_i} \times 100$$

$$Foam\ expansion\ \% = \frac{(V_T - V_i)}{V_i} \times 100$$

$$Foam\ satbility\ \% = \frac{V_{30}}{V_{if}} \times 100$$

Where:

V_T: Total volume of foam and liquid,

V_i : Initial volume of liquid egg white used,

V_{30}: Volume of foam after 30 min of drain,

V_{if} : Volume of initial foam.

2.7.8. Microbiological analysis

The main objective for treating liquid egg product is to extend their shelf life. Microbiological propriety is one of the keys to determine the shelf life. Even with different treatments, some heat-resistant bacteria can survive Necidová et al. (2019). During the storage time, the changes of mesophilic total plate count was studied in day 0, 7, 14, and 21 according to ISO 4833-1:2014. Plate count agar (PCA) was used to enumerate mesophilic total plate count. Samples of liquid egg were poured on nutrient agar from a decimal dilution. The inoculated Petri dishes were incubated for 72 hours at 30 °C. Three parallel measurements were performed per sample.

2.7.9. Statistical analysis

The experimental data were examined using SPSS (Version 27.0, SPSS Inc.). The data were subjected to analysis of variance (ANOVA) and General Linear Model (GLM), then the level of significance was established using post-Hoc test at (P<0.05): according to homogeneity Tukey test is used in case it is accepted if not Games-Howell take a place. Unscrambler 9.0 software was used to statistically analyze the central composite design and generate the surface responses. The mean data ± standard deviation was presented.

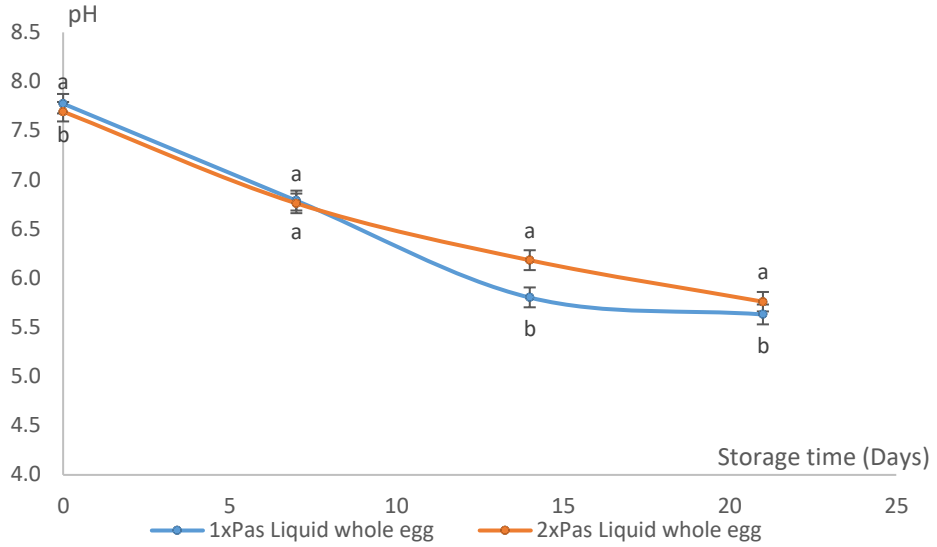
3. RESULTS AND DISCUSSION

3.1. Preliminary studies

3.1.1. Liquid whole egg

3.1.1.1. One- and two-time pasteurized liquid whole egg

Physicochemical properties



a and b mean they are significantly different regarding each other

Figure 6: Changes of pH values for 1xPas and 2xPas LWE during storage

Both, one- and two-time pasteurized LWE showed significant difference of pH values within 21 days storage (P<0.05) only in day 7 the difference between them was not significant (P>0.05). In the first two weeks, pasteurized samples had remarkable close value e.g., day 0 pH values registered 7.77±0.02 and 7.69±0.01 for one-time (1xPas) and two-time (2xPas) pasteurized LWE.

On day 14, 1xPas LWE decreased noticeably while 2xPas LWE continued to decrease gradually but samples showed again close pH value. The evolution of 1xPas and 2xPas LWE pH values is presented in Figure 6. Color parameters are represented in Table 6. L* values of 1xPas LWE showed an insignificant difference from L* values of 2xPas of LWE in the beginning of the measurement (P>0.05) but starting from day 14 the difference is significant till the end of experiment (P<0.05). L* values of both samples showed an increase of brightness during storage comparing to the stable lightness of UHT-pasteurized LWE during same storage time in Liu et al. (2020) research.

Table 6: Changes of color parameters of 1xPas and 2xPas LWE during storage

Color parameter	Sample	Storage time (days)			
		0	7	14	21
L*	1xPas	72.88±0.44a	74.20±0.85a	76.76±0.31a	76.27±0.15a
	2xPas	74.26±0.67a	75.40±0.40a	78.49±0.74b	78.53±0.28b
a*	1xPas	5.63±0.24a	0.32±0.20a	-0.23±0.12a	-0.26±0.04a
	2xPas	5.68±0.22a	1.79±0.16b	-1.40±0.09b	-0.95±0.17b
b*	1xPas	34.59±0.59a	36.87±0.33a	36.75±0.61a	36.77±0.26a
	2xPas	32.02±0.37b	33.42±0.17b	25.84±0.39b	21.25±0.45b
C*	1xPas	35.04±0.62a	36.87±0.33a	36.75±0.61a	36.77±0.26a
	2xPas	32.52±0.35b	33.47±0.17b	25.88±0.39b	21.27±0.45b
*h**	1xPas	1.41±0.00a	1.56±0.01a	-1.56±0.00a	-1.56±0.00a
	2xPas	1.40±0.01a	1.52±0.00b	-1.52±0.00b	-1.53±0.01b

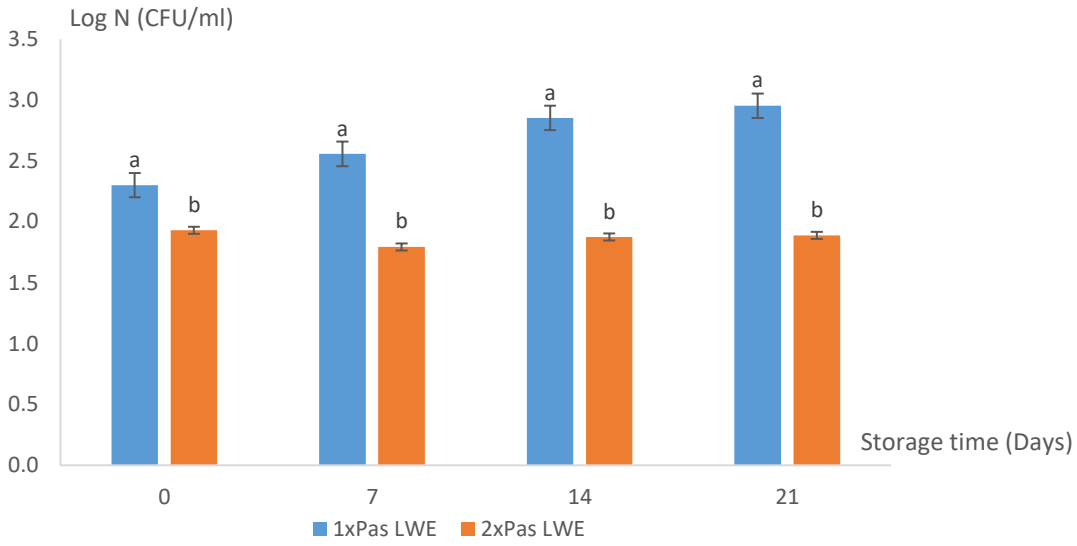
a and b mean they are significantly different regarding each other

*a** values of one-time pasteurized LWE started to be significantly different compared to the *a** values of two-time pasteurized LWE from the 7th day of conservation (P<0.05). Indicating the red when it is positive and the green when it is negative, *a** values of samples decreased all along storage time. After two weeks of storage, *a** values decreased to a negative number which indicates that the color of pasteurized samples started to have some greenish color while *a** values of UHT-pasteurization LWE in Liu et al. (2020) started to decrease 3 weeks and did not reach negative value even after 5 weeks. Hue° values also only started to significantly differ on day 7 (P<0.05). The *h** values increased in the first week thus in the rest of the time of storage it stayed constant.

Throughout refrigeration time, 1xPas LWE *b** values increased in first week then it stayed constant around ≈36 similarly to UHT-pasteurized in case of Liu et al. (2020) research while *b** values of 2xPas LWE showed some fluctuation started with slight increase then decreased in the other 3 storage weeks. The difference between 1xPas LWE and 2xPas LWE was significant all along

measurement time (P<0.05). Chroma parameter had the same pattern of b* values, it had also a significant difference during the storage time (P<0.05). Results showed that a simple pasteurization was sufficient to maintain the bright yellow color of LWE.

Microbiological results



a and b mean they are significantly different regarding each other

Figure 7: Mesophilic total plate count of 1xPas and 2xPas LWE during storage

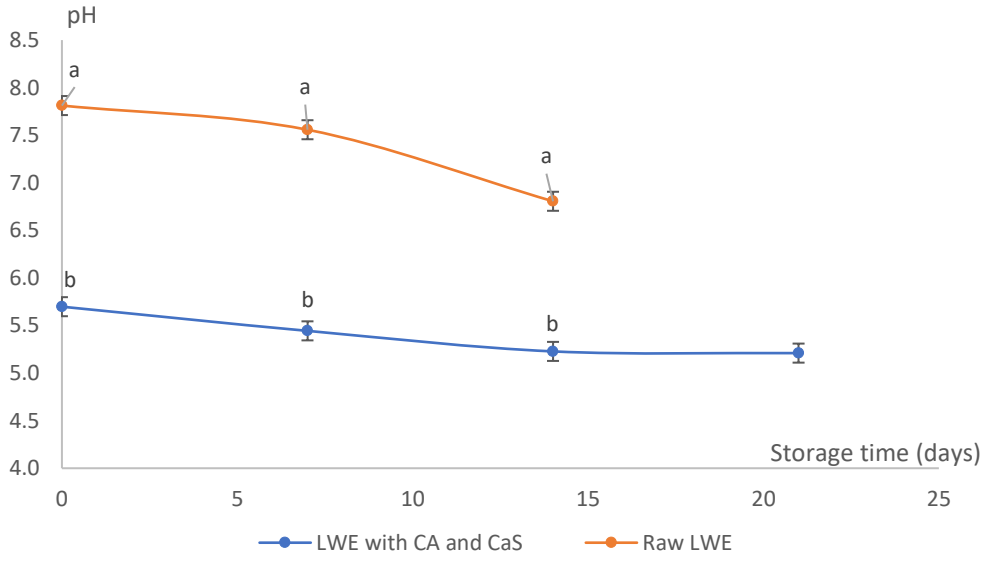
Heat treatment is one of the oldest conservation methods used for liquid egg products to reduce the microbial contamination but due to the sensitivity of egg protein it has been also under evaluation. Total plate count during storage is described in Figure 7. Double pasteurization showed a significant difference during refrigeration storage of LWE from one-time pasteurization (P<0.05). The total plate count of 1xPas LWE is comparable to total plate counts of control group of Necidová et al. (2019) while total plate count of 2xPas LWE was similar to the total plate counts of LWE with 0.25 g nisin per kg in Necidová et al. (2019). Throughout conservation, 1xPas LWE showed increasing values while 2xPas LWE did not only showed lower total plate count, but it retained the microbial growth around 2 Log CFU/ml.

3.1.1.2. Pasteurized liquid whole egg with citric acid and calcium sorbate

Physicochemical properties

The results from the physicochemical properties and color of LWE with CA and CaS are shown in Figure 8 and Table 7. Both pH and color values of LWE showed distinct differences during the storage time.

The pH values of raw LWE and pasteurized LWE with CA and CaS showed a considerable difference starting from the beginning of storage. The two samples showed decrease of pH values. Although the decrease of pH for the pasteurized LWE with CA and CaS was slight compared to the raw LWE. Pasteurized LWE with CA and CaS showed a significant difference all along 21 days compared to the control (P<0.05). The slight maintenance of pH for pasteurized LWE with CA and CaS can be attributed to the heat treatment and presence of citric acid and calcium sorbate by preventing the growth of spoilage microorganism.



a and b mean they are significantly different regarding the control

Figure 8: Changes pH values for raw and pasteurized LWE with CA and CaS during storage

The color of pasteurized LWE with CA and CaS showed significant difference for all the parameters (L*, a*, b*, C*, and *h**) comparing to the control (P<0.05) during the storage period. This main difference is proved by the colour-difference, where ΔE^*_{ab} exceed 12 so the colour-difference between pasteurized LWE with CA and CaS as explained in Table 5, and the control can be seen by unaided eye.

The color parameters (L*, b*, and C*) for pasteurized LWE with CA and CaS were significantly higher than color parameters of raw LWE. The L* values for the samples were slightly fluctuated during the storage, the lightness of treated LWE with CA and CaS is affected, and the sample had a lighter and brighter color comparing to raw LWE. This is suggested to the presence of citric acid and matches the results listed by Marušić Radovčić et al. (2021) where the citric acid affected the light of the sample in contrast with the control during 4 weeks of storage. The *h** values showed a similar pattern to L* values.

Table 7: Changes of color parameters for raw and pasteurized LWE with CA and CaS during storage

Color parameter	Sample	Storage time (days)			
		0	7	14	21
L*	Control	67.79 ± 0.21a	65.67 ± 0.05a	67.21 ± 0.03a	
	LWE	80.78 ± 0.04b	79.05 ± 0.16b	78.78 ± 0.18b	79.91 ± 0.19
a*	Control	4.34 ± 0.07a	5.52 ± 0.07a	5.86 ± 0.04a	
	LWE	-1.28 ± 0.03b	-0.96 ± 0.03b	-1.62 ± 0.10b	-0.96 ± 0.03
b*	Control	29.86 ± 0.22a	29.41 ± 0.13a	29.75 ± 0.04a	
	LWE	39.28 ± 0.27b	39.38 ± 0.64b	39.93 ± 0.27b	39.77 ± 0.17
C*	Control	30.18 ± 0.21a	29.93 ± 0.00a	30.32 ± 0.03a	
	LWE	39.30 ± 0.27b	39.39 ± 0.64b	40.00 ± 0.00b	39.78 ± 0.16
*h**	Control	1.43 ± 0.00a	1.39 ± 0.00a	1.38 ± 0.00a	
	LWE	-1.54 ± 0.00b	-1.55 ± 0.00b	-1.53 ± 0.00b	-1.55 ± 0.00
ΔE^*_{ab}		17.00	17.90	17.13	

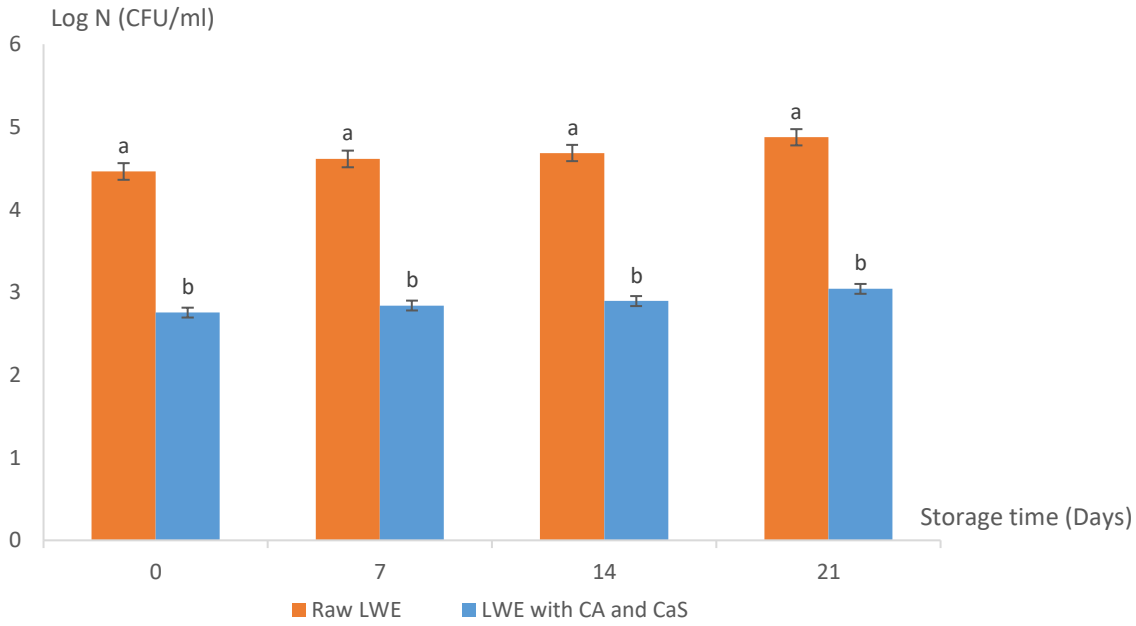
a and b mean they are significantly different regarding the control

The a* values for treated LWE with CA and CaS were noticeably lower than the control values, in fact the color of pasteurized LWE with CA and CaS was more greenish than the control which showed more redness to the yellow color of it. Like the results in Marušić Radovčić et al. (2021), a* values of the control increased throughout the storage period. Tokuşoğlu (2018) and Marušić Radovčić et al. (2021) explained this increase by the formation of complex between conalbumin with the Fe^{3+} which yield a red color; one of the components comes from egg white which is conalbumin and the other component which is the ion of Fe^{3+} comes from egg yolk.

The yellow color is indicated by b* and C* assign the color tone. Pasteurized LWE with CA and CaS presented an observable difference for the two parameters comparing to the control. It induces to conclude that the treated LWE with CA and CaS had brighter and more yellowish color than the raw LWE. The pigmentation responsible for the yellow color of LWE is arriving from the egg yolk. Even though the treatment enhances the yellow color of LWE and its brightness by preserving the pigmentation of egg yolk. It affected the color by the presence of some greenish shade due to the heat treatment.

Microbiological results

The results of mesophilic total plate count during storage are presented by Figure 9. The total plate counts of heat treatment with citric acid, and calcium sorbate of LWE is significantly lower comparing to the raw LWE ($P<0.05$).



a and b mean they are significantly different regarding the control

Figure 9: Mesophilic total plate count for raw and pasteurized LWE with CA and CaS during storage

Throughout the storage, the control presented a higher mesophilic total plate count with a value around 4.5 log CFU/ml. This value is double the total plate count found in the treated sample. In the other hand, the total plate count in pasteurized LWE with CA and CaS increased slightly from around 2.8 log CFU/ml in the first day of storage up to around 3 log CFU/ml in the end of it. The

increase of microbial growth comes with the decreasing pH value. This can be explained by the denaturation of LWE components which are used as a nutriment for the viable cells in LWE.

In accordance with these results, Góngora-Nieto et al. (2003) reported that pasteurizing liquid whole egg by pulsed electric fields (PEF) and citric acid 0.5% diminishes by at least the half the aerobic plate count. Heat pasteurized LWE with citric acid and calcium sorbate had a minimal microbial growth, in opposition the PEF treated LWE showed a sudden increase of microbial growth starting from the 18th day of storage. The presence of calcium sorbate and citric acid with heat pasteurization avoided the sudden microbial growth for the LWE due to decreasing pH of the samples consequently extending the shelf life of LWE to more than two weeks.

3.1.2. Liquid egg white

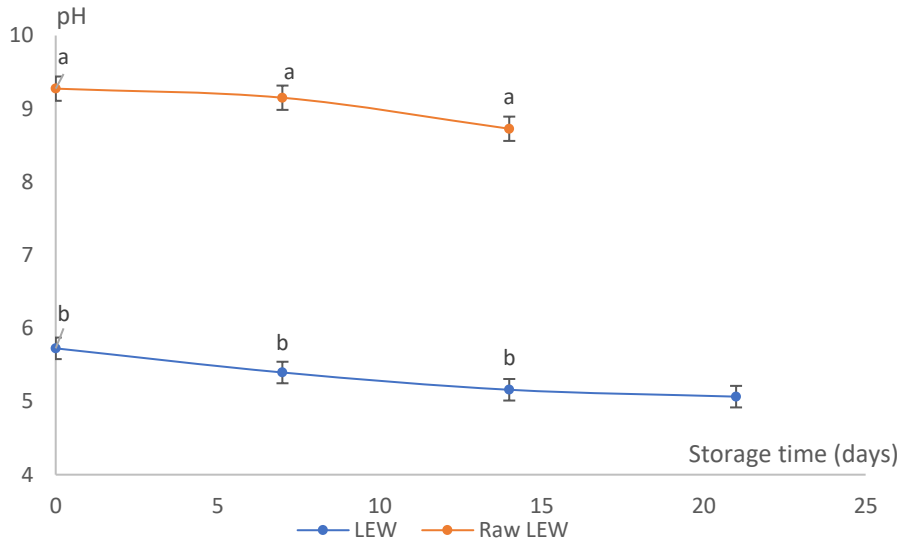
3.1.2.1. Physicochemical properties

The addition of citric acid and calcium sorbate resulted in a lower pH value than the pH value of the control as shown in Figure 10. During the entire of storage time, pH values of pasteurized LEW with CA and CaS showed significantly decreased values in contrast with the raw LEW (P<0.05).

Even though the raw and treated LEW with CA and CaS had a big pH difference, both had the same evolution of pH during of the storage. The two products slightly decreased from 5.73±0.01 and 9.27±0.02 in the beginning to 5.07±0.02 and 8.73±0.01 for LEW with CA and CaS and raw LEW by the final day of conservation, successively. This may be due to that LEW is richer in water (≈ 90%) than nutrient.

The color-difference of the control and the treated LEW with CA and CaS for 14 days ranges from 1.53 to 9.40, showed in Table 8. This suggests that it is easy to spot the difference between the two samples by unaided eye in accord with Table 5. The lowest color-difference was on 14th day of storage, where the difference between the two samples can be seen by close observation.

All along the experiment days, pasteurized LEW with CA and CaS had relatively higher values for color parameters (L*, a*, b*, C*, and *h**) than the control, represented in Table 8. The L* values of treated LEW with CA and CaS showed a significant increase comparing to the raw LEW (P<0.05), only the value on 1st day and 7th day was not significant comparing to the L* value of the control on the 14th day and 7th day, respectively (P>0.05).



a and b mean they are significantly different regarding the control

Figure 10: Changes of pH values for raw and pasteurized LEW with CA and CaS during storage

The L* values of the treated LEW with CA and CaS moderately decreased over time to go from 67.16±0.84 to 56.69±0.84 on the last day of storage. While the raw LEW exhibits some fluctuation and recorded the highest L* value on the 14th day of storage with 66.88 ± 0.80. This high value can be explained by the appearance of some white agglomeration in the raw LEW which makes the liquid egg thicker and more translucent.

The a* values of pasteurized LEW with CA and CaS slightly increased during conservation time and goes from -1.60±0.12 to -1.11±0.18; these a* values significantly increased all along the storage time comparing to the control (P<0.05). At the same time, the a* values of raw LEW fluctuated, and it showed the highest value (-2.58±0.19) on the 7th day.

The same pattern was noticed for the other control colour parameters also (b*, C*, and h*), where they showed a fluctuation during the storage and the highest increased value in the 7th day. Nevertheless, the treated LEW with CA and CaS showed a significant increase comparing to the raw LEW (P<0.05) for b*, C*, and h* values throughout conservation time. The b* values were higher than the ones of the control. Equally freeze- and spray-dried treatment improved color parameters for LEW in Jesús et al. (2013). Results reveal that the pasteurization treatment with preservatives affected the color of LEW and made the LEW thicker and more translucent likewise

in Li, Zhang, et al. (2018) where the color of egg white gels changed from white to amber with presence of NaOH. The explanation for this is the denaturation of LEW protein such as conalbumin (its denaturation temperature is located between 60 to 73 °C).

Table 8: Changes of color parameters for raw and pasteurized LEW with CA and CaS during storage

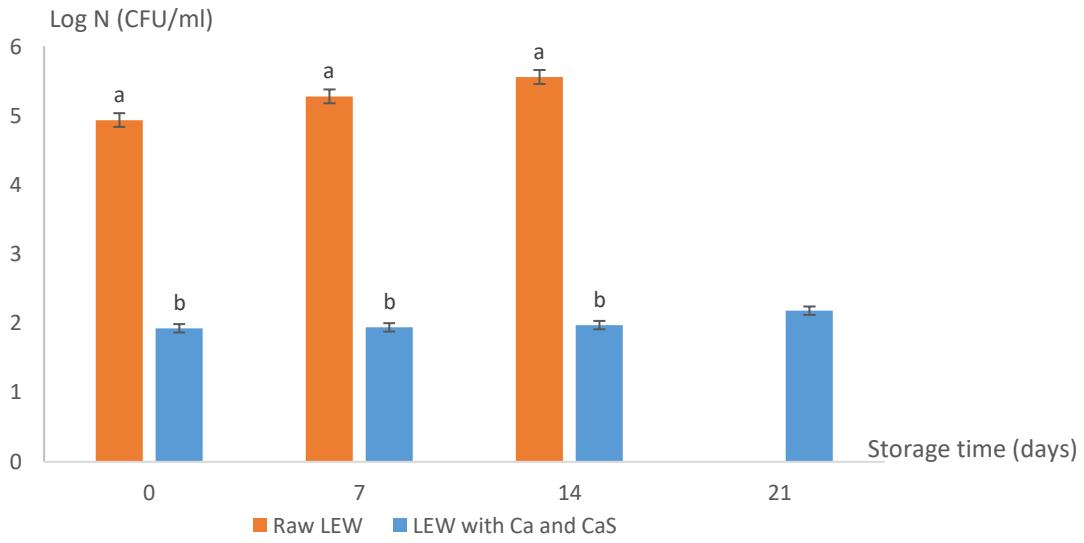
Color parameters	Sample	Storage time (days)			
		0	7	14	21
L*	Control	63.31 ± 0.31a	61.57 ± 0.45a	66.88 ± 0.80a	
	LEW	67.16±0.84b	61.55±0.91a	58.05±0.96b	56.69±0.84
a*	Control	-2.68 ±0.22a	-2.58±0.19a	-2.74±0.07a	
	LEW	-1.60±0.12b	-1.54±0.31b	-1.23±0.15b	-1.11±0.18
b*	Control	14.63±0.33a	15.18±0.12a	14.37±0.36a	
	LEW	16.61±0.09b	16.31±0.42b	17.24±0.25b	16.78±0.47
C*	Control	14.87±0.35a	15.40±0.13a	14.63±0.36a	
	LEW	16.69±0.10b	16.38±0.43b	17.29±0.26b	16.81±0.48
h*	Control	-1.39±0.01a	-1.40±0.01a	-1.38±0.00a	
	LEW	-1.47±0.01b	-1.48±0.02b	-1.50±0.01b	-1.50±0.01
ΔE^*_{ab}		4.47	1.53	9.40	

a and b mean they are significantly different regarding the control

3.1.2.2. Microbiological results

The mesophilic total plate count is represented in Figure 11. The initial total plate count (day 0) of control was around 5 Log CFU/ml and increased to around 5.5 Log CFU/ml on the last day of storage (day 14). These values are higher comparing to the treated LEW with CA and CaS with approximately 3 Log CFU/ml. This high population count can be due to endogenous contamination or the presence of egg yolk in the LEW; it has been mentioned that the presence of even 0.5% of egg yolk is sufficient to allow microbial growth in LEW at permissive temperature Techer et al. (2013). The total plate count values for pasteurized LEW with CA and CaS were significantly lower than for control (P<0.05). In fact, the total plate count of treated LEW with CA and CaS slightly increased in storage time from around 2 log CFU/ml on day 0 to 2.2 log CFU/ml on the 21st day. The increase of microbial growth correlated also with the pH decrease. The increase in total plate count led to a decrease in pH values. The perceptible difference between microbial

results of treated LEW with CA and CaS and the control is another proof for the efficiency of pasteurization in presence of additive in minimizing the microbial growth. In a previous work of Techer et al. (2014), they proved that a pasteurization at 57 °C for 6 min showed an efficiency to reduce the percentage of inoculated samples to total mesophilic bacteria by 64% for aerobic conditions and 50% for anaerobic conditions. This efficiency of pasteurization and preservative can be on behalf of the presence of many of antimicrobial molecules such as lysozyme, ovo-transferrin or conalbumin… (Huopalahti et al. 2007; Techer et al. 2013). Pasteurization of LEW with CA and CaS extend the shelf life to 3 weeks comparing to the control.



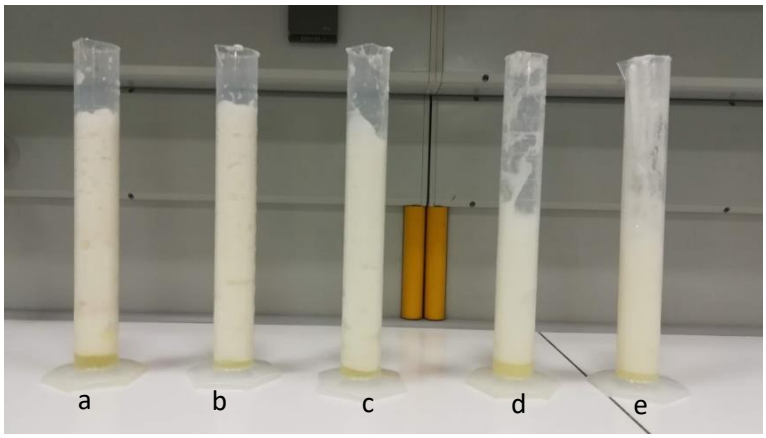
a and b mean they are significantly different regarding the control

Figure 11: Mesophilic total plate count for raw and pasteurized LEW with CA and CaS during storage

3.1.2.3. Foaming property

Foaming propriety is the unique property of LEW which is important for all food products containing egg whites, such as desserts. For this reason, this propriety has been studied by much research. Foam properties are described in Figure 12 and Figure 13. Foam expansion (FE%) which characterizes how much foam in volume is generated from the initial liquid after whipping (Wang and Wang 2009), foam stability (FS%) describe the stability of foam after 30 minutes, and foam

ability (FA%) represent the ability of egg white protein to create foam, of pasteurized LEW with CA and CaS and the raw LEW.



a, b, and c: Foam made from pasteurized LEW with CA and CS

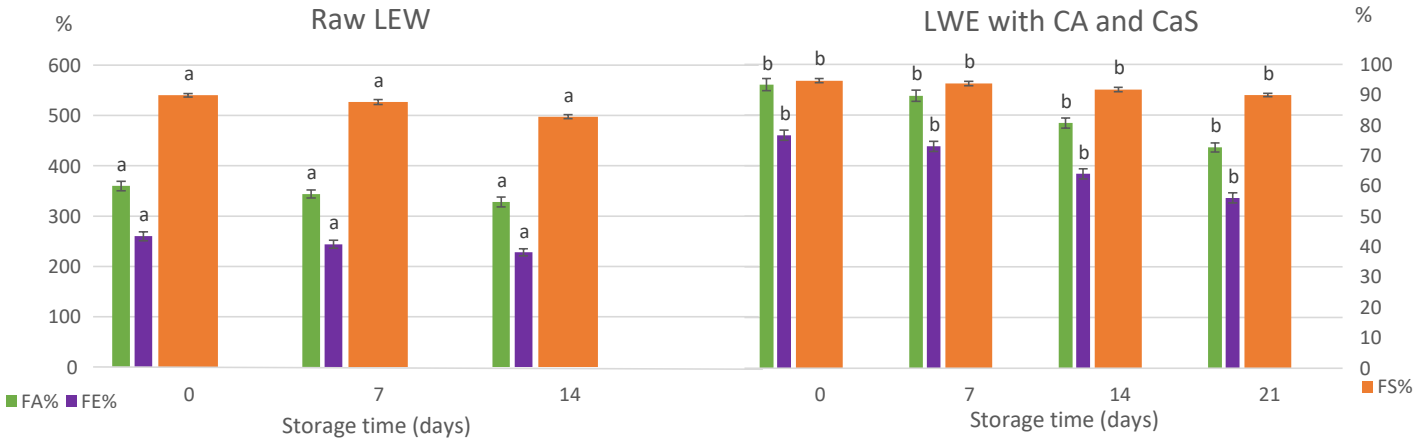
d and e: Foam made from raw liquid egg white.

Figure 12: Some examples of foam test

Figure 13 shows that the pasteurization treatment with the preservative enhanced the foaming ability of treated LEW by 35% compared to the raw LEW (P<0.05). This result is close to the result of Chen and Ma (2020) where the treatment of LEW with 120 µmol/g of gallic acid enhanced the foaming ability by 10.26% without ultrasound treatment and 36.08% for LEW treated with ultrasound containing the same amount of gallic acid. During the conservation time, the foam ability of pasteurized LEW with CA and CaS decreased from 560±12% on day 0 to 436±9% on day 21 while in case of the control decreased from 360±9.5% on day 0 to 328±10.02% on day 14. The foaming ability is related to the propriety of the protein to form a film at the air-water interface (Duan et al. 2018). Furthermore, it has been demonstrated that ovalbumin, which is hierarchically second for the importance of foaming, possesses higher foaming ability in its denatured form because the proteins can adsorb effortlessly to the air bubble surface (Mine 1995; Campbell et al. 2003). This is also proved in the study of Duan et al. (2018) where the foam ability of LEW treated with different concentration of 2,20-azobis (2-amidinopropane) dihydrochloride (AAPH) increased from 86.6% to 91.4% compared with untreated sample.

Similarly, to the foam ability, foam expansion of pasteurized LEW with CA and CaS exhibited a significant increase comparing to the control (P<0.05). Foam expansion showed 460±10% and 260±9% for treated LEW with CA and CaS, and control respectively in the beginning of the

storage time and decreased to 336±9% and 228±7%, respectively in the end. While pasteurization, citric acid and calcium sorbate raised the foam expansion by 43%, the high intensity ultrasound decreased this property by 38.04% (Arzeni et al. 2012). The authors explained this decrease by the effects of the treatment on the apparent viscosity which have been reduced by 13% approximately.



a and b mean they are significantly different regarding the control

Figure 13: Foam properties of raw LEW and pasteurized LEW with CA and CaS during storage

The viscosity is not the only reason for decreasing foam expansion. The presence of egg yolk and its fractions in LEW have an apparent significant reduction effect on the foam expansion capacities (Li et al. 2019).

Foam stability property is the capacity/time of the foam to be stable. In Figure 13, the foam stability of pasteurized LEW with CA and CaS showed significant increase compared to the control (P<0.05) during the days of storage. It was not significant only on 14th day of storage compared to the control on day 0 (P>0.05). Pasteurization treatment and additives increased the foam stability of LEW by 4% going from 90±0.55% for raw LEW up to 94.64±0.71% for treated LEW on the 1st day of the experiment. Surprisingly, these results are not consistent with a previous study where an ultrasonic treatment did not have any significant modification (Sheng et al. 2018) or it was the opposite and the foam stability declined by 10.54% when 120 µmol/g of gallic acid was added to LEW. However, the foam stability of pasteurized LEW with CA and CaS decreased during the storage from 96.64±0.71% to 89.91±0.58% on the last day of storage.

Moreover, the increase of foam stability in this study agrees with recent research of Chen and Ma (2020); Duan et al. (2018) and Li, Wang, et al. (2018). This may be due to many reasons, Li, Wang, et al. (2018) mentioned that a proper acidic condition with pH range of 5.0-7.0 can be favorable to

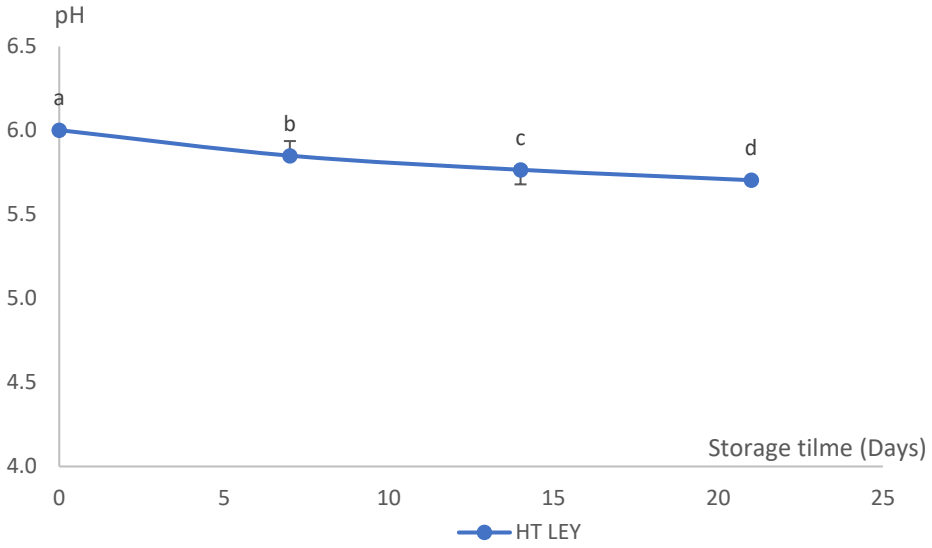
maintain the foam stability of EW proteins and the reason behind the high foam stability is that protein aggregates at low pH environment and stabilize the interfacial films. Consequently, the foam stability is the result of the ability of the proteins at the interface to form a cohesive network by both covalent and noncovalent bidding (Li et al. 2019).

3.1.3. Liquid egg yolk

3.1.3.1. Pasteurized liquid egg yolk

Physicochemical properties

Evolution of pH values of pasteurized liquid egg yolk is presented in Figure 14. At conservation time, pH values showed significant difference between the storage days (P<0.05). In the beginning, the sample showed a pH value equal to 6.00±0.01 then it started to decrease moderately to attain 5.70±0.01 by day 21. In Hidas, Nyulas-Zeke, et al. (2021), where LEY was subjected to other 2 treatments after pasteurization: addition of 5% salt (NaCl) and freezing at -18 °C, pH value evolved differently and increased within 28 storage days.



a, b, c, and d mean they are significantly different regarding the day

Figure 14: Changes of pH values for pasteurized LEY during storage

Only on day 0, color parameters (L*, a*, b*, C*, and *h**) showed a significant difference towards the other days of conservation (P<0.05) while during the remaining time of storage the difference was insignificant (P>0.05). The changes of color parameters are shown in Table 9. It noticed that

independently from the other color parameters, L* started with high value than decreased during storage going down from 72.70±0.89 to 65.04±0.69 in day 0 and day 21 respectively. Decrease of L* value signifies that the brightness of the sample diminishes with time. As regards the other color parameters a*, b* and C* showed increasing values during storage contrary to the hue° values and to L* values, which decreased within 21 days of storage.

The freezing treatment with 5% salt showed same results for L* and b* parameters, where L* value decreased and b* value increased, despite the fact that a* value was the opposite of this experiment and decreased during storage (Hidas, Nyulas-Zeke, et al. 2021). Even though that pasteurization is a heat treatment, it only affected the brightness of LEY and not only maintained but it accentuated the yellow color of liquid egg yolk.

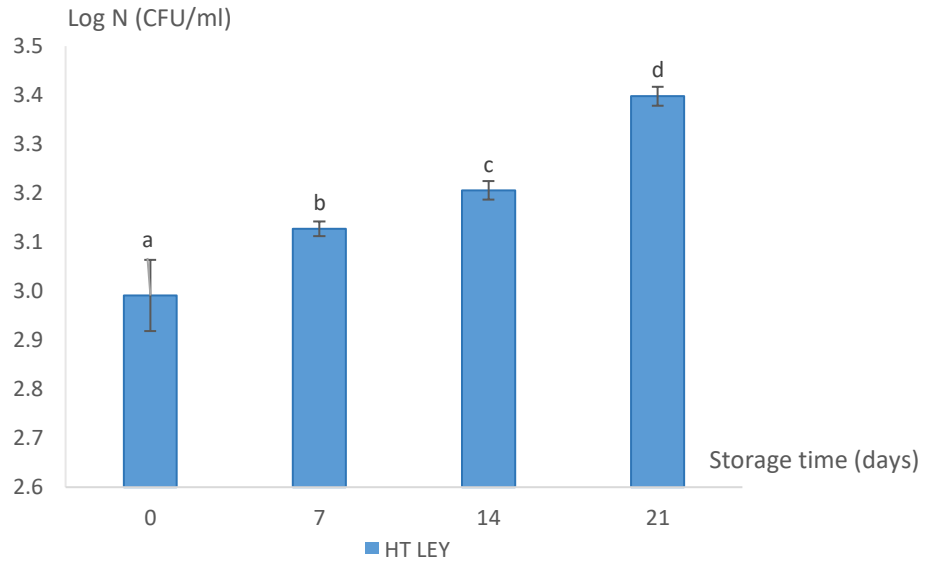
Table 9: Changes of color parameters for pasteurized LEY during storage

Color parameters	Storage time (days)			
	0	7	14	21
L*	72.70±0.89a	67.15±3.07bcd	65.84±0.94bcd	65.04±0.69bcd
a*	7.99±0.22a	11.55±1.88bcd	12.27±0.24bcd	12.59±0.30bcd
b*	38.49±0.72a	51.17±6.97bcd	53.71±0.79bcd	53.42±0.84bcd
C*	39.32±0.70a	52.46±7.21bcd	55.09±0.81bcd	54.88±0.85bcd
*h**	1.37±0.01a	1.35±0.01bcd	1.35±0.00bcd	1.34±0.00bcd

a, b, c, and d mean they are significantly different regarding the day

Microbiological results

The total plate count in pasteurized LEY, presented in Figure 15, started with a relatively high count around 3 Log CFU/ml but close to mesophilic aerobe cell count samples treated with HHP treatment (350 MPa for 5 minutes) in Tóth et al. (2020). Then, it started to increase lightly to attain around 3.5 Log CFU/ml in last day of the study. The microbial growth in day storage showed significant difference during the storage time ($P<0.05$).



a, b, c, and d mean they are significantly different regarding the day

Figure 15: Mesophilic total plate count for pasteurized LEY during storage

Thermodynamic property

Thermograms of pasteurized LEY obtained by differential scanning calorimetry (DSC) are featured in Figure 16, and enthalpy changes and denaturation temperature values are registered in Table 10. Along storage time, denaturation temperature showed a significant difference ($P<0.05$). Likewise, denaturation enthalpy had a significant difference during storage only in day 14 it had an insignificant difference toward day 21 ($P>0.05$).

Table 10: Pasteurization effect on the denaturation enthalpy and denaturation temperature of LEY

Storage time (day)	T_d (°C)		ΔH (J/g)
0	Raw LEY	77.78±0.08a	0.95 ±0.02
	LEY	78.23±0.05a	1.01±0.01a
7	78.03±0.14b		0.97±0.04b
14	77.69±0.18c		0.79±0.11cd
21	76.72±0.16d		0.83±0.01cd

a, b, c, and d mean they are significantly different regarding the day

As mentioned in literature, denaturation of egg yolk protein occurs at a temperature starting at 69 °C. Thermograms of Figure 16 exhibit only a single peak which is explained by the presence of

one protein, accordingly the thermograms are similar to the ones in Xu et al. (2018) regardless they used egg yolk from duck egg not hen eggs. The one peak of raw LEY in day 0 occurs at 77.78±0.08 °C suggesting that it could be a delayed denaturation of α-livetin or LDL protein. The same explanation could be projected also for the thermograms of pasteurized LEY. Highest denaturation enthalpy was registered for the denaturation enthalpy of treated LEY on Day 0. The denaturation temperature of pasteurized LEY is reduced throughout storage days and diminishes only by 1.93%.

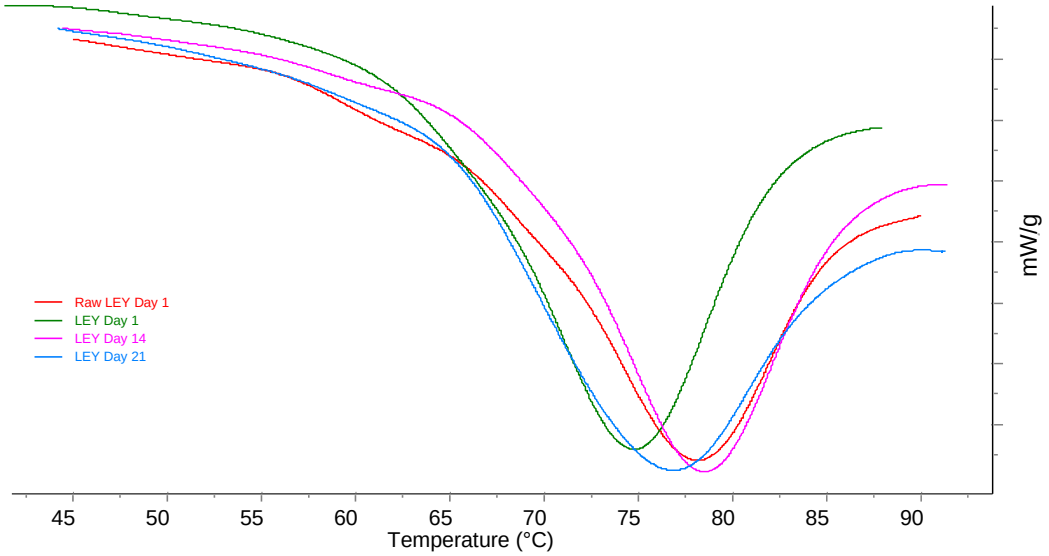


Figure 16: Thermograms of raw LEY and pasteurized LEY during storage

Emulsion stability

Despite the high oil content relative to water, mayonnaise is an oil in-water emulsion (Depree and Savage 2001). And an oil-in-water emulsion system can be broken by the increase of temperature and oil exudation happens as a result Huang et al. (2016). Mayonnaise samples are illustrated in Figure 17. Until the last day of the measurement, all the mayonnaise samples stored at 20°C did not show any texture difference. After one week of storage, raw LEY mayonnaise showed an oil exudation from the first 10 minutes on both temperature 40°C and 60°C. Mayonnaise made by pasteurized LEY exceeds small amount of oil in last 5 minutes at 40°C while for 60°C it exudes oil after 30 minutes. At the same time, mayonnaise made by pasteurized egg yolk then froze did not exude oil at 60°C even after 120 storage day at -18°C (Huang et al. 2016). In last week of storage (21st day), emulsion was damaged by 60 °C heat treatment in the first 10 minutes while

emulsion at 40 °C showed stability till the last minute. Pasteurization treatment of LEY improved the stabilization of emulsion comparing raw LEY during storage.

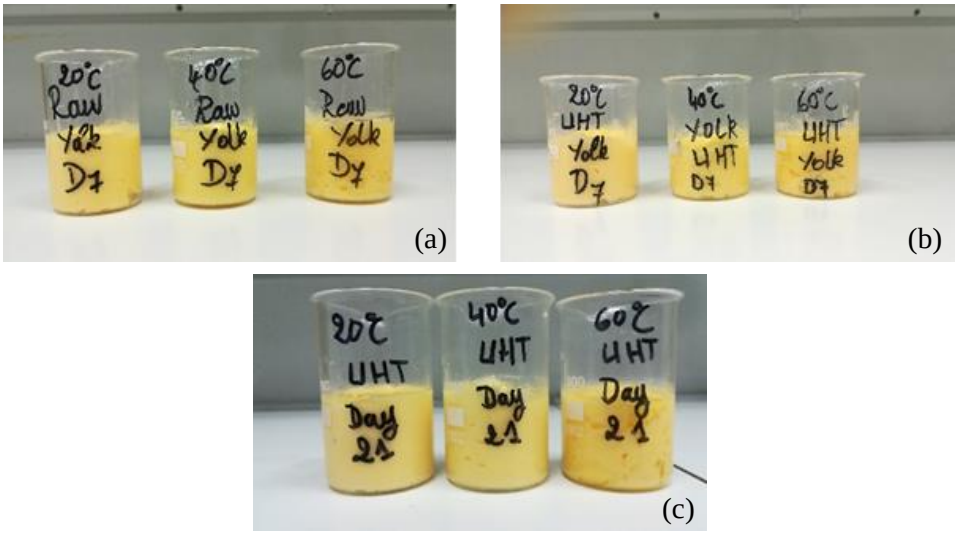


Figure 17: Heat stability of raw (a) and pasteurized LEY mayonnaise (b) on the 7th day and pasteurized LEY on the 21st day (c)

3.1.3.2. Pasteurized liquid egg yolk with citric acid and calcium sorbate

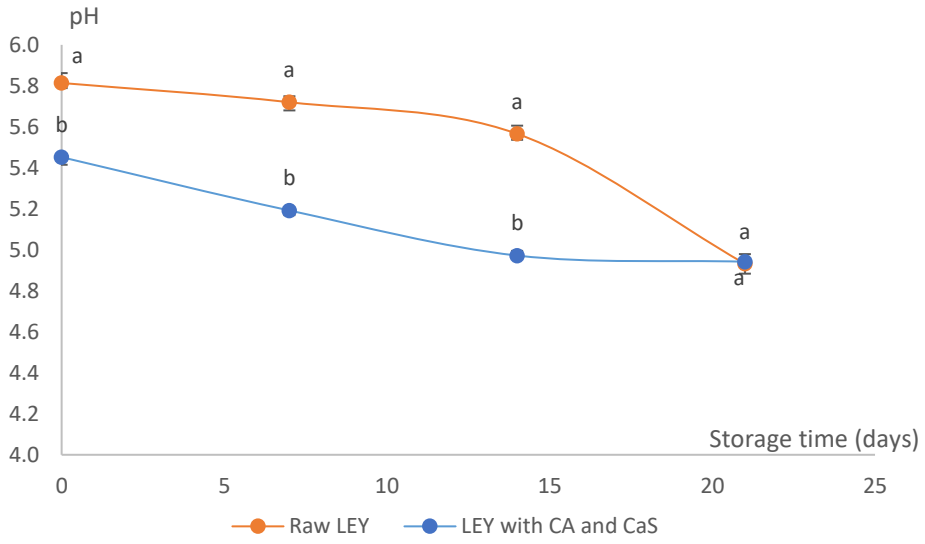
Physicochemical properties

Figure 18 shows the evolution of pH values of liquid egg yolk during the storage time. The pH values of pasteurized LEY with CA and CaS had a significant decrease comparing to the control (P<0.05) till the last day of conservation (P>0.05).

Even though at the beginning of the experiment, heat-treated LEY with CA and CaS had lower pH value than the control 5.45±0.01 and 5.81±0.05 respectively, they ended with the same pH value on the 21st day of storage 4.94±0.04 and 4.93±0.02. All along the preservation time, pasteurized LEY with CA and CaS gradually decreased and showed a pH value under 5 (4.97±0.02) on 14th day in the time the raw LEY still held a pH value equal to 5.57±0.04. The pH decrease may be due to the composition alteration with time.

Color is considered as one of the main characteristics of LEY. ΔE^*_{ab} allow the comparison between the color of raw materials and the treated materials. Just after the treatment the color difference between the two samples recorded the highest value 8.89. After 7 days of storage the ΔE^*_{ab}

decreased to reach 3.36 but back to increase up to 6.70 and 7.99 on days 14 and 21 successively. The ΔE^*_{ab} values indicated that the difference between the heat-treated LEY with CA and CaS, and raw LEY is easily noticed by unaided eye according to Table 5.



a and b mean they are significantly different regarding the control

Figure 18: Changes of pH values for raw and pasteurized LEY with CA and CaS during storage

The color parameters (L*, a*, b*, C*, and *h**) are featured in Table 11. L* values exhibited a significant difference comparing to control (P<0.05) starting from the 7th day of the experiment while the difference was not significant on day 0 (P>0.05). Regardless that the L* value of treated LEY started with a lower value than the control, 67.97±1.24 and 70.55±1.56 consecutively, starting from the 7th day they changed roles and L* values of pasteurized LEY with CA and CaS revealed higher numbers than the control. The L* values of control decreased constantly and reached 58.85±0.77 on the last day. While the L* values of treated LEY stabilized during 7th and 14th day of storage ≈64 to raise up to 65.03±1.45 on the last day. Even though on the 1st day, the control was brighter than the treated sample, it started to fade compared to the pasteurized LEY with CA and CaS in the rest of experiment time.

Pasteurized LEY with CA and CaS a* values showed a significant difference in the two first weeks of storage (P<0.05) than the difference was not any longer significant (P>0.05). The a* values of

the control showed fluctuation in the experiment, and it went from 8.73 ±0.69 on day 0 up to 13.56±0.90 on day 7 to decrease to 11.76±0.27 again in day 14.

Table 11: Changes of color parameters for raw and pasteurized LEY with CA and CaS during storage

Color parameters	Sample	Storage (days)			
		0	7	14	21
L*	Control	70.55±1.56a	61.35± 0.27a	58.80± 0.53a	58.85±0.77a
	LEY	67.97±1.24a	64.20±0.63b	64.82±0.89b	65.03±1.45b
a*	Control	8.73 ±0.69a	13.56±0.90a	11.76±0.27a	12.16±0.89a
	LEY	12.34±0.52b	11.79±0.57b	12.16±0.39a	12.10±0.85a
b*	Control	45.43±2.18a	52.52±1.26a	49.96±0.62a	47.83±1.44a
	LEY	53.13±1.64b	52.60±1.30a	52.88±0.69b	52.89±1.41b
C*	Control	46.27±2.07a	54.25±1.41a	51.33±0.64a	49.35±1.54a
	LEY	54.55±1.63b	53.91±1.39a	54.26±0.76a	54.25±1.56b
*h**	Control	1.38±0.02a	1.32±0.01a	1.34±0.00a	1.32±0.01a
	LEY	1.34±0.01a	1.35±0.01b	1.34±0.00a	1.35±0.01a
ΔE^*_{ab}		8.89	3.36	6.70	7.99

a and b mean they are significantly different regarding the control

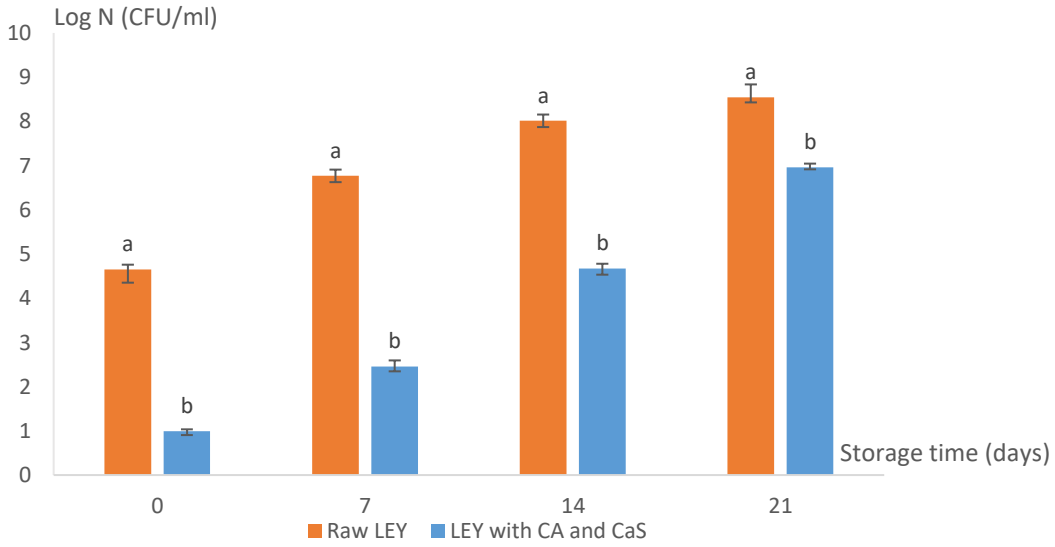
Meanwhile, the a* values of treated LEY exhibited in some way a constant value around 12 all along the storage time. High values of a* showed the presence of red color in the samples instead of the green which determines that color parameters did not get affected by the heat treatment.

The b* values of pasteurized LEY with CA and CaS had a significant difference compared to the control during conservation time (P<0.05) only on the 7th day of storage the difference was not significant (P>0.05). The same pattern was noticed for C* values where their value of treated LEY was only significant compared to the control in the beginning and in the finale storage day (P<0.05). Meantime, the difference between C* values of treated LEY and control was insignificant on storage day 7 and 14 (P>0.05). The b* and C* values of pasteurized LEY with CA and CaS showed also constant values during storage around 52 and 54 respectively. Pasteurized LEY with CA and CaS maintained the bright yellow color with high and constant b* and chroma values. At the same time, the b* and C* values of control showed a vast fluctuation,

and both parameters started with a low value then they went up to close of treated LEY values on day 7 to decrease again below the treated LEY values. Differently from other parameters, the hue values of pasteurized LEY with CA and CaS showed insignificant difference compared to the control (P>0.05) only on day 7 the difference was significant (P<0.05). The results showed that pasteurization treatment with the presence of CA and CaS sustained and improved the yellow color of LEY equally to short wave ultraviolet treatment of LEY with different time of treatment (De Souza and Fernández 2012).

Microbiological results

Mesophilic total plate counts of pasteurized LEY with CA and CaS is presented in the Figure 19. The total plate count of treated LEY decreased significantly comparing to the total plate count of the control (P<0.05). The effect of pasteurization treatment in presence of CA and CaS was noticed from the 1st storage day. The total plate count decreased from around 5 Log CFU/ml before the treatment to 1 Log CFU/ml after it.



a and b mean they are significantly different regarding the control

Figure 19: Mesophilic total plate count for raw and pasteurized LEY with CA and CaS during storage

Furthermore, total plate count of treated LEY continued to increase gradually which goes from around 2.5 Log CFU/ml up to around 5 Log CFU/ml in day 7 and 14 respectively but remained below the control values and was similar to short wave ultraviolet of LEY for 30min (De Souza and Fernández 2012). By the end of the preservation time, both samples showed the highest

amount of total plate count around 7 Log CFU/ml and 9 Log CFU/ml for pasteurized LEY with CA and CaS, and raw LEY respectively. Over time, heat treatment of LEY showed an advantageous result in terms of microorganism destruction and extends the shelf life of LEY for two weeks.

To conclude, LEY With additive had the lowest pH values comparing to raw and pasteurized LEY. After the treatment, color parameters of heat-treated LEY with CA and CaS showed a relatively stable values comparing to raw and pasteurized LEY. Although a* and b* values of raw and pasteurized LEY increased slightly. The presence of additives decreased the initial mesophilic total plate count by 4 log CFU/ml while pasteurization decreased the count by only 1 Log CFU/ml. However, during the storage time the mesophilic total plate count of the pasteurized LEY stayed stable while the mesophilic total plate count slope of LEY with CA and CaS increased significantly.

3.2. Heat treatment of liquid whole egg in presence of various acids

3.2.1. Correlation between acids and pH of liquid egg products

Before starting the measurement, the correlation was determined between added acid quantity and decrease in pH of liquid egg products. Curve was represented in Figure 20 and the coefficient of determination R^2 is presented in Table 12. Since LEY had the lowest starting pH, it used less quantity of acids to reach needed pH and showed the highest R^2 comparing to other liquid egg products. Opposite to LEY, LEW took a higher amount to reach needed pH values due to its high starting pH ≈ 9.27. Thus, R^2 values of LEW was the lowest one for all acids comparing to other liquid egg products. R^2 values of LWE were exceed 0.9 except for acetic acid where R^2 was a slightly lower.

Table 12: Coefficient of determination R^2 between added acid quantity and decrease in pH of liquid egg products

Acid type	Egg product type		
	LWE	LEW	LEY
Citric Acid	0.955	0.826	0.984
Lactic Acid	0.979	0.874	0.989
Acetic Acid	0.886	0.862	0.971
Ascorbic Acid	0.964	0.885	0.977

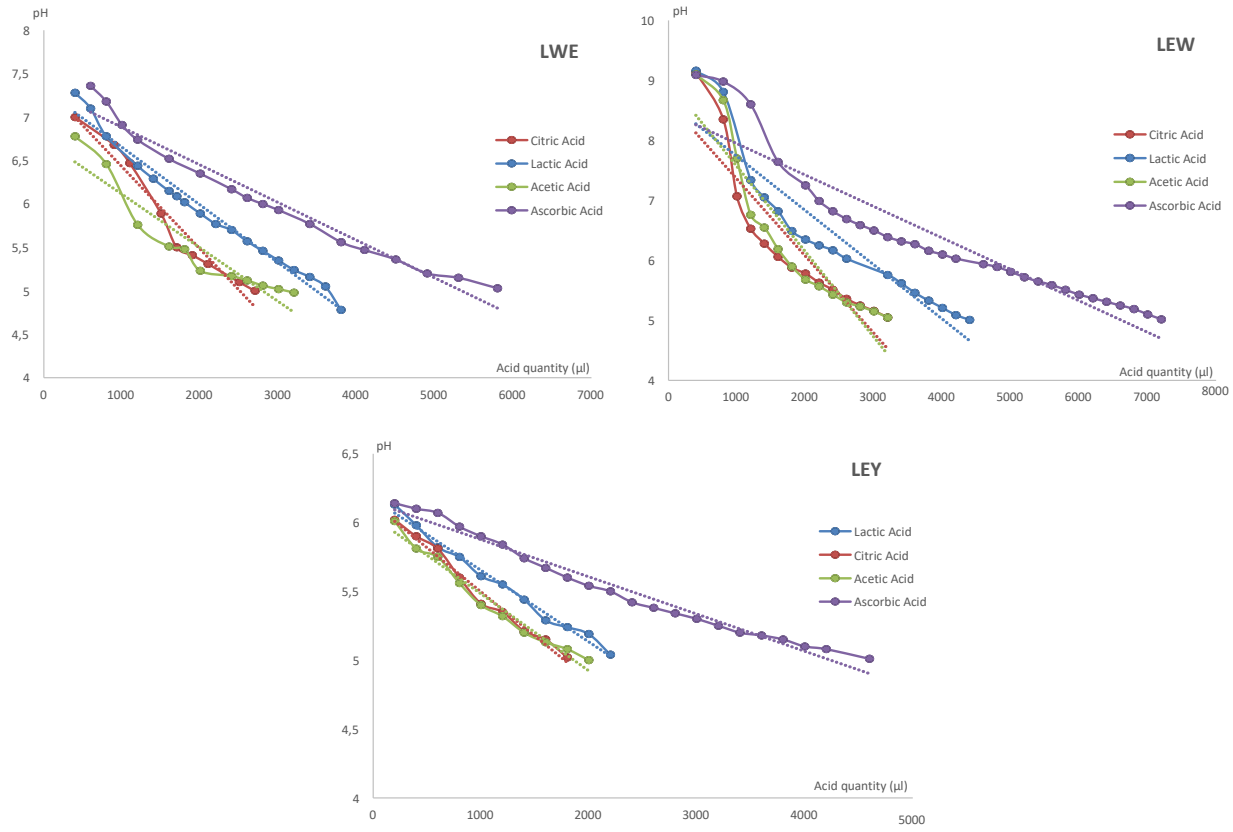


Figure 20: Curves of correlation between added acid quantity and decrease in pH of liquid egg products

3.2.2. Liquid whole with citric acid

3.2.2.1. Physicochemical properties

The change of pH values and color parameters (L*, a*, b*, C*, and *h**) after the heat treatment with citric acid are presented in Table 13. All the samples showed a significant difference compared the control ($P<0.05$). For the 3 first pH (5.0, 5.5 and 6.0), the pH values showed a slight increase after the heat treatment while the other ones unimportantly declined. These minor changes can be the results of protein changes after heat treatment despite the presence of citric acid. Also, the unfolding of egg proteins can be one of the reasons for pH minor change. In fact, after the heat treatment few aggregations appeared in the preservation bags. Due to the presence of the protein of egg white/yolk, it cannot be specified which fraction is responsible for these changes. According to Li, Zhang, et al. (2018), the pH of the egg white gel increased when the concentration of NaOH

increased (0.0, 0.1, 0.2, 0.3, and 0.4%), while when they conserved the same concentration of NaOH (0.3%) and they added different concentration of NaCl (0.3, 0.6, 0.9, and 1.2%) the pH value showed some stability.

After heat treatment of LWE in presence of CA, according to the L* values, treated samples showed higher lightness compared to the control except those pH samples which showed close value to the control one. In fact, all pH showed a significant difference compared to the raw LWE (P<0.05) except of sample pH 7, it showed insignificant difference to the control (P>0.05). Evolution of a* values depended on starting pH values, pH 7 and pH 6.5 samples that showed an increase of redness comparing to control while other samples exhibit a considerable decrease from 4.39±0.12 to -1.80±0.06 for raw LWE and pH 5 samples, respectively. The diminution of a* values indicate the color of samples tendency to green color. a*, b* and C* values exhibited a significant difference compared to the raw LWE after treatment (P<0.05).

Table 13: Effect of CA, different pHs and heat treatment on pH and color parameters of LWE

pH of the sample	pH	L*	a*	b*	C*	*h**
pH 5	5.17±0.01b	81.97±0.37b	-1.80±0.06b	38.08±0.38b	38.12±0.38b	-1.52±0.38b
pH 5.5	5.60±0.01b	73.88±1.75b	-1.89±0.21b	37.12±1.19b	37.16±1.18b	-1.52±0.01b
pH 6	6.13±0.01b	76.20±1.28b	-1.47±0.12b	38.09±1.51b	38.12±1.51b	-1.53±0.00b
pH 6.5	6.49±0.01b	68.69±0.36b	4.97±0.15b	33.65±1.08b	34.02±1.07b	1.42±0.00a
pH 7	6.75±0.01b	67.45±0.39a	6.06±0.16b	32.12±0.59b	32.68±0.61b	1.38±0.00b
Control	7.81±0.06a	67.76±0.28a	4.39±0.12a	29.46±0.87a	29.79±0.87a	1.42±0.00a

a and b mean they are significantly different regarding the control

All b* and C* values of LWE increased after the heat treatment in presence of CA. Samples of pH 6 and 5 showed the highest b* and C* values. Hue° values of pH 6.5 sample were not significantly different from raw LWE but *h** values of other pH were significantly different in contrast with raw LWE. According to the results, the starting pH values affected the color parameters. The presence of CA maintains LWE color, and it accentuated in low starting pH value.

3.2.2.2. Thermodynamic properties

Enthalpy changes and denaturation temperature values are registered in Table 14, and thermograms are exhibited in Figure 21. Thermograms of raw LWE featured three different peaks at 80.98±6.10 for lysozyme or ovalbumin of EW protein or HDL of EY protein, 60.62±4.23, and 50.56±1.15 °C both peaks may present ovo-transferrin, or/and part of γ-livetin egg yolk protein. Untreated LWE in De Souza and Fernández (2013) showed also 3 peaks in thermograms. On the other day, samples of different pH values illustrated only one peak located between 73.06 °C and 78.60 °C.

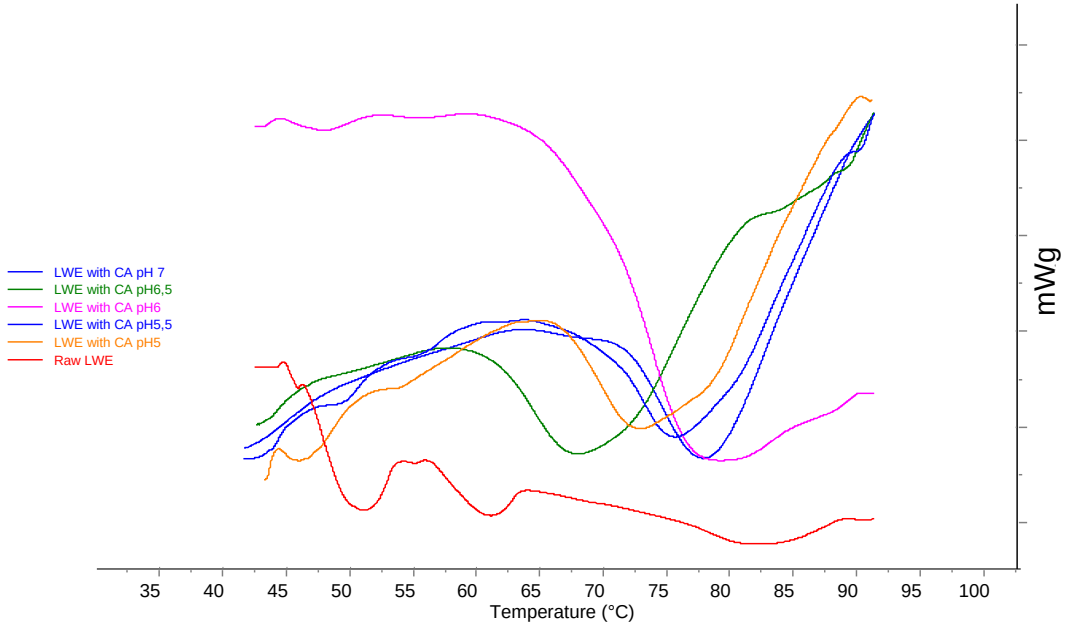


Figure 21: Thermograms of effect of CA, different pHs and heat treatment on LWE

Even with the presence of citric acid, heat treatment affected the sensitive egg proteins and mainly the LEW protein disappeared from thermograms due to their denaturation. T_d of different pH samples did not show any significant difference compared to the control (P>0.05). Highest enthalpy (2.14±0.62 J/g) was registered for sample pH 7 and lowest enthalpy value (0.87±0.15 J/g) was for sample pH 5. Thus, only pH 7 and pH 6.5 samples showed a significant enthalpy difference compared to the control (P<0.05).

Table 14: Effect of CA, different pHs and heat treatment on the denaturation enthalpy and denaturation temperature of LWE

pH of the sample		T_d (°C)	ΔH (J/g)
pH 5		73.28±4.24a	0.87±0.15a
pH 5.5		77.72±0.24a	1.26±0.01a
pH 6		76.60±0.07a	1.18±0.06a
pH6.5		73.06±3.63a	1.58±0.09b
pH7		78.60±0.02a	2.14±0.62b
Control	1st Peak	80.98±6.10a	1.44±0.34a
	2nd peak	60.62±4.23a	0.59±0.41a
	3rd peak	50.56±1.15a	1.16±0.56a

a and b mean they are significantly different regarding the control

3.2.2.3. Rheological properties

Knowledge of the rheological properties of food products is essential for the product development, quality control, sensory evaluation and design and evaluation of the process equipment (Kumbár, Strnková, et al. 2015). The effect of heat treatment combined with the addition of citric acid on the apparent viscosity of liquid whole egg is presented in Figure 22. The Herschel-Bulkley model parameters are presented in Table 15.

Raw LWE showed the lowest apparent viscosity (η), yield stress (τ_0) and consistency coefficient (K) but it showed the highest flow behavior index (n). Even though pH 7 sample showed the highest apparent viscosity (η), it had the lowest yield stress (τ_0) compared to other samples. All the samples had $\tau_0 < 0$ and $n<1$ which mean that they showed pseudoplastic behavior, only raw LWE and pH 5.5 showed a dilatant behavior according to Björn et al. (2012). Augmentation of apparent viscosity can be attributed to the effects of the breakdown of weak linkages between the proteins (De Souza and Fernández 2013; Tang et al. 1993). The whole liquid egg contains lipoprotein (major protein of egg yolk) which when , heated above about 70 °C , becomes more viscous (Nguyen and Burley 1984). Also, the augmentation of apparent viscosity could be the reason for the aggregation of egg white protein. In this study, heat treatment takes place at 70 °C

although De Souza and Fernández (2013) determined 60 °C as the phase transition temperature in egg white.

Table 15: Effect of CA, different pHs and heat treatment on Herschel-Bulkley model parameters of LWE

pH of the sample	Herschel-Bulkley model parameters		
	τ_0 (Pa)	K (Pa s^n)	n
pH7	0.586	0.147	0.671
pH6.5	0.642	0.147	0.677
pH6	0.645	0.154	0.644
pH5.5	0.713	0.008	1.041
pH5	0.630	0.036	0.866
Control	0.066	0.006	1.070

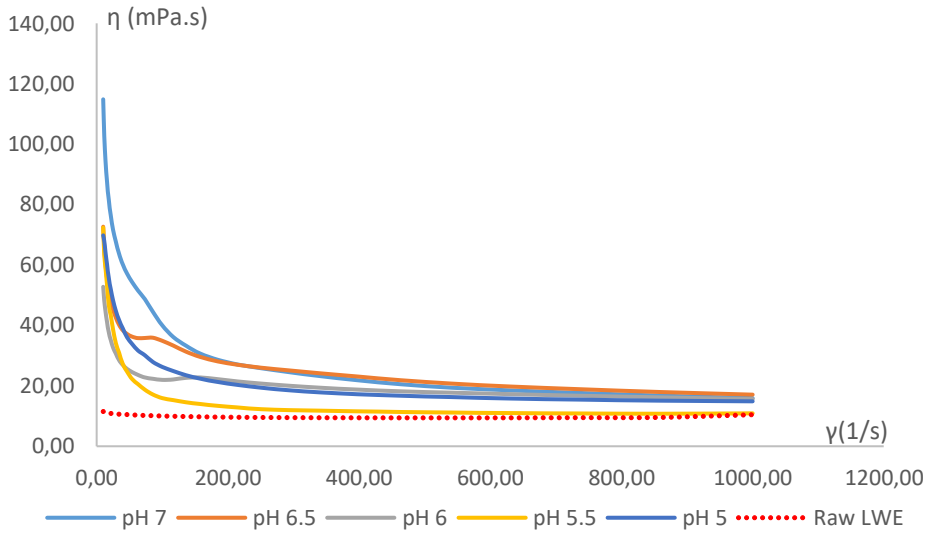


Figure 22: Effect of CA, different pHs and heat treatment on apparent viscosity of LWE

3.2.3. Liquid whole egg with lactic acid

3.2.3.1. Physicochemical properties

Evolution of pH and color parameters are gathered in Table 16. A slight increase can be seen in samples of pH 5, pH 5.5 and pH 6 while the two last samples showed a slight decrease after heat treatment. All pH values of treated samples with LA showed significant difference to pH value of the raw LWE (P<0.05). Lightness of lowest pH (5 and 5.5) showed highest lightness comparing to raw LWE while the other sample ones (pH 6 and 6.5) showed a lower L* value lower than the control and one (pH 7) showed a close L* value to control (67.67±0.25). L* values of heat-treated samples with LA were significantly different from raw LWE ones (P<0.05). After heat treatment with LA, a* values for pH 7 and pH 6.5 increased to 5.06±0.12 and 5.78±1.38, respectively, at the same time a* values of pH 6, pH 5.5 and pH 5 declined to 3.28±0.75, -1.46±0.15 and -2.04±0.04. The lowest a* value was registered by the lowest starting pH value 5. Statistically, treated samples were significantly different from raw LWE (P<0.05). Negative a* value indicates that the yellow color of sample has some green tendency. This greenish tendency is usually because of heat treatment.

Table 16: Effect of LA, different pHs and heat treatment on pH and color parameters of LWE

pH of the sample	Color parameters					
	pH	L*	a*	b*	C*	*h**
pH 5	5.09±0.01b	80.82±0.67b	-2.04±0.04b	37.25±0.61b	37.31±0.60b	-1.52±0.00b
pH 5.5	5.53±0.01b	72.18±2.24b	-1.46±0.15b	38.37±1.75b	38.40±1.74b	-1.53±0.01b
pH 6	6.08±0.01b	62.18±1.46b	3.28±0.75b	29.02±2.16a	29.21±2.22a	1.46±0.02b
pH6.5	6.47±0.01b	55.13±1.58b	5.78±1.38b	23.29±3.43a	24.00±3.64a	1.33±0.03b
pH7	6.79±0.04b	67.67±0.25b	5.06±0.12b	31.33±0.22b	31.74±0.24b	1.41±0.00b
Control	7.81±0.06a	67.76±0.29a	4.39±0.12a	29.46±0.88a	29.79±087a	1.42±0.01a

a and b mean they are significantly different regarding the control

In the same pattern as L* values, lowest starting pH (5 and 5.5) showed highest b* values, other samples showed a close or lower b* values. Samples with starting pH 6 and 6.5 showed lowest b* values and were not significantly different from raw LWE (P>0.05); rest of samples showed a significant difference in contrast with the control (P<0.05). Chroma parameters present the same

statistical difference compared to the control and same evolution as b* values. Hue° values showed a significant difference compared to the control ($P<0.05$). As the result of heat treatment in presence of lactic acid with different starting pH, the color change of LWE depends on starting pH value.

3.2.3.2. Rheological properties

Rheological change of LWE after heat treatment and lactic acid are shown in Figure 23, and Herschel-Bulkley model parameters are presented in Table 17. Apparent viscosity of treated samples had an increase after heat treatment, and highest one was for pH 5.5 sample. The result was confirmed by the Herschel-Bulkley model parameters, where pH 5.5 sample had the highest minimum stress to start the shear $\tau_0 =$2.083 Pa. In fact, the sample showed a dilatant behavior. Only two samples showed pseudoplastic behavior which are the highest and the lowest starting pH (7 and 5); other samples including control showed dilatant behavior. Effects of heat treatment on the rheological properties is noticeable even with the presence of LA, beside decreasing starting pH value boost heat treatment effect.

Table 17: Effect of LA, different pHs and heat treatment on Herschel-Bulkley model parameters of LWE

pH of the sample	Herschel-Bulkley model parameters		
	τ_0 (Pa)	K (Pa s^n)	n
pH7	0.043	0.186	0.660
pH6.5	0.370	0.003	1.153
pH6	0.354	0.000	1.506
pH5.5	2.083	0.003	1.196
pH5	0.998	0.078	0.790
Control	0.066	0.006	1.070

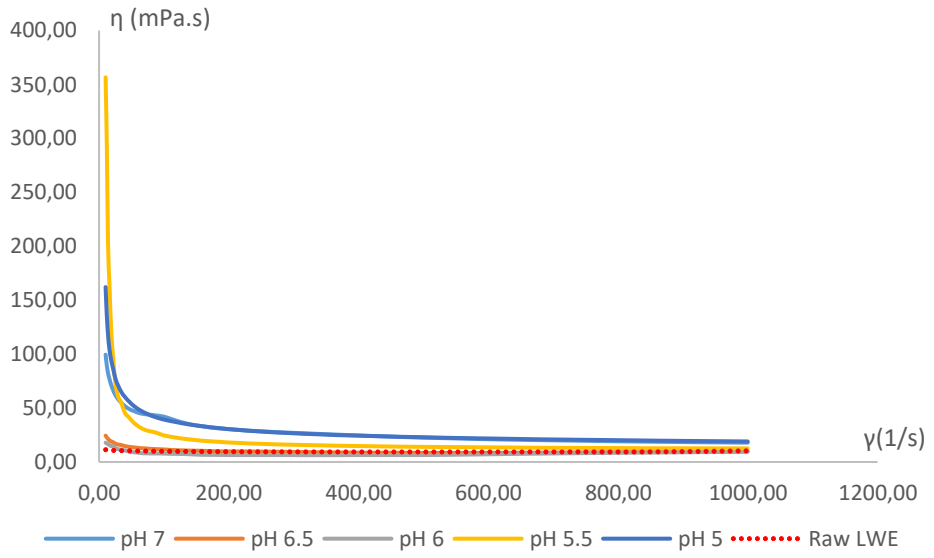


Figure 23: Effect of LA, different pHs and heat treatment on apparent viscosity of LWE

3.2.4. Liquid whole egg with Acetic Acid

3.2.4.1. Physicochemical properties

Slight increase was noticed in pH value of the samples after heat treatment. The changes of pH value and color parameters are described in Table 18. The difference between pH value of heat-treated samples with acetic acid (AcA) presence and control was significant ($P<0.05$). Lowest starting pH value (5 and 5.5) recorded higher lightness compared to control but L* values of it were not significantly different to the raw LWE ($P>0.05$).

Correspondingly, pH 7 sample did not have any significant difference compared to the control ($P>0.05$) and its lightness decreased after treatment. Samples with starting pH 6 and 6.5 had adjacent L* values, 65.83±0.60 and 65.33±0.45 consecutively. L* values of both samples diminished after heat treatment. It can be noticed that starting pH value affects the lightness of LWE. Low pH value led to increase lightness at the same time higher pH value led to decrease lightness comparing to control. Redness of LWE color heightened after treatment for pH 7 and 6.5 samples while other pHs declined and a* value for pH 5 sample took a negative value (-0.61±0.26) which indicates that the color of the sample tended to be green. According to the raise of b* value of treated LWE, it can be noticed that AcA enhanced yellow color of samples. Likewise, C* value increased after treatment. Not only the presence of AcA but also starting pH affected pH and color parameters of LWE.

Table 18: Effect of AcA, different pHs and heat treatment on pH and color parameters of LWE

pH of the sample	Color parameters					
	pH	L*	a*	b*	C*	*h**
pH 5	5.13±0.02b	71.12±1.91a	-0.61±0.26b	37.70±2.34b	37.71±2.34b	-1.55±0.01b
pH 5.5	5.58±0.01b	68.63±1.85a	0.71±0.11b	37.77±1.97b	37.77±1.97b	1.55±0.00b
pH 6	6.08±0.01b	65.83±0.60b	3.43±0.45b	34.40±0.91b	34.58±0.92b	1.47±0.01b
pH6.5	6.59±0.02b	65.33±0.45b	8.16±0.43b	33.81±0.80b	34.78±0.78b	1.33±0.01b
pH7	7.04±0.02b	64.45±1.73a	7.52±0.41b	32.08±0.36b	32.95±0.38b	1.34±0.01b
Control	7.81±0.06a	67.76±0.29a	4.39±0.12a	29.46±0.88a	29.79±087a	1.42±0.001a

a and b mean they are significantly different regarding the control

3.2.4.2. Rheological properties

Apparent viscosity of heat-treated samples with acetic acid was close to raw LWE, presented in Figure 24. As it noticed in the Figure 24, sample pH 5 showed highest apparent viscosity compared to control ones.

This is proved by Herschel-Bulkley model parameters, regrouped in Table 19, where pH 5 sample registered the highest yield stress τ_0 comparing to other samples including control. Besides, it is the only sample which showed a pseudoplastic behavior and highest consistency coefficient K. Apparent viscosity of pH 7, 6 and 5.5 samples showed lessened values comparing to the control, moreover their behavior was dilatant as control behavior. Sample of starting pH 6, even if it exhibited a slight increase of apparent viscosity comparing to raw LWE, it showed the same dilatant behavior as the raw LWE.

Table 19: Effect of AcA, different pHs and heat treatment on Herschel-Bulkley model parameters of LWE

pH of the sample	Herschel-Bulkley model parameters		
	τ_0 (Pa)	K (Pa s^n)	n
pH7	0.169	0.002	1.213
pH6.5	0.160	0.003	1.168
pH6	0.123	0.003	1.173
pH5.5	0.188	0.002	1.253
pH5	0.648	0.160	0.571
Control	0.066	0.006	1.070

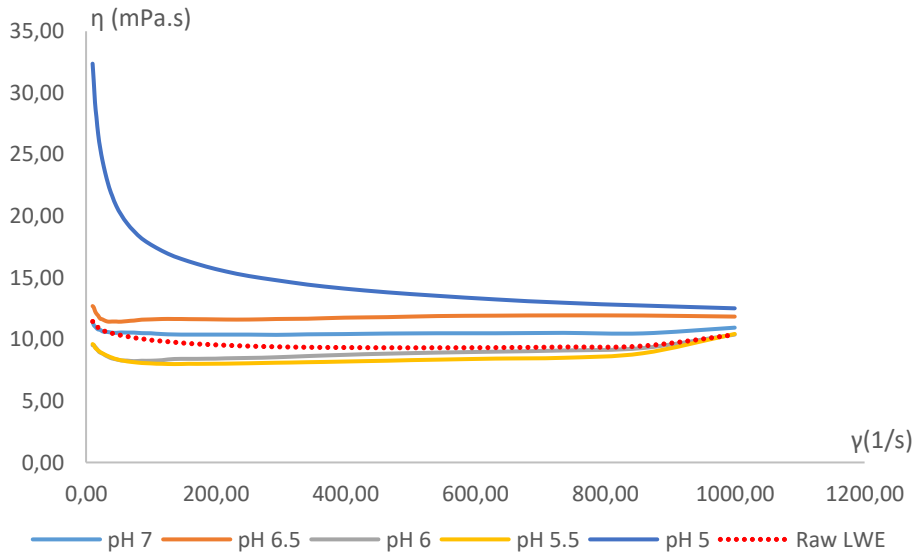


Figure 24: Effect of AcA, different pHs and heat treatment on apparent viscosity on LWE

3.2.5. Liquid whole egg with ascorbic acid

3.2.5.1. Physicochemical properties

Difference between pH value of heat-treated samples in presence of ascorbic acid (AscA) and pH value of raw LWE was significant (P<0.05). After heat treatment the pH value of treated samples rose slightly. Changes of pH value and color parameters are presented in Table 20. Samples with

starting pH above 5 had a fluctuating L* value but were still lower than the L* value of control. Only pH 5 sample showed a greater lightness comparing to control even though that its L* value does not have a significant difference compared to the L* value of raw LWE (P>0.05). Two highest starting pH (7 and 6.5) presented highest a* values after treatment compared to the control. Other samples showed lower a* values indicating that color of sample showed less redness. Sample pH 5 had a negative a* value referring to that color of the sample tended to be green. Nevertheless, b* values improved and increased from 29.46±0.88 for control to 36.20±0.62 for pH 5 samples. The chroma values also increased after treatment. Both parameters showed a significant difference compared to the control (P<0.05). The Hue° values of pH 7, pH 6.5 and pH 5 sample decreased, and the pH 5 sample showed the lowered *h** value. It can be noticed that pH 6 and pH 5.5 showed a slight increase of *h** value. Hue° value of samples showed a significant difference comparing *h** value of the control (P<0.05).

However, in the presence of ascorbic acid and high starting pH value, yellow color of samples increased with more tendency to red. Although, with lower starting pH value, yellow color enhanced but it tends to be greenish. The green color is result of effect of heat treatment.

Table 20: Effect of AscA, different pHs and heat treatment on pH and color parameters of LWE

pH of the sample	Color parameters					
	pH	L*	a*	b*	C*	*h**
pH 5	5.07±0.02b	69.20±0.85a	-0.27±0.09b	36.20±0.62b	36.20±0.62b	-1.56±0.0b
pH 5.5	5.57±0.01b	64.94±1.15b	1.22±0.16b	36.92±3.65b	36.95±3.64b	1.54±0.01b
pH 6	6.28±0.40b	66.16±0.49b	4.23±0.44a	36.03±0.86b	36.28±0.88b	1.45±0.01b
pH6.5	6.74±0.01b	62.46±0.99b	7.81±0.39b	32.82±0.88b	33.74±0.90b	1.34±0.01b
pH7	7.05±0.01b	65.94±0.43b	7.24±0.16b	33.82±0.85b	34.59±0.86b	1.36±0.00b
Control	7.81±0.06a	67.76±0.29a	4.39±0.12a	29.46±0.88a	29.79±087a	1.42±0.01a

a and b mean they are significantly different regarding the control

3.2.5.2. Rheological properties

Herschel-Bulkley model parameters are described in Table 21, and apparent viscosity is featured in Figure 25. Lowest starting pH (5) had the highest apparent viscosity. It also recorded the grater

τ_0 comparing to the control. This applies to LWE with starting pH 5.5 which exhibit high yield stress compared to other samples. In fact, the two samples showed pseudoplastic behavior while other samples including control showed dilatant behavior. Other samples revealed a superior apparent viscosity too. Heat treatment influenced apparent viscosity though AscA and different starting pH affected the behavior of LWE.

Table 21: Effect of AscA, different pHs and heat treatment on Herschel-Bulkley model parameters of LWE

pH of the sample	Herschel-Bulkley model parameters		
	τ_0 (Pa)	K (Pa s^n)	n
pH7	0.1638	0.0045	1.1432
pH6.5	0.0538	0.0075	1.0478
pH6	0.1598	0.0024	1.2001
pH5.5	0.6466	0.0124	0.6290
pH5	0.2161	0.0241	0.9033
Control	0.066	0.006	1.070

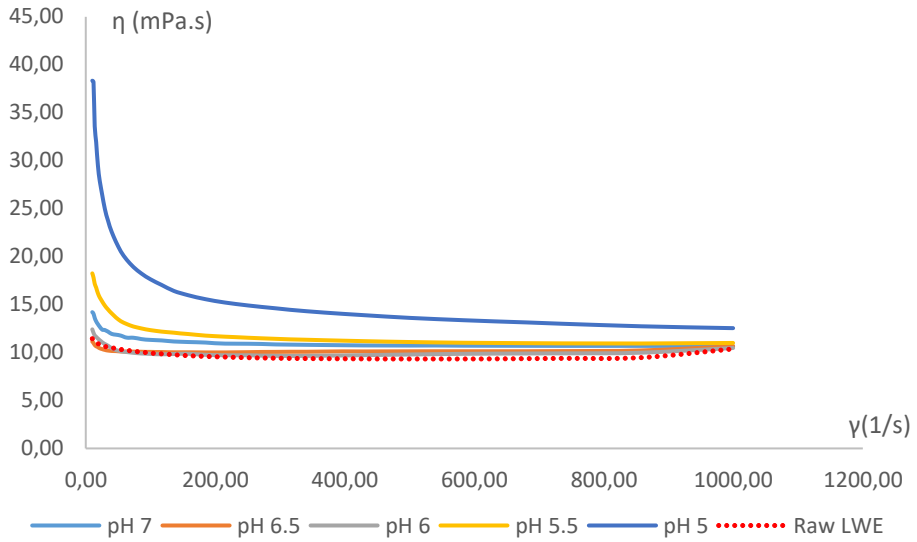


Figure 25: Effect of AscA, different pHs and heat treatment on apparent viscosity on LWE

3.2.6. Sensory analysis for LWE with various acids

Main sensory characters to outline the sensory profile are color, smell or odor, taste, and texture. Some of tasted muffin's samples are presented in Figure 26. Scores attributed to different samples are highlighted in spider chart illustrated in Figure 27. It can be noticed from the charts, that CA and LA samples were more desirable to the consumers than AcA and AscA.

For color propriety, CA and LA samples showed the highest registered score around 9 compared to the control and the two other acids. The lowest score for color was for AscA with starting pH 5, consumers scored its color 3.42±1.52. Despite this, the entire samples showed a higher score than raw LWE. Statistically, only CA samples showed a significant difference in contrast with the color of the control ($P<0.05$).

Odor or smell is an important sensory characteristic for egg products. High ranking score was for LA sample with starting pH 7 (8.86±1.95), and the lowest ranking was for AscA sample with starting pH 5 (2.86±1.07). Mainly, sample pH 7 of CA and LA had a better rank than the control; other samples showed either slight improvement or a lower rank than raw LWE.

Consumer opinion about the taste of LWE omelet with various acids is important as the product will be commercialized. According to spider charts, raw LWE exhibits the highest rank compared to treated samples. In De Souza and Fernández (2012), the difference between untreated and UV-C treated for 25 minutes whole egg was easily spotted by the panelist. Only two samples showed recorded close score to control which are: LA sample with starting pH 7 (7.43±1.51) and AcA sample with starting pH 6 (7.14±2.79). Remaining samples had a lower rank than the control.

Further, texture of omelet prepared with LWE and AscA had an equivalent score to the control. Yet other samples revealed higher rank than the raw LWE. Mostly, pH 7, pH 6, and pH 6.5 samples showed a greater score than the raw LWE.

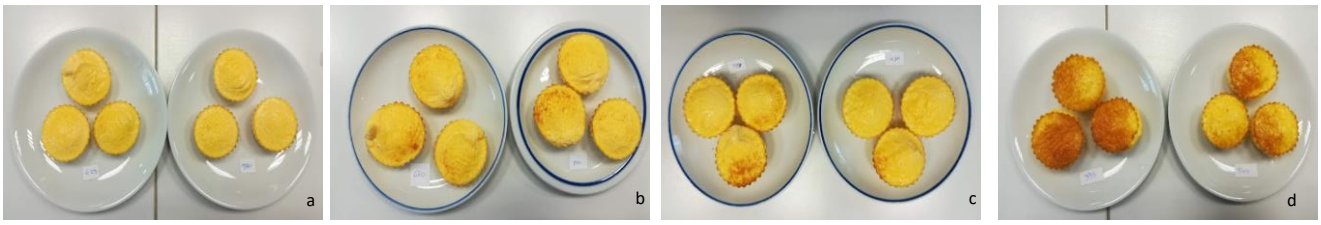


Figure 26: Muffin egg with CA pH5 (a), pH5.5 (b), pH6 (c) and pH6.5 (d)

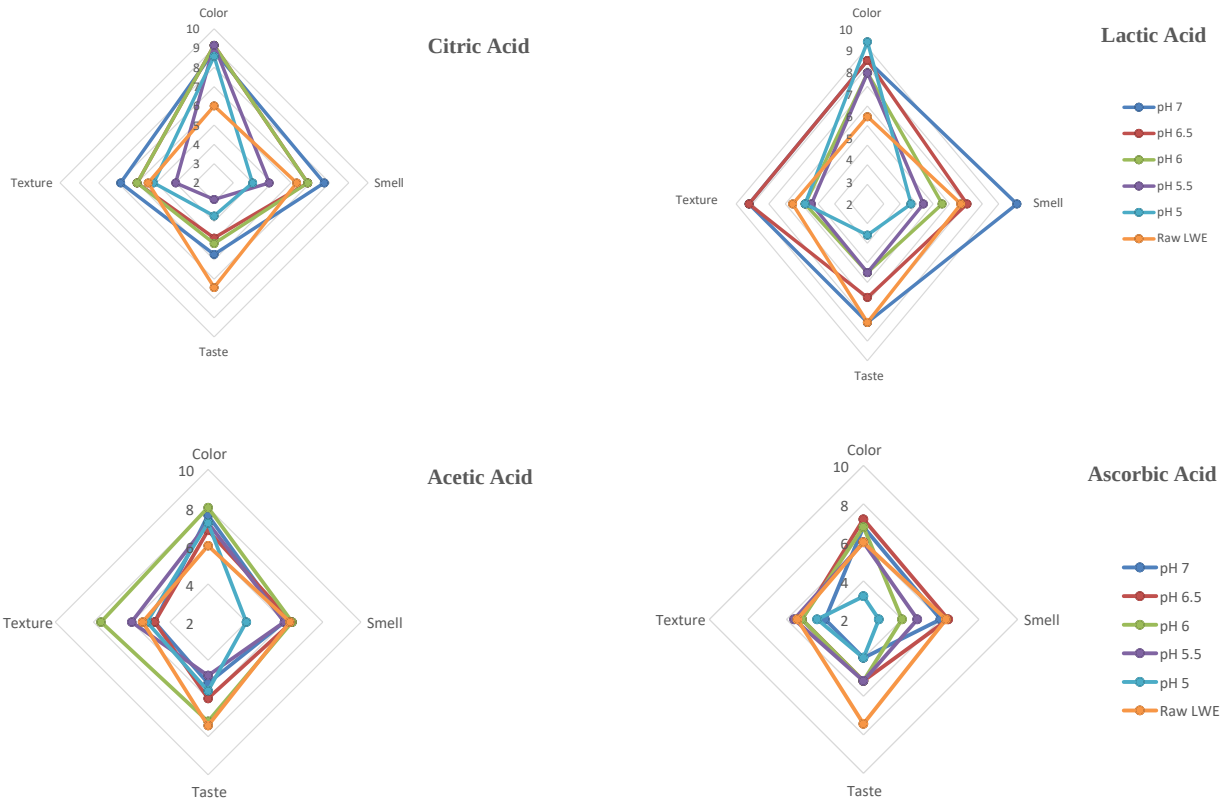


Figure 27: Spider chart of effect of different acids and pHs on LWE sensory profile

3.3. Effect of citric acid/lactic acid with heat treatment during storage

According to the sensorial proprieties (color, smell, taste, and texture), LWE muffin samples with citric acid or lactic acid took the lead and had highest score comparing the LWE muffins with acetic and ascorbic acid. For these reasons, further investigation was held to study the impact of this acids on LWE during storage time.

3.3.1. Effect of citric acid and heat treatment on LWE during storage

3.3.1.1. Physicochemical properties

Results of pH and color parameters of heat treated LWE with CA and control are summarized in Table 22. The pH values of different samples showed a significant difference compared to the control in beginning of storage but in the 7th day pH 6.5 did not show a notable change toward control. The difference between samples in the last week of storage was significant.

Table 22: Effect of citric acid, different pHs and heat treatment on pH and color parameters of LWE

Sample	Storage days		
	0	7	15
pH			
C	7.81±0.06a	6.81±0.02a	-
pH 5	5.31±0.05b	5.33±0.02b	5.29±0.01
pH 5.5	5.59±0.07b	5.70±0.03b	5.63±0.02
pH 6	6.21±0.10b	6.35±0.02b	6.28±0.03
pH 6.5	6.60±0.06b	6.81±0.03a	6.77±0.02
pH 7	7.21±0.07b	7.32±0.04b	7.27±0.02
*L**			
C	67.76±0.29a	67.20±0.96a	-
pH 5	84.20±0.16b	86.29±2.56b	86.30±0.16
pH 5.5	79.29±1.16b	84.50±0.71b	85.54±0.40
pH 6	74.93±0.63b	76.62±0.41b	76.42±2.30
pH6.5	64.39±0.51b	67.85±0.35a	71.83±1.50
pH7	71.59±0.17b	71.47±0.17b	68.24±0.35
*a**			
C	4.39±0.12a	5.87±0.17a	-
pH 5	-3.99±0.10b	-3.69±0.12b	-3.65±0.08
pH 5.5	-4.14±0.26b	-3.71±0.18b	-3.48±0.14
pH 6	-3.42±0.07b	-3.34±0.04b	-3.08±0.31
pH6.5	4.33±0.07a	4.44±0.11b	2.87±0.31
pH7	2.98±0.16b	3.62±0.08b	4.46±0.16
*b**			
C	29.46±0.88a	29.76±0.75a	-
pH 5	37.76±0.18b	36.92±0.76b	36.17±0.16
pH 5.5	40.45±0.92b	37.95±0.83b	36.20±0.36
pH 6	38.93±0.32b	37.84±0.49b	35.75±1.08
pH6.5	34.06±0.16b	30.50±1.39a	31.14±1.89
pH7	31.45±0.15b	29.33±0.10a	28.32±0.27
*C**			
C	29.79±0.87a	30.33±0.71a	-
pH 5	37.97±0.17b	37.10±0.76b	36.36±0.16
pH 5.5	40.66±0.88b	38.13±0.85b	36.36±0.36
pH 6	39.08±0.32b	37.99±0.48b	35.88±1.10
pH6.5	34.33±0.16b	30.82±1.39a	31.28±1.88
pH7	31.59±0.16b	29.55±0.11a	28.67±0.26

*h**			
C	1.42±0.01a	1.38±0.01a	-
pH 5	-1.47±0.00b	-1.47±0.00b	-1.47±0.00
pH 5.5	-1.47±0.01b	-1.47±0.00b	-1.47±0.00
pH 6	-1.48±0.00b	-1.48±0.00b	-1.48±0.01
pH6.5	1.44±0.00b	1.43±0.00b	1.48±0.01
pH7	1.48±0.00b	1.45±0.00b	1.41±0.01

a and b mean they are significantly different regarding the control

Treated sample with CA showed slight increase in the beginning of experiment then decreased marginally, this is also seen in the pH evolution of Marušić Radovčić et al. (2021) where LWE was pasteurized at 66 °C and an amount of 300, 400 and 500 mg/L CA were added. It was mentioned by Rêgo et al. (2012) and Marušić Radovčić et al. (2021) that pH value of commercially pasteurized eggs decreases after 2 weeks. The pH value of pasteurized eggs in both studies decreased after 2 weeks of storage.

The addition of CA and different starting pH value affected color parameters of LWE. Treated sample showed a lighter color comparing to the control and L* value of samples ranged between 64.39±0.51 and 84.20±0.16 and control L* value is 67.76±0.29. The difference between control and treated samples was significant in the 1st week, until 7th day pH 6.5 did not have any significant differences in contrast with control. Through the rest of the storage days, treated samples presented a significant difference between each other. Differently, pH 7 sample diminished throughout storage time reaching 68.24±0.35 in the last day while other samples showed an increase of L* values.

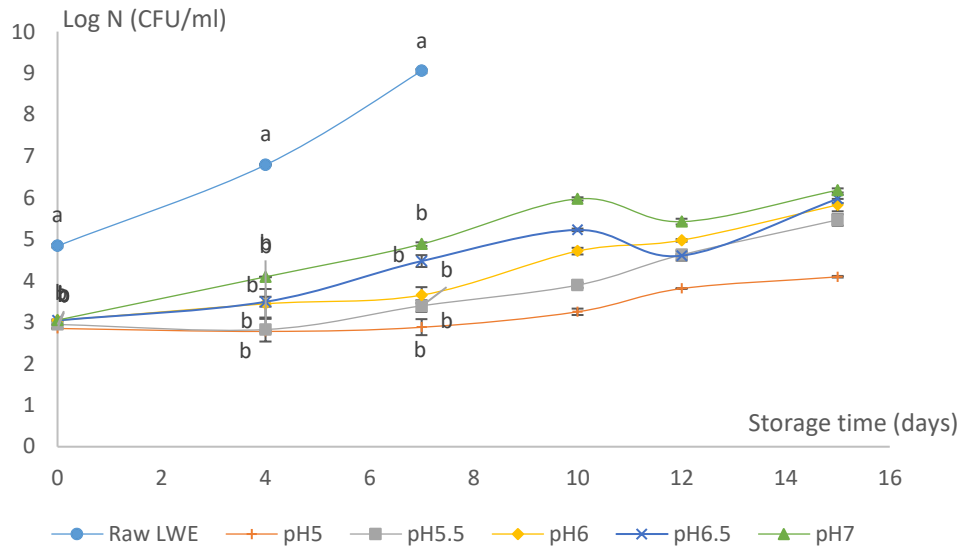
The effect citric acid on LWE a* value according to starting pH can be noticed. While control slightly improved within 1 week of storage, depending on the starting pH citric acid influenced a* value of heat treated LWE. For lower starting pH (5, 5.5, and 6), a* value manifested negative value which indicated that the color of the samples tends to green. Though, starting pH (6.5 and 7) exhibit a* value close to the control. Samples with low pH starting value slightly increased through conservation. Meantime, a* value of pH 6.5 sample improved in the first 7 days of storage, but it reduced in the 15 days to 2.87±0.31. Besides, a* value of pH 7 sample started with a low value compared to control but it augmented during experiment and showed a value close to the control one.

Setting b* value of control and treated LWE samples side by side, it can be noticed that the addition of CA and using different starting pH affected yellow color of LWE. The b* value of treated samples was markedly higher than the control. Yet, both samples devalued during the conservation time. Lower b* value was recorded for pH 7 sample where it exhibited by the second week b* value close to the control one, 29.33±0.10 and 29.76±0.75, respectively. C* value showed a similar pattern to b* value. Referring to the saturation of color, *h** value of heat treated LWE with CA showed a slight increase in contrast with raw LWE. The color of LWE was affected by citric acid addition resulting the augmentation of light and yellow color and depending on pH CA prevented the color loss. For low pH value, CA did not prevent the greening caused by heat treatment. But with a pH close to the raw LWE, CA preserves the redness of the sample. Moreover, it protected the yellow color of LWE to not fade during storage. These results are comparable to Marušić Radovčić et al. (2021) ones where research mention that color changes are due to the variation of pH which affected by addition of different CA concentrations (300, 400, 500 mg/L).

3.3.1.2. Microbiological results

Microbiology highlights the safety of food products. In Figure 28, the mesophilic total plate count was illustrated. Certainly, the effect of CA and heat treatment on LWE can be spotted. It diminished the total plate count by 2 Log CFU/ml approximately. The initial mesophilic total plate count was around 5 Log CFU/ml for raw LWE and decreased to around 3 Log CFU/ml just after the treatment.

Through storage period, total plate count of heat-treated samples with CA augmented considerably but it did not outrun total plate count of raw LWE. According to starting pH, total plate count differentiated. Sample with starting pH 7 showed the highest microorganism count while lowest starting pH 5 exhibit lowest microbial count. CA addition aided to prevent microbiological proliferation during conservation time.



a and b mean they are significantly different regarding the control

Figure 28: Effect of CA and different pHs on mesophilic total plate count of heat-treated LWE during storage

3.3.1.3. Rheological properties

Rheological properties were determined by Herschel-Bulkley model and summarized in Table 23 and apparent viscosity were illustrated in Figure 29. The correlation was relatively high (0.809 to 0.999). The lowest correlation coefficient is attributed to pH 5 sample in the 15th day of storage. Compared to the control, treated samples presented higher yield stress during fifteen days of storage. Throughout this period, pH 5 and pH 5.5 exhibit the highest yield stress. It decreased constantly and went from 18.561 and 1.874 in the beginning of the experiment to 60.581 and 11.218 at the last day of experiment for pH 5 and pH 5.5 samples.

Table 23: Effect of CA and different pHs on Herschel-Bulkley model parameters of heat-treated LWE and control during storage

Sample	Storage days		
	0	7	15
τ_0 (Pa)			
C	0.000	0.000	-
pH 5	18.561	21.001	60.581
pH 5.5	1.874	4.119	11.218
pH 6	0.091	0.055	0.204
pH6.5	0.000	0.000	0.959
pH7	5.898	0.055	0.000

K (Pa s^n)			
C	0.010	0.011	-
pH 5	0.054	0.727	0.007
pH 5.5	0.054	0.174	2.056
pH 6	0.089	0.124	0.187
pH6.5	0.390	1.325	1.178
pH7	1.848	2.117	1.087
n			
C	1.014	1.012	-
pH 5	0.937	0.614	1.254
pH 5.5	0.873	0.774	0.495
pH 6	0.803	0.774	0.753
pH6.5	0.584	0.444	0.534
pH7	0.468	0.443	0.504
Correlation			
C	0.999	0.999	-
pH 5	0.968	0.989	0.809
pH 5.5	0.999	0.997	0.980
pH 6	0.999	0.999	0.999
pH6.5	0.986	0.994	0.998
pH7	0.999	0.999	0.997

In fact, not only these samples showed a high yield stress in the beginning, but pH 7 sample also showed a high τ_0 value then it started to decrease to overlap with τ_0 value of the control. Both samples of pH 6 and 6.5 started with low τ_0 value then increased. These results are confirmed by apparent viscosity graphs where pH 5, 5.5 and 7 samples showed the highest apparent viscosity for day 0 and 7, and in day 15 beside pH 5, 5.5 samples pH 6.5 illustrated the highest apparent viscosity. Using Ostwald-de Waele law to evaluate apparent viscosity, Marušić Radovčić et al. (2021) mentioned that the highest apparent viscosity in first week of storage belonged to the control beside apparent viscosity of samples with CA did not have any significant difference in contrast with control.

Samples showed a shear-thinning (pseudoplastic) rheological behavior. Most treated samples had a flow behavior index ranged between 0 and 1 ($0<n<1$). Raw LWE showed dilatant behavior ($n>1$) during week of storage. In opposition with other treated samples, pH 5 sample revealed a dilatant rheological behavior in the last day of experiment. In Marušić Radovčić et al. (2021) study, LWE with citric acid showed a dilatant behavior which accords with other study of liquid egg white of Radványi et al. (2012).

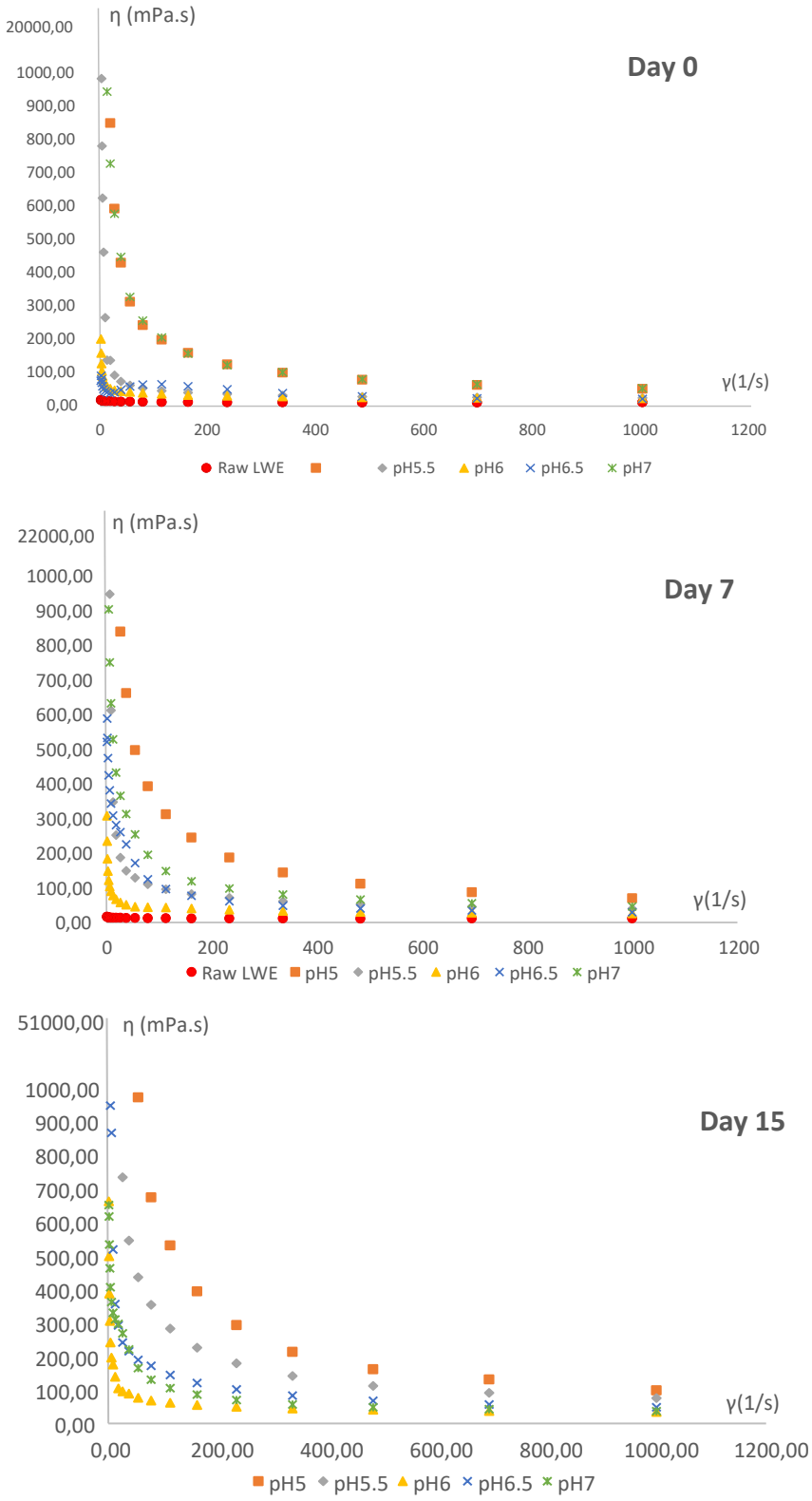


Figure 29: Effect of citric acid and different pHs on apparent viscosity of heat-treated LWE during day 0, 7 and 15

Raw LWE as a control showed the lowest consistency coefficient whilst pH 7 sample presented the highest one after one week of storage. Heat treated LWE sample with CA revealed higher consistency compared to the control. Through conservation, K values increased distinctly. Addition of CA maintained the pseudoplastic rheological behavior of samples, only low pH sample by the end of conservation time shifted to dilatant fluid. This may be explained by intern change of LWE composition.

3.3.1.4. Sensory analysis

Addition of CA, changing of pH, and conservation caused many changes to LWE. To evaluate these changes from consumers perspective sensory analysis was held and results were presented in spider chart illustrated in Figure 30. Regardless that control had the highest score for taste (7.6±1.83), it showed the lowest score for the odor (5.8±2.39) in the 1st day of experiment.

The color of food is important to consumers as it is the first property that the eye can catch. Interestingly, three of the treated samples had the highest score around 8 which are pH 5.5, 6, and 6.5 samples. The other samples had a lower score, and pH 7 sample resulted the lowest score for the first day. Sample with starting pH 7 showed the lowest score for color property and samples with pH 5.5 and pH 6 had the highest score.

Smell or odor is one of the main sensory features for eggs. Raw LWE showed lowest score (5.8±2.39) but then in 4th and 7th day consumers accepted the smell and attribute to it higher scores, 6±1.63 and 7±1.42 consecutively. For treated samples, pH 5 sample exhibited the lowest score through storage time while pH 6 and pH 6.5 had the high ranking comparing to other samples. This suggest that the consumers liked the smell of egg muffins made from pH 6 and pH 6.5 samples. Accordingly, in the work of Jesús et al. (2013) the smell of pudding obtained by a freeze-dried or spray-dried LWE was overall accepted by the consumers because drying treatment concentrated the aroma components by eliminating the water , thereby increasing the strength of its smell and flavor.

Regarding the taste of egg muffins, raw LWE sample was liked by the panelist in the beginning of experiment, and they gave the highest score to it. After that in the 4th day the score diminished to 6.4±1.83. Despite being disliked by the consumers on the 1st day, pH 6.5 had a high rank in the rest of storage.

Overall, texture of egg muffins exhibited some flocculation throughout the conservation time. On the 1st week of conservation, consumers attributed to pH 5 egg muffin samples the lowest score in contrast with all other samples. Only in the 7th day the sample had a score equal to 7±2.16. Similarly, to other sensory proprieties, pH 7 sample had the lowest score compared to other samples.

Through storage time, consumers started to attribute lower scores to the properties, and this can be due to the deterioration of physicochemical properties such as pH changes and microbiological contamination.

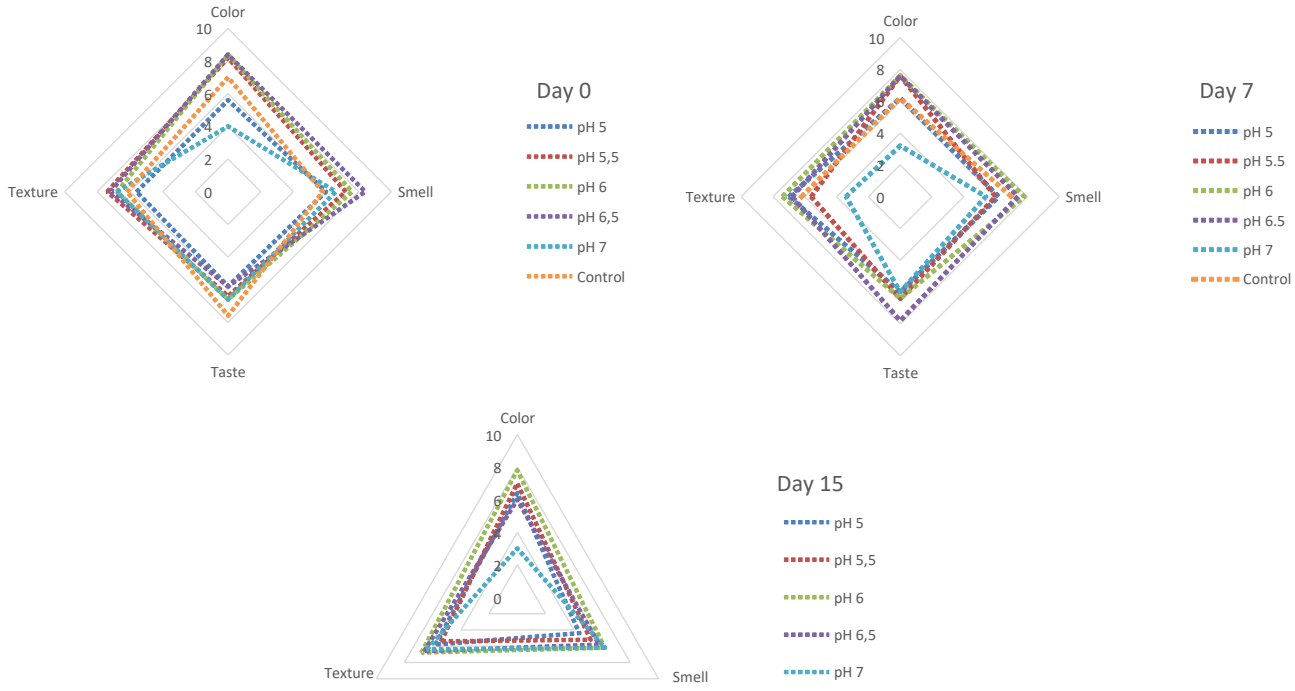


Figure 30: Spider chart of effect of citric acid and different pHs on heat-treated LWE sensorial profile during day 0, 7 and 15

3.3.2. Effect of lactic acid and heat treatment during storage

3.3.2.1. Physicochemical proprieties

Impacts of lactic acid, different pHs and heat treatment on pH and color parameters of LWE and control are presented in Table 24. Directly after heat treatment, pH values of treated samples increased slightly. All through conservation time, treated samples with the control started to decrease moderately.

Table 24: Effect of lactic acid, different pHs on pH and color parameters of heat-treated LWE and control

Sample	Storage days		
	0	7	15
pH			
C	7.81±0.06a	6.81±0.02a	-
pH 5	5.03±0.01b	4.97±0.02b	4.91±0.02
pH 5.5	5.56±0.03b	5.41±0.02b	5.31±0.04
pH 6	6.35±0.02b	6.21±0.01b	6.17±0.03
pH6.5	6.75±0.01b	6.66±0.02b	6.61±0.05
pH7	7.31±0.02b	7.21±0.03b	7.26±0.06
L*			
C	67.76±0.29a	67.20±0.96a	-
pH 5	81.99±1.08b	82.05±0.64b	82.71±0.89
pH 5.5	76.61±0.60b	77.73±0.49b	78.52±0.38
pH 6	69.67±0.85b	65.19±1.93a	74.47±0.37
pH6.5	68.08±1.05a	64.01±1.72b	65.60±0.94
pH7	71.16±0.32b	69.26±0.20b	67.89±1.49
a*			
C	4.39±0.12a	5.87±0.17a	-
pH 5	-2.96±0.24b	-3.12±0.11b	-2.97±0.11
pH 5.5	-2.89±0.16b	-3.24±0.10b	-2.78±0.05
pH 6	2.39±0.16b	1.19±0.24b	1.13±0.06
pH6.5	5.06±0.19b	6.60±0.75b	4.80±0.22
pH7	4.41±0.27a	4.57±0.09b	4.63±0.08
b*			
C	29.46±0.88a	29.76±0.75a	-
pH 5	34.93±1.06b	34.70±0.56b	31.76±0.72
pH 5.5	36.09±0.56b	34.90±0.38b	32.54±0.59
pH 6	31.47±0.75b	29.42±1.50a	28.61±0.47
pH6.5	28.98±1.17a	29.86±2.22a	24.12±1.51
pH7	28.79±0.27a	27.50±0.27b	23.73±1.58

C*			
C	29.79±0.87a	30.33±0.71a	-
pH 5	35.06±1.07b	34.84±0.56b	31.90±0.73
pH 5.5	36.21±0.56b	35.05±0.38b	32.66±0.59
pH 6	31.56±0.74a	29.44±1.50a	28.64±0.47
pH6.5	29.42±1.18a	30.59±2.27a	24.60±1.52
pH7	29.13±0.26a	27.88±0.28b	24.18±1.54
*h**			
C	1.42±0.01a	1.38±0.01a	-
pH 5	-1.49±0.00b	-1.48±0.00b	-1.48±0.00
pH 5.5	-1.49±0.00b	-1.48±0.00b	-1.49±0.00
pH 6	1.50±0.01b	1.53±0.01b	1.53±0.00
pH6.5	1.40±0.00b	1.35±0.02b	1.37±0.01
pH7	1.42±0.01a	1.41±0.00b	1.38±0.02

a and b mean they are significantly different regarding the control

In the first week of storage, treated samples showed a significant difference in contract with the control ($P<0.05$), over time of storage treated samples had a significant difference also compared to each other ($P<0.05$). After two weeks of storage, pH values of treated samples decreased compared to the beginning, but it did not reach starting pH values. Presence of LA before heat treatment resulted in a slight increase of pH values.

L* values represent the lightness of the samples. Following the heat treatment, samples with LA revealed higher lightness compared to control. Highest lightness was for pH5 and pH5.5 sample and it enhanced during storage. The L* values are related to L* values of freeze- and spray-dried LWE in Jesús et al. (2013) where they registered successively 72.64 ± 1.17 and 86.45 ± 0.48.

The a* values indicate the redness of the color when it is positive and the greenness when it is negative. Samples with low pH values (5 and 5.5) had negative a* values indicating that their color tends to green comparing to control and other treated samples. The values decreased in the first week of storage then returned to the starting a* values in the second week. For other samples, they exhibited positive a* values indicating the redness of samples. Highest a* values throughout storage were for pH 6.5 sample while pH 6 sample showed the lowest value and then lightly declined. Only pH 7 sample exhibited a relatively constant a* value during conservation. With a low pH value, LA did not eliminate greenish coloration of samples which occur due to heat treatment. At 6.5 pH, LA enhanced a* value thus the redness of LWE. The redness enhancing

takes place with freeze- and spray-dry as well, which concentrates the remaining components after eliminating water (Jesús et al. 2013).

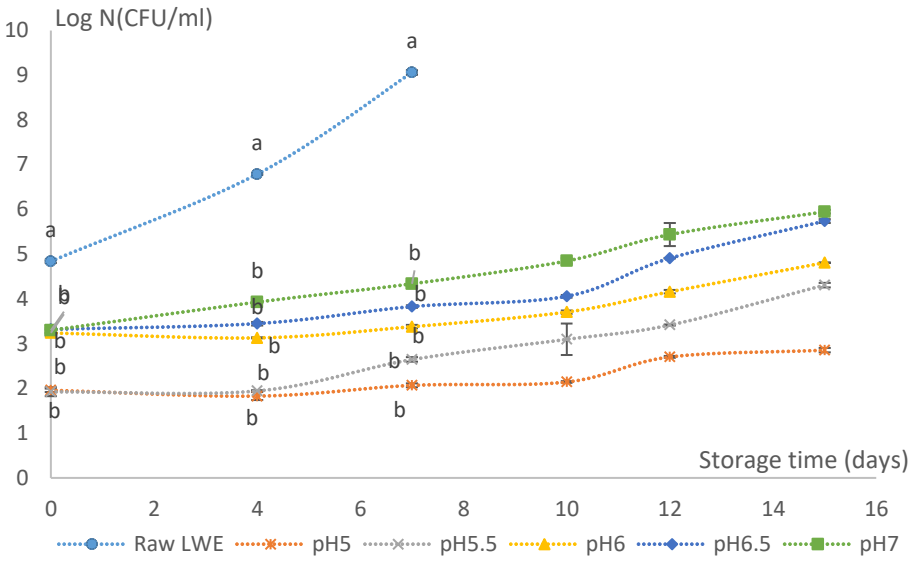
Yellowness is evaluated by b* values when it is positive, negative b* value indicates blueness. Dissimilarly to a* values, lower pH value samples (5, 5.5, and 6) showed higher b* value which means that yellowness of these samples was more intense than the control. Besides, a* values of pH 5 and pH 5.5 samples were negative, making the color of these samples yellow tending to green and pH 6 sample had a positive a* values make their color yellow tending to red. Throughout the experiment, b* values of these samples diminished. As follows, higher pH value samples (6.5 and 7) had b* values close to the control ones. Sample of pH 6.5 increased on the 7th day of storage then declined on the last day of conservation from 29.86±2.22 to 24.12±1.51 consecutively. In contrast, pH 7 sample decreased with conservation time. Though, both samples showed similar b* values as spray-dry LWE while other samples had b* values related to freeze-dried LWE (Jesús et al. 2013).

C* values describe the intensity or saturation. C* values had a similar pattern as the b* values. According to the evolution of chroma values, yellow color of the samples did not fade after heat treatment, in contrary for low pH value it enhanced the intensity of color. Hue° values had a similar pattern as a* values. Such as pH, effect of LA in color parameters depends on the starting pH value. For a low starting pH, LA improved yellow color for LWE but it did not prevent it from the greenish color caused by heat treatment.

3.3.2.2. Microbiological properties

Total plate count during the experiment is illustrated in Figure 31. From beginning to end, mesophilic total counts of heat-treated samples with LA showed a significant difference toward raw LWE and in comparison, to each other. Presence of LA in the samples, as a preservative, diminished remarkably the microbial load. Lowest starting pH samples (pH 5 and pH 5.5) showed the lowest total plate count in the beginning of storage, then over time total plate count increased and still pH 5 sample exhibited the lowest total plate count during the experiment. Yet, the presence of LA and low starting pH value reduced the total plate count by 3 Log CFU/ml comparing to raw LWE. For other samples with relatively higher starting pH value (pH 6, 6.5, and 7), presence of LA diminished the microbial load by 2 Log CFU/ml compared to the control. The result is related to Necidová et al. (2019) research where Benzoate-sorbate, Defence JB, Galimax Flavor V50 were

used as preservative for pasteurized LWE, and these preservatives diminished the lactic acid bacteria count at least by the half. Overall, effect of LA is clear on the total plate count and the intensity of the effect depends on the starting pH, the lower the pH, the more intense the effect.



a and b mean they are significantly different regarding the control

Figure 31: Effect of lactic acid and different pHs on mesophilic total plate count of heat-treated LWE during storage

3.3.2.3. Rheological properties

Evolution of rheological properties is illustrated in Table 25 and Figure 32. Correlation of samples to Herschel-Bulkley model was high (0.929 to 0.999). Only pH 5.5 sample was not highly correlated to the model in the 7th day of storage (0.595).

During the whole time of storage, samples with LA showed higher yield stress in contrast with the control. In fact, raw LWE did not need any yield stress to start to shear while low starting pH samples need a high yield stress to start it. Sample of pH 5 showed the highest τ_0 compared to other samples and it augmented during storage similarly to pH 5 sample with CA. Samples with starting pH 5.5 and pH 6 exhibit also an elevated τ_0 initially but then τ_0 of pH 5.5 sample increased from 3.017 to 13.598 on the 7th day to decrease in the last day of conservation to 8.734. Whilst τ_0 of pH 6 sample decreased after a week of conservation to 0.695 and increased again to 10.041 after two weeks of storage at 4 °C.

Table 25: Effect of lactic acid and different pHs on Herschel-Bulkley model parameters of heat-treated LWE during storage

Sample	Storage days		
	0	7	15
τ_0 (Pa)			
C	0.042	0.035	-
pH 5	9.268	18.188	26.003
pH 5.5	3.017	13.598	8.734
pH 6	1.495	0.695	10.041
pH 6.5	0.189	0.181	0.531
pH 7	0.573	0.000	0.174
K (Pa s^n)			
C	0.009	0.009	-
pH 5	0.111	0.117	0.048
pH 5.5	0.017	0.000	0.012
pH 6	0.026	1.353	0.002
pH6.5	0.054	0.038	0.020
pH7	0.258	0.644	0.617
n			
C	1.028	1.032	-
pH 5	0.794	0.830	0.993
pH 5.5	1.017	2.187	1.119
pH 6	0.910	0.333	1.323
pH6.5	0.866	0.872	0.964
pH7	0.673	0.578	0.562
Correlation			
C	0.999	0.999	-
pH 5	0.988	0.972	0.948
pH 5.5	0.997	0.595	0.942
pH 6	0.999	0.929	0.967
pH6.5	0.999	0.997	0.999
pH7	0.999	0.999	0.998

LWE samples with starting pH 6.5 and pH 7 showed the lowest yield stress. Yield stress of pH 6.5 increased during time while simultaneously τ_0 of pH 7 sample had the same pattern as τ_0 of pH 6 sample with LA and the one with CA also. The yield stress changes during the experiment can be observed also in the apparent viscosity figures. According to behavior index (n) in Table 25, all samples including the raw LWE exhibited a non-Newtonian behavior ($n \neq 1$) but with different characteristic.

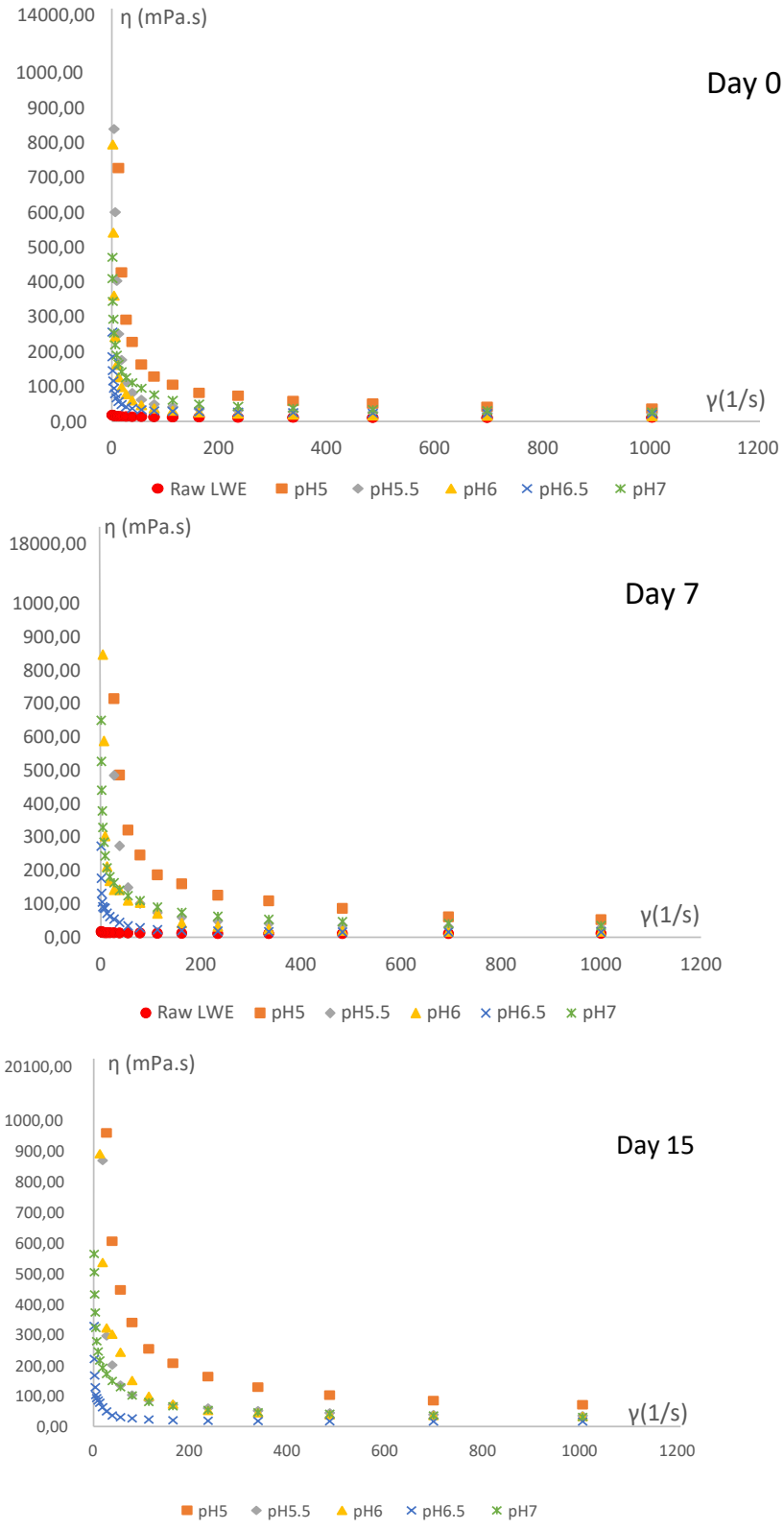


Figure 32: Effect of lactic acid and different pHs on apparent viscosity of heat-treated LWE during Day 0, 7, and 15

Control and pH 5.5 sample showed a dilatant behavior throughout storage time ($1.017<n<2.187$) which is different from pH 5.5 sample with CA which showed pseudoplastic behavior ($n<1$) but similar to Marušić Radovčić et al. (2021) study where LWE with CA showed a dilatant behavior and accords with other study of liquid egg white of Radványi et al. (2012). Meanwhile, samples with starting pH 5, pH 6.5, and pH 7 had a pseudoplastic behavior in the same way as pH 6.5 and pH 7 samples with CA.

Behavior index of LA sample with starting pH 6 showed the same pattern as the lowest starting pH sample with CA where they showed in the first week of storage pseudoplastic behavior then they change to dilatant behavior. Part of this results similar to Singh et al. (2014) where the LWE where prepared from egg stored at room (20-24 °C) and refrigeration (6 °C) temperature and showed a pseudoplastic behavior according Ostwald-de Waele law (Power Law) during 56 days of storage.

Herschel-Bulkley model showed that the values of consistency index (K) are situated between 0.009 and 1.353. Lowest K value was recorded for pH 5.5 after one week of storage, in the same day pH 6 showed the highest K value. Sample with starting pH 5 had a constant K value in first week of storage then in the second week it decreased to approximately the half. Alike, K value of pH 6.5 sample started to decrease after one week of conservation. In the other side, K values increased during storage for pH 7 sample going from 0.258 in the beginning of the experience then stabilized at 0.644 by the second week. Eventually, the effect of LA depends on the starting pH of the sample.

3.3.2.4. Sensory analysis

Scores of the color, smell, taste, and texture attributed by the consumers to heat-treated LWE with LA during storage are illustrated in the spider chart Figure 33. In contrary to LWE with CA, sample of LA with starting pH 7 showed the lowest score for all sensory analysis in the 1st day of the experiment.

For two weeks, sample with starting pH 6 had the highest score for color propriety. In mean time, pH 7 showed the lowest color score for same period time and was the less favorable to the consumers. The difference between color score of pH 7 sample and the other samples including control is distinctive and it is also expressed on b* values where pH 7 sample had the lowest values.

The color scores for other heat-treated samples with LA are relatively high and superposed with the control. By one week of storge, sample with starting pH 5.5 had a similar score as pH 6 sample. Both samples were favorably scored in the 7th day of storage. Arriving to the last day of storage, treated samples showed close scores. In the beginning of the experiment, pH 7 sample had the lowest score of smell (5±2.04) then starting from the 4th day consumers started to attribute higher score for it (5.8±2.08). Sample with starting pH 6.5 had the highest score in the first day and last week of storage while after one week of storage sample with starting pH 7 and control had the highest smell score attributed by the panelist. In Sedoski et al. (2012), cooked whole egg had the favorable odor scored by the panelist comparing to egg sticks with canola, flaxseed, menhaden, and algae oil.

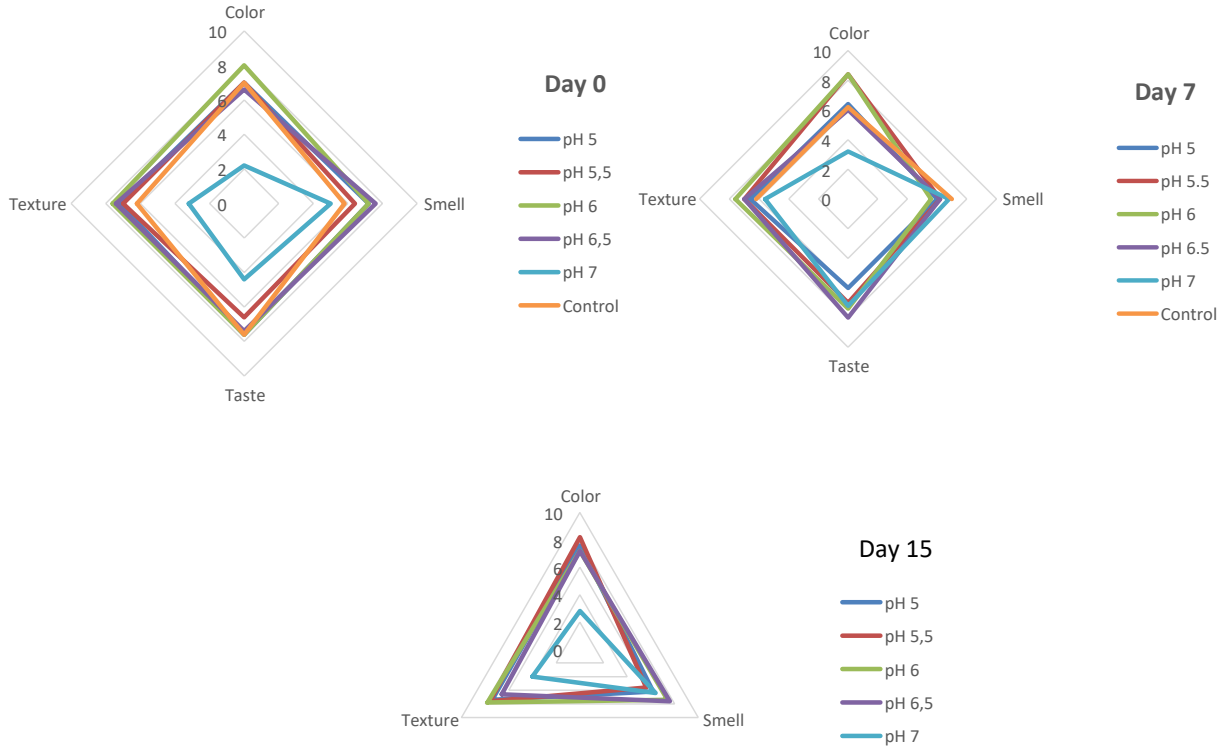


Figure 33: Spider chart of effect of lactic acid and different pHs on heat-treated LWE sensory profile during day 0, 7 and 15

For color property, pH 7 sample showed the least favorable taste score compared to other samples in the first day of storage (4.4±2.49) but starting from the 4th day consumers attribute to it higher score (5.8±1.88). Samples with starting pH 5 and pH 6 beside the control were the favorable scored

in the first day by the panelist but by the 4th day of storage pH 6.5 sample take the lead and has the highest score for taste property through conservation time.

In addition to color, smell, and taste, panelist scored the texture of egg muffin in the mouth. Heat-treated sample with starting pH 6 was the favorable sample for the panelist throughout the experiment time in texture terms. Contrary to Sedoski et al. (2012) where the favorable scored sample for texture between fingers was the one cooked with no-treated whole egg and the least favorable sample was egg sticks with canola. The most disliked sample during storage time was the egg muffin cooked from pH 7 sample. At the same time, other samples showed close scores to each other.

3.4. Effect of HHP treatment with nisin and lysozyme during storage

High Hydrostatic Pressure (HHP) technology, held at refrigeration, ambient or moderate heating temperature, is an emerging, eco-friendly food process that during the last few decades has been applied to food products to improve microbiological safety and extend shelf life with preservation of food quality characteristics and retain its organoleptic and nutritional qualities to those of fresh unprocessed products (Monfort et al. 2012; Naderi, Pouliot, et al. 2017).

3.4.1. Effects on liquid whole egg

3.4.1.1. Physicochemical properties

Evolution of the physicochemical proprieties and color parameters during the storage time is summarized in annex 3. In Figure 34, response surface (RS) without curvature of pH in day 0, 14, and 21 (response surface for other parameters are grouped in annex 3) is presented. The model of HHP treatment with N and Lys was not significantly different ($P>0.05$) on pH values during the two first week of storage. On the 21st day of storage the model had a significant difference on pH value where the pressure of HHP treatment, quantity of N had significant effect on pH values ($P<0.05$). Samples treated with high-pressure (400-435 MPa) showed constant pH values during storage, meanwhile pH values of samples treated with pressure between 226-300 MPa started to decrease after the 14th day of storage. As high-pressure samples, samples treated with 350 MPa kept a constant pH value till the last day where they decreased, and the diminished values depended on the added quantity of N firstly then the added quantity of Lys. Based on the work of Bi et al. (2020) generally the pH values of samples did not show a difference before and after treatment contrary to sample treated with ultrasound and Lys where the pH values indicated slight but

significant increase which can be explained by the release of CO_2 after the break down of a part of carbonic acid in egg white. In the first week of the experiment, the treatment model did not have significant effect on L* values of LWE (P>0.05). Then starting from second week of storage, model of treatment, pressure of HHP treatment and quantity of added N showed a significant difference on L* values of LWE (P<0.05) but after three week the effect of N was not significant anymore (P>0.05). LWE samples with high added quantity of N (5 mg) had grater L* values so they had a lighter color then the other LWE samples. Mainly, L* values reduced in storage similarly to L* values UHT-pasteurized LWE of Liu et al. (2020).

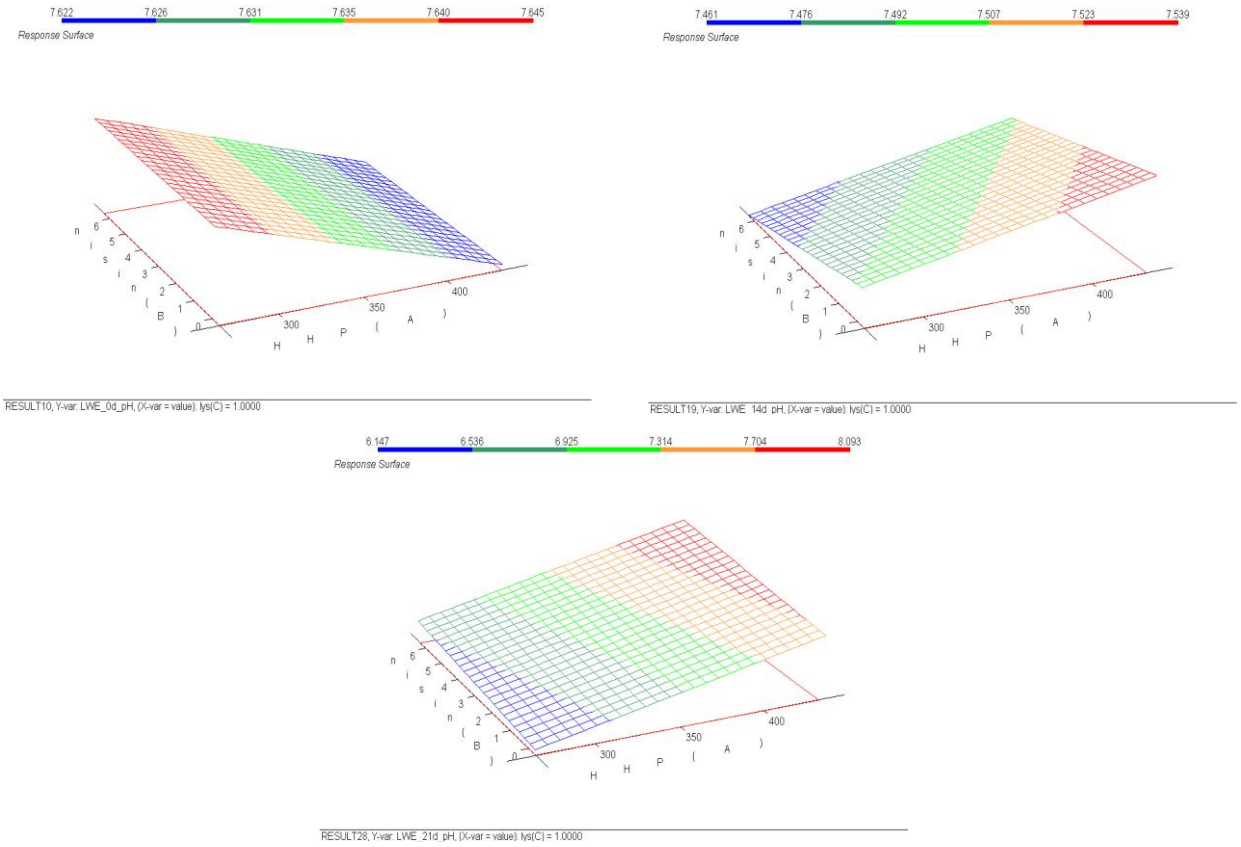


Figure 34: Response surface of the effect of different HHP pressure, nisin and lysozyme on pH values of LWE during day 0, 14 and 21

Just after treatment, sample treated with 226 MPa pressure with 3 mg of N and 1mg of Lys and 350 MPa pressure with 6.35 mg of N and 1 mg of Lys showed a modest rise a* values comparing to other samples. Most a* values of the samples slightly increased or stabilized during two first weeks of storage, few of them reduced in the 21st day. The effect of whole treatment was significant

only on the 14th day accompanied with effect of different pressure of HHP. While HHP treatment with N and Lys preserve the redness of LWE color, UHT pasteurization and ultrasound with Lys only decreased the redness of LWE through storage as it was presented by Bi et al. (2020) and Liu et al. (2020).

Yellowness of LWE color is indicated by b* values. Sample treated with lowest pressure had the highest b* value compared to other samples. Model of treatment and different pressure used for HHP had a significant effect on the yellowness of LWE (P<0.05) through conservation period. Almost all b* values increased throughout the storage time. HHP treatment in presence of N and Lys enhanced yellow color of LWE may be due to the presence of Lys as in Bi et al. (2020) ultrasound treatment with Lys improved b* values after treatment. Chroma and Hue° values had the same pattern as b* values through conservation time. HHP treatment with the presence of N and Lys preserved the color parameters for LWE during storage time.

3.4.1.2. Microbiological results

Growth of mesophilic total plate count of treated LWE through conservation period is illustrated on Figure 35. The RS are represented in the appendix 2. Model of treatment and different pressure used for HHP treatment had a significant influence on the development of total plate count during first week then starting form the second conservation week all parameters including added quantity of N and Lys had a significant effect on microbial growth (P<0.05). Directly after treatment, LWE with lowest pressure (226 MPa) treatment had maximum of microbial growth (around 5 Log CFU/ml) while the lowest growth was for samples treated with high HHP pressure (435 MPa). LWE treated with high pressure showed the lowest bacterial growth and for a pressure of 435 MPa LWE had eventually a constant microbial load (around 3 Log CFU/ml). (Lee et al. (2003) ; Monfort et al. (2012) and Wang et al. (2013) agreed that HHP treatment can have an effect on *S*. enterica and *E*. coli under specific conditions, and Lee et al. (2003) showed that combining the N with HHP treatment of LWE develop lethal effect against Gram-positive *Listeria*. Similarly to these results, Bi et al. (2020) recommended a heat treatment for LWE treated by ultrasound treatment combined with Lys which showed an inactivation of *S*. Typhimurium.

All along two weeks of storage, samples did not exceed the maximum count for mesophilic total plate count. In day 21, samples with low Lys concentrations exceeded the maximum count of

mesophilic total plate. Generally, HHP treatment of LWE in presence of N and Lys restrained the microbiological proliferation for 2 weeks.

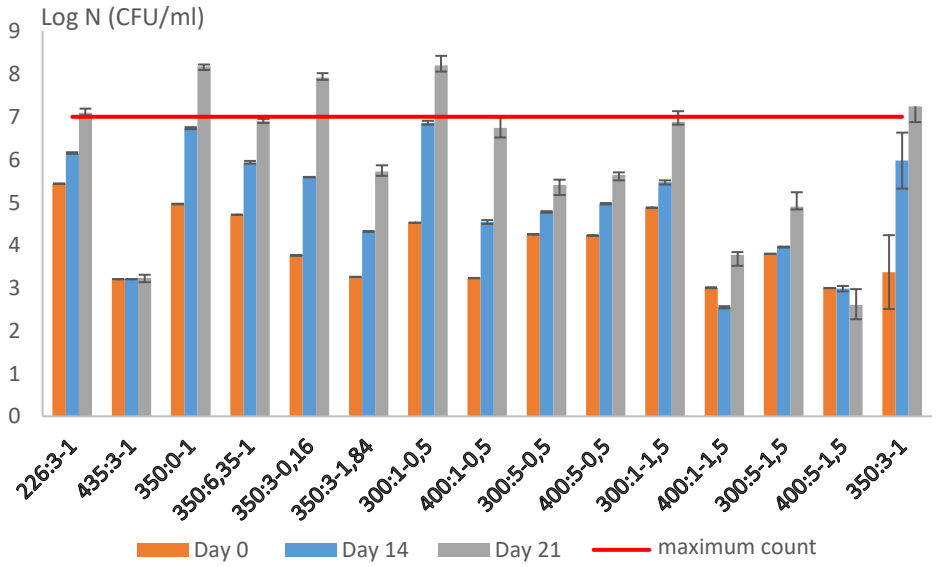


Figure 35: Effect of different HHP pressure, Nisin and Lysozyme on mesophilic total plate count on LWE during storage

3.4.1.3. Thermodynamic properties

The progress of denaturation temperature and enthalpy changes are summarized in Table 26. Thermograms and response surface are added in the annex 3. In the beginning of experiment, model of treatment and added N had a significant effect in both T_d and ΔH (P<0.05); this effect is faded in the second week for both. By the third storage week, model of treatment and added Lys had a significant effect on denaturation temperature of LWE while model of treatment and different pressure of HHP treatment significantly impacted the enthalpy (P<0.05).

All samples showed a single peak in thermograms. This can be explained by the denaturation of most LWE proteins by the treatment. The protein present can be egg yolk protein (LDL, α-, and γ-livetins) due to their high T_d between 63 °C and 76 °C according to Xu et al. (2019) research on egg yolk and its products. The absent peaks can be linked to egg white proteins because their T_d is low compared to LEY.

Table 26: Effect of different HHP pressure, Nisin and Lysozyme on thermal proprieties of LWE proteins

Sample	Storage days		
	0	14	21
T_d (°C)			
226:3-1	77.36±0.08	77.00±0.23	75.29±1.53
435:3-1	77.38±0.07	77.25±0.04	67.96±1.29
350:0-1	77.55±0.04	76.70±0.02	76.51±0.56
350:6.35-1	63.53±0.12	76.90±0.34	76.68±0.89
350:3-0.16	69.96±7.27	77.17±0.04	65.92±1.53
350:3-1.84	77.48±0.04	73.10±7.51	76.16±1.75
300:1-0.5	78.45±0.04	77.29±0.03	64.74±1.20
400:1-0.5	77.82±0.15	72.75±3.83	76.10±1.71
300:5-0.5	72.78±4.51	76.62±0.31	74.46±0.87
400:5-0.5	77.78±0.19	71.55±6.11	68.02±0.28
300:1-1.5	76.94±0.31	76.81±0.52	74.12±1.72
400:1-1.5	77.74±0.02	77.15±0.23	78.62±1.20
300:5-1.5	77.25±0.04	72.65±3.46	63.46±1.97
400:5-1.5	77.56±0.02	77.43±0.09	77.21±2.48
350:3-1	74.84±5.73	72.19±5.80	75.18±0.47
ΔH (J/g)			
226:3-1	0.23±0.00	0.24±0.01	0.22±0.02
435:3-1	0.18±0.04	0.23±0.00	0.32±0.03
350:0-1	0.22±0.00	0.22±0.00	0.37±0.04
350:6.35-1	0.39±0.00	0.22±0.00	0.37±0.04
350:3-0.16	0.30±0.16	0.23±0.00	0.22±0.04
350:3-1.84	0.22±0.01	0.25±0.04	0.23±0.03
300:1-0.5	0.14±0.04	0.22±0.00	0.10±0.02
400:1-0.5	0.21±0.01	0.23±0.02	0.14±0.02
300:5-0.5	0.23±0.04	0.21±0.00	0.25±0.01
400:5-0.5	0.23±0.01	0.30±0.07	0.33±0.05
300:1-1.5	0.16±0.06	1.57±1.16	0.16±0.01
400:1-1.5	0.26±0.00	0.21±0.01	0.33±0.01
300:5-1.5	0.19±0.00	0.29±0.04	0.08±0.02
400:5-1.5	0.24±0.00	0.17±0.03	0.26±0.04
350:3-1	0.23±0.05	0.29±0.06	0.22±0.05

Sample treated with 350 MPa HHP pressure and containing high amounts of N (6.35 mg) and 1 mg of Lys exhibited the lowest T_d peak (63.53±0.12 °C) may correspond to albumin or γ-livetins

while the highest T_d peak was observed for LWE sample treated with 300 MPa pressure and contained 1 mg of N and 0.5 mg of Lys (78.45±0.04 °C) may corresponded to T_d of ovalbumin (Andrássy et al. 2006; Naderi, House, et al. 2017). Practically, T_d of most of samples slightly diminished during conservation period. Contrary to this, in both studies of Tóth et al. (2017) and Tóth et al. (2020), DSC thermograms of LWE after HHP treatment had two peaks, that may represent ovo-transferrin of egg white and LDL of egg yolk.

3.4.1.4. Rheological properties

Parameters of rheological properties are determined by Herschel-Bulkley model and summarized in Table 27 and apparent viscosity were illustrated in annex 3. The correlation with Herschel-Bulkley model was relevant (between 0.894 and 0.999). Throughout storage period, LWE sample treated with 223 MPa pressure and containing 3 mg of N and 1 mg of Lys showed a dilatant behavior (n>1) similarly to LWE treated with 200-250 MPa in Ahmed et al. (2003).

Though, other samples showed pseudoplastic behavior (0<n<1) only in the last day sample treated with 350 MPa and containing 3 mg of N and 1 mg of Lys showed a dilatant behavior in 21st day of refrigeration. With an eyesight, technically all samples showed a relatively high consistency coefficient, only LWE sample treated with the lowest pressure showed a low K coefficient. Just after the treatment, only LWE sample treated with the highest pressure (435 MPa) exhibited highest yield stress then it decreased during storage. In the last week of storage, τ_0 of samples treated with 300 MPa increased. The presence of different τ_0 is illustrated by the apparent viscosity graphs. In fact, apparent viscosity increased during the experiment. This can be explained by the component change of LWE such as protein denaturation because of breakdown of protein network links occurred during HHP treatment and with the presence of egg yolk in LWE, which could also be effected the structure of fat globules (De Souza and Fernández 2013). Accordingly, the treatment did not have an effect on the shear thinning, which liquid egg products are known to be (Severa et al. 2010; De Souza and Fernández 2013). Graphs of apparent viscosity of Bi et al. (2020) showed that the viscosity increased by using ultrasound treatment with and without Lys compared to the control and by using the power law model it have been proved that LWE in that trial showed also a pseudoplastic characteristics of a shear-thinning fluid.

Table 27: Effect of different HHP pressure, Nisin and Lysozyme on Herschel-Bulkley model parameters of heat-treated LWE during storage

Sample	Storage days		
	0	14	21
τ_0 (Pa)			
226:3-1	0.000	0.147	0.000
435:3-1	0.669	0.003	0.000
350:0-1	0.000	0.000	0.000
350:6.35-1	0.000	0.000	0.000
350:3-0.16	0.000	0.000	0.000
350:3-1.84	0.000	0.000	0.000
300:1-0.5	0.000	0.000	0.379
400:1-0.5	0.000	0.000	0.000
300:5-0.5	0.000	0.000	0.121
400:5-0.5	0.000	0.000	0.000
300:1-1.5	0.000	0.000	0.330
400:1-1.5	0.000	0.000	0.000
300:5-1.5	0.000	0.000	0.065
400:5-1.5	0.000	0.000	0.000
350:3-1	0.000	0.000	0.000
K (Pa s^n)			
226:3-1	0.002	0.001	0.002
435:3-1	5.726	5.126	5.949
350:0-1	4.927	1.158	1.075
350:6.35-1	4.898	2.698	1.973
350:3-0.16	3.827	2.133	1.061
350:3-1.84	3.599	1.790	1.554
300:1-0.5	0.555	0.459	0.103
400:1-0.5	5.406	4.726	3.991
300:5-0.5	0.496	1.287	0.182
400:5-0.5	6.546	5.371	3.828
300:1-1.5	0.923	0.485	0.088
400:1-1.5	5.408	4.167	4.073
300:5-1.5	0.262	0.301	0.120
400:5-1.5	6.090	5.613	4.082
350:3-1	4.694	2.598	0.001

n			
226:3-1	1.215	1.428	1.220
435:3-1	0.273	0.294	0.264
350:0-1	0.261	0.448	0.423
350:6.35-1	0.247	0.328	0.345
350:3-0.16	0.278	0.334	0.426
350:3-1.84	0.270	0.362	0.384
300:1-0.5	0.475	0.469	0.704
400:1-0.5	0.270	0.300	0.315
300:5-0.5	0.505	0.339	0.623
400:5-0.5	0.255	0.286	0.304
300:1-1.5	0.405	0.463	0.721
400:1-1.5	0.267	0.300	0.311
300:5-1.5	0.583	0.533	0.678
400:5-1.5	0.256	0.286	0.321
350:3-1	0.269	0.323	1.318
Correlation			
226:3-1	0.996	0.995	0.996
435:3-1	0.894	0.926	0.916
350:0-1	0.911	0.980	0.984
350:6.35-1	0.892	0.943	0.966
350:3-0.16	0.924	0.952	0.987
350:3-1.84	0.915	0.967	0.973
300:1-0.5	0.984	0.988	0.999
400:1-0.5	0.929	0.934	0.941
300:5-0.5	0.981	0.976	0.996
400:5-0.5	0.920	0.924	0.950
300:1-1.5	0.971	0.987	0.999
400:1-1.5	0.922	0.931	0.934
300:5-1.5	0.991	0.993	0.998
400:5-1.5	0.915	0.912	0.941
350:3-1	0.919	0.939	0.995

3.4.2. Effects on Liquid Egg white

3.4.2.1. Physicochemical properties

Color parameters and pH values evolution of LEW during the experiment are grouped in table with their RS in annex 4. Figure 36 shows the RS the effect of the treatment on the pH values of LEW during day 0, 14 and 21. Over conservation time, pH values of LEW slightly diminished.

Model of treatment, pressure of HHP treatment, various quantity of Lys and the interaction between N and Lys had a significant impact on pH values changes after the treatment ($P<0.05$) and the RS with curvature of it showed a minimum point. Therefore, none of the parameters had any significant effect on pH values of LEW in the rest of conservation time ($P>0.05$). L* values indicated the lightness of the samples, considering that LEW is a lucid transparent fluid its L* value should be high. L* values of treated LEW was included between 43 and 63 which close of L* values if LEW treated with ultraviolet in De Souza and Fernández (2012). It was significantly affected by treatment and pressure in first day and by treatment and interaction between N and Lys in third week of storage. But the lightness of LEW was enhanced by freeze- and spray-dry treatment in Jesús et al. (2013) research.

As it was expected a* values were low and even negative, and it indicates the greenness when it is negative. Model treatment and pressure of HHP had a significant effect on a* values over storage time and on 21st day interaction of pressure and nisin quantity was added also ($P<0.05$). The effect can be seen on the RS of a* values where RS present a plan with curvature. The RS with maximum point illustrates that if pressure of HHP treatment reduced and the quantity of nisin was around 1 mg, the a* value diminished. The same effect was induced by spray-dry treatment while with freeze-dry treatment a* values was positive (1.31 ± 0.29) comparably to ultraviolet treated EW (De Souza and Fernández 2012; Jesús et al. 2013). At the beginning, LEW treated with 300 MPa pressure showed lowest a* values compared to other samples then it increased in a similar way to the other samples. Therefore, b* values of LEW exhibited relatively high values then decreased during storage. LEW samples treated with 400 MPa showed the lowest b* values compared to other samples. The b* values were significantly lower than b* values in De Souza and Fernández (2012) and Jesús et al. (2013). HHP pressure had a significant effect on b* values all along refrigeration time while N influenced b* values only on second week. Chroma and Hue° values had same pattern as b* values during storage time. These results imply that LEW is white transparent liquid that tends to green.

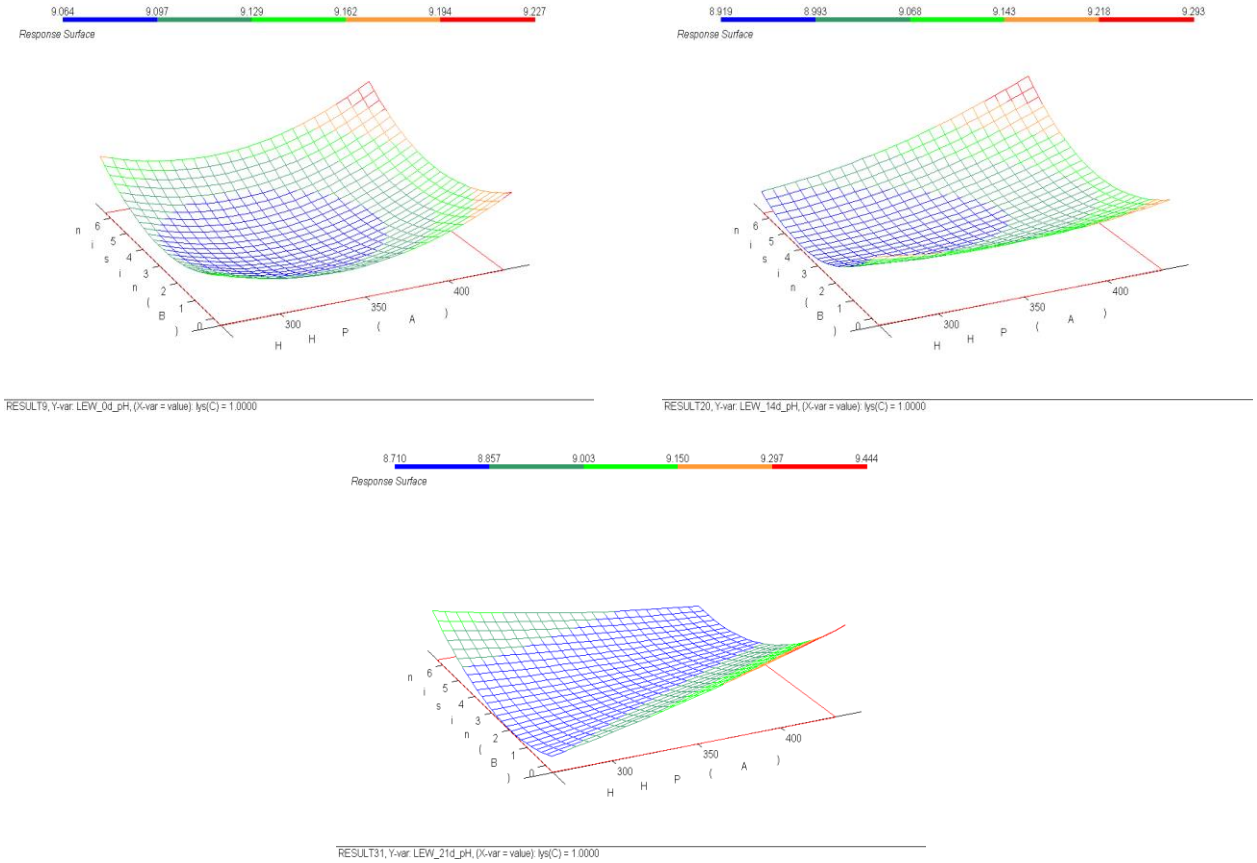


Figure 36: Response surface of effect of different HHP pressure, Nisin and Lysozyme on pH values of LEW during day 0, 14 and 21

3.4.2.2. Microbiological results

Highest mesophilic total plate count was found in three samples which were treated by 300 MPa pressure (around 5 Log CFU/ml) although lowest value was noticed in sample treated with 350 MPa and contained 3 mg of N and 1 mg of Lys (around 4 Log CFU/ml). Evolution of total plate count over storage time is illustrated by Figure 37. The RS with curvature had a minimum point explaining that minimizing pressure and N value increased the microbial growth which is added in annex 4. Over storage time, microbial load increased until the 21st day when the load is on its stationary growth phase. In first week of storage, model of treatment, different parameters, and the interaction of pressure-nisin had significant effect on microbial load of LEW. After 14 days of storage only model and pressure had significant impact on microbial growth. The HPP treatment of LEW in presence of N and Lys was close of short-wave ultraviolet treatment of LEW for 5 minutes while for 30 minutes of same treatment the total aerobic counts was significantly reduced (De Souza and Fernández 2013; De Souza and Fernández 2012). All samples did not exceed the

maximum mesophilic total plate count during the experiment which lead that the treatment prevent growth of the microbiological load.

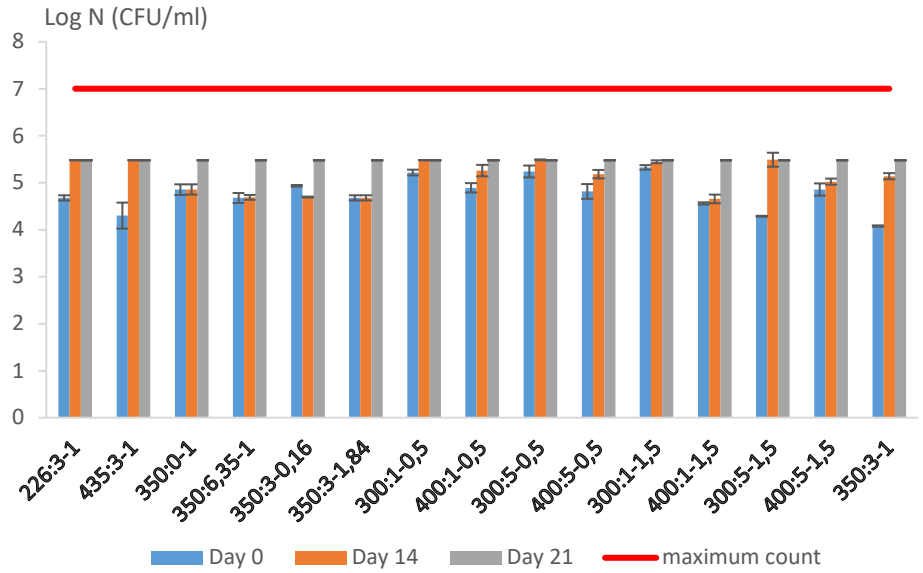


Figure 37: Effect of different HHP pressure, Nisin and Lysozyme on mesophilic total plate count of LEW during storage

3.4.2.3. Thermodynamic properties

Table 28 shows evolution of T_d and enthalpy change of LEW proteins during storage after HHP treatment in presence of N and Lys. Thermograms and RS are added in the annex 4. All DSC thermograms showed presence of two peaks between 61 °C and 76 °C like the control DSC peak curves. The first peak may present ovo-transferrin which had a T_d around 60 °C and the second peak may characterize ovalbumin which had a T_d around 76 °C (Mizutani et al. 2006; Mohammadi Nafchi et al. 2013). During storage, T_d for ovo-transferrin slightly reduced and T_d of ovalbumin reduced slightly or increased depending on pressure and quantity of N and Lys present. However, the enthalpy of ovo-transferrin started with a low $\Delta H_{1denaturation}$, increased remarkably after two weeks for majority of samples (mainly sample treated with 350 MPa pressure), and then decreased again in the last week of storage. $\Delta H_{1denaturation}$ of ovalbumin showed a stability for two weeks of storage then increased by the third week.

Table 28: Effect of different HHP pressure, nisin and lysozyme on thermal proprieties of LEW proteins during storage

Sample	Storage days		
	0	14	21
T_{d1} (°C)			
226:3-1	62.51±0.96	61.33±0.49	57.50±0.26
435:3-1	61.77±0.16	61.31±0.08	66.08±1.02
350:0-1	62.49±0.31	62.24±0.26	62.02±0.10
350:6.35-1	62.24±0.29	61.72±0.47	62.40±0.31
350:3-0.16	62.21±0.22	61.76±0.03	62.34±0.05
350:3-1.84	62.16±0.31	61.76±0.01	62.33±0.01
300:1-0.5	57.23±0.29	61.76±0.00	62.32±0.00
400:1-0.5	62.60±0.35	62.80±0.16	62.28±0.28
300:5-0.5	63.15±1.04	62.33±0.37	62.27±0.03
400:5-0.5	63.03±0.16	62.44±0.06	62.23±0.03
300:1-1.5	62.51±0.53	62.06±0.10	62.59±0.22
400:1-1.5	62.64±0.76	62.34±0.25	62.60±0.16
300:5-1.5	62.33±0.36	62.13±0.30	61.52±0.26
400:5-1.5	62.68±0.25	62.23±0.01	61.68±0.13
350:3-1	62.05±0.11	62.22±0.04	61.68±0.03
T_{d2} (°C)			
226:3-1	76.28±0.24	75.59±0.34	71.35±0.25
435:3-1	76.14±0.10	75.52±0.07	71.35±0.04
350:0-1	75.85±0.65	76.73±0.43	75.54±0.31
350:6.35-1	75.35±0.08	76.04±1.71	75.46±0.07
350:3-0.16	75.52±0.47	76.03±0.29	75.45±0.02
350:3-1.84	75.27±0.33	75.86±0.05	75.44±0.00
300:1-0.5	66.70±0.27	75.84±0.01	75.44±0.00
400:1-0.5	75.68±0.40	76.24±0.27	76.21±0.38
300:5-0.5	76.03±0.45	76.28±0.14	76.15±0.08
400:5-0.5	75.26±0.10	76.27±0.03	76.13±0.02
300:1-1.5	75.78±0.25	76.14±0.25	76.57±0.15
400:1-1.5	75.74±0.46	75.74±0.30	76.47±0.11
300:5-1.5	76.70±0.82	75.97±0.16	76.59±0.16
400:5-1.5	75.75±0.35	75.86±0.01	76.56±0.02
350:3-1	75.71±0.11	75.88±0.02	76.57±0.02

ΔH_1 (J/g)			
226:3-1	0.08±0.00	0.56±0.03	0.05±0.01
435:3-1	0.06±0.04	0.55±0.01	0.05±0.00
350:0-1	0.07±0.01	0.05±0.02	0.07±0.01
350:6.35-1	0.07±0.01	0.38±0.08	0.07±0.00
350:3-0.16	0.07±0.00	0.34±0.03	0.07±0.00
350:3-1.84	0.07±0.00	0.35±0.00	0.07±0.00
300:1-0.5	0.04±0.01	0.35±0.00	0.07±0.00
400:1-0.5	0.06±0.00	0.05±0.02	0.08±0.01
300:5-0.5	0.09±0.01	0.07±0.03	0.08±0.00
400:5-0.5	0.09±0.00	0.06±0.00	0.08±0.00
300:1-1.5	0.05±0.01	0.04±0.03	0.06±0.01
400:1-1.5	0.07±0.01	0.07±0.01	0.06±0.01
300:5-1.5	0.08±0.01	0.06±0.03	0.07±0.01
400:5-1.5	0.09±0.00	0.06±0.00	0.07±0.00
350:3-1	0.09±0.01	0.06±0.00	0.07±0.00
ΔH_2 (J/g)			
226:3-1	0.09±0.00	0.16±0.02	0.24±0.01
435:3-1	0.08±0.00	0.16±0.00	0.24±0.00
350:0-1	0.16±0.07	0.14±0.04	0.20±0.01
350:6.35-1	0.13±0.04	0.15±0.00	0.21±0.00
350:3-0.16	0.13±0.02	0.14±0.00	0.21±0.00
350:3-1.84	0.12±0.01	0.15±0.00	0.21±0.00
300:1-0.5	0.14±0.02	0.15±0.00	0.21±0.00
400:1-0.5	0.15±0.04	0.12±0.03	0.16±0.03
300:5-0.5	0.15±0.01	0.18±0.02	0.16±0.01
400:5-0.5	0.14±0.02	0.16±0.01	0.16±0.00
300:1-1.5	0.13±0.02	0.14±0.02	0.31±0.01
400:1-1.5	0.13±0.02	0.14±0.03	0.13±0.03
300:5-1.5	0.18±0.01	0.16±0.03	0.24±0.02
400:5-1.5	0.14±0.04	0.15±0.00	0.21±0.01
350:3-1	0.16±0.02	0.16±0.00	0.22±0.01

RS for T_{d1} shows that with high HHP pressure in presence of N can increase T_d of ovo-transferrin. In fact, N was the only parameter which had a significant effect on T_{d1} in the beginning (P<0.05). T_d of ovalbumin increased in case of high HHP pressure in presence of low quantity N content and in the opposite case (low HHP pressure and high N presence). All parameters and their interactions had significant impact on ovalbumin T_d (P<0.05) then the effect faded. Therefore, ovo-transferrin

enthalpy was significantly affected by N presence in first week of storage and by the Lys in third week whereas ovalbumin enthalpy was only significantly affected by the HHP pressure, Lys presence, then interaction between them and the interaction between pressure and N in third week of storage. Eventually, HHP treatment in presence of N and Lys did not affect the presence ovo-transferrin for most of the samples, it stabilized but combined the peak with lysozyme. These results are similar to other results mentioned by Van der Plancken et al. (2006); Modugno et al. (2018) and Zhao et al. (2019). Yet, Mizutani et al. (2006) indicated that T_d of ovo-transferrin can be stabilized and increased by thermostabilizing its pure form in presence of anion (sodium sulfate, sodium citrate, sodium phosphate, sodium bicarbonate or sodium chloride) or sorbitol.

Consequently, denaturation enthalpy is related with remaining secondary structure of a protein and a net value of a combination of endothermic reactions and exothermic processes, such as the breakup of hydrophobic interactions or protein aggregation (Van der Plancken et al. 2006).

3.4.2.4. Rheological proprieties

Effect of HHP treatment in presence of N and Lys on rheological parameters of LEW is reported in Table 29 while the curves of apparent viscosity are illustrated in the annex 4. According to the graphs illustrated in annex 4, apparent viscosity of treated LEW mainly diminished over time. The rheological parameters were determined by Herschel–Bulkley model. The correlation to the model was high (0.891 and 0.998). All LEW samples exhibit a shear-thinning with a pseudoplastic rheological behavior during storage ($0<n<1$). Only the sample which was treated by the lowest HHP pressure (226 MPa) showed a shear-thinning with a dilatant behavior starting from second storage week ($n>1$). This behavior is detected also in the albumen treated 0.101 and 250 MPa (down curve of thixotropy) (Ahmed et al. 2003). Consequently, it is the only sample with yield stress through storage time while sample treated with 300 MPa and contained 1 mg of N, 0.5 mg of Lys had a relatively high yield stress only after the treatment. In the same pattern, consistency coefficient had high values, and samples treated with 226 MPa pressure had a low consistency coefficient value.

The result is contrary to the results of Ahmed et al. (2003) where HHP treated albumen had a τ_0 and K similar to 226 MPa treated sample but in case of ultraviolet treatment, LEW had high K values determined by power-law model (De Souza and Fernández 2013). The common result between this results and Ahmed et al. (2003) and De Souza and Fernández (2012) results is that

LEW are generally shear thinning fluid with pseudoplastic behavior but can have a dilatant behavior under certain conditions.

The effect is usually generated by breakdown of weak linkage between the proteins so the breaking of network which result in shear thinning that decreases apparent viscosity (De Souza and Fernández 2013). Usually, the breakdown of linkage means the collapsing of disulfide bonds resulting in the unfolding and aggregation of protein (Ahmed et al. 2003).

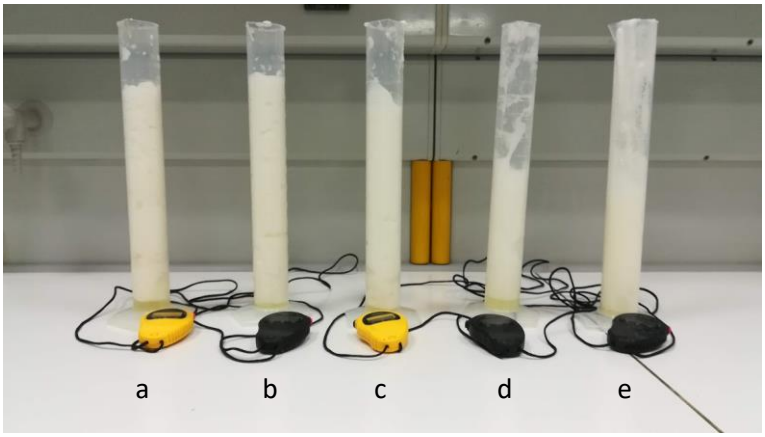
Table 29: Effect of different HHP pressure, nisin and lysozyme on Herschel-Bulkley model parameters of heat-treated LEW during storage

Sample	Storage days		
	0	14	21
τ_0 (Pa)			
226:3-1	0.644	0.217	0.254
435:3-1	0.000	0.000	0.000
350:0-1	0.000	0.000	0.000
350:6.35-1	0.000	0.000	0.000
350:3-0.16	0.000	0.000	0.000
350:3-1.84	0.000	0.000	0.000
300:1-0.5	0.929	0.000	0.000
400:1-0.5	0.000	0.000	0.000
300:5-0.5	0.000	0.000	0.000
400:5-0.5	0.000	0.000	0.000
300:1-1.5	0.000	0.000	0.000
400:1-1.5	0.000	0.000	0.000
300:5-1.5	0.000	0.000	0.000
400:5-1.5	0.000	0.000	0.000
350:3-1	0.000	0.000	0.000
K (Pa s^n)			
226:3-1	0.050	0.001	0.001
435:3-1	5.547	5.894	6.598
350:0-1	4.043	2.028	1.744
350:6.35-1	2.493	3.390	3.232
350:3-0.16	0.895	1.659	1.450
350:3-1.84	3.668	2.382	4.131
300:1-0.5	0.342	0.922	1.149
400:1-0.5	3.880	2.175	1.663
300:5-0.5	0.977	0.211	0.192
400:5-0.5	3.252	3.871	1.093
300:1-1.5	0.922	0.125	0.029

400:1-1.5	3.316	2.608	2.596
300:5-1.5	1.078	0.267	0.068
400:5-1.5	3.784	3.306	2.079
350:3-1	3.760	3.467	2.357
n			
226:3-1	0.672	1.258	1.406
435:3-1	0.279	0.260	0.254
350:0-1	0.213	0.296	0.300
350:6.35-1	0.286	0.205	0.217
350:3-0.16	0.451	0.359	0.376
350:3-1.84	0.216	0.262	0.178
300:1-0.5	0.475	0.343	0.313
400:1-0.5	0.243	0.329	0.368
300:5-0.5	0.337	0.554	0.568
400:5-0.5	0.266	0.234	0.415
300:1-1.5	0.344	0.627	0.841
400:1-1.5	0.261	0.288	0.283
300:5-1.5	0.323	0.522	0.724
400:5-1.5	0.250	0.261	0.337
350:3-1	0.210	0.220	0.257
Correlation			
226:3-1	0.968	0.998	0.997
435:3-1	0.933	0.920	0.918
350:0-1	0.891	0.971	0.980
350:6.35-1	0.955	0.932	0.946
350:3-0.16	0.988	0.966	0.964
350:3-1.84	0.906	0.963	0.927
300:1-0.5	0.986	0.981	0.972
400:1-0.5	0.931	0.950	0.969
300:5-0.5	0.974	0.982	0.980
400:5-0.5	0.933	0.910	0.987
300:1-1.5	0.979	0.984	0.993
400:1-1.5	0.925	0.945	0.953
300:5-1.5	0.980	0.981	0.992
400:5-1.5	0.927	0.923	0.952
350:3-1	0.891	0.938	0.950

3.4.2.5. Foaming property

As the foaming ability is a desirable property for eggs and it is determined by the content and interaction of egg white proteins, the effect of HHP treatment of LEW in presence of Lys and N on FA, FE and FS is presented in Table in annex 4 and the experience is illustrated in Figure 38.



a: 300:1-0.5 sample b: 226:3-1 sample
c: 350:3-1 sample d: 435: 3-1 sample
e: 400:1-0.5 sample

Figure 38: Some examples of foam test of HHP treated LEW in presence of N and Lys in day 0

The table in annex 4 shows that sample treated with the minimum pressure 226MPa in presence of 3 mg of N and 1 mg of Lys had the highest FA while the sample with the minimum FA is the one treated with the highest pressure (435MPa) in the presence of the same amount of N and Lys. The effect of the HHP treatment was significant on FA characteristic while the presence of additive was insignificant. The FA of the samples decreased during the storage time in the same way as the FA of HT LEW with CA and CaS in the beginning of the study. According to the results, FA of HT LEW with CA and CaS showed higher results than the HHP treated one.

In parallel, FE showed similar results as the FA and the different pressure of HHP treatment had a significant effect of it. Samples treated with 435 MPa-400 MPa showed a lower FE compared to other HHP treated samples.

However, FS of HHP treated samples showed a stable foam during the storage time and the different pressure of HHP showed a significant effect on the stability of the foams. The FS of HHP treated LEW is higher than FS of the HT LEW with CA and CaS. These results suggest that the HHP treatment in presence of N and Lys decreased the FA, but it increased the FS of LEW.

3.1.1. Effects on Liquid Egg yolk

3.1.1.1. Physicochemical proprieties

Color parameters development and pH values changes of LEY through storage are summarized in table in annex 5 with their RS. Figure 39 is the RS with curvature which illustrates the effect of parameters and their interaction on pH values. During the first two week of storage, model, pressure of HHP treatment and quality of Lys had a significant impact on pH values. According to the RS high pressure with low N quantity elevated the pH values in the first week while a pressure around 350 MPa with presence of N increased pH values in the second week. This can be seen on pH values of LEY samples treated with pressure around 350-400 MPa where in second storage week they had constant or slightly elevated pH values compared to first week. Thus, in last week of experiment, pH values diminished and only Lys and the interaction between HHP pressure/N and N/Lys had a significant impact. These results are in contrast with the results of Hidas, Nyulas-Zeke, et al. (2021) and Hidas et al. (2020), where frozen storage of LEY or the cryogenic freezing resulted in an increase in pH values.

An important characteristic of LEY is color. According to the results, HHP pressure and quantity of Lys are the main parameters which had significant effect on lightness of LEY samples. L* values diminished over storage time equivalently to the decrease of L* values in Hidas, Nyulas-Zeke, et al. (2021) even though they were significantly higher. The a* values were positive after treatment which indicated that the color of LEY tends to red but then it decreased during conservation time. The effect of HHP treatment of LEY in presence of N and Lys was not significant on a* values in first day of storage then Lys quantity and the interaction of HHP pressure and N had a significant effect on it in second and third week respectively. The effect of treatment is noticeable by comparing a* values to the one of LEY treated with short-wave ultraviolet treatment by (De Souza and Fernández 2012). At the same time, post-processing with HHP treatment of hard-cooked peeled egg could improve a* values of egg yolk which indicated that the color of hard egg yolk tends to green (Shahbaz et al. 2018). Egg yolk is characterized by its yellow color which is indicted by b* values.

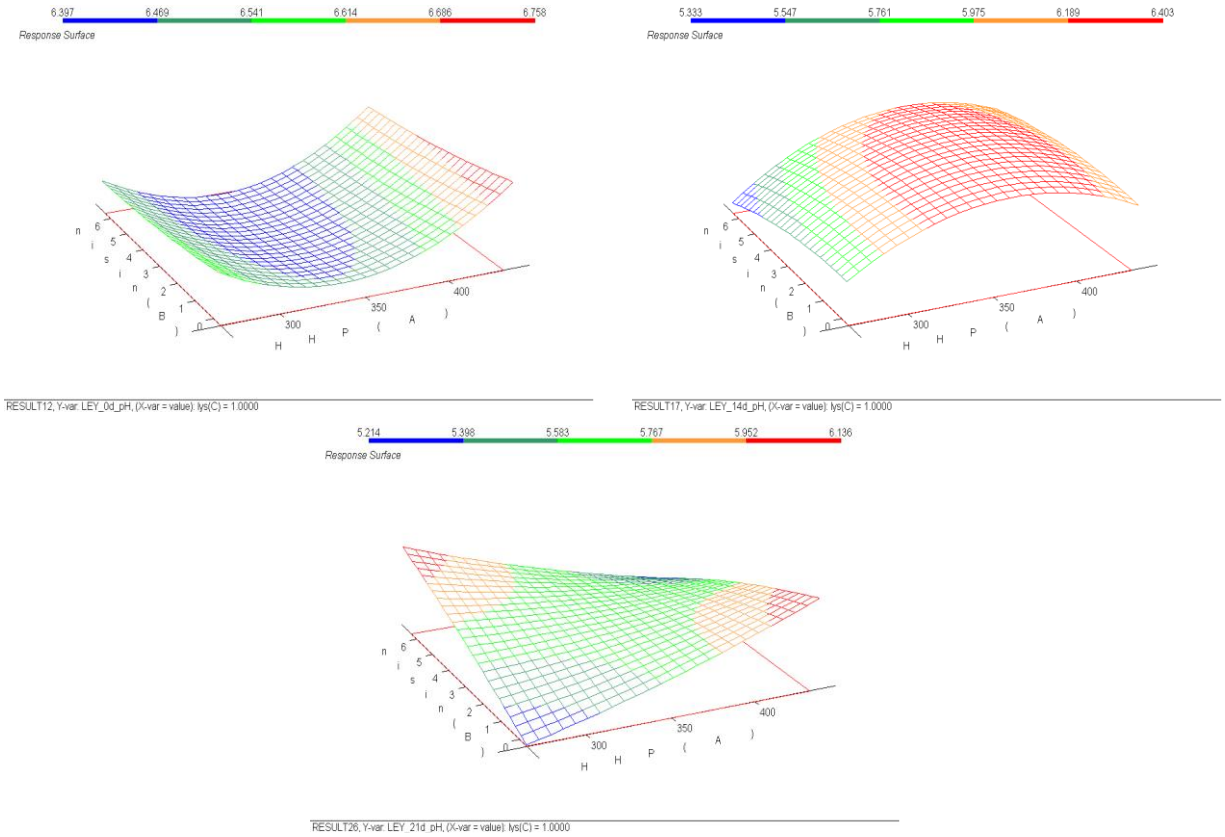


Figure 39: Response surface of effect of different HHP pressure, nisin and lysozyme on pH values of LEY during day 0, 7 and 21

The b* values of LEY declined over storage and exhibit values lower than the one of (De Souza and Fernández 2012; Hidas, Nyulas-Zeke, et al. 2021; Hidas, Németh, et al. 2021) but correlated to b* values of hard-cooked peeled egg which had a post-processing treatment with HHP (300-600MPa) by Shahbaz et al. (2018) and Naderi, Pouliot, et al. (2017) where yellow color of EY faded after HHP treatment. These changes cannot be due to modification of carotenoids which are the pigment responsible of yellow color in EY because according to Oey et al. (2008) and Naderi, Pouliot, et al. (2017) they are stable but HP cause denaturation and aggregation of proteins (Monfort et al. 2012; Naderi, Pouliot, et al. 2017). Chroma and Hue° had the same pattern as b* values. The results indicated that because of the treatment, LEY lost some of its lightness and its yellow color tends to red, but it faded over storage.

3.1.1.2. Microbiological results

Mmesophilic total plate count of LEY is illustrated in Figure 40, the RS are added in the annex 5. Figure 40 of microbial load of treated LEY showed that only three samples (226 MPa and 350

MPa with 3mg N and 1 mg Lys, and 400 MPa with 1 mg N and 1.5 mg Lys) had significantly lower values, according to this none of the parameters had significant impact on the microorganism population only the interaction between N and Lys had significant effect after the treatment. The three samples were treated with different HHP pressure (226, 350, and 435MPa) but they had the same amount of N and Lys (3 mg and 1 mg respectively).

Even with refrigeration storage at 4 °C, microbial load increased starting from the second week, and LEY samples had a constant mesophilic total plate count which means that the microbial population of the samples is in stationary phase, but it did not exceed the maximum count. This may be due to the use of relatively low HHP pressure used. In case of the work of Shahbaz et al. (2018) where they used a high HHP pressure (500-600 MPa) for a post treatment for hard-cooked eggs, total viable count was not detectable until a minimum of 24 storage days. The results were similar to LEY treated with both dynamic and static short-wave ultraviolet by De Souza and Fernández (2013).

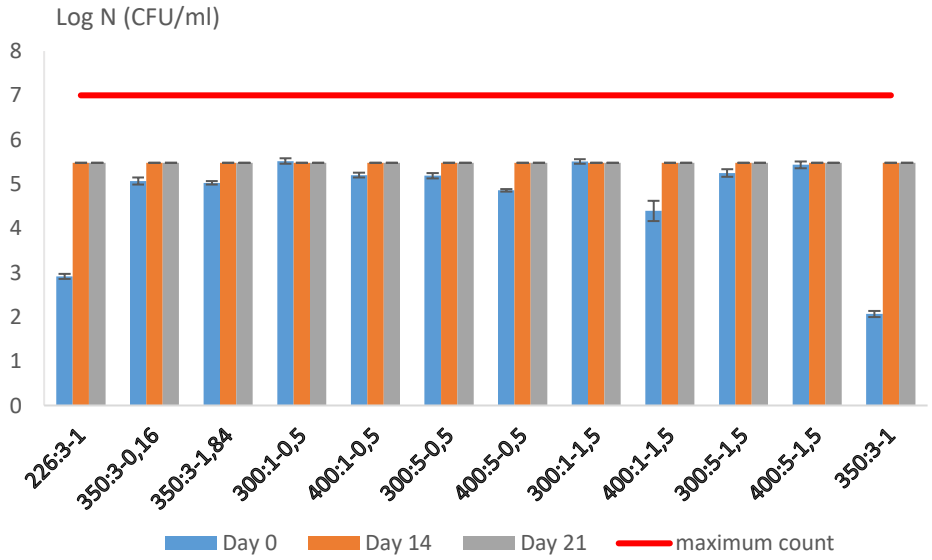


Figure 40: Effect of different HHP pressure, nisin and lysozyme on mesophilic total plate count of LEY during storage

3.1.1.3. Thermodynamic properties

To determine the effect of HHP treatment on LEY protein in presence of N and Lys, thermograms obtained by DSC were added in annex 5 and values of maximum peak of T_d and enthalpy changes

ΔH were grouped in Table 30. According to illustrated thermograms, only one peak was shown during conservation time. Most samples had a peak around 76 °C which can present either LDL complexes or α-livetin (Xu et al. 2019), the other can be delayed peak of them due to HHP treatment effect in presence of N and Lys. Consequently, T_d peak diminished which imply the deterioration of proteins quantity in samples but it cannot determine whether it is LDL or α-livetin because DSC cannot separate into fraction (Cordobés et al. 2004). These results correlate with Hidas, Németh, et al. (2021) results where cryogenic freezing and storage at -18 °C of LEY was examined. Thus, in the results of Ibanoglu and Erçelebi (2007); De Souza and Fernández (2013) and Hidas, Nyulas-Zeke, et al. (2021) the only peak showed in their results was around 85 °C representing high-density lipoproteins, phosvitin and low-density lipoproteins. In the publication of Naderi, Pouliot, et al. (2017), studied diluted egg yolk protein profile after HHP treatment by gel-electrophoresis, comparison between the native and SDS-PAGE profile of LEY provided evidence for the formation of large aggregates via disulfide and hydrophobic interactions and according to the electrophoresis profile only phosvitin line appeared. These results are correlated with RS of HHP treated LEY in presence of N and Lys which highlight that with low HHP pressure and moderate quantity of N, denaturation temperature is elevated.

Meanwhile, enthalpy values decreased by half or more in some cases over storage time. HHP pressure and Lys quantity had a significant impact on ΔH just after treatment, while on the second week all the parameters had an impact on enthalpy changes but in the last day only HHP pressure and N quantity had significant effect.

Table 30: Effect of different HHP pressure, nisin and lysozyme on thermal proprieties of LEY proteins

Sample	Storage days		
	0	14	21
T_d (°C)			
226:3-1	75.66±0.49	73.47±0.31	69.05±0.46
435:3-1	73.39±0.36	73.09±0.15	67.96±0.63
350:0-1	72.72±0.28	60.51±0.58	58.41±0.67
350:6.35-1	71.56±0.57	62.89±0.67	60.89±0.67
350:3-0.16	70.90±0.57	70.23±0.35	68.73±0.35
350:3-1.84	72.02±0.52	70.87±0.69	70.12±0.69
300:1-0.5	75.48±0.20	73.31±0.32	61.03±0.09
400:1-0.5	76.59±0.41	64.50±1.03	63.48±1.03

300:5-0.5	75.61±0.35	71.89±0.49	70.87±0.49
400:5-0.5	75.69±0.32	62.75±0.36	76.29±0.20
300:1-1.5	76.07±0.20	75.61±0.36	74.68±0.36
400:1-1.5	76.48±0.47	63.96±0.61	62.95±0.61
300:5-1.5	76.04±0.33	73.78±0.47	71.77±0.47
400:5-1.5	76.44±1.20	61.89±0.69	60.99±0.69
350:3-1	76.54±0.08	75.60±0.43	74.27±0.43
ΔH (J/g)			
226:3-1	0.24±0.02	0.19±0.02	0.17±0.04
435:3-1	0.36±0.01	0.20±0.06	0.16±0.02
350:0-1	0.53±0.05	0.06±0.02	0.04±0.02
350:6.35-1	0.39±0.06	0.10±0.01	0.08±0.01
350:3-0.16	0.35±0.05	0.15±0.02	0.10±0.02
350:3-1.84	0.48±0.05	0.22±0.03	0.11±0.03
300:1-0.5	0.12±0.03	0.13±0.01	0.12±0.02
400:1-0.5	0.35±0.11	0.10±0.03	0.04±0.03
300:5-0.5	0.12±0.02	0.17±0.02	0.14±0.02
400:5-0.5	0.24±0.08	0.15±0.02	0.12±0.01
300:1-1.5	0.11±0.01	0.13±0.02	0.09±0.02
400:1-1.5	0.21±0.07	0.13±0.01	0.12±0.01
300:5-1.5	0.42±0.09	0.20±0.01	0.14±0.01
400:5-1.5	0.50±0.16	0.11±0.01	0.09±0.01
350:3-1	0.84±0.03	0.12±0.03	0.11±0.03

3.1.1.4. Rheological properties

LEY showed the highest correlation to Herschel–Bulkley model, where all samples had a value of 0.999. Parameters of Herschel–Bulkley model are grouped in Table 31, and apparent viscosity curves are illustrated in annex 5. During storage time, apparent viscosity of LEY mainly diminished. According to the results presented in Table 31, all samples showed a yield stress all along refrigeration time yet the τ_0 changes can be seen on apparent viscosity curves. LEY sample treated with low HHP pressure had the lowest τ_0. These values are higher than τ_0 values of non-treated LEY reported in the work of Kumbár, Nedomová, et al. (2015), while they are much lower than determined τ_0 for cryogenic freeze LEY in the experiment of Hidas, Németh, et al. (2021). Most of LEY samples had a high K value (>1) with the exception of samples treated with low HHP pressure (226-300 MPa) and one sample of 350 MPa pressure treated with 350 MPa and 6.35 mg of N and 1 mg of Lys. Over time, majority of K values diminished but the consistency coefficient

for some samples increased. This fluctuation was seen also in Kumbár, Nedomová, et al. (2015) and Hidas, Németh, et al. (2021) results. Thus, K values of De Souza and Fernández (2013) and Hidas, Németh, et al. (2021) were significantly higher than K values of HHP treated LEY in presence of N and Lys. Independently of other liquid egg products, all treated LEY showed n values less than 1, indicating behavior of shear thinning fluid with pseudoplastic behavior. The highest n values were for LEY sample treated with 226 MPa HHP pressure which showed a dilatant behavior in other liquid egg products.

Table 31: Effect of different HHP pressure, nisin and lysozyme on Herschel-Bulkley model parameters of heat-treated LEY during storage

Sample	Storage days		
	0	14	21
τ_0 (Pa)			
226:3-1	0.909	0.946	0.722
435:3-1	9.199	6.419	0.464
350:0-1	7.249	7.237	8.648
350:6.35-1	5.730	4.833	5.169
350:3-0.16	7.079	5.906	5.463
350:3-1.84	8.520	8.130	7.722
300:1-0.5	3.017	2.626	2.299
400:1-0.5	6.470	6.424	5.256
300:5-0.5	3.229	2.977	2.726
400:5-0.5	8.828	7.583	6.394
300:1-1.5	2.837	3.535	2.627
400:1-1.5	4.928	4.288	3.750
300:5-1.5	4.176	4.232	3.133
400:5-1.5	6.795	4.696	3.561
350:3-1	9.458	6.363	6.076
K (Pa s^n)			
226:3-1	0.404	0.169	0.151
435:3-1	2.960	3.646	5.640
350:0-1	1.576	1.518	1.645
350:6.35-1	0.827	0.827	0.684
350:3-0.16	1.509	1.536	1.479
350:3-1.84	1.554	1.638	1.723
300:1-0.5	0.964	0.637	0.642

400:1-0.5	2.582	2.514	2.460
300:5-0.5	0.827	0.928	1.029
400:5-0.5	2.757	2.821	2.657
300:1-1.5	0.977	0.856	1.027
400:1-1.5	2.594	2.597	2.534
300:5-1.5	0.924	0.888	0.926
400:5-1.5	2.719	2.700	2.625
350:3-1	1.750	1.776	1.784
n			
226:3-1	0.877	0.935	0.938
435:3-1	0.673	0.644	0.583
350:0-1	0.729	0.731	0.729
350:6.35-1	0.787	0.787	0.811
350:3-0.16	0.735	0.732	0.737
350:3-1.84	0.734	0.730	0.726
300:1-0.5	0.778	0.797	0.798
400:1-0.5	0.676	0.678	0.678
300:5-0.5	0.787	0.781	0.776
400:5-0.5	0.676	0.679	0.681
300:1-1.5	0.778	0.785	0.777
400:1-1.5	0.675	0.671	0.673
300:5-1.5	0.780	0.785	0.776
400:5-1.5	0.670	0.668	0.670
350:3-1	0.726	0.720	0.719
Correlation			
226:3-1	0.999	0.999	0.999
435:3-1	0.999	0.999	0.999
350:0-1	0.999	0.999	0.999
350:6.35-1	0.999	0.999	0.999
350:3-0.16	0.999	0.999	0.999
350:3-1.84	0.999	0.999	0.999
300:1-0.5	0.999	0.999	0.999
400:1-0.5	0.999	0.999	0.999
300:5-0.5	0.999	0.999	0.999
400:5-0.5	0.999	0.999	0.999
300:1-1.5	0.999	0.999	0.999
400:1-1.5	0.999	0.999	0.999
300:5-1.5	0.999	0.999	0.999
400:5-1.5	0.999	0.999	0.999
350:3-1	0.999	0.999	0.999

Conclusion and recommendations

Heat treatment was and is still one of the oldest ways to preserve food. Since not all food components are heat resistant, to reduce heat effect, heat treatment was combined with other minimal processing methods. Effect of heat treatment on LEP depended on the treated fraction. Double pasteurization of LWE resulted in a decrease of pH values which was associated with significant reduction of mesophilic total plate count during storage at 4 °C (around 2 Log CFU/ml) but at the same time it was the reason of color changes comparing to once pasteurized LWE.

To reduce heat effect on LEP, citric acid and calcium sorbate were added to LEP before pasteurization. For LWE, the addition of CA and CaS reduced pH values which was able to cause a significant reduction of the mesophilic total plate count through storage (around 2 Log CFU/ml) similar to Góngora-Nieto et al. (2003) results, but it did not prevent color damage and yellow color of LWE tended to green (negative a* values) compared to raw LWE. This effect was similar to the effect that was induced on LEW, where pH values significantly reduced, therefore the mesophilic total plate count was significantly lowered in contrast with the raw LEW (around 3 Log CFU/ml) and the addition of CA and CaS enhanced the color of LEW during storage by increasing a* values. The presence of citric acid and calcium sorbate augmented the foam ability approximately by 35%, foam expansion by 43%, and foam stability by 4% of LEW. Pasteurized LEY relatively maintained a constant pH values during 15 storage days with yellow color tended to red (high a* and b* values). Simultaneously, mesophilic total plate count of treated LEY started with a relative high count (3 Log CFU/ml) then increased all along storage time. Thermograms of heat-treated LEY showed the effect of the treatment on LEY proteins where only one peak could be seen representing one of LEY proteins. On the first day, it was close to raw LEY peak, then the peak delayed which can be explained by protein denaturation during storage. The protein denaturation during conservation time can also be highlighted by the results of emulsion stability of mayonnaise where it lost its stability with time. Adding CA and CaS to LEY before pasteurization reduced pH values significantly compared to raw LEY thus reducing mesophilic total plate count by approximately 4 Log CFU/ml and it prevented the loss of color with higher lightness and yellowness color comparing to raw LEY. The CA and CaS had different effect on LEP, but it mainly reduced the mesophilic total plate count growth by reducing the pH values and prevented color loss for LEW and LEY.

The correlation between various acids and LEP was demonstrated before starting the research, proving that LEY had the highest correlation to CA, LA, AcA, and AscA compared to LEW and LWE. Just after the treatment, CA significantly influenced pH values and color parameters, where samples with low starting pH values had increased the lightness and yellow color of LWE, but it did not prevent the decrease of a* values due to heat effect which can be attributed to the yellow greenish color of the samples. The CA effect and different starting pH values had also an effect on colorimetric and rheological properties. All samples including the control had yield stress point and pH 5.5 sample showed the highest τ_0 and apparent viscosity and it exhibited same dilatant rheological behavior as the control where other samples exhibited pseudoplastic behavior.

Thermograms of LWE with CA showed only one peak which may present ovo-transferrin of EW protein or LDL and/or livetins of EY proteins. LA also had a significant effect on LWE samples with different starting pH values similar to the CA effect. However, the presence of LA increased the lightness only for low starting pH samples (5 and 5.5), but it did not prevent the greenish effect of heat treatment comparing to other samples (6, 6.5, and 7) which had positive a* values so their yellow color tended to red. For rheological properties, LA had a significant effect on low starting pH sample (pH 5) where it exhibited the highest τ_0 and apparent viscosity. Contrary to other acids, AcA increased pH and b* values of LWE samples but it affected only the lightness and redness of lowest starting pH value (5) as a result its bright yellow color tended to green. This also impacted the rheological properties where it showed the highest yield stress and apparent viscosity. Similar to AcA, AscA slightly increased pH, a*, and b* values but only sample with lowest starting pH value (5) had a low a* value making it the only sample with greenish effects. The effect of AscA on rheological properties of LWE is significantly observed in samples with low starting pH (5 and 5.5) where both samples showed shear thinning with pseudoplastic behavior and sample with starting pH 5 exhibited the highest apparent viscosity while sample with starting pH 5.5 had the highest yield stress.

It was important to illustrate the sensory profile of the samples to perceive consumer opinion. For the color of egg muffins, consumers agreed that the color of muffins made with raw egg was unpleasant where for the muffins made with LWE containing CA and LA with low starting pH were considered pleasant. Accordingly, the panelist approved that the smell of sample with starting pH 5 had an unpleasant smell in all muffins egg despite the added acid while samples with starting

pH 7 were the favorable muffin for them. Despite the smell, panelist preferred the taste of egg muffins made with raw eggs. Though, they agreed that the muffins made with starting pH 5 of LA and AcA and muffins made with starting pH 5.5 of CA and AscA were unpleasant to taste. Therefore, regardless the added acid, egg muffins made with starting pH 5.5 had unpleasant texture in the mouth and the ones made with starting pH 7 was the preferred one for the panelist. Since CA and LA egg muffins had the highest rank for taste after raw egg yolk muffins, they were the subject of next experiments.

Generally, during the storage period, CA had a significant effect on the physicochemical properties and color parameters of LWE with different starting pH values. It increased pH, L*, a* and b* values and as in the previous experiment only samples with starting pH 5, 5.5, and 6 had negative a* values which means that low starting pH values do not prevent the greenish effect of heat treatment. From microbiological point of view, it reduced by approximately around 2 Log CFU/ml the starting mesophilic total plate count and even if total plate count increased during storage, it was significantly lower than the total plate count of the control. The effect of CA and different starting pH values was significantly higher in the rheological properties of LWE where samples showed shear thinning with pseudoplastic behavior throughout the storage period contrary to the control and sample with starting pH 5, which showed dilatant behavior on the last day of storage. The addition of CA caused the appearance of yield stress on most samples, and the sample with starting pH 5 showed the highest τ_0 and apparent viscosity during 15 days of storage. For sensorial profile of the samples, egg muffins made with sample with starting pH 6.5 were the favorable sample for the panelist for its color, smell, and texture while for the taste raw egg muffins were the pleasant one after the treatment but starting from second week of storage the pleasant muffins for the consumers was the one made with starting pH 6.5.

Differently than CA, pH and L* values of samples with LA increased after the treatment but it decreased during storage, while b* values increased during storage period. Similar to CA, low starting pH values (5 and 5.5) exhibited negative a* values, while the other samples showed relatively stable values. The effect of LA on mesophilic total plate count was like the effect of CA when the starting pH value of the sample is relatively high (pH 6, 6.5, and 7) but for low starting pH value the mesophilic total plate count is reduce by approximately 3 Log CFU/ml comparing to raw LWE. All samples had a yield stress point and pH 5 sample had the highest τ_0 and apparent

viscosity for 15 days. In Contrast to the control, only pH 5.5 samples showed shear thinning with pseudoplastic behavior and pH 6 sample showed shear thinning with dilatant behavior in last day. Sensory, pH 6.5 sample was the most pleasant sample for the panelist for its smell, texture, and taste but for color pH 6 was the favorable one for them. Consequently, according to ranking by consumers, egg muffins made with starting pH 6.5 LWE for both acids were the favorable ones. Even with addition of additives, heat treatment still has unpleasant effect on egg products. Nowadays, consumers are more aware about additives in food products, hence companies are more oriented to use minimal processing combined with natural active compounds. HHP is considered as a novel, non-thermal process where food undergoes under a pressure above 100 up to 900 MPa (Naderi, Doyen, et al. 2017) and to ensure the microbial safety of products active compounds were added such as nisin and lysozyme.

LEP were the subject of HHP treatment combined with N and Lys to reduce the damages effects of thermal treatment. Effect of different HHP pressure and different quantities of N and Lys on LWE was not significant on pH values and color parameters in the beginning of treatment although during storage samples treated with low HHP pressure (226-300MPa) has shown low pH values and high L* values after three storage weeks. Accordingly, a* and b* parameters showed relatively high values. Consequently, different HHP pressure and N and Lys had an impact on mesophilic total plate count of LWE where lowest microbial growth was noticed in high HHP pressure (400-435MPa) treated samples and the highest one in low HHP pressure (226 MPa). Simultaneously, thermograms of LWE showed only one peak during day 21 of storage, but because of presence of EW and EY the present protein can be either ovalbumin from EW protein or LDL and/or livetins from EY proteins. HHP treatment with N and Lys effect can be noticed in rheological properties where all samples showed shear thinning with pseudoplastic behavior only samples treated with low HHP pressure showed shear thinning with dilatant behavior.

For LEW, HHP treatment combined with N and Lys did not have any significant effect on pH values although it decreased during storage but mainly samples treated with 400 MPa pressure showed stable pH values. Eventually, HHP with N and Lys had a significant impact on color parameters of LEW and showed relatively high L* values but it exhibited negative a* values which increased over time. The color of samples after HHP treatment was distinguishable. After the treatment, all parameters had a significant effect on the mesophilic total plate count but on the

second week only the different HHP pressure had significant impact on the mesophilic total plate count. Thermograms of LEW illustrate the presence of two peaks during storage period. The first one suggests the presence of ovo-transferrin and the second one suggest ovalbumin. Significant impact of HHP treatment with N and Lys was observed in yield stress of sample treated with low HHP pressure where it showed high τ_0 for 21 days, beside the sample which was treated with 300 MPa and 1 mg N and 0.5 mg Lys presenting highest τ_0 only after the treatment. Also, all samples showed shear thinning with pseudoplastic behavior with the exception of sample treated with 226 MPa, which showed a shear thinning with dilatant behavior starting on the second week of storage.

Different HHP pressure and quantity of Lys had significant impact on pH values of LEY and the values mainly decreased during storage. Lightness of HHP treated LEY with N and Lys was high and L* values of samples treated with 400 MPa increased during 21 days of storage. The a* and b* values also mainly decreased and HHP pressure and quantity of Lys had the main significant effect on it, thus the color of LEY was pale yellow which tended to red. The effect of treatment was significant only after the treatment, but the mesophilic total plate count stabilized starting from second week. The treatment significantly affected the mesophilic total plate count of the three samples treated with different HHP pressures but with the same quantity of additives (3mg of N and 1mg of Lys). Thermograms of LEY illustrated one peak with denaturation temperature around 70-76 °C which may suggest the presence LDL or/and livetins proteins. Effect of treatment on rheological properties could be noticed by unaided eye. LEY was thicker after the treatment which indicated the presence of yield stress for all the samples and the lowest values was noticed in sample treated with low HHP pressure (226 MPa) and the highest was for sample treated with the highest HHP pressure (435 MPa). Despite that LEY looked thicker by unaided eye according flow behavior index it showed shear thinning with pseudoplastic behavior. Consequently, LEY had the highest correlation with the Herschel-Bulkley model. Effectiveness of HHP treatment with N and Lys on the microbiological destruction depended on the egg fraction. Egg yolk showed the lowest microbial reduction, then egg white, then whole egg. It could be noticed that HHP treatment with N and Lys restrained the mesophilic total plate count growth of whole egg.

To resume, HHP treatment and addition of additives (CA with CaS, CA, and LA) reduced microbiological load of LWE with a favorable result for HHP treatment combined with N and Lys.

Though, HHP treatment preserved color quality of LWE during the storage similarly to LWE samples with CA and LA and starting pH values between 6 and 7, it increased the apparent viscosity and both, low and high pressure, changed the rheological behavior of LWE. It is recommended to study the effects of 300-350 MPa pressure of HHP treatment combined with N and Lys or CA or LA since they had a positive impact to sensory qualities according to the panelist. For LEW, it is recommended to study to effectiveness of HHP treatment with pressure between 350 and 400 MPa combined with 1-5 mg of N and 1-5 mg of Lys or to combine it with CA with CaS due to their effect on foamability of LEW. Accordingly, higher HHP pressure (starting from 350 MPa) combined with 1-5 mg of N and 1-5 mg of Lys can be used for LEY and N and Lys can be changed by CA with CaS for their ability to reduce microbiological load and preventing the break of emulsion.

New scientific results

1. Pasteurization (65 °C for 10min) and double pasteurization (65 °C for 20 min) had a significant effect on pH values of liquid whole egg during storage. Double pasteurization did not prevent color change (greenish effect) of heat treatment and altered the yellow color comparing of single pasteurization of samples. Double pasteurization showed a stable microbiological load (2 log CFU/ml) for 3 weeks storage comparing to single pasteurization.
2. Combination of heat treatment (70 °C for 3min) with 0.5% CA and 0.3% calcium sorbate significantly decreased pH values of liquid whole egg compared to the control which led to the decrease of the microbiological load with 2 log CFU/ml compared to raw liquid whole egg, and it showed a stable microbiological load (≈3log CFU/ml) for 3 weeks storage. The presence of 0.5% citric acid and 0.3% calcium sorbate enhanced the lightness and yellow color of LWE, but it did not prevent the greening caused by heat treatment.
3. Combination of heat treatment (56 °C for 3min) with 0.5% citric acid and 0.3 % reduced pH values of liquid egg white from 9.3 to 5.7 enabling the decrease of microbiological population by 3 log CFU/ml comparing to the raw liquid egg white. Presence of 0.5% citric acid and 0.3% calcium sorbate enhanced color parameters of liquid egg white and improved its foaming ability 35% compared to the control.
4. Combination of heat treatment (67 °C for 3 min) with 0.5% citric acid and 0.3% of calcium sorbate reduced pH values of liquid egg yolk significantly compared to the raw product. This concentration of citric acid and calcium sorbate significantly decreased microbiological load of liquid egg yolk from 5 log CFU/ml to 1 log CFU/ml and enhanced its yellow color, stabilized its L* and a* values.
5. Combination of heat treatment with citric acid or lactic acid stabilized pH values of samples of liquid whole egg with different starting pH for two weeks storage. Presence of citric and lactic significantly reduced the mesophilic total plate count of liquid whole egg minimum by approximately 2 log CFU/ml. Samples with low starting pH value (5-6) had discoloration but it enhanced the color parameters (lightness and yellow color) and caused changes in flow behavior from pseudoplastic to dilatant behavior. Besides, samples with citric acid and lactic acid had a favorable ranking for sensorial parameters comparing to other acids.
6. Combination of HHP treatment with nisin and lysozyme stabilized pH values of liquid whole egg during 3 weeks in case of high pressure (350-435 MPa) treated samples while in case of

lower pressure (226-300 MPa) treatments it started to decrease after 2 weeks. High HHP pressure (350-435 MPa) in presence of 3 mg of nisin and 1 mg of lysozyme significantly decreased the microbiological population but 435 MPa pressure led to high apparent viscosity. Pressure of 300 MPa and 5 mg of nisin and 1.5 mg of lysozyme showed low microbiological load and its growth was minimal during storage.

7. Combination of HHP treatment with nisin and lysozyme stabilized pH values of liquid egg white during 3 weeks for high HHP pressure while for lower pressure start to decrease after 2 weeks. Sample treated with HHP pressure 350MPa in presence of 3 mg of nisin and 1 mg lysozyme had the minimal microbiological growth and less discoloration for 3 weeks of storage.
8. Combination of HHP treatment with nisin and lysozyme had a significant effect on pH value of liquid egg yolk where high HHP pressure samples had stable pH value during refrigeration while pH value of other samples started to decrease just after one week. Sample treated with HHP pressure 350 MPa in presence of 3 mg of nisin and 1 mg lysozyme showed minimal microbiological growth, but it had the highest apparent viscosity.

Summary

Nowadays, expectation of customers toward food products and industries are high. To meet this expectation much research has been held. This research aimed also to improve qualities and shelf life of food products. Such as diary and alcohol products take an enormous part in our daily life, egg and mainly its products started to have same enormous part. Egg products are considered as challenging products because they get perishable easily. To prevent the alteration of egg products, one of the oldest preservations were applied which is heat treatment. Usually, temperatures around 65 to 68 °C for 5 to 6 min for both whole egg and egg yolk are applied. Because of the thermo-labile protein, egg white is treated with a milder temperature around 55 to 57 °C for 2 to 5 min. Unfortunately, heat treatment caused damage in thermo-labile proteins of egg. To encounter these damages, heat treatment was combined with other conservation methods applying acids and bioactive compounds. Other minimal process methods have also been introduced in the egg products industry such as high hydrostatic pressure (HHP), ultraviolet radiation, and high-intensity ultrasound combined or no with other conservation methods.

The aim of this study was to investigate the effects of heat treatment combined with different type of acids and HHP treatment combined with antimicrobial peptides (nisin and lysozyme) on rheological, physicochemical, and sensory proprieties of liquid egg products.

The different liquid egg products were supplied by the production line of Capriovus Ltd (Szigetcsép, Hungary) after homogenization. Before heat-treatment, 0.5% citric acid and 0.3% calcium sorbate per kg were added to liquid egg products. In separate experiments citric acid, lactic acid, acetic acid, and ascorbic acid were added to liquid whole egg until it reached different pH values (pH 5.0, 5.5, 6.0, 6.5, and 7.0). Similarly, before HHP treatment (266 MPa - 435 MPa), nisin (0 mg - 6.35 mg) and lysozyme (0.16 mg - 1.5 mg) were added to different liquid egg products. Accordingly, after each treatment samples were stored at refrigeration room (4 °C) for approximately 3 weeks. During the storage time, pH, color parameters, calorimetric properties, apparent viscosity, and microbiological properties were studied.

Heat treatment combined with 0.5% citric acid and 0.03% calcium sorbate significantly decreased pH values of liquid whole egg and with this decrease it reduced the microbiological load with 2 log CFU/ml compared to raw liquid whole egg and prevented its growth by maintaining

approximately same microbiological load for 21 days, but some discoloration occurred even if they enhanced lightness of liquid whole egg. Thus, the effect of the same amount of citric acid and calcium sorbate is more noticeable on pH values, and on the microbial growth of liquid egg white by decreasing it by 3 log CFU/ml comparing to the control. Additionally, it enhanced the color proprieties of liquid whole egg and its foaming ability by 35% comparing to raw. Like for other liquid egg products, the combination of heat treatment with 0.5% citric acid and 0.3% calcium sorbate diminished the pH values of liquid egg yolk, enhanced its a* and b* values and stabilized its lightness and the color difference between the treated sample and the raw could be noticed by unaided eye. The additives decreased the microbial load by 3 log CFU/ml comparing to the control but during the storage time it decreased and the difference between the treated and non-treated samples was only 1 log CFU/ml in the last day of storage.

Heat treatment combined with different acids and different starting pH values for liquid egg products was also studied. It could be noticed that the pH values of liquid whole egg were the least affected ones by acids (citric acid, lactic caid, acetic acid and ascorbic acid 20%) compared to other liquid egg products. For the same acid, acetic acid 20% had the lowest effect on liquid egg products. Combination of heat treatment with citric acid or lactic acid stabilized pH values of samples of liquid whole egg with different starting pH. Microbial load of treated samples with citric acid reduced the initial microbial load by 2 log CFU/ml for all different starting pH values. Lactic acid reduced the same microbiological load for samples with starting pH value between 6-7 while for samples with low starting pH values (5 - 5.5) it reduced the initial microbiological load by 3 Log CFU/ml. Samples with low starting pH values had discoloration, but it enhanced the color parameters for other starting pH value samples and caused some changes in flow behavior. In sensory analysis, both samples had a favorable ranking comparing to other acids. For heat treated samples with acetic acid or ascorbic acid pH values of samples of liquid whole egg with different starting pH were stabilized after the treatment, and the presence of both acids enhanced the color of liquid whole egg, but samples with low starting pH value greening effect of heat treatment occurred with a change in flow behavior.

Different pressure of HHP treatment and different concentrations of nisin and lysozyme influenced pH values of liquid whole egg, in the beginning it stabilized pH values of liquid whole egg during 3 weeks for high HHP pressure treated samples, while for lower pressure started to decrease after

2 weeks but it prevented the discoloration. High HHP pressure (350 MPa – 435 MPa) in presence of 3 mg nisin and 1mg lysozyme significantly decreased microbiological population but 435 MPa pressure led to high apparent viscosity. Pressure of 300 MPa and 5 mg nisin and 1.5 mg of lysozyme showed low microbiological load and its growth was minimal during storage. The combined treatment also stabilized the pH values of liquid whole egg during 3 weeks for high HHP pressure treated samples while for lower pressure values it started to decrease after 2 weeks. Effect of different pressure of HHP treatment with nisin and lysozyme was noticed directly after the treatment. During storage, the effect vanished. Sample treated with 350 MPa HHP pressure in presence of 3 mg nisin and 1 mg lysozyme had minimal microbial growth and less discoloration for 3 weeks of storage, but it had the highest apparent viscosity. Contrary to other liquid egg products, high HHP pressure treated samples of liquid egg yolk in presence of nisin and lysozyme had stable pH value during refrigeration while pH value of other samples started to decrease after 7 days. Like liquid whole egg, effect of different pressure of HHP treatment, nisin and lysozyme on liquid egg yolk samples was noticed directly after the treatment but during storage the effect vanished.

Appendices

Annex 1 References

Abeyrathne, E.D.N.S., Lee, H.Y. and Ahn, D.U. (2013). Egg white proteins and their potential use in food processing or as nutraceutical and pharmaceutical agents—A review. *Poultry Science*, 92(12), pp.3292–3299.

Aganovic, K., Hertel, C., Vogel, R., Johne, R., Schlüter, O., Schwarzenbolz, U., Jäger, H., Holzhauser, T., Bergmair, J., Roth, A., Sevenich, R., Bandick, N., Kulling, S., Knorr, D., Engel, K.-H. and Heinz, V. (2021). Aspects of high hydrostatic pressure food processing: Perspectives on technology and food safety. *Comprehensive Reviews in Food Science and Food Safety*, 20.

Ahmed, J., Ramaswamy, H.S., Alli, I. and Ngadi, M. (2003). Effect of high pressure on rheological characteristics of liquid egg. *LWT - Food Science and Technology*, 36(5), pp.517–524.

Alamprese, C., Casiraghi, E. and Rossi, M. (2012). Mechanical and rheological properties of fresh egg pasta as affected by shell egg production factors. *International Journal of Food Science & Technology*, 47(12), pp.2503–2509.

Alleoni, A.C.C. (2006). Albumen protein and functional properties of gelation and foaming. *Scientia Agricola*, 63(3), pp.291–298.

Ameen, S. and Caruso, G. (2017). *Lactic Acid in the Food Industry*.

Andrássy, É., Farkas, J., Seregély, Zs., Dalmadi, I., Tuboly, E. and Lebovics, V. (2006). Changes of hen eggs and their components caused by non-thermal pasteurizing treatments II. Some non-microbiological effects of gamma irradiation or hydrostatic pressure processing on liquid egg white and egg yolk. *Acta Alimentaria*, 35(3), pp.305–318.

Anton, M. (2013). Egg yolk: structures, functionalities and processes. *Journal of the Science of Food and Agriculture*, 93(12), pp.2871–2880.

Arzeni, C., Pérez, O.E. and Pilosof, A.M.R. (2012). Functionality of egg white proteins as affected by high intensity ultrasound. *Food Hydrocolloids*, 29(2), pp.308–316.

Atılgan, M.R. and Unluturk, S. (2008). Rheological Properties of Liquid Egg Products (LEPS). *International Journal of Food Properties*, 11(2), pp.296–309.

Ayivi, R.D., Gyawali, R., Krastanov, A., Aljaloud, S.O., Worku, M., Tahergorabi, R., Silva, R.C. da and Ibrahim, S.A. (2020). Lactic Acid Bacteria: Food Safety and Human Health Applications. *Dairy*, 1(3), pp.202–232.

Aymerich, T., Picouet, P.A. and Monfort, J.M. (2008). Decontamination technologies for meat products. *Meat Science*, 78(1–2), pp.114–129.

Baron, F. and Jan, S. (2011). Egg and egg product microbiology. In: *Improving the Safety and Quality of Eggs and Egg Products*. Elsevier, pp.330–350. Available from:

https://linkinghub.elsevier.com/retrieve/pii/B9781845697549500140 [accessed 28 December 2021].

Bi, X., Wang, X., Chen, Y., Chen, L., Xing, Y. and Che, Z. (2020). Effects of combination treatments of lysozyme and high power ultrasound on the Salmonella typhimurium inactivation and quality of liquid whole egg. *Ultrasonics Sonochemistry*, 60, p.104763.

Björn, A., Karlsson, A., Ejlertsson, J., Svensson, B.H. and Segura de La Monja, P. (2012). Rheological Characterization. , 2012, p.16.

Blume, K., Dietrich, K., Lilienthal, S., Ternes, W. and Drotleff, A.M. (2015). Exploring the relationship between protein secondary structures, temperature-dependent viscosities, and technological treatments in egg yolk and LDL by FTIR and rheology. *Food Chemistry*, 173, pp.584–593.

Calderón-Miranda, M.L., Barbosa-Cánovas, G.V. and Swanson, B.G. (1999). Inactivation of Listeria innocua in liquid whole egg by pulsed electric fields and nisin. *International Journal of Food Microbiology*, 51(1), pp.7–17.

Campbell, L., Raikos, V. and Euston, S.R. (2003). Modification of functional properties of egg-white proteins. *Food / Nahrung*, 47(6), pp.369–376.

Chalamaiah, M., Esparza, Y., Temelli, F. and Wu, J. (2017). Physicochemical and functional properties of livetins fraction from hen egg yolk. *Food Bioscience*, 18, pp.38–45.

Chen, Y. and Ma, M. (2020). Foam and conformational changes of egg white as affected by ultrasonic pretreatment and phenolic binding at neutral pH. *Food Hydrocolloids*, 102, p.105568.

Chung, Y.-K., Vurma, M., Turek, E.J., Chism, G.W. and Yousef, A.E. (2005). Inactivation of Barotolerant Listeria monocytogenes in Sausage by Combination of High-Pressure Processing and Food-Grade Additives. *Journal of Food Protection*, 68(4), pp.744–750.

Cordobés, F., Partal, P. and Guerrero, A. (2004). Rheology and microstructure of heat-induced egg yolk gels. *Rheologica Acta*, 43(2), pp.184–195.

Corry, J.E.L. (2007). Microbiological analysis of eggs and egg products. In: *Microbiological Analysis of Red Meat, Poultry and Eggs*. Elsevier, pp.183–201. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9781845690595500097 [accessed 3 December 2021].

Cwiková, O. and Nedomová, Š. (2014). Microbiological quality of egg liquid products. *Potravinarstvo Slovak Journal of Food Sciences*, 8(1), pp.114–118.

Damodaran, S. (2005). Protein Stabilization of Emulsions and Foams. *Journal of Food Science*, 70(3), pp.R54–R66.

Dawson, P.L. and Martinez-Dawson, R. (1998). Using response surface analysis to optimize the quality of ultrapasteurized liquid whole egg. *Poultry Science*, 77(3), pp.468–474.

De Souza, P.M., Müller, A., Beniaich, A., Mayer-Miebach, E., Oehlke, K., Stahl, M., Greiner, R. and Fernández, A. (2015). Functional properties and nutritional composition of liquid egg products treated in a coiled tube UV-C reactor. *Innovative Food Science & Emerging Technologies*, 32, pp.156–164.

De Souza, P.M. and Fernández, A. (2012). Consumer acceptance of UV-C treated liquid egg products and preparations with UV-C treated eggs. *Innovative Food Science & Emerging Technologies*, 14, pp.107–114.

De Souza, P.M. and Fernández, A. (2013). Rheological properties and protein quality of UV-C processed liquid egg products. *Food Hydrocolloids*, 31(1), pp.127–134.

Deleu, L.J., Wilderjans, E., Van Haesendonck, I., Courtin, C.M., Brijs, K. and Delcour, J.A. (2015). Storage induced conversion of ovalbumin into S-ovalbumin in eggs impacts the properties of pound cake and its batter. *Food Hydrocolloids*, 49, pp.208–215.

Delves-Broughton, J. (2012). Natural antimicrobials as additives and ingredients for the preservation of foods and beverages. , 2012, p.35.

Depree, J.A. and Savage, G.P. (2001). Physical and flavour stability of mayonnaise. *Trends in Food Science & Technology*, 12(5–6), pp.157–163.

Donovan, J.W. (1977). A study of the baking process by differential scanning calorimetry. *Journal of the Science of Food and Agriculture*, 28(6), pp.571–578.

Doseděl, M., Jirkovský, E., Macáková, K., Krčmová, L., Javorská, L., Pourová, J., Mercolini, L., Remião, F., Nováková, L., Mladěnka, P., and on behalf of The OEMONOM. (2021). Vitamin C—Sources, Physiological Role, Kinetics, Deficiency, Use, Toxicity, and Determination. *Nutrients*, 13(2), p.615.

Drabik, K., Batkowska, J., Próchniak, T. and Horecka, B. (2021). Citric acid as a factor limiting changes in the quality of table eggs during their storage. *Poultry Science*, 100(4), p.100995.

Driessen, A.J.M., van den Hooven, H.W., Kuiper, W., Van de Camp, M., Sahl, H.-G., Konings, R.N.H. and Konings, W.N. (1995). Mechanistic studies of lantibiotic-induced permeabilization of phospholipid vesicles. *Biochemistry*, 34(5), pp.1606–1614.

Duan, X., Li, M., Shao, J., Chen, H., Xu, X., Jin, Z. and Liu, X. (2018). Effect of oxidative modification on structural and foaming properties of egg white protein. *Food Hydrocolloids*, 75, pp.223–228.

Elez-Martínez, P., Martín-Belloso, O., Rodrigo, D. and Sampedro, F. (2007). Impact of pulsed electric fields on food enzymes and shelf-life. In: *Food Preservation by Pulsed Electric Fields*. Elsevier, pp.212–246. Available from: https://linkinghub.elsevier.com/retrieve/pii/B978184569058850014X [accessed 29 December 2021].

Ethiraj, S. (2012). Lantibiotic nisin: Natural preservative from Lactococcus lactis. , 2012. Available from: https://www.academia.edu/2141837/Lantibiotic_nisin_Natural_preservative_from_Lactococcus_lactis [accessed 17 October 2019].

Farahnaky, A., Gray, D.A., Mitchell, J.R. and Hill, S.E. (2003). Ascorbic acid and hydrogen peroxide (Fenton's reagent) induced changes in gelatin systems. *Food Hydrocolloids*, 17(3), pp.321–326.

Fialová, J., Chumchalová, J., Miková, K. and Hrůšová, I. (2008). Effect of food preservatives on the growth of spoilage lactobacilli isolated from mayonnaise-based sauces. *Food Control*, 19(7), pp.706–713.

Fu, Xuan, Fu, Xing, Wang, Q., Sheng, L., Huang, X., Ma, M. and Cai, Z. (2017). Fluorescence switch biosensor based on quantum dots and gold nanoparticles for discriminative detection of lysozyme. *International Journal of Biological Macromolecules*, 103, pp.1155–1161.

Ghoush, M.A., Samhouri, M., Al-Holy, M. and Herald, T. (2008). Formulation and fuzzy modeling of emulsion stability and viscosity of a gum–protein emulsifier in a model mayonnaise system. *Journal of Food Engineering*, 84(2), pp.348–357.

Ginsau, M.A. (2015). High Pressure Processing: A Novel Food Preservation Technique. *IOSR Journal of Environmental Science, Toxicology and Food Technology*, 9(5), pp.109–113.

Gmach, O., Bertsch, A., Bilke-Krause, C. and Kulozik, U. (2019). Impact of oil type and pH value on oil-in-water emulsions stabilized by egg yolk granules. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 581, p.123788.

Gomes, R.J., Borges, M. de F., Rosa, M. de F., Castro-Gómez, R.J.H. and Spinosa, W.A. (2018). Acetic Acid Bacteria in the Food Industry: Systematics, Characteristics and Applications. *Food Technology and Biotechnology* [online], 56(2). Available from: http://www.ftb.com.hr/images/pdfarticles/2018/April_June/FTB-56-139.pdf [accessed 3 January 2022].

Góngora-Nieto, M.M., Pedrow, P.D., Swanson, B.G. and Barbosa-Cánovas, G.V. (2003). Energy analysis of liquid whole egg pasteurized by pulsed electric fields. *Journal of Food Engineering*, 57(3), pp.209–216.

Gullo, M., Verzelloni, E. and Canonico, M. (2014). Aerobic submerged fermentation by acetic acid bacteria for vinegar production: Process and biotechnological aspects. *Process Biochemistry*, 49(10), pp.1571–1579.

Gumudavelli, V., Subbiah, J., Thippareddi, H., Velugoti, P. r. and Froning, G. (2007). Dynamic Predictive Model for Growth of Salmonella Enteritidis in Egg Yolk. *Journal of Food Science*, 72(7), pp.M254–M262.

Hauben, K.J.A., Wuytack, E.Y., Soontjens, C.C.F. and Michiels, C.W. (1996). High-Pressure Transient Sensitization of Escherichia coli to Lysozyme and Nisin by Disruption of Outer-Membrane Permeability. *Journal of Food Protection*, 59(4), pp.350–355.

Hester, P., ed. (2017). *Egg innovations and strategies for improvements*. London, United Kingdom ; San Diego, CA: Academic Press, an imprint of Elsevier.

Hidas, K.I., Visy, A., Csonka, J., Nyulas-Zeke, I.C., Friedrich, L., Pásztor-Huszár, K., Alpár, B., Hitka, G., Felföldi, J., Fehér, O. and Gere, A. (2020). Development of a Novel Gluten-Free Egg Pie Product: Effects of Sensory Attributes and Storage. *Sustainability*, 12(24), p.10389.

Hidas, K.I., Nyulas-Zeke, I.C., Visy, A., Baranyai, L., Nguyen, L.P.L., Tóth, A., Friedrich, L., Nagy, A. and Németh, C. (2021). Effect of Combination of Salt and pH on Functional Properties of Frozen-Thawed Egg Yolk. *Agriculture*, 11(3), p.257.

Hidas, K.I., Németh, C., Nguyen, L.L.P., Visy, A., Tóth, A., Barkó, A., Friedrich, L., Nagy, A. and Nyulas-Zeke, I.C. (2021). Effect of cryogenic freezing on the rheological and calorimetric properties of pasteurized liquid egg yolk. *Czech Journal of Food Sciences*, 39(No. 3), pp.181–188.

Hite, B.H. (1899). *The effect of pressure in the preservation of milk : a preliminary report* [online]. West Virginia University Agricultural Experiment Station. Available from: https://researchrepository.wvu.edu/wv_agricultural_and_forestry_experiment_station_bulletins/58 [accessed 22 November 2021].

Hofstetter, S., Gebhardt, D., Ho, L., Gänzle, M. and McMullen, L.M. (2013). Effects of nisin and reutericyclin on resistance of endospores of Clostridium spp. to heat and high pressure. *Food Microbiology*, 34(1), pp.46–51.

Huan, Y., Kong, Q., Mou, H. and Yi, H. (2020). Antimicrobial Peptides: Classification, Design, Application and Research Progress in Multiple Fields. *Frontiers in Microbiology*, 11, p.2559.

Huang, L., Wang, T., Han, Z., Meng, Y. and Lu, X. (2016). Effect of egg yolk freezing on properties of mayonnaise. *Food Hydrocolloids*, 56, pp.311–317.

Huopalahti, R., European Cooperation in the Field of Scientific and Technical Research (Organization) and European Science Foundation, eds. (2007). *Bioactive egg compounds*. Berlin ; New York: Springer.

Ibanoglu, E. and Erçelebi, E.A. (2007). Thermal denaturation and functional properties of egg proteins in the presence of hydrocolloid gums. *Food Chemistry*, 101(2), pp.626–633.

Jesús, M.N. de, Zanqui, A.B., Valderrama, P., Tanamati, A., Maruyama, S.A., Souza, N.E. de and Matsushita, M. (2013). Sensory and physico-chemical characteristics of desserts prepared with egg products processed by freeze and spray drying. *Food Science and Technology*, 33(3), pp.549–554.

Johnson, T.M. and Zabik, M.E. (1981). Egg Albumen Proteins Interactions in an Angel Food Cake System. *Journal of Food Science*, 46(4), pp.1231–1236.

Johnston, C.S., Steinberg, F. and Rucker, R. (2013). Ascorbic Acid. *Handbook of Vitamins*, 29 July 2013, pp.489–520.

Jones, D.R. (2007). Egg Functionality and Quality During Long-Term Storage. *International Journal of Poultry Science*, 6, pp.157–162.

Kalchayanand, N., Sikes, T., Dunne, C.P. and Ray, B. (1994). Hydrostatic Pressure and Electroporation Have Increased Bactericidal Efficiency in Combination with Bacteriocins. *Applied and Environmental Microbiology*, 60(11), pp.4174–4177.

Kalchayanand, N., Sikes, A., Dunne, C.P. and Ray, B. (1998). Interaction of Hydrostatic Pressure, Time and Temperature of Pressurization and Pediocin AcH on Inactivation of Foodborne Bacteria. *Journal of Food Protection*, 61(4), pp.425–431.

Kamysz, W., Okrój, M. and Łukasiak, J. (2003). Novel properties of antimicrobial peptides. *Acta Biochimica Polonica*, 50(2), pp.461–469.

Khan, I., Tango, C.N., Miskeen, S., Lee, B.H. and Oh, D.-H. (2017). Hurdle technology: A novel approach for enhanced food quality and safety – A review. *Food Control*, 73, pp.1426–1444.

Kirimura, K., Honda, Y. and Hattori, T. (2011). Citric Acid. In: *Comprehensive Biotechnology*. Elsevier, pp.135–142. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780080885049001690 [accessed 29 December 2021].

Kościuczuk, E.M., Lisowski, P., Jarczak, J., Strzałkowska, N., Jóźwik, A., Horbańczuk, J., Krzyżewski, J., Zwierzchowski, L. and Bagnicka, E. (2012). Cathelicidins: family of antimicrobial peptides. A review. *Molecular Biology Reports*, 39(12), pp.10957–10970.

Kovalcuks, A., Straumite, E. and Duma, M. (2016). The Effect of Egg Yolk Oil on The Chemical, Physical and Sensory Properties of Mayonnaise. *Rural Sustainability Research*, 35(330), pp.24–31.

Kumbár, V., Nedomová, Š., Strnková, J. and Buchar, J. (2015). Effect of egg storage duration on the rheology of liquid egg products. *Journal of Food Engineering*, 156, pp.45–54.

Kumbár, V., Strnková, J., Nedomová, Š. and Buchar, J. (2015). Fluid dynamics of liquid egg products. *Journal of Biological Physics*, 41(3), pp.303–311.

Lechevalier, V., Guérin-Dubiard, C., Anton, M., Beaumal, V., David Briand, E., Gillard, A., Le Gouar, Y., Musikaphun, N., Tanguy, G., Pasco, M., Dupont, D. and Nau, F. (2017). Pasteurisation of liquid whole egg: Optimal heat treatments in relation to its functional, nutritional and allergenic properties. *Journal of Food Engineering*, 195, pp.137–149.

Lee, D.U., Heinz, V. and Knorr, D. (2003). Effects of combination treatments of nisin and high-intensity ultrasound with high pressure on the microbial inactivation in liquid whole egg. *Innovative Food Science & Emerging Technologies*, 4(4), pp.387–393.

Lei, J., Sun, L., Huang, S., Zhu, C., Li, P., He, J., Mackey, V., Coy, D.H. and He, Q. (2019). The antimicrobial peptides and their potential clinical applications. *American Journal of Translational Research*, 11(7), pp.3919–3931.

Leistner, L. (2000). Basic aspects of food preservation by hurdle technology. *International Journal of Food Microbiology*, 55(1–3), pp.181–186.

Leistner, L. and Gorris, L.G.M. (1995). Food preservation by hurdle technology. *Trends in Food Science & Technology*, 6(2), pp.41–46.

Li, J., Zhang, Y., Fan, Q., Teng, C., Xie, W., Shi, Y., Su, Y. and Yang, Y. (2018). Combination effects of NaOH and NaCl on the rheology and gel characteristics of hen egg white proteins. *Food Chemistry*, 250, pp.1–6.

Li, J., Wang, C., Li, X., Su, Y., Yang, Y. and Yu, X. (2018). Effects of pH and NaCl on the physicochemical and interfacial properties of egg white/yolk. *Food Bioscience*, 23, pp.115–120.

Li, X., Li, J., Chang, C., Wang, C., Zhang, M., Su, Y. and Yang, Y. (2019). Foaming characterization of fresh egg white proteins as a function of different proportions of egg yolk fractions. *Food Hydrocolloids*, 2019, p.8.

Liang, Y. and Kristinsson, H.G. (2007). Structural and foaming properties of egg albumen subjected to different pH-treatments in the presence of calcium ions. *Food Research International*, 40(6), pp.668–678.

Liu, T., Lv, B., Zhao, W., Wang, Y., Piao, C., Dai, W., Hu, Y., Liu, J., Yu, H. and Sun, F. (2020). Effects of Ultrahigh Temperature Pasteurization on the Liquid Components and Functional Properties of Stored Liquid Whole Eggs. *BioMed Research International*, 2020, pp.1–10.

Llave, Y., Fukuda, S., Fukuoka, M., Shibata-Ishiwatari, N. and Sakai, N. (2018). Analysis of color changes in chicken egg yolks and whites based on degree of thermal protein denaturation during ohmic heating and water bath treatment. *Journal of Food Engineering*, 222, pp.151–161.

López-Pedemonte, T.J., Roig-Sagués, A.X., Trujillo, A.J., Capellas, M. and Guamis, B. (2003). Inactivation of Spores of Bacillus cereus in Cheese by High Hydrostatic Pressure with the Addition of Nisin or Lysozyme. *Journal of Dairy Science*, 86(10), pp.3075–3081.

Marušić Radovčić, N., Karlović, S., Medić, H. and Režek Jambrak, A. (2021). Effect of citric acid addition on functional properties of pasteurized liquid whole eggs. *Journal of Food Science and Technology*, 58(3), pp.985–995.

Masschalck, B., García-Graells, C., Van Haver, E. and Michiels, C.W. (2000). Inactivation of high pressure resistant Escherichia coli by lysozyme and nisin under high pressure. *Innovative Food Science & Emerging Technologies*, 1(1), pp.39–47.

Masso-Silva, J. and Diamond, G. (2014). Antimicrobial Peptides from Fish. *Pharmaceuticals*, 7(3), pp.265–310.

Mine, Y. (1995). Recent advances in the understanding of egg white protein functionality. *Trends in Food Science & Technology*, 6(7), pp.225–232.

Mine, Y. and Yang, M. (2010). Functional Properties of Egg Components in Food Systems. In: Guerrero-Legarreta, I., ed. *Handbook of Poultry Science and Technology*. Hoboken, NJ, USA: John Wiley & Sons, Inc., pp.579–630. Available from: https://onlinelibrary.wiley.com/doi/10.1002/9780470504451.ch29 [accessed 16 November 2021].

Miranda, J.M., Anton, X., Redondo-Valbuena, C., Roca-Saavedra, P., Rodriguez, J.A., Lamas, A., Franco, C.M. and Cepeda, A. (2015). Egg and Egg-Derived Foods: Effects on Human Health and Use as Functional Foods. *Nutrients*, 7(1), pp.706–729.

Mizutani, K., Chen, Y., Yamashita, H., Hirose, M. and Aibara, S. (2006). Thermostabilization of Ovotransferrin by Anions for Pasteurization of Liquid Egg White. *Bioscience, Biotechnology, and Biochemistry*, 70(8), pp.1839–1845.

Modugno, C., Loupiac, C., Bernard, A., Jossier, A., Neiers, F., Perrier-Cornet, J.-M. and Simonin, H. (2018). Effect of high pressure on the antimicrobial activity and secondary structure of the bacteriocin nisin. *Innovative Food Science & Emerging Technologies*, 47, pp.9–15.

Mohammadi Nafchi, A., Tabatabaei, R.H., Pashania, B., Rajabi, H.Z. and Karim, A.A. (2013). Effects of ascorbic acid and sugars on solubility, thermal, and mechanical properties of egg white protein gels. *International Journal of Biological Macromolecules*, 62, pp.397–404.

Monfort, S., Ramos, S., Meneses, N., Knorr, D., Raso, J. and Álvarez, I. (2012). Design and evaluation of a high hydrostatic pressure combined process for pasteurization of liquid whole egg. *Innovative Food Science & Emerging Technologies*, 14, pp.1–10.

Montejano, J.G., Hamann, D.D. and Lanier, T.C. (1984). Thermally Induced Gelation of Selected Comminuted Muscle Systems?Rheological Changes during Processing, Final Strengths and Microstructure. *Journal of Food Science*, 49(6), pp.1496–1505.

Mudannayaka, A.I., Rajapaksha, D.S.W. and Kodithuwakku, A.H.T. (2016). Effect of Beeswax, Gelatin and Aloe vera Gel Coatings on Physical Properties and Shelf Life of Chicken Eggs Stored at 30°C. , 2016, p.8.

Muñoz, A., Dominguez-Gasca, N., Jimenez-Lopez, C. and Rodriguez-Navarro, A.B. (2015). Importance of eggshell cuticle composition and maturity for avoiding trans-shell Salmonella contamination in chicken eggs. *Food Control*, 55, pp.31–38.

Murray, B.S. (2007). Stabilization of bubbles and foams. *Current Opinion in Colloid & Interface Science*, 12(4–5), pp.232–241.

Naderi, N., House, J.D., Pouliot, Y. and Doyen, A. (2017). Effects of High Hydrostatic Pressure Processing on Hen Egg Compounds and Egg Products. *Comprehensive Reviews in Food Science and Food Safety*, 16(4), pp.707–720.

Naderi, N., Pouliot, Y., House, J.D. and Doyen, A. (2017). High hydrostatic pressure effect in extraction of 5-methyltetrahydrofolate (5-MTHF) from egg yolk and granule fractions. *Innovative Food Science & Emerging Technologies*, 43, pp.191–200.

Naderi, N., Doyen, A., House, J.D. and Pouliot, Y. (2017). The use of high hydrostatic pressure to generate folate-enriched extracts from the granule fraction of hen's egg yolk. *Food Chemistry*, 232, pp.253–262.

Nakimbugwe, D., Masschalck, B., Anim, G. and Michiels, C.W. (2006). Inactivation of gram-negative bacteria in milk and banana juice by hen egg white and lambda lysozyme under high hydrostatic pressure. *International Journal of Food Microbiology*, 112(1), pp.19–25.

Natoli, S., Markovic, T., Lim, D., Noakes, M. and Kostner, K. (2007). Unscrambling the research: Eggs, serum cholesterol and coronary heart disease. *Nutrition & Dietetics*, 64(2), pp.105–111.

Necidová, L., Bursová, Š., Ježek, F., Haruštiaková, D., Vorlová, L. and Golian, J. (2019). Effect of preservatives on the shelf-life and sensory characteristics of pasteurized liquid whole egg stored at 4°C. *Poultry Science*, 98(11), pp.5940–5948.

Neira, C., Laca, Amanda, Laca, Adriana and Díaz, M. (2017). Microbial diversity on commercial eggs as affected by the production system. A first approach using PGM. *International Journal of Food Microbiology*, 262, pp.3–7.

Németh, C., Friedrich, L., Zeke, I. and Balla, C. (2010). A new liquid egg product. *Review of Faculty of Engineering (Analecta Technica Szegediensis)*, 2010, pp.165–170.

Németh, C., Mráz, B., Friedrich, L., Suhajda, A., Janzsó, B. and Balla, C. (2011). Microbiological measurements for the development of a new preservation procedure for liquid egg. *Czech Journal of Food Sciences*, 29(No. 6), pp.569–574.

Nguyen, T. and Burley, R. (1984). Studies on the Apoproteins of the Major Lipoprotein of the Yolk of Hen's Eggs. V. Protein Sulfhydryl Groups: Accessibility in the Lipoprotein and the Effect of Temperature. *Australian Journal of Biological Sciences*, 37(2), p.7.

Oey, I., Van der Plancken, I., Van Loey, A. and Hendrickx, M. (2008). Does high pressure processing influence nutritional aspects of plant based food systems?. *Trends in Food Science & Technology*, 19(6), pp.300–308.

Oladejo, D. (2015). Application of Osmotic Dehydration for Shelf Life Extension of Fresh Poultry Eggs. , 2(6), p.5.

Patrignani, F., Vannini, L., Sado Kamdem, S.L., Hernando, I., Marco-Molés, R., Guerzoni, M.E. and Lanciotti, R. (2013). High pressure homogenization vs heat treatment: Safety and functional properties of liquid whole egg. *Food Microbiology*, 36(1), pp.63–69.

Patterson, M.F., Ledward, D.A. and Rogers, N. (2006). High Pressure Processing. In: Brennan, J. G., ed. *Food Processing Handbook*. Weinheim, FRG: Wiley-VCH Verlag GmbH & Co. KGaA,

pp.173–200. Available from: https://onlinelibrary.wiley.com/doi/10.1002/3527607579.ch6 [accessed 23 November 2021].

Picart-Palmade, L., Cunault, C., Chevalier-Lucia, D., Belleville, M.-P. and Marchesseau, S. (2019). Potentialities and Limits of Some Non-thermal Technologies to Improve Sustainability of Food Processing. *Frontiers in Nutrition*, 5, p.130.

Ponce, E., Pla, R., Sendra, E., Guamis, B. and Mor-Mur, M. (1998). Combined effect of nisin and high hydrostatic pressure on destruction of Listeria innocua and Escherichia coli in liquid whole egg. *International Journal of Food Microbiology*, 43(1), pp.15–19.

Pou, K.R.J. and Raghavan, V. (2020). Recent Advances in the Application of High Pressure Processing-Based Hurdle Approach for Enhancement of Food Safety and Quality. *Journal of Biosystems Engineering*, 45(3), pp.175–187.

Radványi, D., Juhász, R., Németh, Cs., Suhajda, A., Balla, Cs. and Barta, J. (2012). Evaluation of the stability of whipped egg white. *Czech Journal of Food Sciences*, 30(No. 5), pp.412–420.

Rahman, M.S. (2015). Hurdle Technology in Food Preservation. In: Siddiqui, M. W. and Rahman, M. S., eds. *Minimally Processed Foods*. Food Engineering Series. Cham: Springer International Publishing, pp.17–33. Available from: http://link.springer.com/10.1007/978-3-319-10677-9_2 [accessed 28 October 2021].

Rannou, C., Queveau, D., Beaumal, V., David-Briand, E., Le Borgne, C., Meynier, A., Anton, M., Prost, C., Schuck, P. and Loisel, C. (2015). Effect of spray-drying and storage conditions on the physical and functional properties of standard and n-3 enriched egg yolk powders | Elsevier Enhanced Reader. *Journal of Food Engineering* [online], 2015. Available from: https://reader.elsevier.com/reader/sd/pii/S0260877414004695?token=CB24C505206492B6E9DED17EC539BAEEAE42C165730361A3BA95EDB4372CC795D002671B493BC17A1180029D42C700FA [accessed 17 October 2019].

Rayner, M., Marku, D., Eriksson, M., Sjöö, M., Dejmek, P. and Wahlgren, M. (2014). Biomass-based particles for the formulation of Pickering type emulsions in food and topical applications. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 458, pp.48–62.

Rêgo, I.O.P., Menezes, L.D.M., Figueiredo, T.C., Oliveira, D.D., Rocha, J.S.R., Lara, L.J.C., Lima, A.L., Souza, M.R. and Cançado, S.V. (2014). Bioactive amines and microbiological quality in pasteurized and refrigerated liquid whole egg1. *Poultry Science*, 93(4), pp.1018–1022.

Rêgo, I.O.P., Cançado, S.V., Figueiredo, T.C., Menezes, L.D.M., Oliveira, D.D., Lima, A.L., Caldeira, L.G.M. and Esser, L.R. (2012). Influência do período de armazenamento na qualidade do ovo integral pasteurizado refrigerado. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, 64, pp.735–742.

Renzetti, S., van den Hoek, I.A.F. and van der Sman, R.G.M. (2020). Amino acids, polyols and soluble fibres as sugar replacers in bakery applications: Egg white proteins denaturation controlled by hydrogen bond density of solutions. *Food Hydrocolloids*, 108, p.106034.

Rima, Mariam, Rima, Mohamad, Fajloun, Z., Sabatier, J.-M., Bechinger, B. and Naas, T. (2021). Antimicrobial Peptides: A Potent Alternative to Antibiotics. *Antibiotics*, 10(9), p.1095.

Robinson, D.S. and Monsey, J.B. (1972). Changes in the composition of ovomucin during liquefaction of thick egg white. *Journal of the Science of Food and Agriculture*, 23(1), pp.29–38.

Rossi, M., Casiraghi, E., Primavesi, L., Pompei, C. and Hidalgo, A. (2010). Functional properties of pasteurised liquid whole egg products as affected by the hygienic quality of the raw eggs. *LWT - Food Science and Technology*, 43(3), pp.436–441.

Ruiz, A., Williams, S.K., Djeri, N., Hinton, A. and Rodrick, G.E. (2009). Nisin, rosemary, and ethylenediaminetetraacetic acid affect the growth of Listeria monocytogenes on ready-to-eat turkey ham stored at four degrees Celsius for sixty-three days. *Poultry Science*, 88(8), pp.1765–1772.

Santos, H.O., de Moraes, W.M.A.M., da Silva, G.A.R., Prestes, J. and Schoenfeld, B.J. (2019). Vinegar (acetic acid) intake on glucose metabolism: A narrative review. *Clinical Nutrition ESPEN*, 32, pp.1–7.

Sedoski, H.D., Beamer, S.K., Jaczynski, J., Partington, S. and Matak, K.E. (2012). Sensory evaluation and quality indicators of nutritionally-enhanced egg product with ω-3 rich oils. *LWT*, 47(2), pp.459–464.

Severa, L., Nedomová, Š. and Buchar, J. (2010). Influence of storing time and temperature on the viscosity of an egg yolk. *Journal of Food Engineering*, 96(2), pp.266–269.

Shahbaz, H.M., Jeong, B., Kim, J.U., Ha, N., Lee, H., Ha, S.-D. and Park, J. (2018). Application of high pressure processing for prevention of greenish-gray yolks and improvement of safety and shelf-life of hard-cooked peeled eggs. *Innovative Food Science & Emerging Technologies*, 45, pp.10–17.

Sheng, L., Wang, Y., Chen, J., Zou, J., Wang, Q. and Ma, M. (2018). Influence of high-intensity ultrasound on foaming and structural properties of egg white. *Food Research International*, 108, pp.604–610.

Singh, J., Sharma, H.K., Premi, M. and Kumari, K. (2014). Effect of storage conditions of egg on rheological properties of liquid whole egg. *Journal of Food Science and Technology*, 51(3), pp.543–550.

Smelt, J.P.P.M. (1998). Recent advances in the microbiology of high pressure processing. *Trends in Food Science & Technology*, 9(4), pp.152–158.

Smith, R. (2004). Regulation (EC) No 883/2004 of the European Parliament and of the Council of 29 April 2004. In: *Core EU Legislation*. London: Macmillan Education UK, pp.288–318. Available from: http://link.springer.com/10.1007/978-1-137-54482-7_27 [accessed 18 November 2021].

Stewart, C.M., Dunne, C.P. and Keener, L. (2016). Pressure-Assisted Thermal Sterilization Validation. In: Balasubramaniam, V. M., Barbosa-Cánovas, G. V., and Lelieveld, H. L. M., eds. *High Pressure Processing of Food*. Food Engineering Series. New York, NY: Springer New York, pp.687–716. Available from: http://link.springer.com/10.1007/978-1-4939-3234-4_29 [accessed 23 November 2021].

Sudagidan, M. and Yemenicioğlu, A. (2012). Effects of Nisin and Lysozyme on Growth Inhibition and Biofilm Formation Capacity of Staphylococcus aureus Strains Isolated from Raw Milk and Cheese Samples. *Journal of Food Protection*, 75(9), pp.1627–1633.

Sun, J., Chang, C., Su, Y., Gu, L., Yang, Y. and Li, J. (2022). Impact of saccharides on the foam properties of egg white: Correlation between rheological, interfacial properties and foam properties. *Food Hydrocolloids*, 122, p.107088.

Sunwoo, H.H. and Gujral, N. (2014). Chemical Composition of Egg and Egg Products | SpringerLink. , 2014, pp.1–27.

Sweis, I.E. and Cressey, B.C. (2018). Potential role of the common food additive manufactured citric acid in eliciting significant inflammatory reactions contributing to serious disease states: A series of four case reports. *Toxicology Reports*, 5, pp.808–812.

Takeuchi, J. and Nagashima, T. (2010). Chemical and Physical Characterization of Dromaius novaehollandiae (Emu) Eggs. *Food Science and Technology Research*, 16(2), pp.149–156.

Tam, J., Wang, S., Wong, K. and Tan, W. (2015). Antimicrobial Peptides from Plants. *Pharmaceuticals*, 8(4), pp.711–757.

Tang, Q., Munro, P.A. and McCarthy, O.J. (1993). Rheology of whey protein concentrate solutions as a function of concentration, temperature, pH and salt concentration. *Journal of Dairy Research*, 60(3), pp.349–361.

Techer, C., Daoud, A., Jan, S., Gautier, M. and Baron, F. (2014). Microbial investigation of industrial liquid egg white. , juillet 2014.

Techer, C., Baron, F. and Jan, S. (2013). Microbial spoilage of eggs and egg products. , 69, p.6.

Tokuşoğlu, Ö. (2018). The Improving Quality and Shelf Life of Table Eggs. , 2018, p.30.

Tolik, D., Poawska, E., Charuta, A., Nowaczewski, S. and Cooper, R. (2014). Characteristics of Egg Parts, Chemical Composition and Nutritive Value of Japanese Quail Eggs – a Review. *Folia Biologica*, 62(4), pp.287–292.

Tóth, Németh, Cs., Pintér, R., Ayari, E., Noori, K. and Friedrich, L. (2020). Effects of High Hydrostatic Pressure's Holding Time on Protein Structure of Liquid Egg Products. *Hungarian Agricultural Engineering*, 2020, pp.73–79.

Tóth, A., Németh, C., Zeke, I., Barkói, A., Hidas, K. and Friedrich, L. (2020). Effects of combined HHP and heat treatment on viscosity attributes and microbiological condition of liquid egg yolk. *Progress in Agricultural Engineering Sciences*, 16(S1), pp.67–74.

Tóth, A., Németh, C., Palotás, P., Surányi, J., Zeke, I., Csehi, B., Castillo, L.A., Friedrich, L. and Balla, C. (2017). HHP treatment of liquid egg at 200-350 MPa. *Journal of Physics: Conference Series*, 950, p.042008.

Toyosaki, T. (2010). Rheological properties, oxidative stability, and tocopherol content during storage of fried dough made with Silky fowl egg: Comparison with hen egg. *Poultry science*, 89, pp.1009–14.

Uysal, R.S., Boyacı, İ.H., Soykut, E.A. and Ertaş, N. (2017). Effects of heat treatment parameters on liquid whole egg proteins. *Food Chemistry*, 216, pp.201–208.

Valenti, P., Visca, P., Antonini, G. and Orsi, N. (1986). Interaction between lactoferrin and ovotransferrin and *Candida* cells. *FEMS Microbiology Letters*, 33(2–3), pp.271–275.

Van der Plancken, I., Van Loey, A. and Hendrickx, M.E. (2006). Effect of heat-treatment on the physico-chemical properties of egg white proteins: A kinetic study. *Journal of Food Engineering*, 75(3), pp.316–326.

Varnam, A., Sutherland, J.M. and Sutherland, J.P. (1995). *Meat and Meat Products: Technology, Chemistry and Microbiology*. Springer Science & Business Media.

Varvara, M., Bozzo, G., Celano, G., Disanto, C., Pagliarone, C.N. and Celano, G.V. (2016). The Use of Ascorbic Acid as a Food Additive: Technical-Legal Issues. *Italian Journal of Food Safety*, 5(1), p.4313.

Wang, C.-Y., Hsu, C.-P., Huang, H.-W. and Yang, B.B. (2013). The relationship between inactivation and morphological damage of Salmonella enterica treated by high hydrostatic pressure. *Food Research International*, 54(2), pp.1482–1487.

Wang, G. and Wang, T. (2009). Improving Foaming Properties of Yolk-Contaminated Egg Albumen by Basic Soy Protein. *Journal of Food Science*, 74(8), pp.C581–C587.

Wee, Y.-J., Kim, J.-N. and Ryu, H.-W. (2006). Biotechnological Production of Lactic Acid and Its Recent Applications. , 2006, p.10.

Wilderjans, E., Lagrain, B., Brijs, K. and Delcour, J.A. (2010). Impact of Potassium Bromate and Potassium Iodate in a Pound Cake System. *Journal of Agricultural and Food Chemistry*, 58(10), pp.6465–6471.

Wouters, A.G.B., Rombouts, I., Fierens, E., Brijs, K., Blecker, C., Delcour, J.A. and Murray, B.S. (2018). Foaming and air-water interfacial characteristics of solutions containing both gluten hydrolysate and egg white protein. *Food Hydrocolloids*, 77, pp.176–186.

Wu, J. (2014). Eggs and Egg Products Processing. In: Clark, S., Jung, S., and Lamsal, B., eds. *Food Processing*. Chichester, UK: John Wiley &amp Sons, Ltd, pp.437–455. Available from: https://onlinelibrary.wiley.com/doi/10.1002/9781118846315.ch19 [accessed 16 November 2021].

Wu, Q., Patočka, J. and Kuča, K. (2018). Insect Antimicrobial Peptides, a Mini Review. *Toxins*, 10(11), p.461.

Xu, L., Zhao, Y., Xu, M., Yao, Y., Nie, X., Du, H. and Tu, Y. (2018). Changes in aggregation behavior of raw and cooked salted egg yolks during pickling. *Food Hydrocolloids*, 80, pp.68–77.

Xu, L., Zhao, Y., Xu, M., Yao, Y., Wu, N., Du, H. and Tu, Y. (2019). Changes in physico-chemical properties, microstructure, protein structures and intermolecular force of egg yolk, plasma and granule gels during salting. *Food Chemistry*, 275, pp.600–609.

Yang, Z.X., Wei, S.Y., Dai, X.Q., Wang, M. and Zhang, T. (1995). The Electronic Properties of a Finite sp-Hybrid chain. *Communications in Theoretical Physics*, 23(4), pp.405–410.

Zaheer, K. (2015). An Updated Review on Chicken Eggs: Production, Consumption, Management Aspects and Nutritional Benefits to Human Health. *Food and Nutrition Sciences*, 06(13), pp.1208–1220.

Zhang, L. and Gallo, R.L. (2016). Antimicrobial peptides. *Current Biology*, 26(1), pp.R14–R19.

Zhao, M., Cai, H., Liu, M., Deng, L., Li, Y., Zhang, H. and Feng, F. (2019). Dietary glycerol monolaurate supplementation for the modification of functional properties of egg white protein. *Journal of the Science of Food and Agriculture*, 99(8), pp.3852–3859.

Zhou, M., Khen, K., Wang, T., Hu, Q., Xue, J. and Luo, Y. (2018). Chemical crosslinking improves the gastrointestinal stability and enhances nutrient delivery potentials of egg yolk LDL/polysaccharide nanogels. *Food Chemistry*, 239, pp.840–847.

Internet sources:

1- http://huto.etk.szie.hu/bemutatkozas/infrastuktura/technologiai-berendezesek November 2021

Annex 2

Table summarize the applied measurement for each liquid egg product

Preliminary study		LWE		LEW	LEY	
		1xPas and 2xPas	CA and CaS	CA and CaS	Pas	CA and CaS
Heat treatment	pH	+	+	+	+	+
	Color	+	+	+	+	+
	Microbiology	+	+	+	+	+
	Foamability			+		
	Emulsion				+	
	DSC				+	

LWE with acids		Citric Acid	Lactic Acid	Acetic Acid	Ascorbic Acid	Citric Acid with storage	Lactic Acid with storage
Heat treatment	pH	+	+	+	+	+	+
	Color	+	+	+	+	+	+
	Viscosity	+	+	+	+	+	+
	Sensory analysis	+	+	+	+	+	+
	DSC	+					
	Microbiology					+	+

Nisin and Lysozyme		LWE	LEW	LEY
HHP Treatment	pH	+	+	+
	Color	+	+	+
	Microbiology	+	+	+
	Viscosity	+	+	+
	DSC	+	+	+
	Foamability		+	

Annex 3

Effect of HHP pressure, nisin and lysozyme on evolution physicochemical properties and color parameters of LWE during the storage

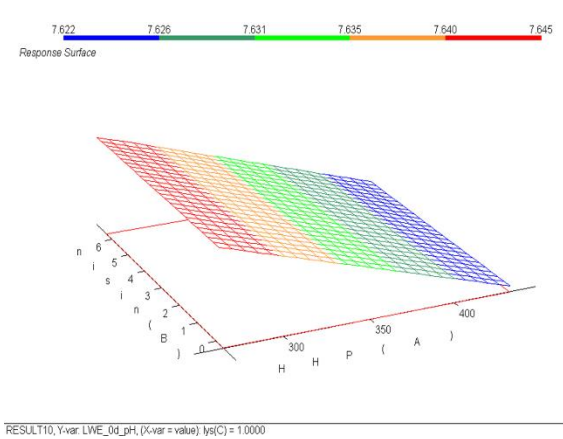
Sample	Storage days		
	0	14	21
pH			
226:3-1	7.61±0.03	7.23±0.03	6.28±0.02
435:3-1	7.70±0.04	7.46±0.01	7.45±0.01
350:0-1	7.61±0.06	7.55±0.01	7.37±0.02
350:6.35-1	7.62±0.01	7.54±0.03	7.51±0.02
350:3-0.16	7.61±0.02	7.50±0.02	7.47±0.02
350:3-1.84	7.65±0.02	7.50±0.02	7.48±0.01
300:1-0.5	7.64±0.03	7.50±0.05	6.03±0.02
400:1-0.5	7.62±0.01	7.54±0.01	7.48±0.00
300:5-0.5	7.66±0.02	7.57±0.02	6.91±0.05
400:5-0.5	7.61±0.03	7.46±0.05	7.53±0.01
300:1-1.5	7.71±0.03	7.58±0.01	6.12±0.04
400:1-1.5	7.61±0.02	7.55±0.01	7.44±0.03
300:5-1.5	7.70±0.01	7.57±0.03	6.73±0.04
400:5-1.5	7.61±0.01	7.46±0.03	7.47±0.00
350:3-1	7.60±0.02	7.49±0.02	7.26±0.27
*L**			
226:3-1	69.42±0.10	68.27±0.07	70.61±0.13
435:3-1	69.49±0.16	67.58±0.69	67.15±0.96
350:0-1	68.78±1.47	66.83±1.63	67.68±0.19
350:6.35-1	67.09±2.58	66.72±0.42	66.46±0.20
350:3-0.16	68.63±0.31	67.11±0.38	65.88±0.79
350:3-1.84	67.74±0.80	67.11±0.16	66.17±0.73
300:1-0.5	69.44±0.15	69.00±0.72	72.54±0.15
400:1-0.5	68.56±0.14	66.60±0.81	66.47±0.71
300:5-0.5	71.16±0.25	70.30±0.48	69.64±0.65

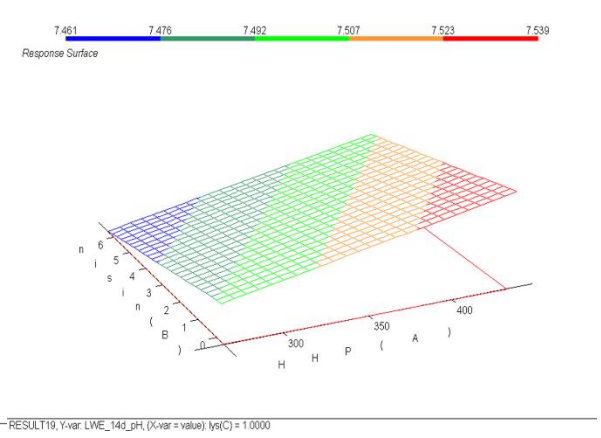
400:5-0.5	70.38±0.11	66.56±1.50	66.86±0.15
300:1-1.5	69.90±0.16	67.79±1.11	69.80±1.52
400:1-1.5	67.71±1.32	66.33±0.35	66.70±0.43
300:5-1.5	70.75±0.24	69.89±0.93	70.25±1.51
400:5-1.5	70.15±1.04	67.69±0.27	66.98±0.20
350:3-1	69.85±0.44	67.80±0.41	68.27±1.57
*a**			
226:3-1	13.62±0.05	14.24±0.05	12.64±0.07
435:3-1	12.86±0.07	12.41±0.16	12.52±0.34
350:0-1	12.70±0.15	12.81±0.45	12.89±0.03
350:6.35-1	13.07±0.14	13.04±0.30	13.38±0.36
350:3-0.16	12.72±0.20	13.06±0.15	13.76±0.27
350:3-1.84	12.72±0.30	12.99±0.08	13.45±0.13
300:1-0.5	12.63±0.13	13.35±0.17	10.22±0.26
400:1-0.5	12.72±0.03	13.15±0.41	13.00±0.32
300:5-0.5	12.59±0.05	13.15±0.06	13.41±0.15
400:5-0.5	12.24±0.11	12.84±0.18	12.73±0.06
300:1-1.5	12.54±0.01	13.53±0.35	11.58±1.24
400:1-1.5	12.64±0.14	13.33±0.16	12.76±0.22
300:5-1.5	12.29±0.05	13.26±0.49	13.57±0.26
400:5-1.5	12.50±0.07	12.72±0.04	12.50±0.04
350:3-1	12.83±0.20	12.91±0.35	11.23±3.12
*b**			
226:3-1	31.90±0.14	31.08±0.14	30.50±0.20
435:3-1	25.81±0.12	25.33±0.64	25.49±0.95
350:0-1	25.05±0.36	27.45±0.98	26.55±0.36
350:6.35-1	25.93±1.43	28.56±0.96	28.16±0.78
350:3-0.16	26.28±0.74	27.47±0.40	28.39±0.61
350:3-1.84	26.45±0.92	27.18±0.10	28.49±0.70
300:1-0.5	27.23±0.67	28.26±0.26	29.72±0.42
400:1-0.5	26.19±0.13	26.98±1.48	25.52±1.86

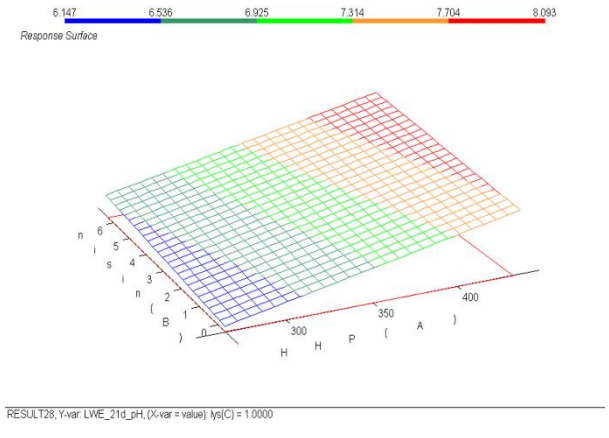
300:5-0.5	26.89±0.12	27.81±0.41	28.15±0.18
400:5-0.5	25.19±0.40	25.69±1.15	26.24±0.19
300:1-1.5	27.62±0.05	29.05±1.04	30.30±1.40
400:1-1.5	26.06±0.35	27.38±0.68	26.20±0.98
300:5-1.5	26.57±0.36	28.03±1.26	29.25±0.28
400:5-1.5	24.45±0.11	26.15±1.18	25.61±0.09
350:3-1	25.61±0.44	26.95±0.81	28.80±2.38
*C**			
226:3-1	34.69±0.41	34.19±0.14	33.02±0.17
435:3-1	28.84±0.12	28.21±0.63	28.40±1.00
350:0-1	28.09±0.37	30.30±0.79	29.52±0.34
350:6.35-1	29.04±1.34	31.39±0.99	31.17±0.86
350:3-0.16	29.20±0.75	30.42±0.42	31.55±0.64
350:3-1.84	29.35±0.95	30.12±0.06	31.51±0.68
300:1-0.5	30.02±0.66	31.25±0.29	31.43±0.33
400:1-0.5	29.11±0.12	30.02±1.48	28.65±1.80
300:5-0.5	29.69±0.13	30.76±0.40	31.18±0.22
400:5-0.5	28.01±1.31	28.72±1.11	29.17±0.17
300:1-1.5	30.33±0.05	32.05±1.09	32.46±1.28
400:1-1.5	28.97±0.35	30.45±0.68	29.14±0.97
300:5-1.5	29.28±0.34	31.01±1.34	32.24±0.30
400:5-1.5	27.46±0.11	29.09±1.08	28.49±0.09
350:3-1	28.64±0.47	29.88±0.82	31.11±1.43
*h**			
226:3-1	1.17±0.00	1.14±0.00	1.18±0.00
435:3-1	1.11±0.00	1.12±0.01	1.11±0.00
350:0-1	1.10±0.00	1.13±0.02	1.12±0.00
350:6.35-1	1.10±0.02	1.14±0.00	1.13±0.00
350:3-0.16	1.12±0.01	1.13±0.00	1.12±0.01
350:3-1.84	1.12±0.01	1.12±0.00	1.13±0.01
300:1-0.5	1.14±0.01	1.13±0.00	1.24±0.01

400:1-0.5	1.12±0.00	1.12±0.01	1.10±0.02
300:5-0.5	1.13±0.00	1.13±0.00	1.13±0.00
400:5-0.5	1.12±0.02	1.11±0.01	1.12±0.01
300:1-1.5	1.14±0.00	1.13±0.00	1.21±0.04
400:1-1.5	1.12±0.00	1.12±0.01	1.12±0.01
300:5-1.5	1.14±0.00	1.13±0.00	1.14±0.01
400:5-1.5	1.10±0.00	1.12±0.02	1.12±0.00
350:3-1	1.11±0.00	1.12±0.01	1.20±0.12

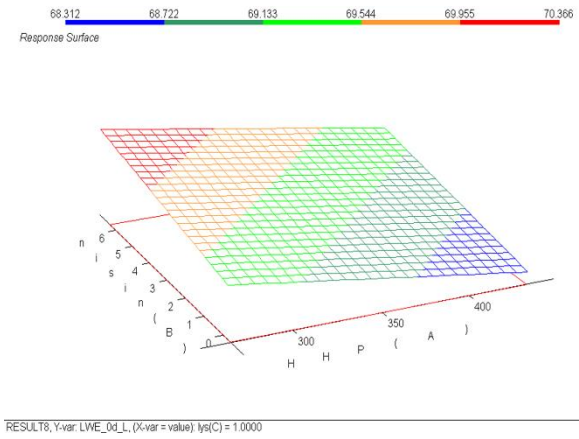
Response surface for pH values of HHP treated liquid whole egg with nisin and lysozyme during storage

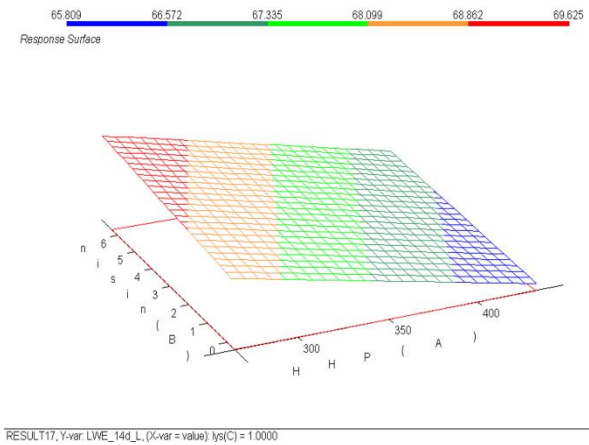


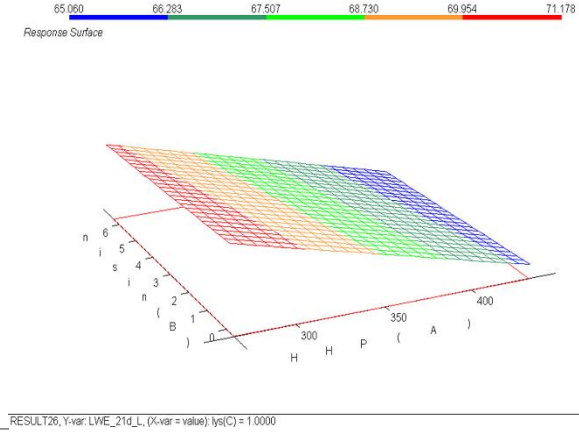




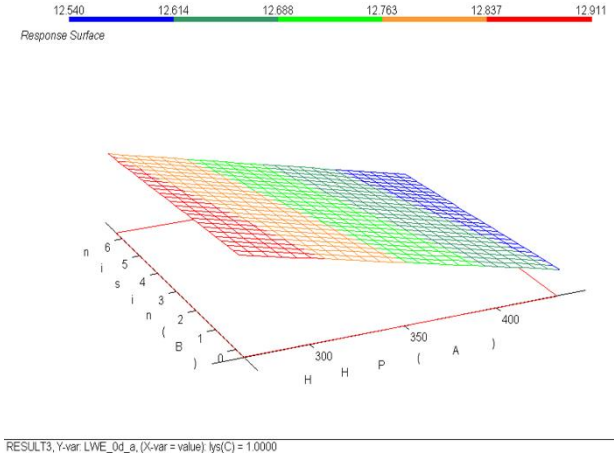
Response surface for L* of HHP treated liquid whole egg with nisin and lysozyme during storage

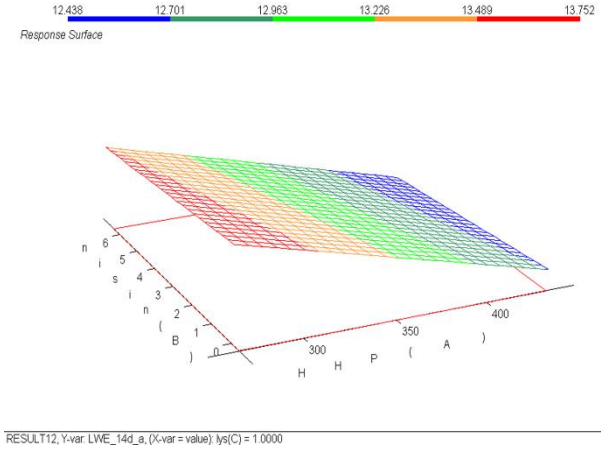


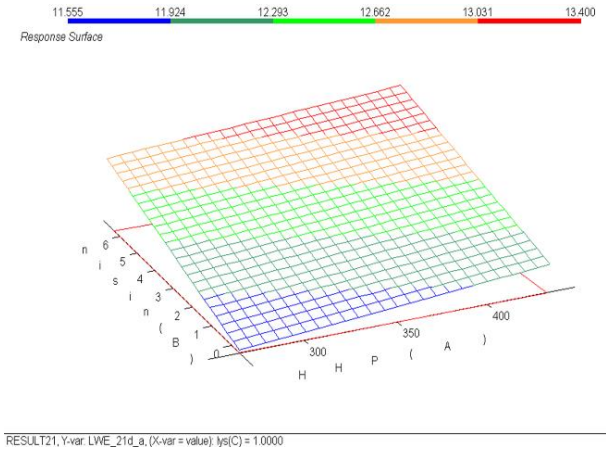




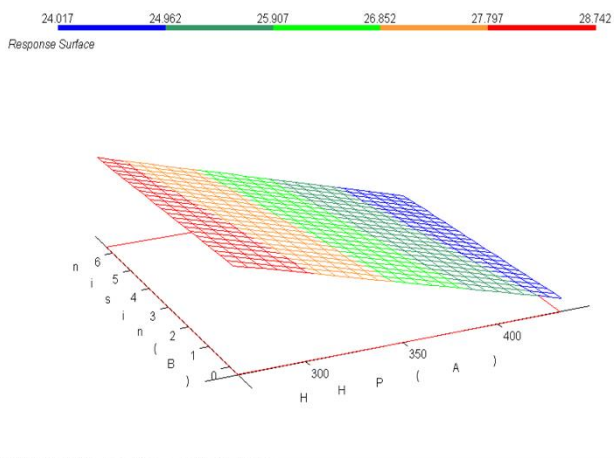
Response surface for a* of HHP treated liquid whole egg with nisin and lysozyme during storage

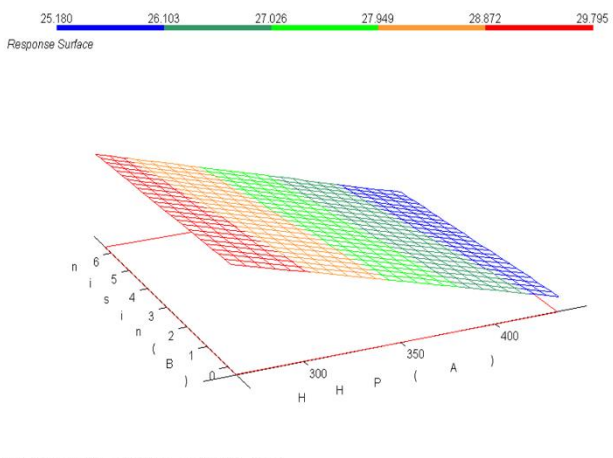


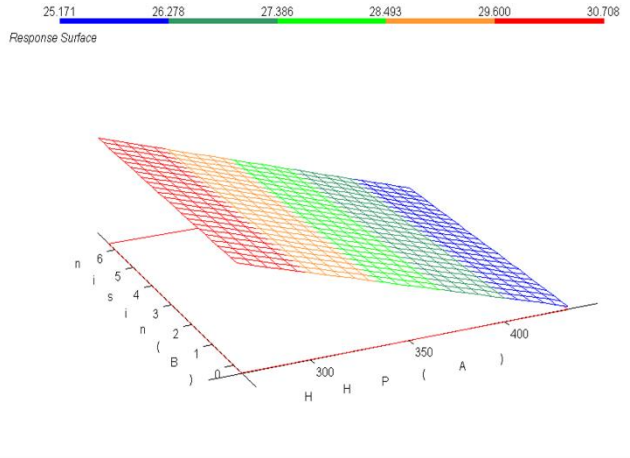




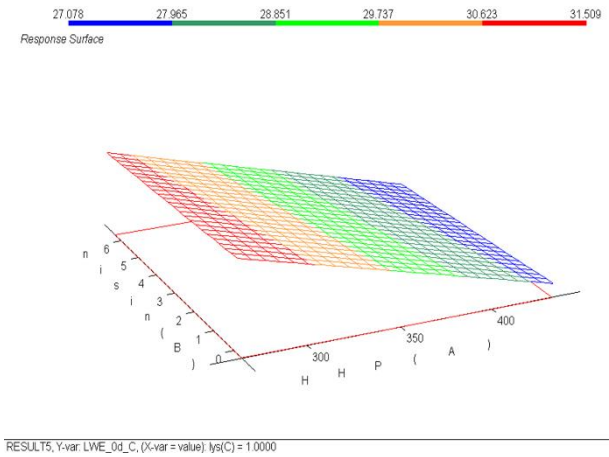
Response surface for b* of HHP treated liquid whole egg with nisin and lysozyme during storage

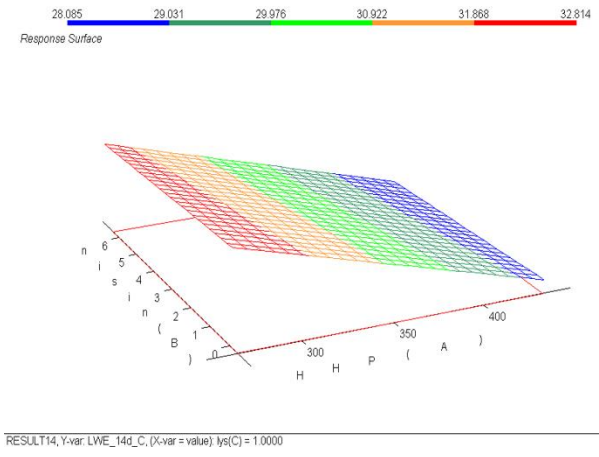


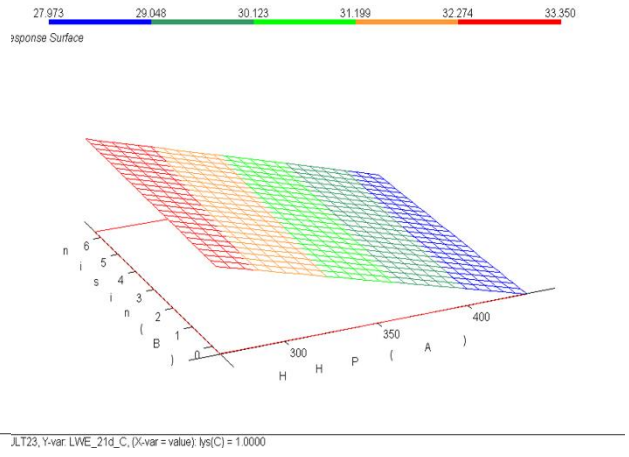




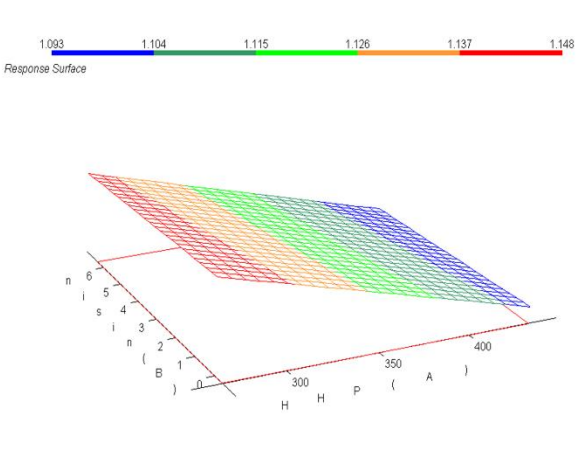
Response surface for C* of HHP treated liquid whole egg with nisin and lysozyme during storage

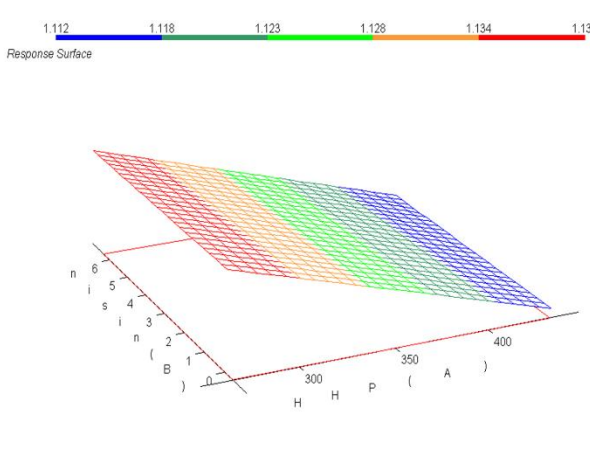


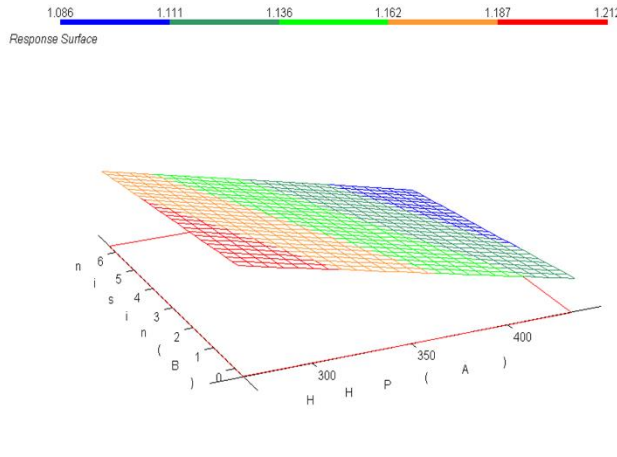




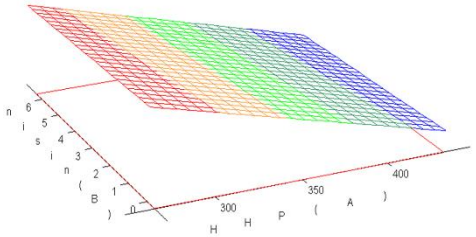
Response surface for h* of HHP treated liquid whole egg with nisin and lysozyme during storage

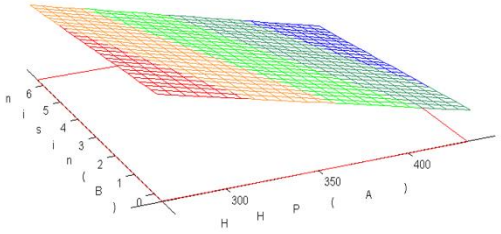


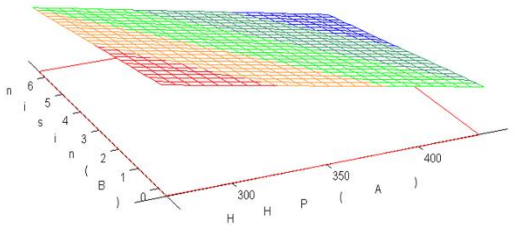




Response surface for mesophilic total plate count of HHP treated liquid whole egg with nisin and lysozyme during storage







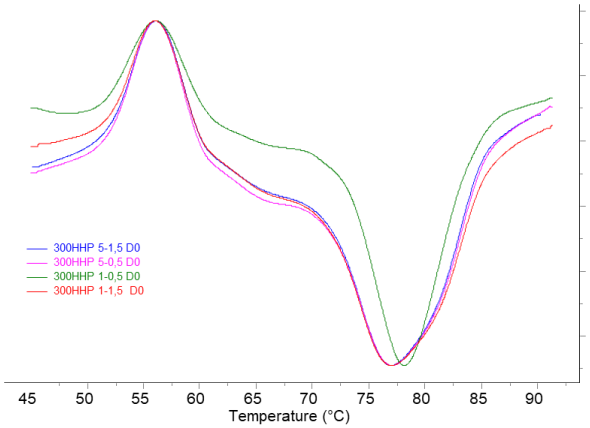
RESULT9, Y-var: LWE_0d_logCFU, (X-var = value): lys(C) = 1.0000

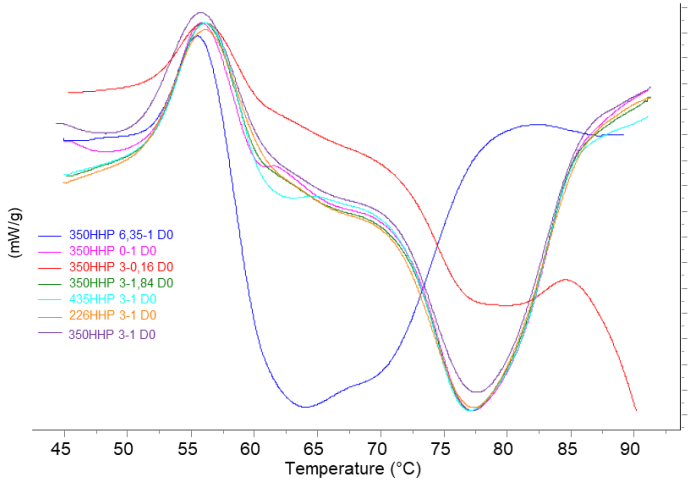
RESULT18, Y-var: LWE_14d_logCFU, (X-var = value): lys(C) = 1.0000

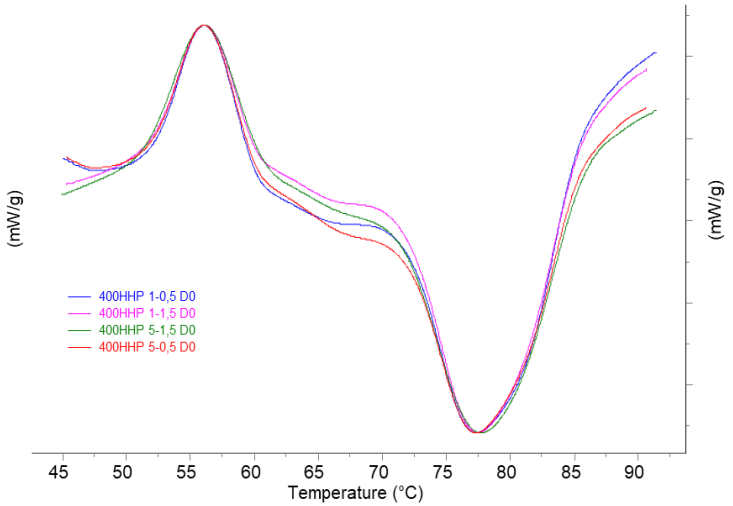
RESULT27, Y-var: LWE_21d_logCFU, (X-var = value): lys(C) = 1.0000

Thermograms of HHP treated liquid whole egg with nisin and lysozyme during storage

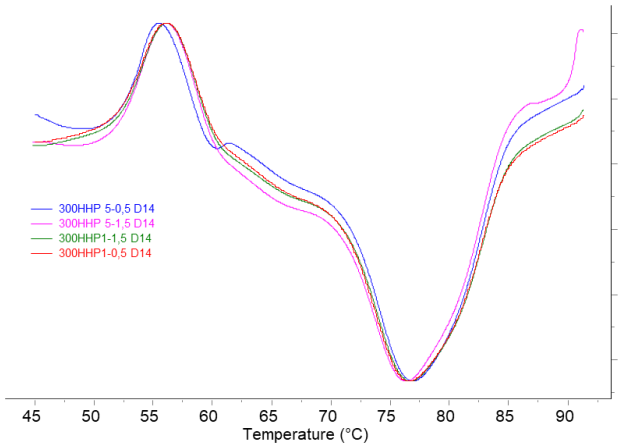
Day 0

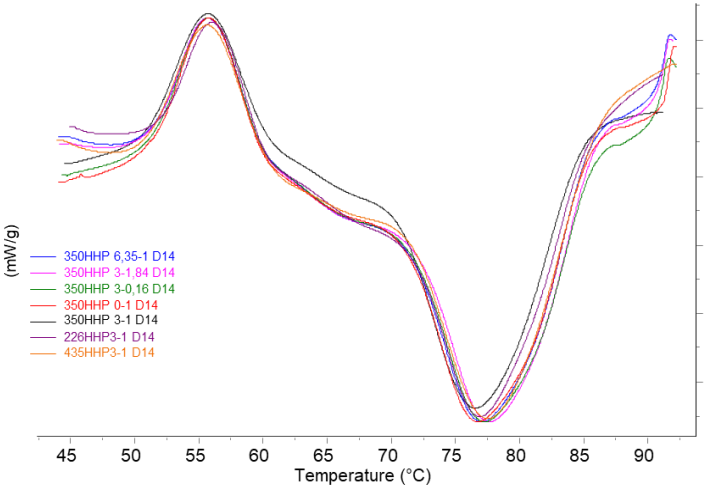


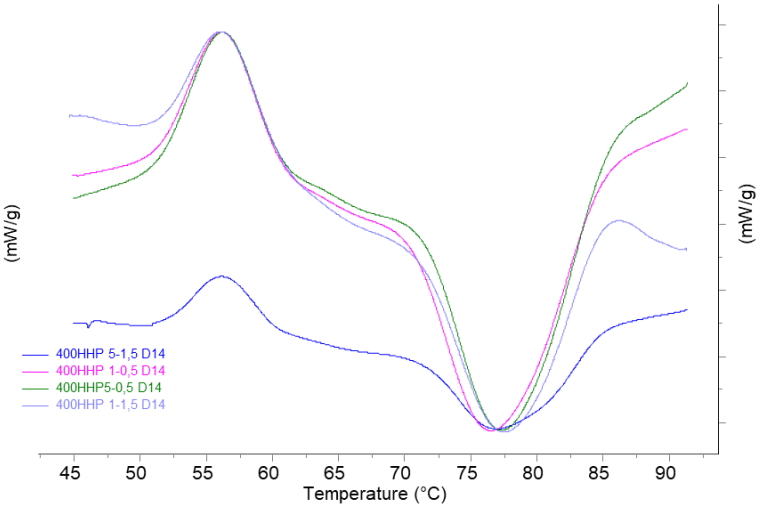




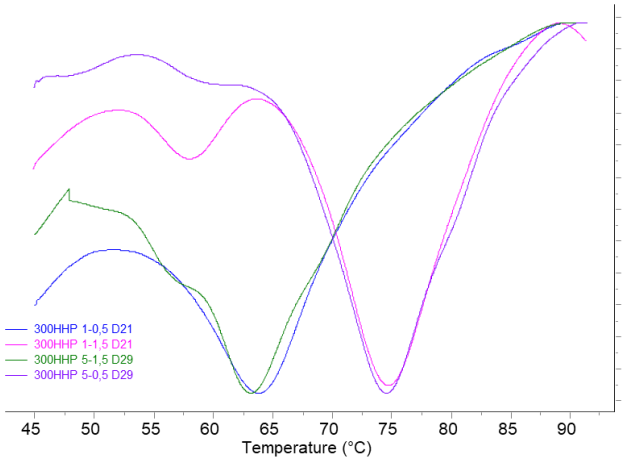
Day 14

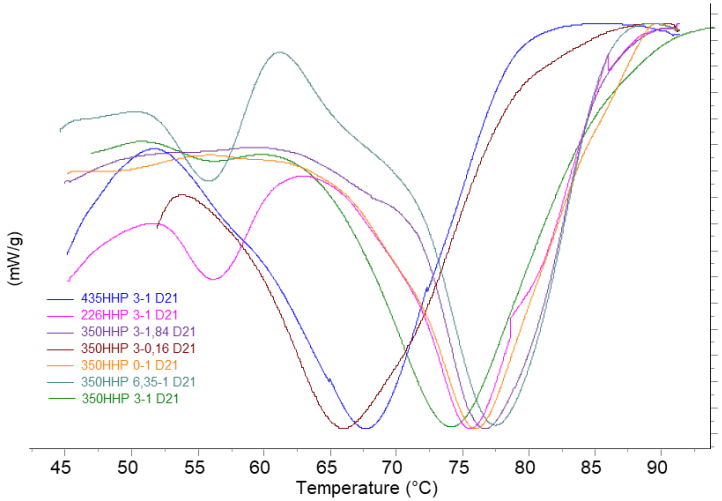

300HHP 5-0,5 D14
300HHP 5-1,5 D14
300HHP1-1,5 D14
300HHP1-0,5 D14
(mW/g)
45 50 55 60 65 70 75 80 85 90
Temperature (°C)

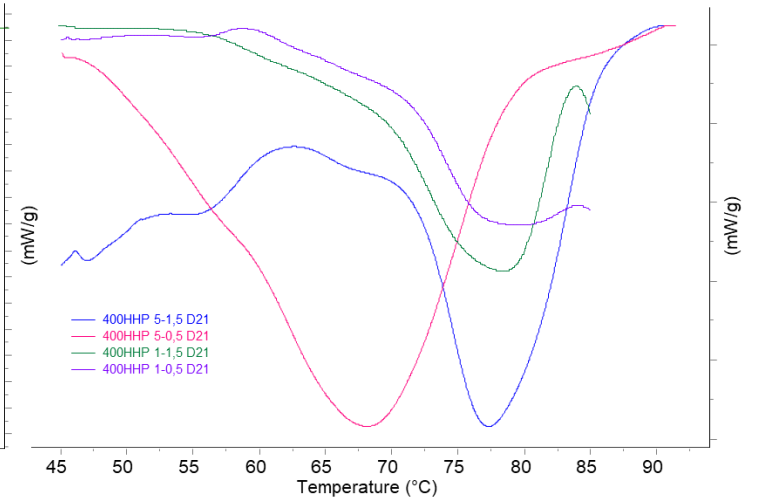

350HHP 6,35-1 D14
350HHP 3-1,84 D14
350HHP 3-0,16 D14
350HHP 0-1 D14
350HHP 3-1 D14
226HHP3-1 D14
435HHP3-1 D14
(mW/g)
45 50 55 60 65 70 75 80 85 90
Temperature (°C)


400HHP 5-1,5 D14
400HHP 1-0,5 D14
400HHP5-0,5 D14
400HHP 1-1,5 D14
(mW/g)
45 50 55 60 65 70 75 80 85 90
Temperature (°C)

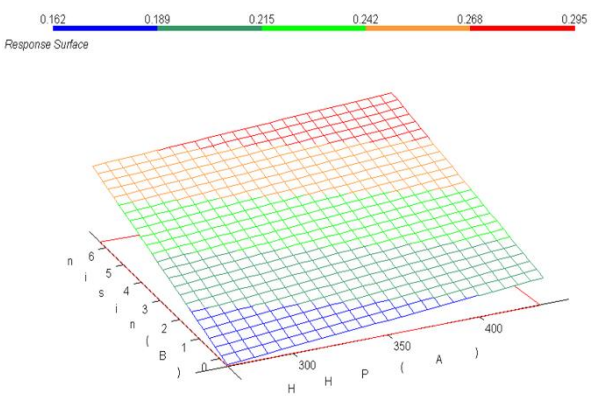
Day 21

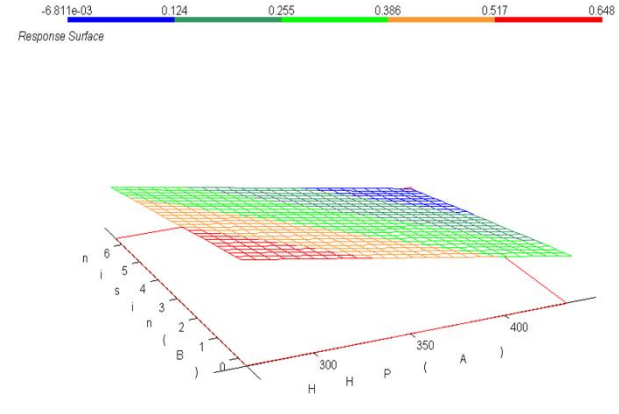

300HHP 1-0,5 D21
300HHP 1-1,5 D21
300HHP 5-1,5 D29
300HHP 5-0,5 D29
(mW/g)
45 50 55 60 65 70 75 80 85 90
Temperature (°C)

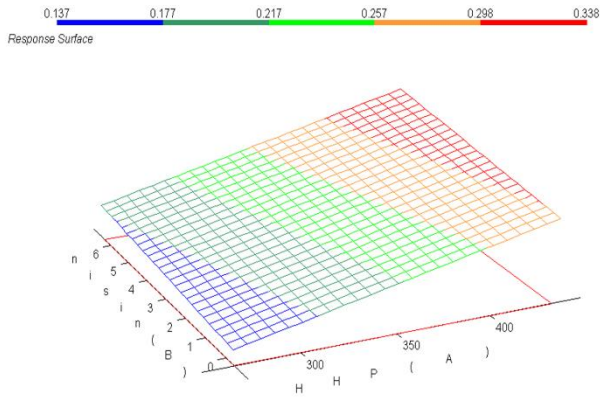

435HHP 3-1 D21
226HHP 3-1 D21
350HHP 3-1,84 D21
350HHP 3-0,16 D21
350HHP 0-1 D21
350HHP 6,35-1 D21
350HHP 3-1 D21
(mW/g)
45 50 55 60 65 70 75 80 85 90
Temperature (°C)


400HHP 5-1,5 D21
400HHP 5-0,5 D21
400HHP 1-1,5 D21
400HHP 1-0,5 D21
(mW/g)
45 50 55 60 65 70 75 80 85 90
Temperature (°C)

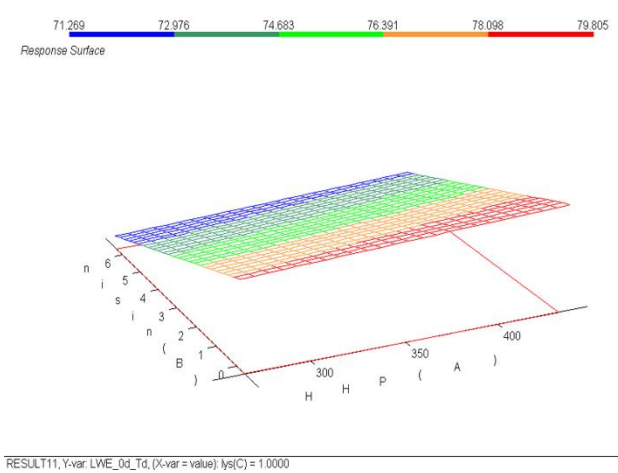
Response surface for denaturation enthalpy of HHP treated liquid whole egg with nisin and lysozyme during storage

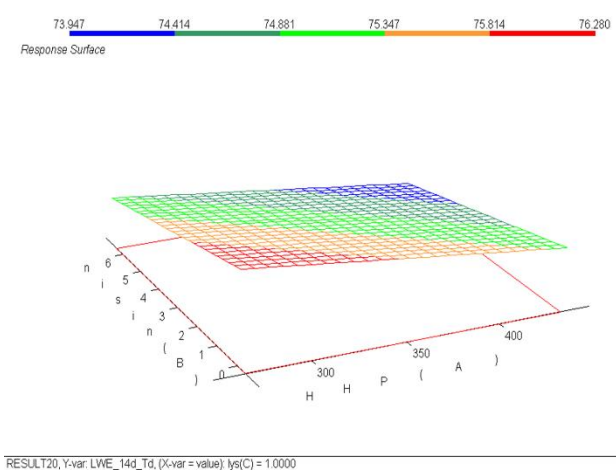


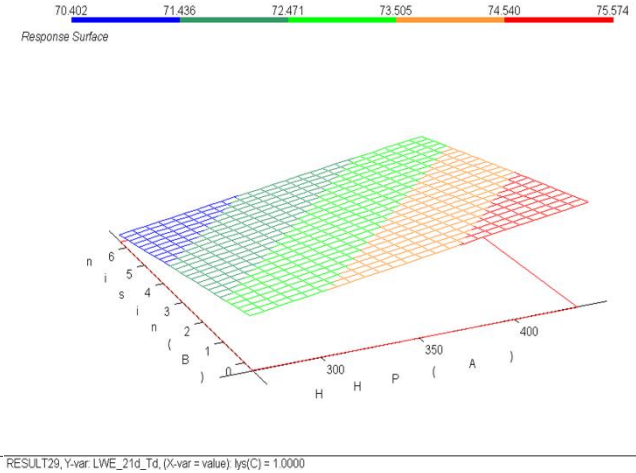




Response surface for denaturation temperature of HHP treated liquid whole egg with nisin and lysozyme during storage

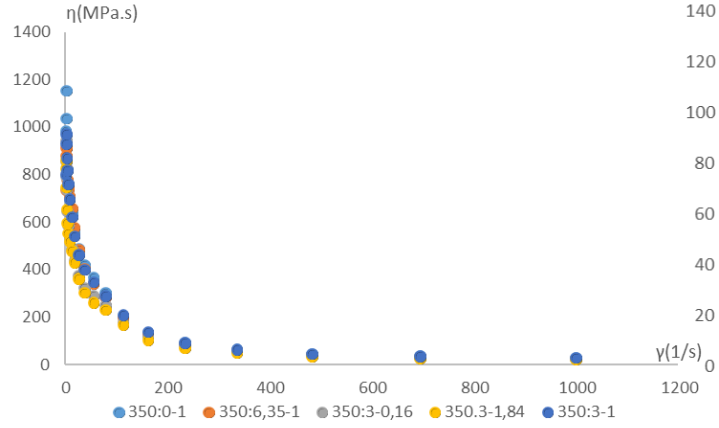


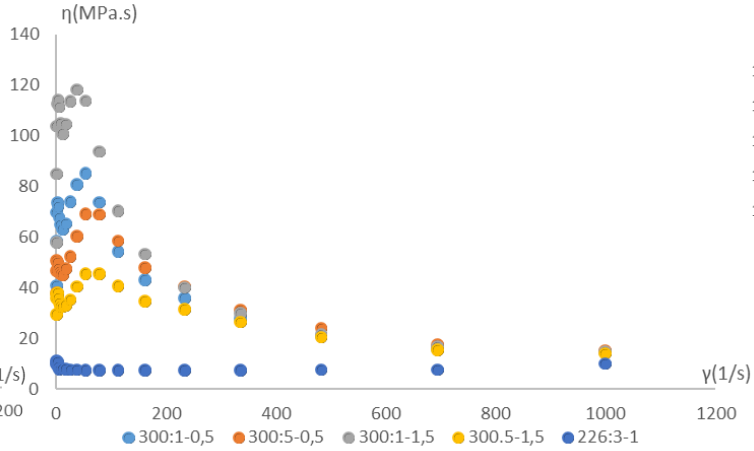


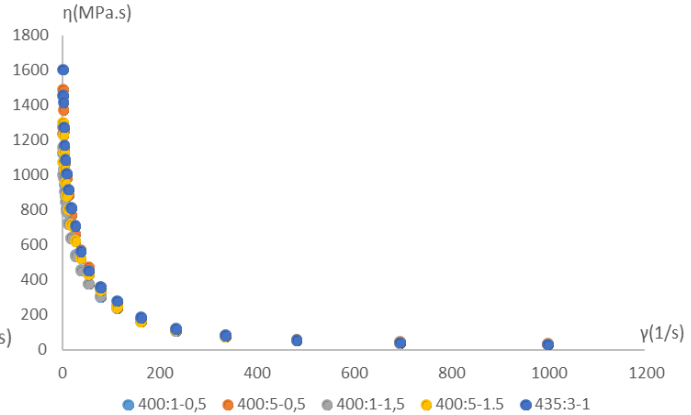


Apparent viscosity of HHP treated liquid whole egg with nisin and lysozyme during storage

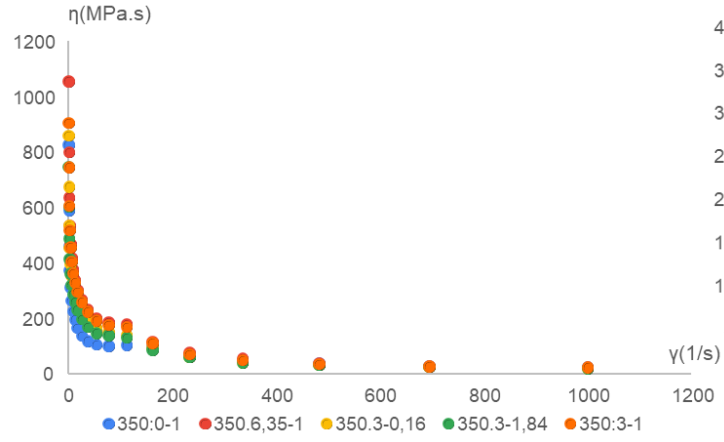
Day 0

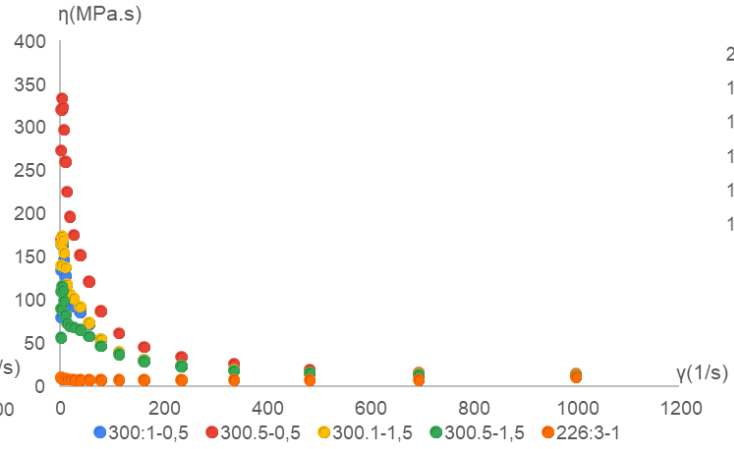


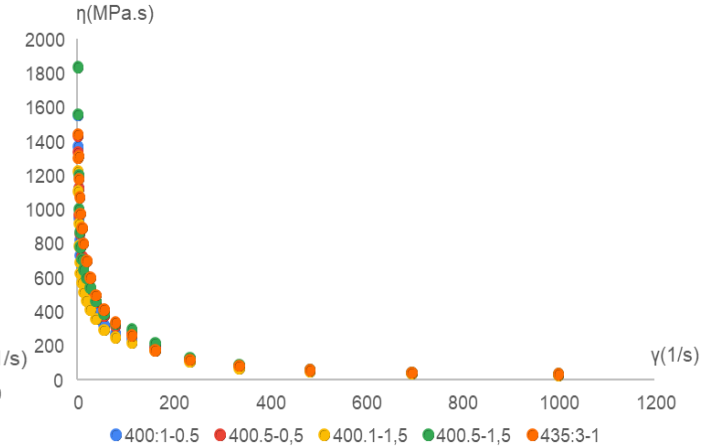




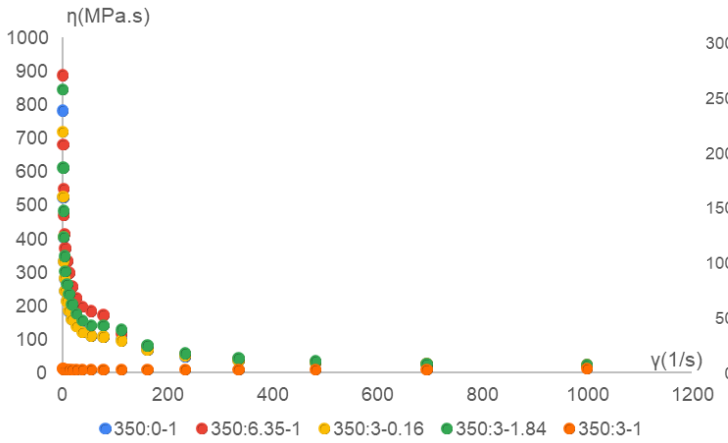
Day 14

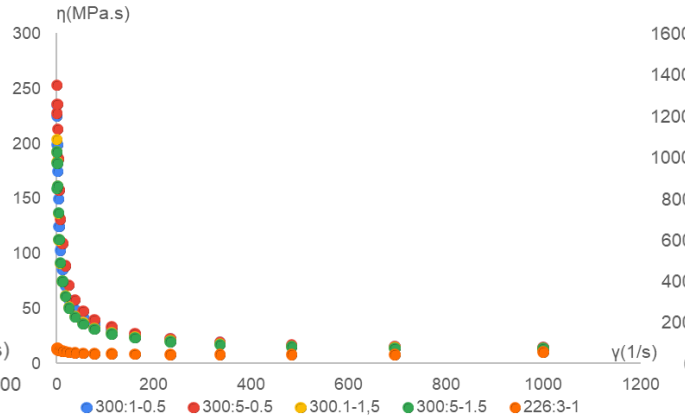


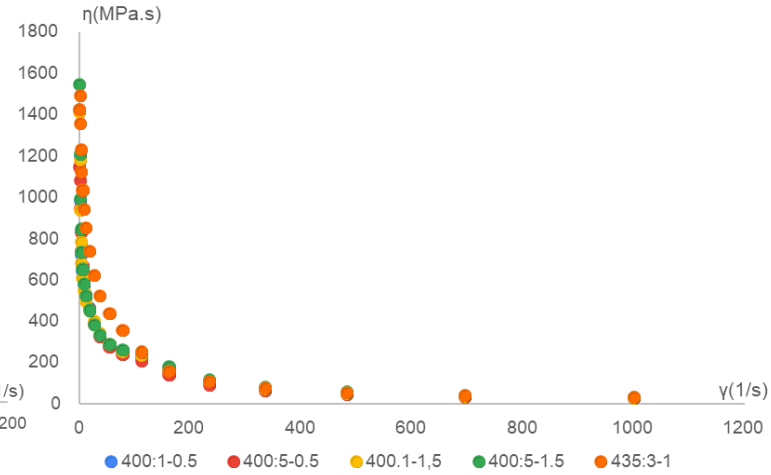




Day 21


η(MPa.s)
1000
900
800
700
600
500
400
300
200
100
0
0
200
400
600
800
1000
1200
γ(1/s)
350:0-1
350:6.35-1
350:3-0.16
350:3-1.84
350:3-1


η(MPa.s)
300
250
200
150
100
50
0
0
200
400
600
800
1000
1200
γ(1/s)
300:1-0.5
300:5-0.5
300.1-1,5
300:5-1.5
226:3-1


η(MPa.s)
1800
1600
1400
1200
1000
800
600
400
200
0
0
200
400
600
800
1000
1200
γ(1/s)
400:1-0.5
400:5-0.5
400.1-1,5
400:5-1.5
435:3-1

Annex 4

Effect of HHP pressure, nisin and lysozyme on evolution physicochemical proprieties and color parameters of liquid egg white during the storage

Sample	Storage days		
	0	14	21
pH			
226:3-1	9.08±0.04	8.92±0.03	8.57±0.05
435:3-1	9.10±0.01	8.92±0.01	8.59±0.41
350:0-1	9.09±0.01	9.09±0.01	8.97±0.02
350:6.35-1	9.09±0.04	8.95±0.06	8.60±0.31
350:3-0.16	9.06±0.02	8.59±0.41	8.14±0.12
350:3-1.84	9.14±0.02	9.08±0.02	8.97±0.02
300:1-0.5	9.13±0.01	9.01±0.02	8.97±0.00
400:1-0.5	9.18±0.04	9.12±0.01	9.23±0.08
300:5-0.5	9.12±0.02	8.97±0.02	9.22±0.02
400:5-0.5	9.14±0.03	9.15±0.04	9.11±0.01
300:1-1.5	9.13±0.02	9.03±0.02	8.80±0.01
400:1-1.5	9.16±0.02	9.14±0.02	9.14±0.05
300:5-1.5	9.17±0.01	9.01±0.05	8.82±0.10
400:5-1.5	9.24±0.04	9.25±0.03	8.85±0.04
350:3-1	9.08±0.01	8.99±0.03	8.83±0.01
*L**			
226:3-1	51.58±4.66	53.96±3.58	56.09±4.50
435:3-1	57.46±2.11	53.61±0.68	60.63±0.64
350:0-1	53.84±5.64	63.08±3.91	50.89±1.20
350:6.35-1	52.26±2.97	43.99±3.77	50.98±0.22
350:3-0.16	51.08±1.07	46.45±1.60	51.03±0.05
350:3-1.84	51.10±2.14	57.50±1.53	48.98±4.37
300:1-0.5	58.21±4.52	48.06±2.80	50.11±1.01
400:1-0.5	46.17±1.65	45.48±1.76	50.35±4.45
300:5-0.5	57.16±1.66	48.07±1.28	50.43±0.72

400:5-0.5	49.89±1.94	44.65±0.71	49.41±1.25
300:1-1.5	62.42±6.51	43.50±4.19	52.47±3.13
400:1-1.5	50.52±0.86	45.90±1.11	47.38±1.11
300:5-1.5	56.31±4.36	47.56±0.96	55.89±9.35
400:5-1.5	45.62±1.62	44.40±0.90	55.80±1.96
350:3-1	46.41±1.65	47.38±2.03	56.96±0.74
*a**			
226:3-1	-2.04±0.42	-0.93±0.48	-1.66±0.71
435:3-1	-1.28±0.25	-0.96±0.08	-1.82±0.14
350:0-1	-1.40±0.30	-1.28±0.45	-0.63±0.08
350:6.35-1	-1.27±0.44	-0.64±0.14	-0.65±0.02
350:3-0.16	-1.19±0.08	-0.68±0.03	-0.65±0.00
350:3-1.84	-1.19±0.03	-0.87±0.45	-0.38±0.45
300:1-0.5	-2.35±0.17	-0.96±0.07	-0.49±0.10
400:1-0.5	-1.73±0.47	-1.92±0.24	-1.57±0.06
300:5-0.5	-2.08±0.12	-0.60±0.30	-1.58±0.01
400:5-0.5	-1.35±0.36	-2.19±0.07	-1.44±0.44
300:1-1.5	-2.31±0.24	-0.75±0.47	-1.01±0.21
400:1-1.5	-1.22±0.14	-1.94±0.18	-1.60±0.12
300:5-1.5	-2.10±0.34	-1.17±0.29	-1.25±0.70
400:5-1.5	-1.98±0.43	-2.05±0.11	-1.35±0.11
350:3-1	-0.99±0.07	-0.41±0.03	-1.39±0.03
*b**			
226:3-1	15.02±0.98	12.38±2.30	13.50±3.65
435:3-1	14.23±0.86	12.46±0.37	14.37±0.76
350:0-1	15.02±1.01	15.31±0.49	11.28±0.74
350:6.35-1	14.57±2.15	7.41±2.16	11.14±0.14
350:3-0.16	14.05±2.44	8.50±0.70	11.12±0.02
350:3-1.84	14.59±1.53	13.42±0.17	9.38±0.02
300:1-0.5	17.02±0.79	13.75±0.43	9.90±0.45
400:1-0.5	2.10±0.29	4.24±0.90	4.12±1.17

300:5-0.5	19.19±0.93	11.99±1.79	8.79±0.56
400:5-0.5	6.57±2.66	0.61±0.68	4.52±0.73
300:1-1.5	16.63±0.70	11.09±2.08	12.94±2.13
400:1-1.5	7.43±0.35	4.71±0.91	1.52±0.88
300:5-1.5	17.79±0.49	16.79±0.47	13.68±2.10
400:5-1.5	2.06±2.75	0.40±0.28	12.17±1.48
350:3-1	10.88±1.48	10.10±1.66	12.47±0.22
C*			
226:3-1	15.16±1.01	12.41±2.33	13.60±3.17
435:3-1	14.29±0.86	12.50±0.37	14.49±0.77
350:0-1	15.09±1.03	15.37±0.50	11.30±0.73
350:6.35-1	14.63±2.17	7.44±2.16	11.16±0.14
350:3-0.16	14.11±2.43	8.53±0.70	11.14±0.02
350:3-1.84	14.64±1.53	13.45±0.20	9.39±2.03
300:1-0.5	17.18±0.80	13.78±0.44	9.91±0.45
400:1-0.5	2.75±0.28	7.92±5.20	4.43±1.07
300:5-0.5	19.30±0.93	12.00±1.80	8.93±0.55
400:5-0.5	6.76±2.46	2.34±0.17	4.77±0.55
300:1-1.5	16.79±0.66	11.12±2.11	12.98±2.14
400:1-1.5	7.53±0.32	5.10±0.90	2.26±0.69
300:5-1.5	17.92±0.46	16.83±0.48	13.75±2.14
400:5-1.5	3.39±1.65	2.10±0.16	12.24±1.48
350:3-1	10.92±1.48	10.11±1.66	12.55±0.22
*h**			
226:3-1	-1.44±0.02	-1.50±0.02	-1.45±0.02
435:3-1	-1.48±0.02	-1.50±0.00	-1.44±0.00
350:0-1	-1.48±0.01	-1.49±0.03	-1.51±0.01
350:6.35-1	-1.48±0.02	-1.48±0.02	-1.51±0.00
350:3-0.16	-1.48±0.02	-1.49±0.00	-1.51±0.00
350:3-1.84	-1.49±0.01	-1.51±0.03	-1.54±0.04
300:1-0.5	-1.43±0.01	-1.50±0.00	-1.52±0.01

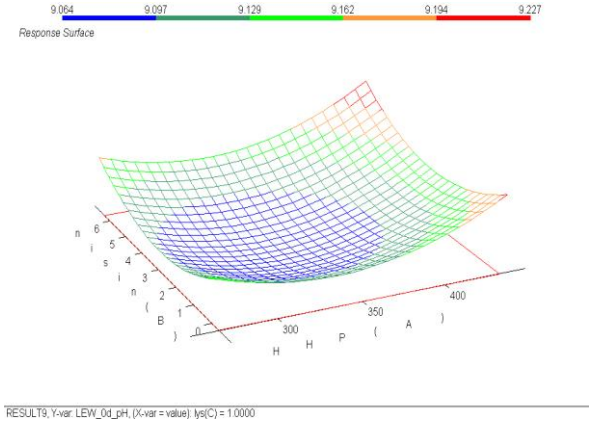
400:1-0.5	-0.89±0.17	-1.13±0.12	-1.19±0.12
300:5-0.5	-1.46±0.00	-1.52±0.02	-1.39±0.01
400:5-0.5	-1.32±0.19	-0.26±0.28	-1.25±0.14
300:1-1.5	-1.43±0.02	-1.51±0.03	-1.49±0.00
400:1-1.5	-1.41±0.03	-1.18±0.04	-0.71±0.25
300:5-1.5	-1.45±0.02	-1.50±0.02	-1.48±0.04
400:5-1.5	-0.58±0.71	-0.19±0.12	-1.46±0.01
350:3-1	-1.48±0.01	-1.53±0.01	-1.46±0.00

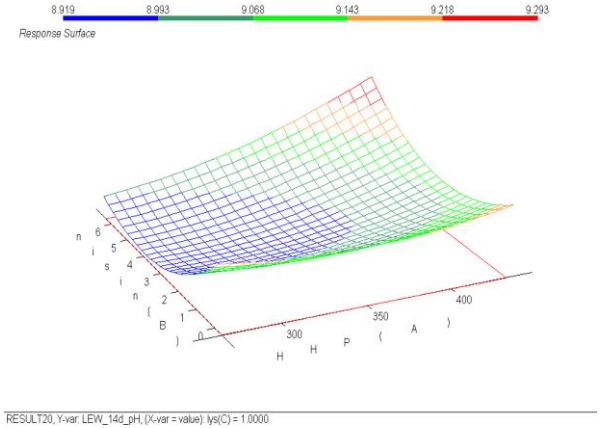
Effect of HHP pressure, nisin and lysozyme on FA, FE, and FS of liquid egg white during the storage

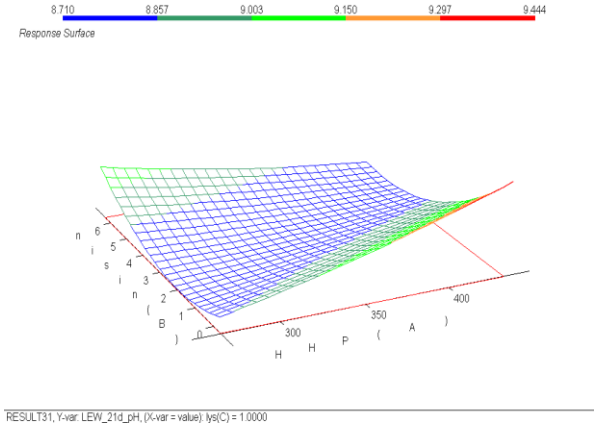
Sample	Storage days		
	0	14	21
FA (%)			
226:3-1	462±20	420±9	364±13
435:3-1	230±16	200±11	164±17
350:0-1	402±21	394±15	374±19
350:6.35-1	420±19	390±17	360±20
350:3-0.16	406±14	386±19	348±11
350:3-1.84	400±17	374±11	342±10
300:1-0.5	440±19	418±9	384±14
400:1-0.5	300±8	282±13	276±17
300:5-0.5	456±14	412±12	386±19
400:5-0.5	316±15	278±16	244±11
300:1-1.5	428±9	398±18	366±10
400:1-1.5	330±10	294±20	240±9
300:5-1.5	290±17	266±13	238±11
400:5-1.5	310±13	270±11	232±16
350:3-1	394±12	364±10	338±19
FE (%)			
226:3-1	362±11	320±9	264±17
435:3-1	130±15	100±11	64±11
350:0-1	302±18	294±18	274±14
350:6.35-1	320±19	290±19	260±18
350:3-0.16	306±11	286±11	248±13
350:3-1.84	300±9	274±10	242±16
300:1-0.5	340±19	318±17	284±18

400:1-0.5	200±10	182±16	176±20
300:5-0.5	356±11	312±11	286±13
400:5-0.5	216±14	178±10	144±16
300:1-1.5	328±11	298±15	266±14
400:1-1.5	230±10	194±19	140±13
300:5-1.5	190±9	166±11	138±16
400:5-1.5	210±14	170±10	132±
350:3-1	294±11	264±15	238±
FS (%)			
226:3-1	92±2	91±3	90±8
435:3-1	90±7	79±6	70±9
350:0-1	95±2	95±9	83±5
350:6.35-1	88±9	87±2	82±9
350:3-0.16	90±4	91±1	90±7
350:3-1.84	90±7	90±5	82±5
300:1-0.5	86±3	88±9	85±3
400:1-0.5	83±2	77±6	70±9
300:5-0.5	88±6	85±1	82±9
400:5-0.5	94±7	73±2	70±2
300:1-1.5	95±4	83±6	84±4
400:1-1.5	83±5	80±7	74±6
300:5-1.5	85±3	84±3	85±5
400:5-1.5	82±6	74±7	57±3
350:3-1	87±4	81±6	76±9

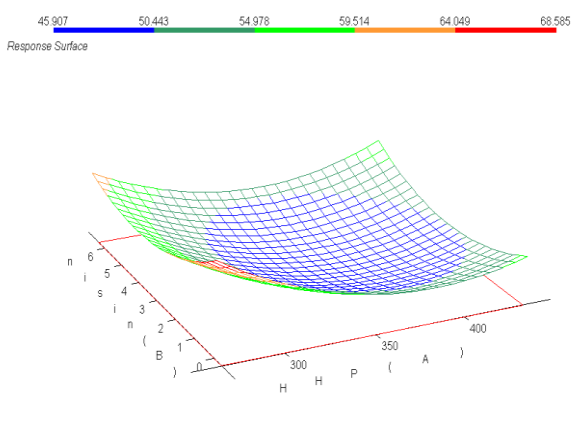
Response surface for pH values of HHP treated liquid egg white with nisin and lysozyme during storage

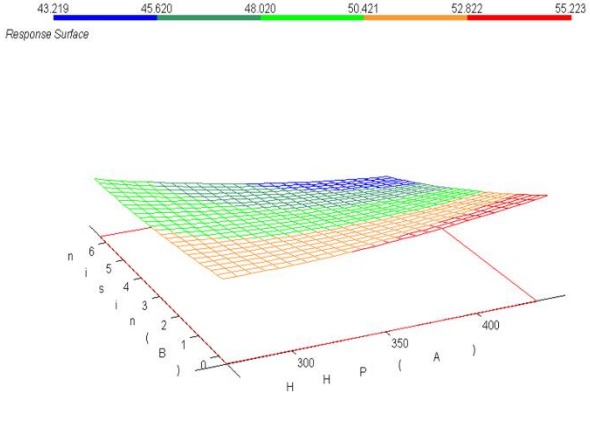


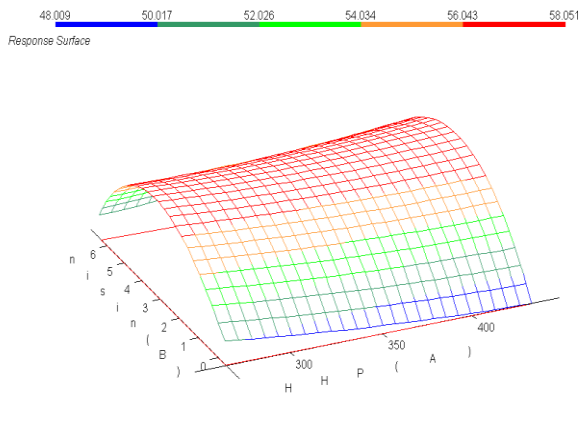




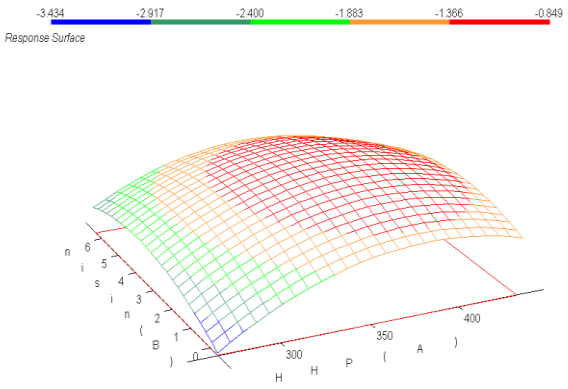
Response surface for *L** of HHP treated liquid egg white with nisin and lysozyme during storage

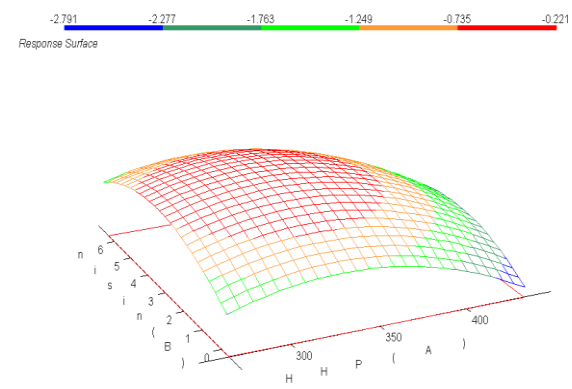


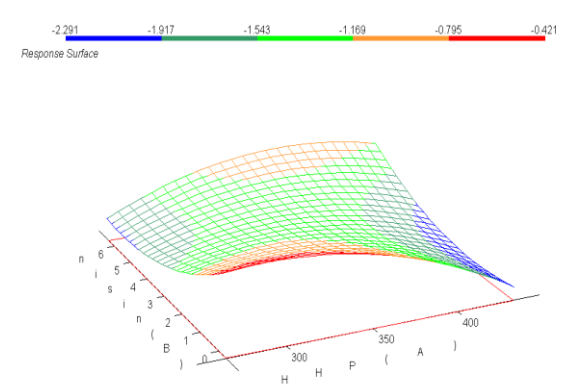




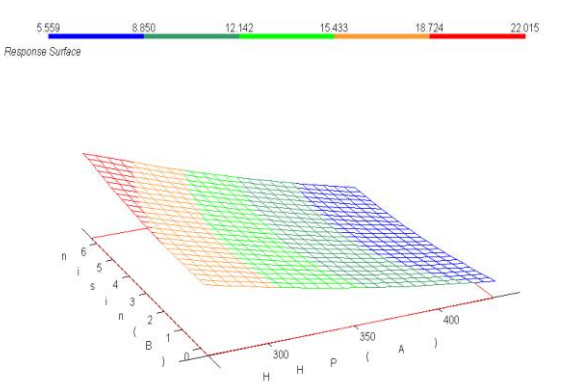
Response surface for *a** of HHP treated liquid egg white with nisin and lysozyme during storage

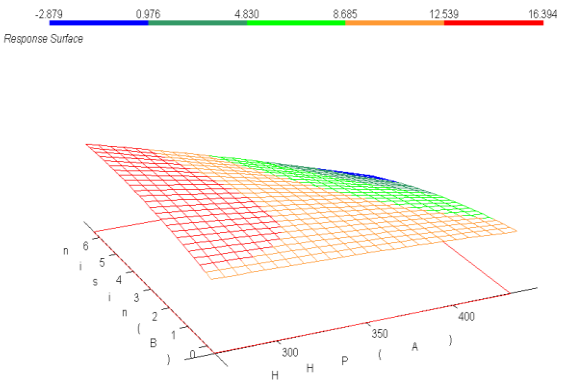


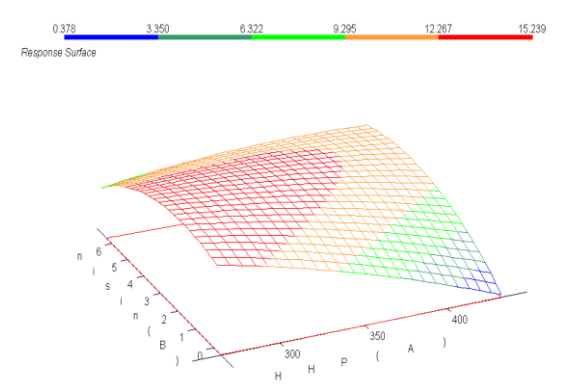




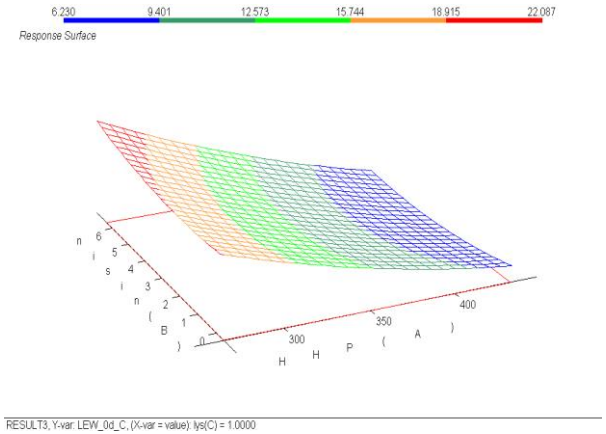
Response surface for *b** of HHP treated liquid egg white with nisin and lysozyme during storage

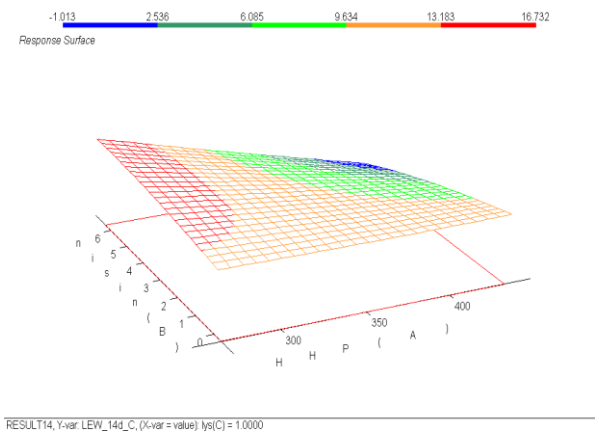


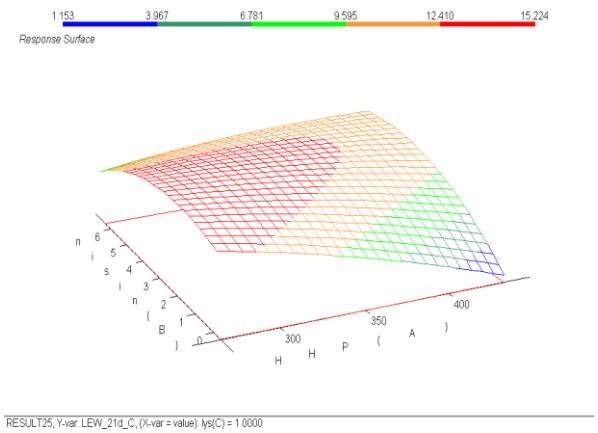




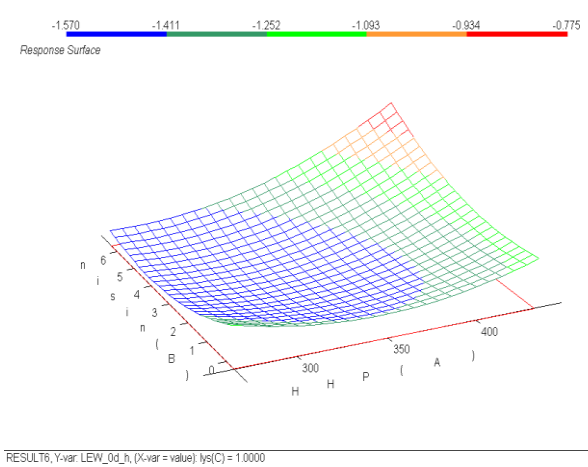
Response surface for C* of HHP treated liquid egg white with nisin and lysozyme during storage

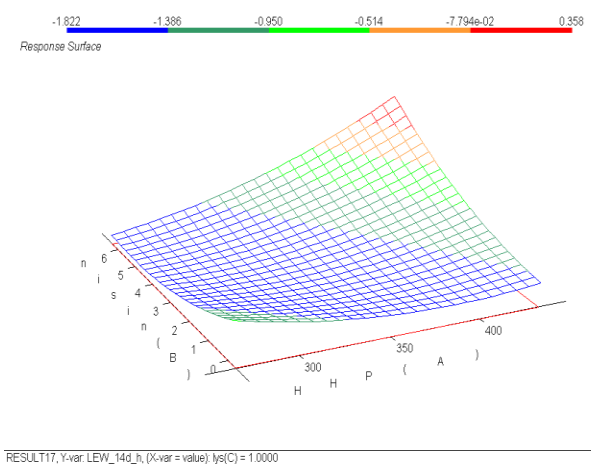


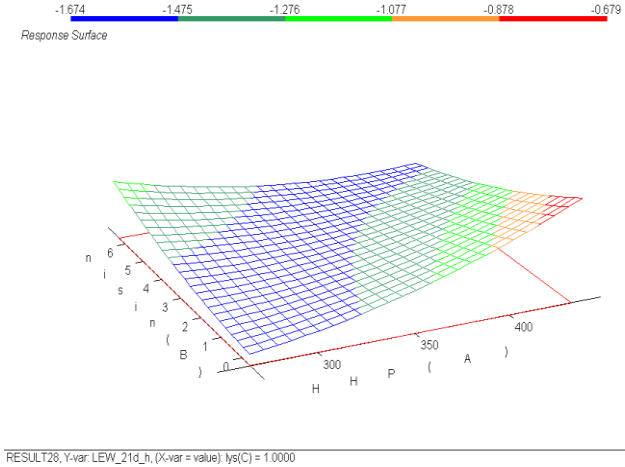




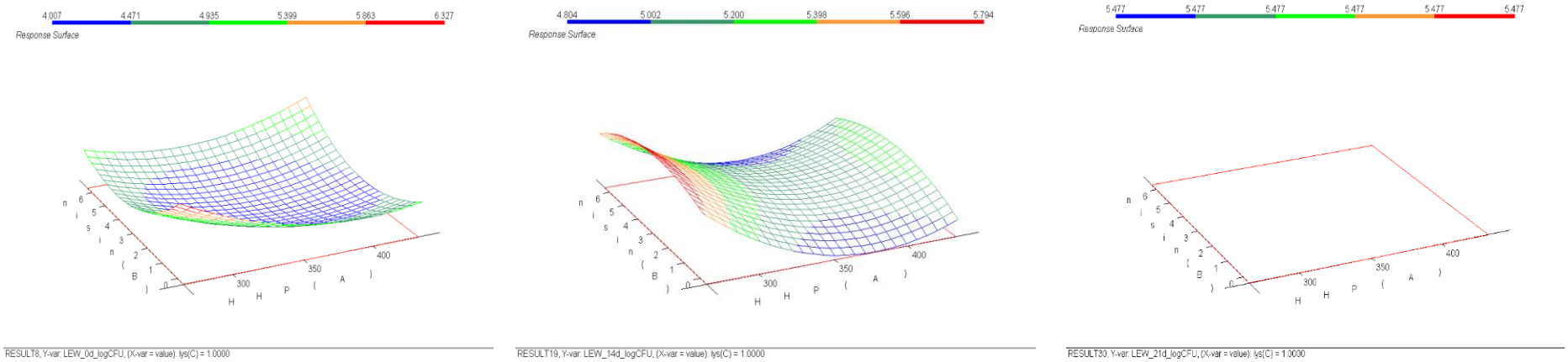
Response surface for *h** of HHP treated liquid egg white with nisin and lysozyme during storage





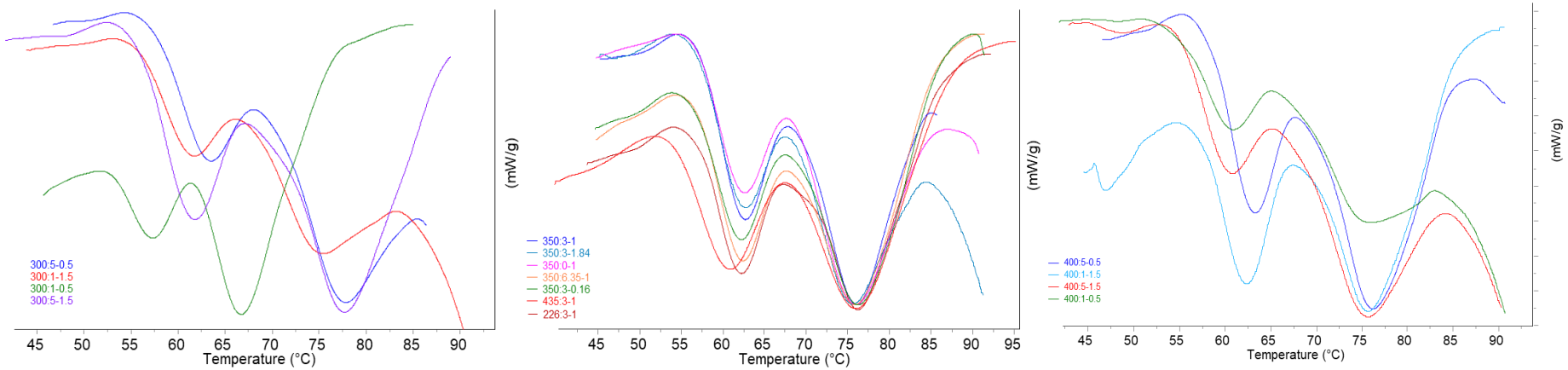


Response surface for mesophilic total plate count of HHP treated liquid egg white with nisin and lysozyme during storage

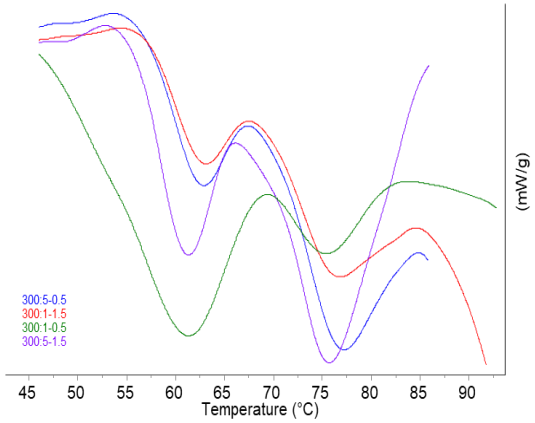


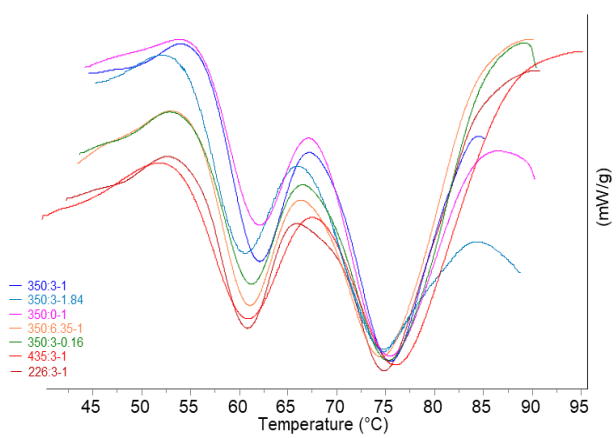
Thermograms of HHP treated liquid egg white with nisin and lysozyme during storage

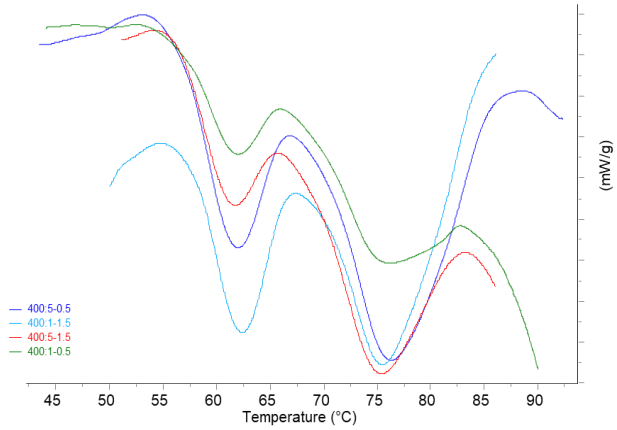
Day 0



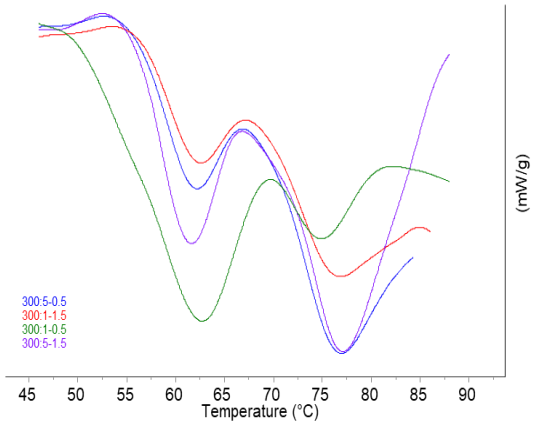
Day 14

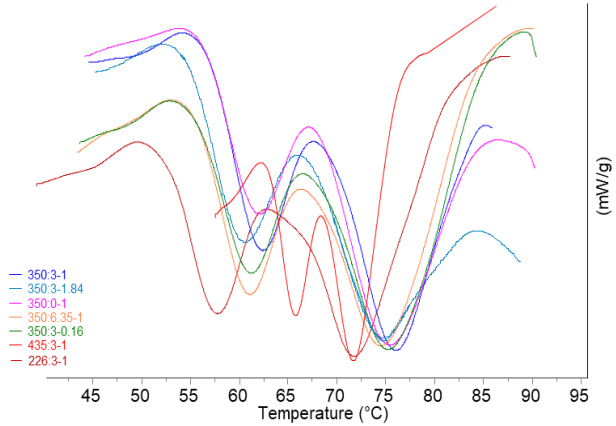

300:5-0.5
300:1-1.5
300:1-0.5
300:5-1.5
(mW/g)
45 50 55 60 65 70 75 80 85 90
Temperature (°C)

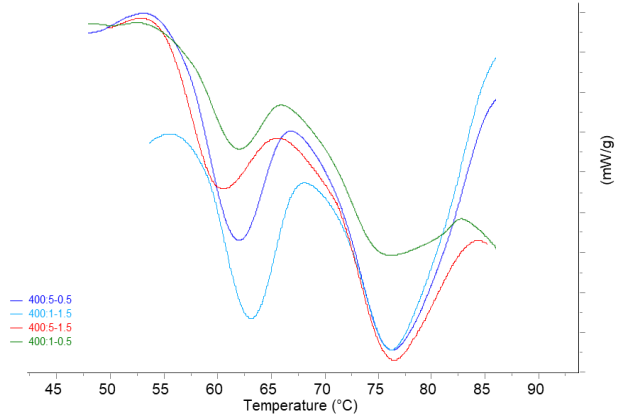

350:3-1
350:3-1.84
350:0-1
350:6.35-1
350:3-0.16
435:3-1
226:3-1
(mW/g)
45 50 55 60 65 70 75 80 85 90 95
Temperature (°C)


400:5-0.5
400:1-1.5
400:5-1.5
400:1-0.5
(mW/g)
45 50 55 60 65 70 75 80 85 90
Temperature (°C)

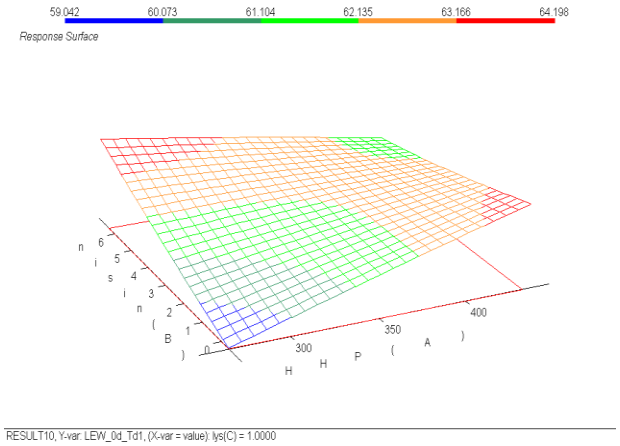
Day 21

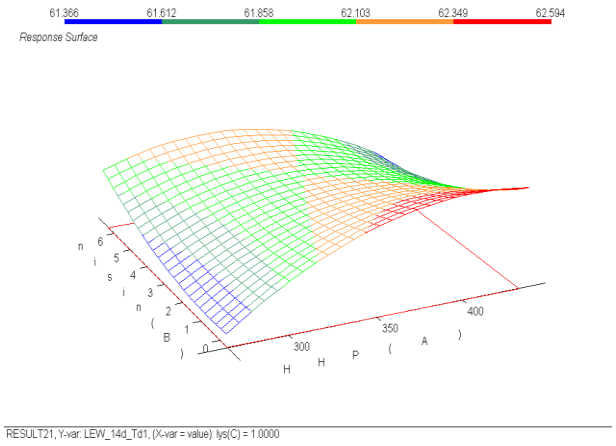

300:5-0.5
300:1-1.5
300:1-0.5
300:5-1.5
(mW/g)
45 50 55 60 65 70 75 80 85 90
Temperature (°C)

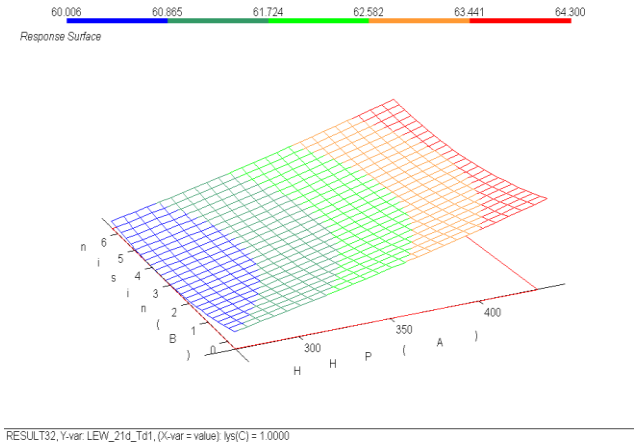

350:3-1
350:3-1.84
350:0-1
350:6.35-1
350:3-0.16
435:3-1
226:3-1
(mW/g)
45 50 55 60 65 70 75 80 85 90 95
Temperature (°C)


400:5-0.5
400:1-1.5
400:5-1.5
400:1-0.5
(mW/g)
45 50 55 60 65 70 75 80 85 90
Temperature (°C)

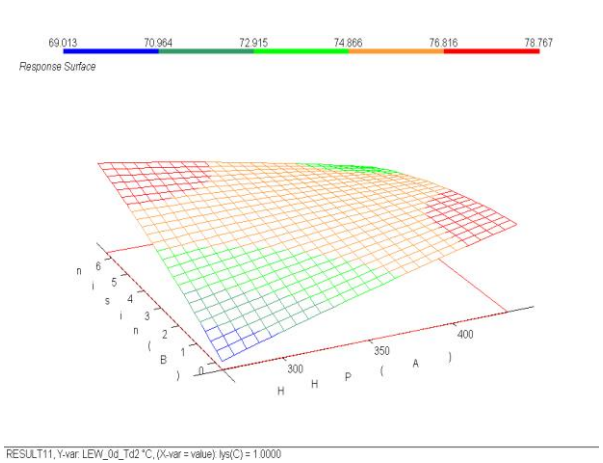
Response surface for 1st peak denaturation temperature of HHP treated liquid egg white with nisin and lysozyme during storage

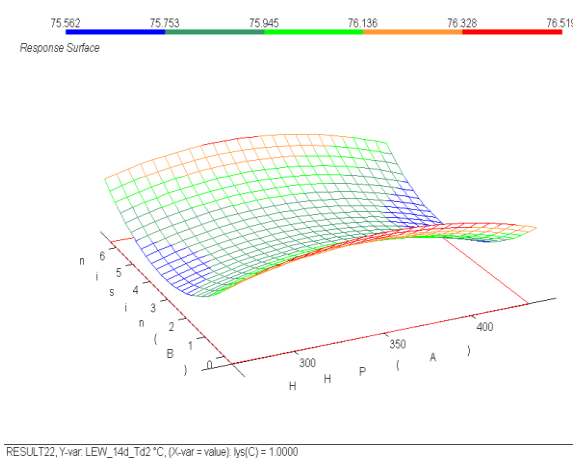


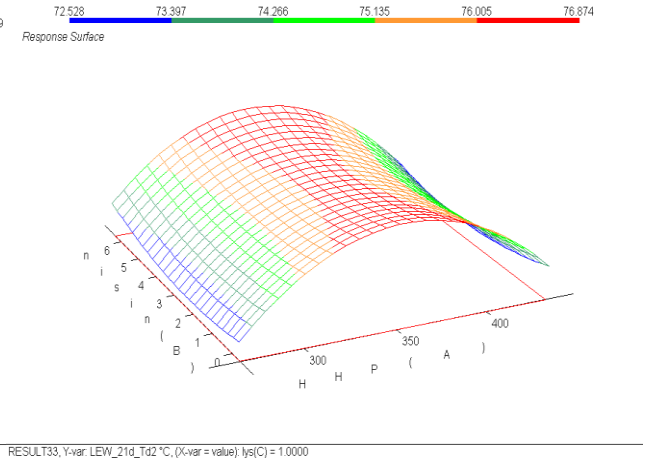




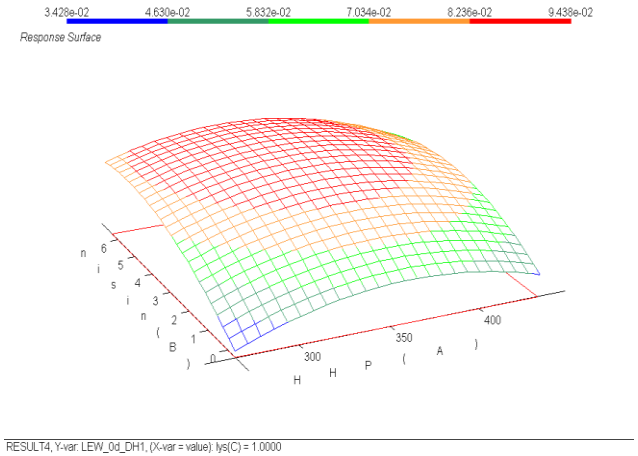
Response surface for 2nd peak denaturation temperature of HHP treated liquid egg white with nisin and lysozyme during storage

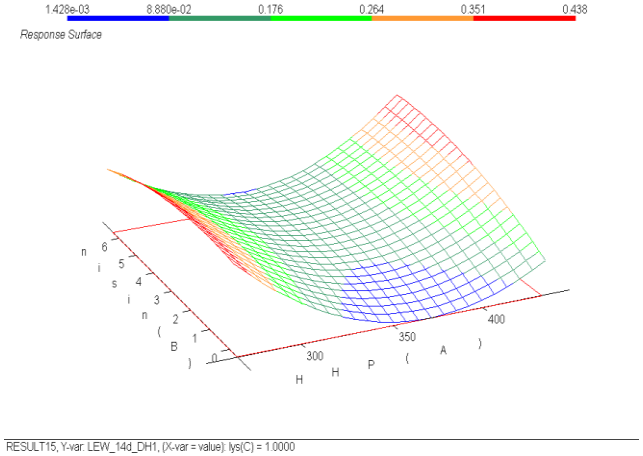


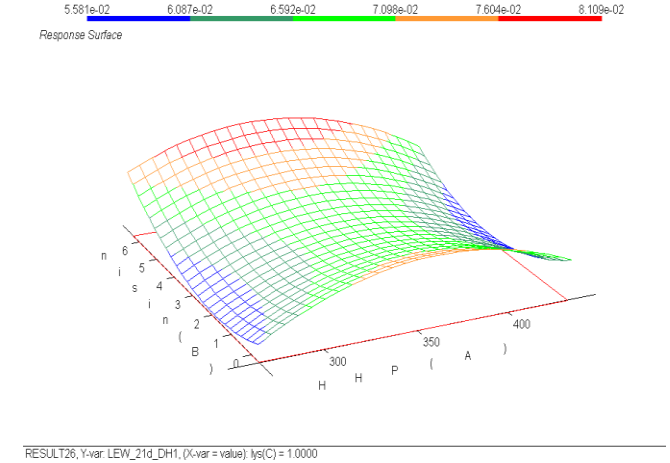




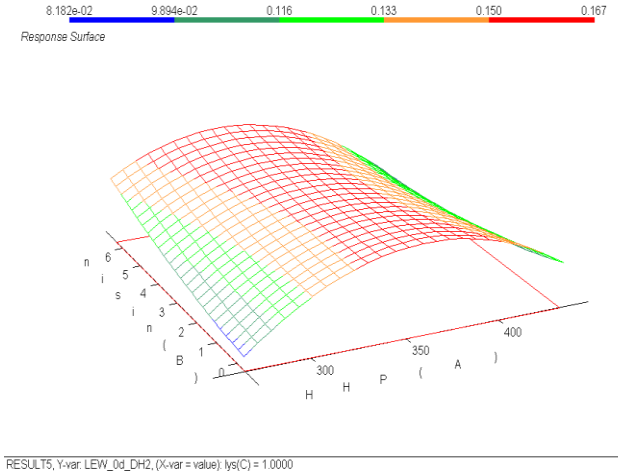
Response surface for 1st peak denaturation enthalpy of HHP treated liquid egg white with nisin and lysozyme during storage

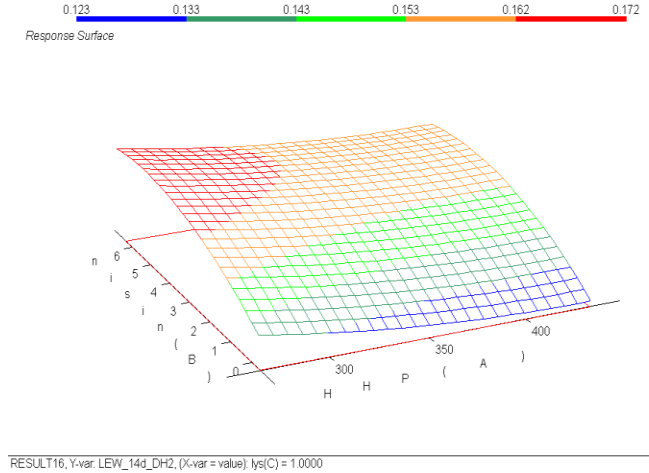


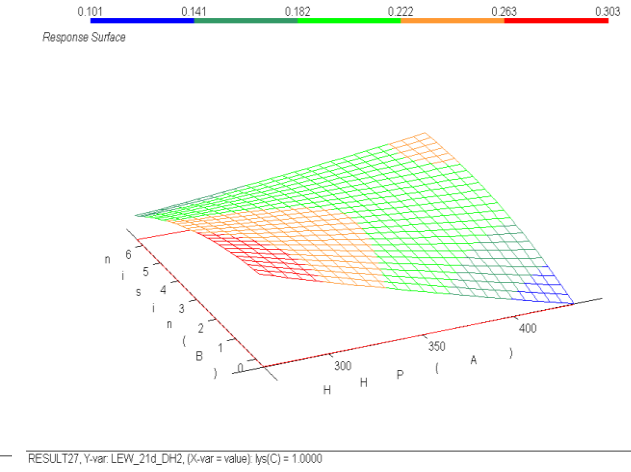




Response surface for 2nd peak denaturation enthalpy of HHP treated liquid egg white with nisin and lysozyme during storage

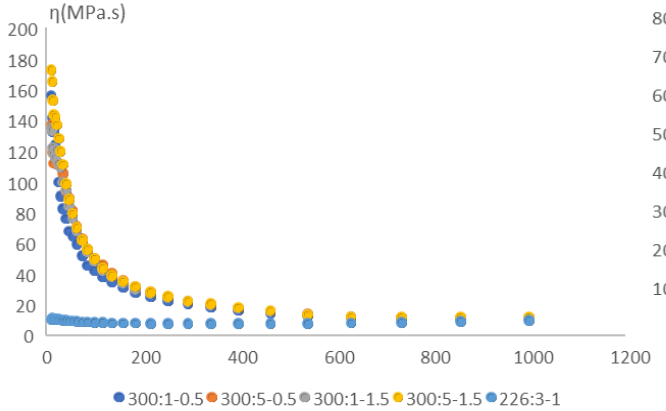


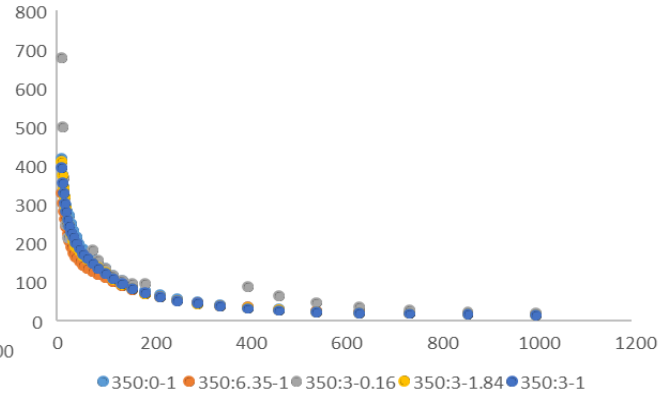


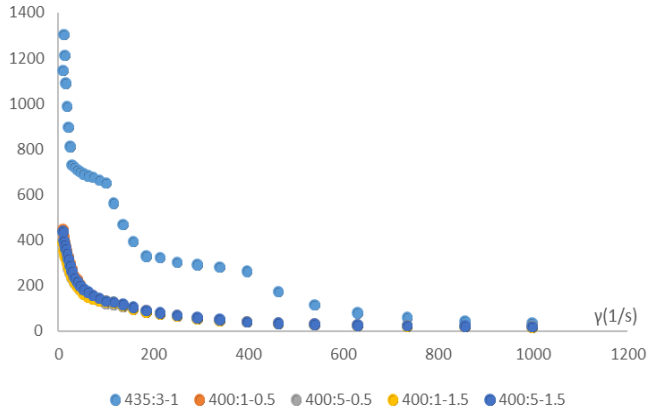


Apparent viscosity of HHP treated liquid egg white with nisin and lysozyme during storage

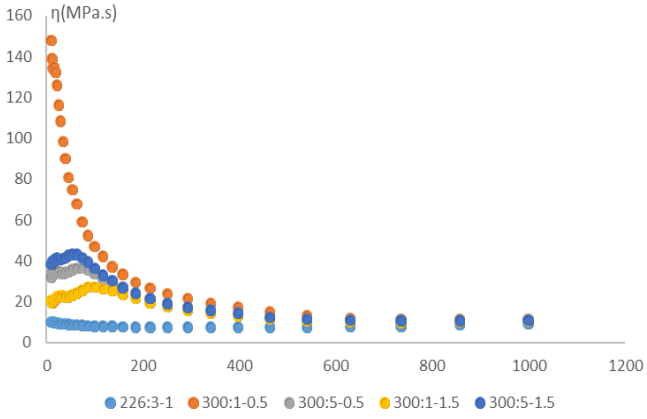
Day 0

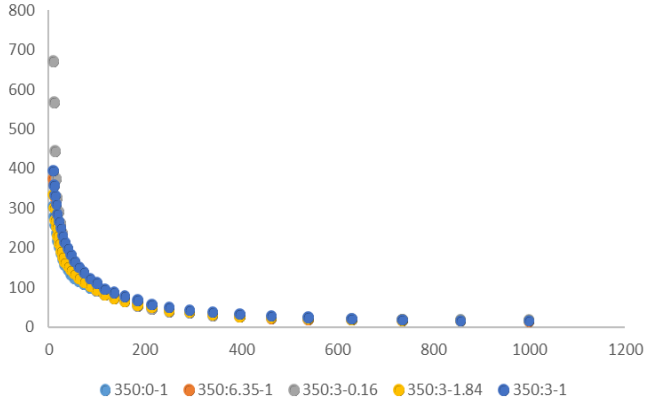


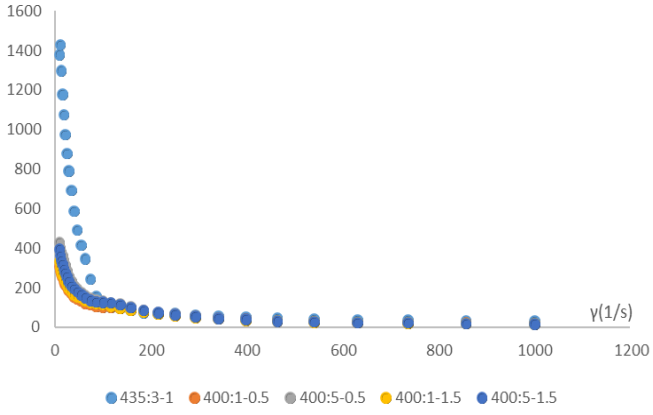




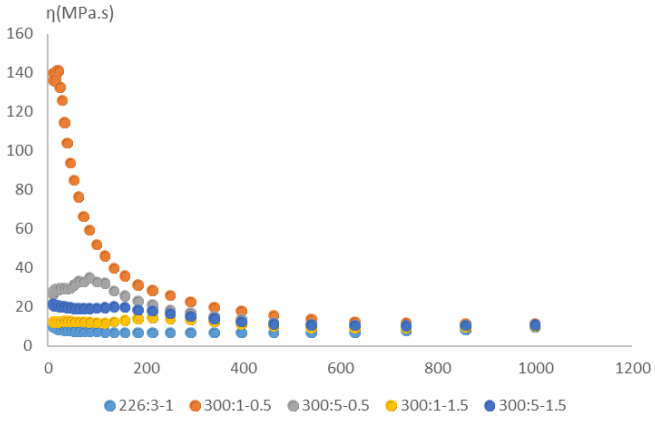
Day 14

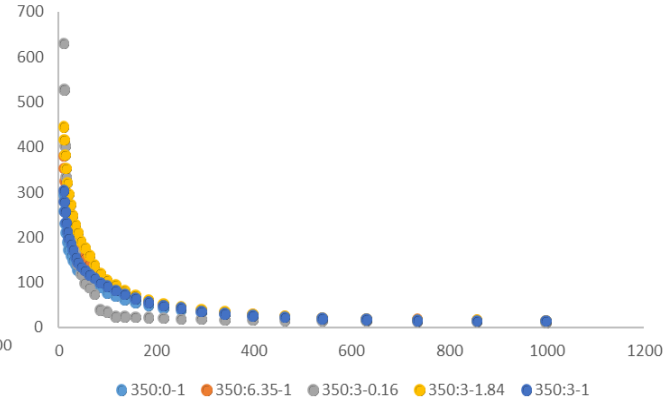


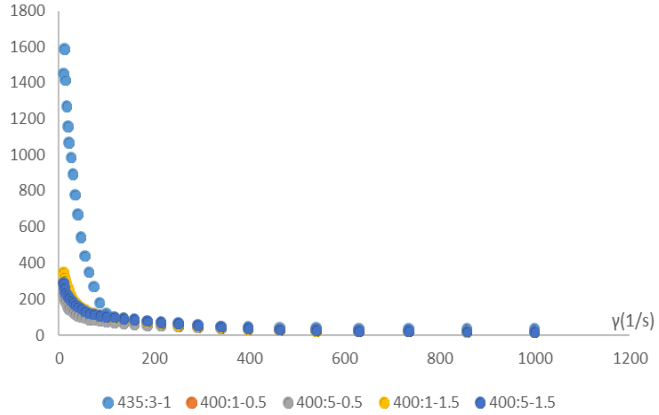




Day 21


η(MPa.s)
160
140
120
100
80
60
40
20
0
0
200
400
600
800
1000
1200
226:3-1
300:1-0.5
300:5-0.5
300:1-1.5
300:5-1.5


700
600
500
400
300
200
100
0
0
200
400
600
800
1000
1200
350:0-1
350:6.35-1
350:3-0.16
350:3-1.84
350:3-1


1800
1600
1400
1200
1000
800
600
400
200
0
γ(1/s)
0
200
400
600
800
1000
1200
435:3-1
400:1-0.5
400:5-0.5
400:1-1.5
400:5-1.5

Annex 5

Effect of HHP pressure, nisin and lysozyme on evolution physicochemical proprieties and color parameters of liquid egg yolk during the storage

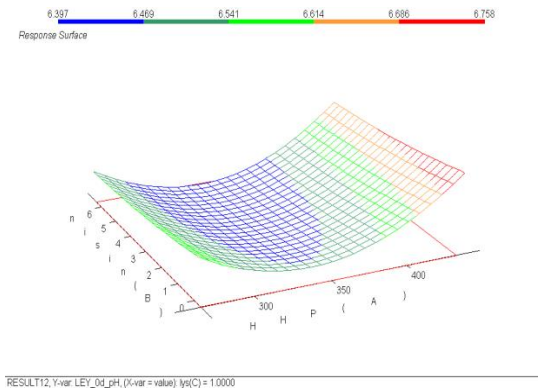
Sample	Storage days		
	0	14	21
pH			
226:3-1	6.51±0.04	5.74±0.03	5.80±0.04
435:3-1	6.64±0.02	5.85±0.09	5.29±0.71
350:0-1	6.50±0.13	6.40±0.02	5.14±0.04
350:6.35-1	6.27±0.05	5.94±0.44	5.73±0.09
350:3-0.16	6.15±0.03	5.83±0.12	5.79±0.17
350:3-1.84	6.45±0.03	6.22±0.10	5.65±0.19
300:1-0.5	6.49±0.07	5.86±0.03	5.83±0.15
400:1-0.5	6.60±0.06	6.26±0.15	6.01±0.02
300:5-0.5	6.50±0.02	5.68±0.27	6.17±0.04
400:5-0.5	6.49±0.05	6.44±0.04	6.40±0.04
300:1-1.5	6.45±0.04	6.04±0.04	5.60±0.16
400:1-1.5	6.51±0.07	6.33±0.08	6.60±0.17
300:5-1.5	6.48±0.09	6.04±0.09	5.45±0.15
400:5-1.5	6.65±0.04	6.24±0.10	5.19±0.07
350:3-1	6.45±0.05	6.39±0.03	5.75±0.11
*L**			
226:3-1	60.48±0.16	60.96±0.62	59.63±0.32
435:3-1	63.44±1.25	61.79±0.66	62.35±0.70
350:0-1	62.13±0.25	64.04±0.39	58.65±0.68
350:6.35-1	58.96±1.30	45.94±0.32	56.42±0.37
350:3-0.16	63.26±0.30	45.21±0.71	56.05±0.70
350:3-1.84	62.01±0.10	55.85±0.35	49.74±0.85
300:1-0.5	62.05±0.56	65.85±0.84	64.16±0.20
400:1-0.5	55.85±0.76	60.86±0.58	60.49±0.89
300:5-0.5	62.43±0.66	62.31±0.71	64.11±0.82

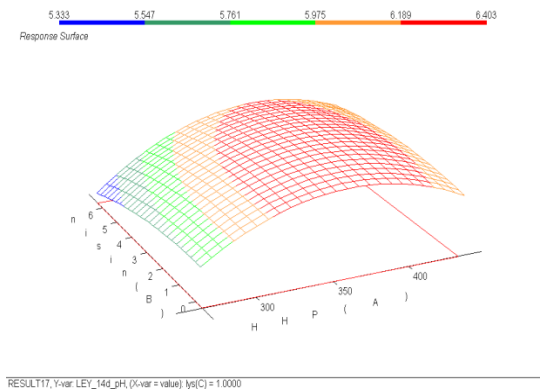
400:5-0.5	61.47±0.39	63.42±0.09	61.35±0.40
300:1-1.5	62.42±0.03	65.59±0.32	61.73±0.36
400:1-1.5	44.01±1.15	68.41±1.20	52.02±0.47
300:5-1.5	51.47±3.68	67.34±0.98	57.14±1.02
400:5-1.5	44.83±2.04	65.03±0.81	58.95±0.90
350:3-1	62.21±0.10	64.24±0.60	57.44±1.25
*a**			
226:3-1	10.26±0.50	9.59±0.09	8.57±0.16
435:3-1	12.09±0.92	10.70±0.23	9.27±0.25
350:0-1	10.02±0.11	10.44±0.09	10.04±0.17
350:6.35-1	10.94±0.54	11.67±0.36	9.92±0.33
350:3-0.16	9.69±0.90	10.87±0.19	9.57±0.47
350:3-1.84	9.57±0.42	10.85±0.27	9.74±0.33
300:1-0.5	9.75±0.16	9.86±0.13	8.91±0.11
400:1-0.5	8.81±0.34	9.27±0.13	8.43±0.57
300:5-0.5	9.85±0.24	10.57±0.31	8.73±0.30
400:5-0.5	9.63±0.09	10.20±0.10	9.87±0.17
300:1-1.5	9.91±0.16	9.63±0.13	8.38±0.25
400:1-1.5	9.28±0.31	9.10±0.15	8.49±0.17
300:5-1.5	8.71±0.40	8.58±0.06	8.63±0.38
400:5-1.5	8.76±0.30	8.03±0.55	8.90±0.16
350:3-1	10.20±0.09	10.34±0.26	9.12±0.09
*b**			
226:3-1	43.19±0.20	47.05±1.47	37.60±1.05
435:3-1	44.29±1.79	46.61±0.21	36.02±0.75
350:0-1	43.89±0.85	45.60±1.10	33.62±0.68
350:6.35-1	44.44±1.81	39.21±0.57	33.80±0.31
350:3-0.16	42.99±0.46	40.24±0.30	33.33±0.22
350:3-1.84	41.53±0.67	46.36±0.47	30.69±1.05
300:1-0.5	44.06±0.61	48.93±1.38	43.06±0.67
400:1-0.5	40.43±1.10	40.12±0.76	37.28±1.21

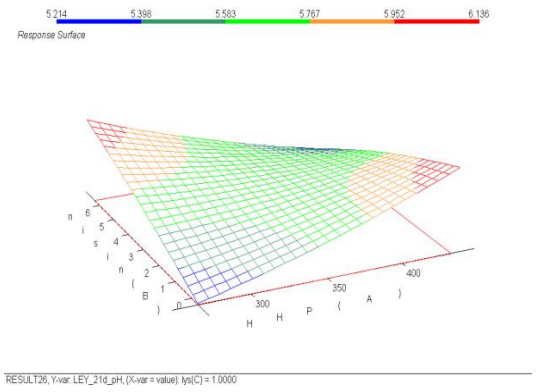
300:5-0.5	42.21±2.31	44.49±1.13	42.55±0.96
400:5-0.5	38.44±0.23	43.89±0.89	39.54±1.42
300:1-1.5	42.37±0.32	48.16±1.07	42.75±1.40
400:1-1.5	44.71±2.15	39.58±0.57	32.97±1.46
300:5-1.5	40.66±0.45	48.82±0.52	40.75±1.54
400:5-1.5	39.87±0.43	35.40±1.03	39.08±0.58
350:3-1	44.43±0.53	42.00±2.49	40.15±1.11
C*			
226:3-1	44.39±0.31	48.02±1.44	38.56±1.06
435:3-1	45.92±1.50	47.82±0.25	37.19±0.76
350:0-1	45.02±0.85	46.78±1.09	35.08±0.61
350:6.35-1	45.77±1.64	40.91±0.64	35.23±0.20
350:3-0.16	44.08±0.36	41.68±0.34	34.68±0.26
350:3-1.84	42.62±0.58	47.62±0.51	32.20±0.90
300:1-0.5	45.13±0.60	49.91±1.36	43.97±0.68
400:1-0.5	41.38±1.10	41.18±0.73	38.22±1.17
300:5-0.5	43.35±2.29	45.73±1.05	43.44±1.00
400:5-0.5	39.62±0.22	45.06±0.88	40.75±1.40
300:1-1.5	43.52±0.33	49.11±1.07	43.56±1.40
400:1-1.5	45.67±2.06	40.61±0.58	34.05±1.45
300:5-1.5	41.58±0.40	49.56±0.51	41.65±1.43
400:5-1.5	40.82±0.48	36.30±1.09	40.08±0.54
350:3-1	45.59±0.52	43.26±2.46	41.17±1.09
*h**			
226:3-1	1.34±0.01	1.37±0.01	1.35±0.00
435:3-1	1.30±0.03	1.35±0.00	1.32±0.01
350:0-1	1.35±0.00	1.35±0.00	1.28±0.01
350:6.35-1	1.33±0.02	1.28±0.01	1.29±0.01
350:3-0.16	1.35±0.02	1.31±0.00	1.29±0.01
350:3-1.84	1.34±0.01	1.34±0.00	1.26±0.02
300:1-0.5	1.35±0.00	1.37±0.00	1.37±0.00

400:1-0.5	1.36±0.01	1.34±0.01	1.35±0.02
300:5-0.5	1.34±0.01	1.34±0.01	1.37±0.00
400:5-0.5	1.33±0.00	1.34±0.00	1.33±0.01
300:1-1.5	1.34±0.00	1.37±0.00	1.38±0.01
400:1-1.5	1.37±0.02	1.34±0.00	1.32±0.01
300:5-1.5	1.36±0.01	1.40±0.00	1.36±0.02
400:5-1.5	1.35±0.01	1.35±0.01	1.35±0.01
350:3-1	1.35±0.00	1.33±0.01	1.35±0.01

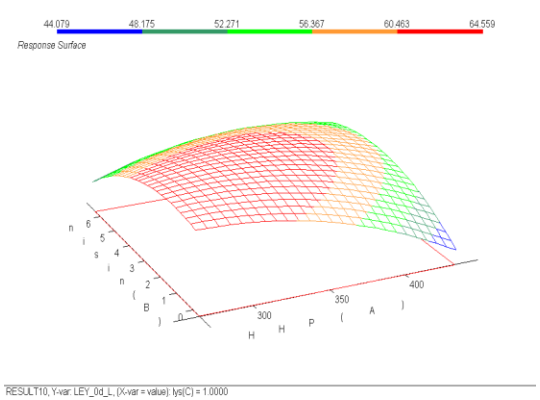
Response surface for pH values of HHP treated liquid egg yolk with nisin and lysozyme during storage

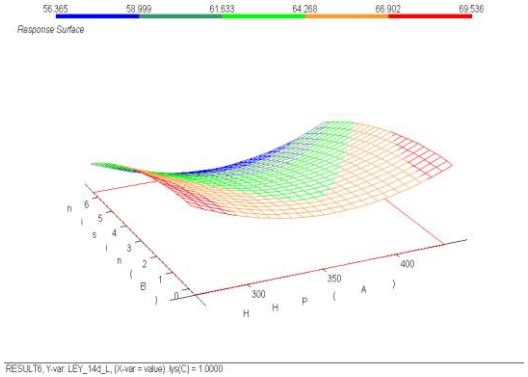


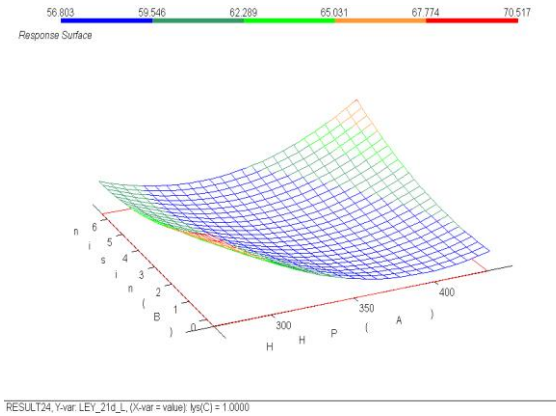




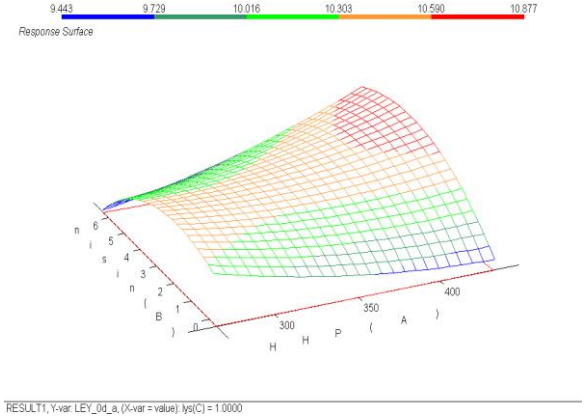
Response surface for *L** of HHP treated liquid egg yolk with nisin and lysozyme during storage

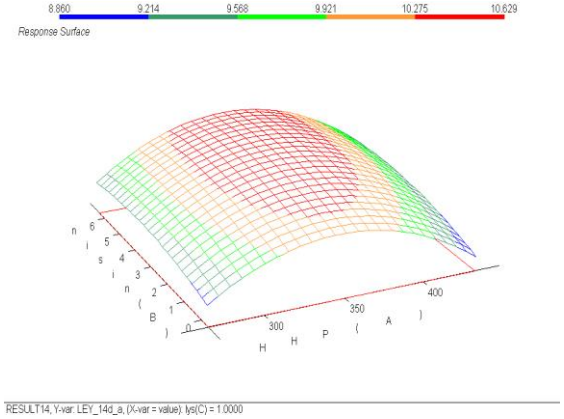


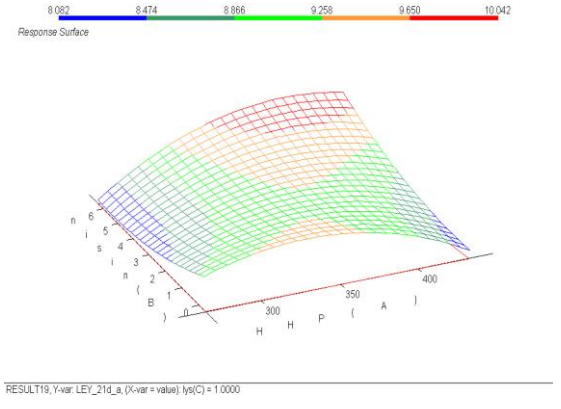




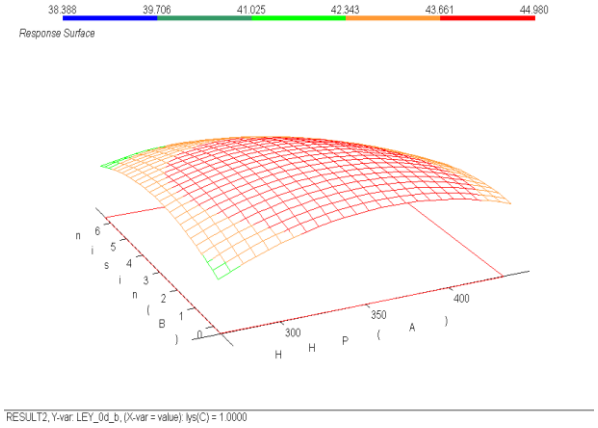
Response surface for *a** of HHP treated liquid egg yolk with nisin and lysozyme during storage

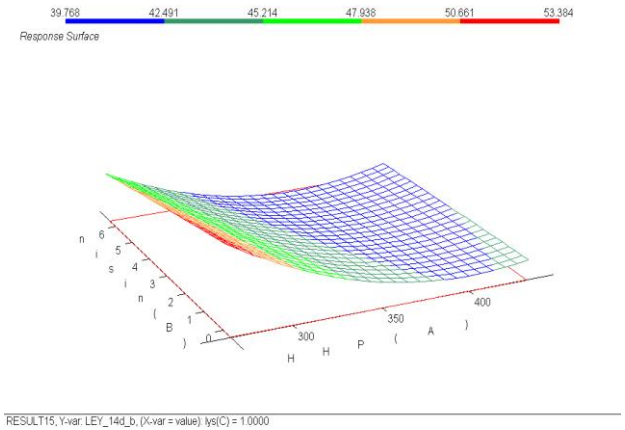


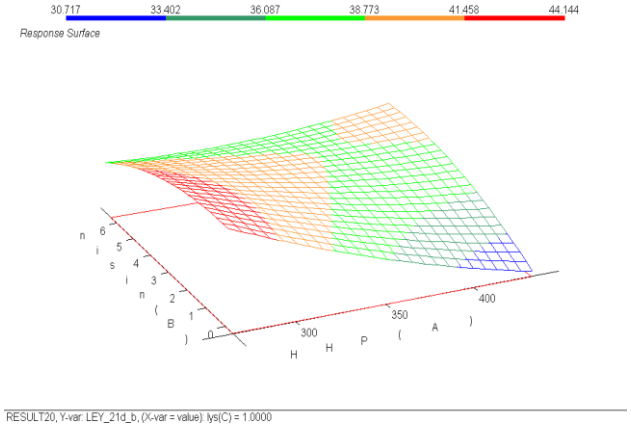




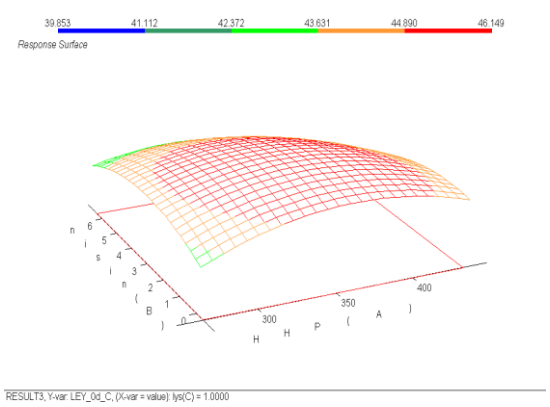
Response surface for *b** of HHP treated liquid egg yolk with nisin and lysozyme during storage

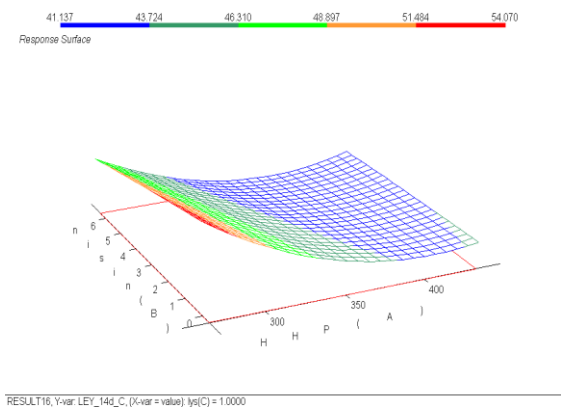


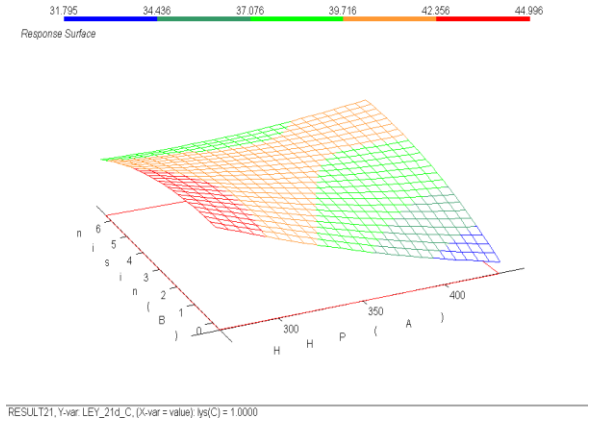




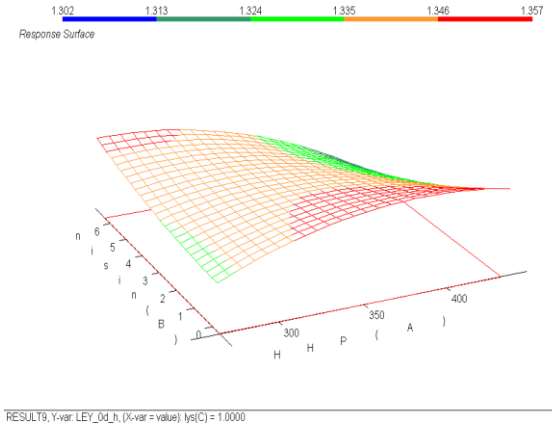
Response surface for C* of HHP treated liquid egg yolk with nisin and lysozyme during storage

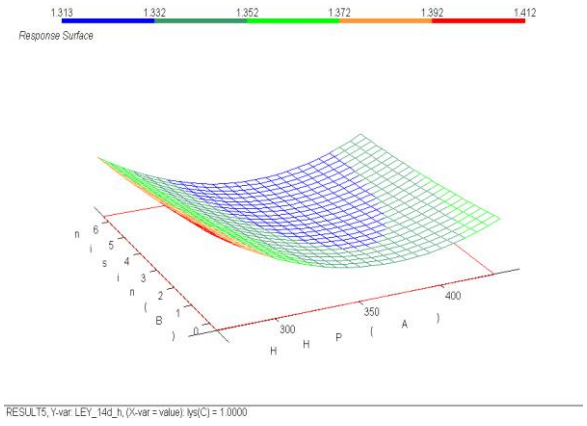


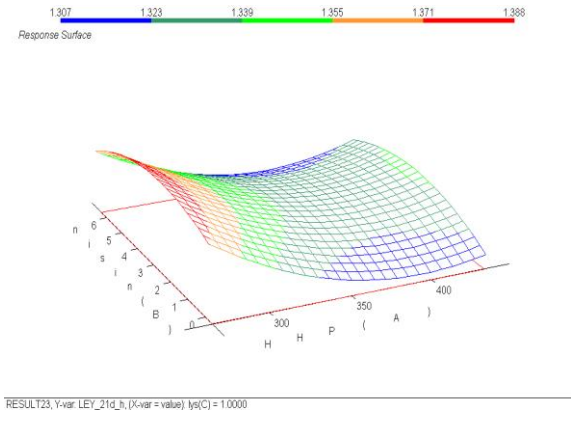




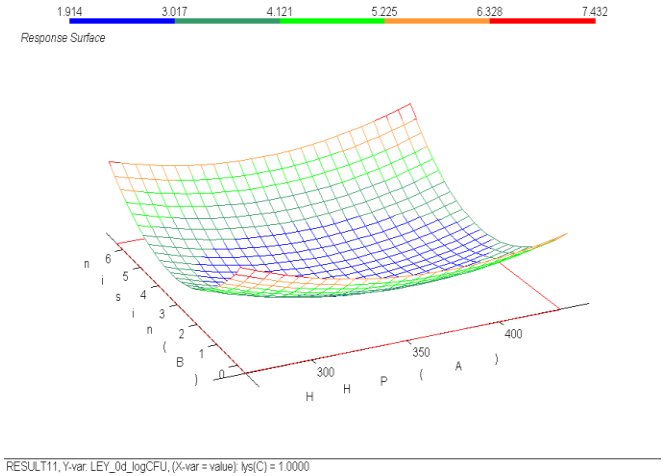
Response surface for *h** of HHP treated liquid egg yolk with nisin and lysozyme during storage

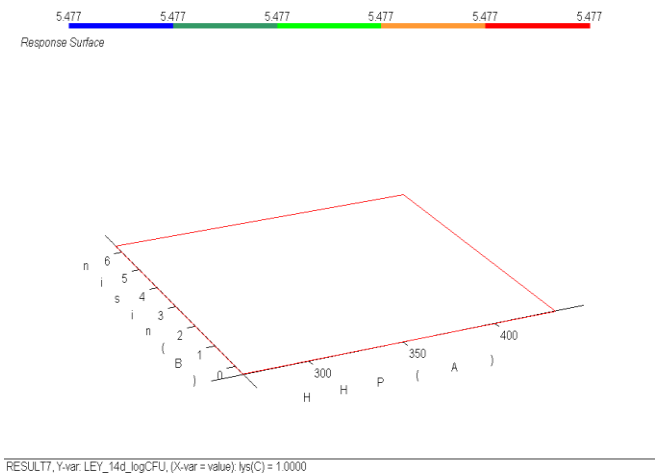


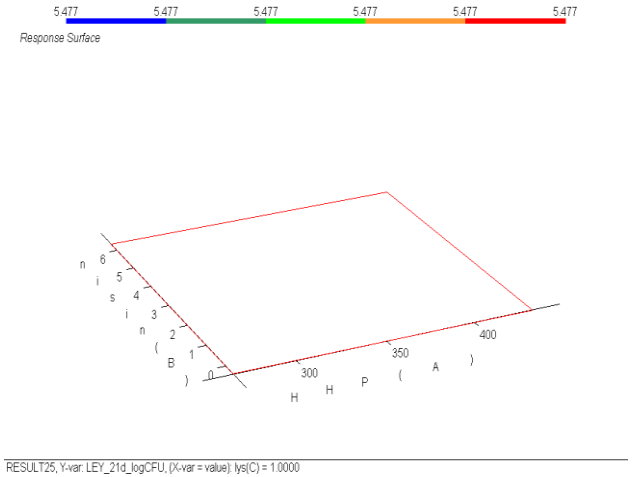




Response surface for mesophilic total plate count of HHP treated liquid egg yolk with nisin and lysozyme during storage

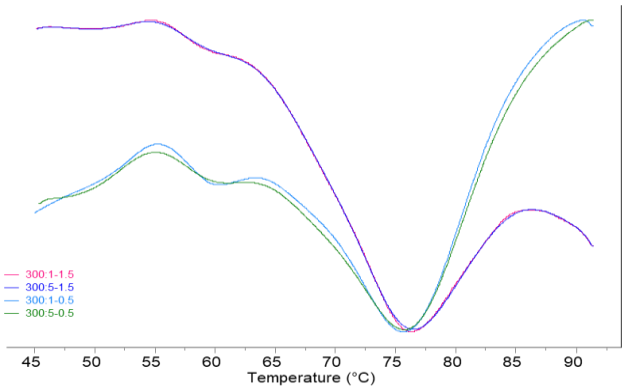


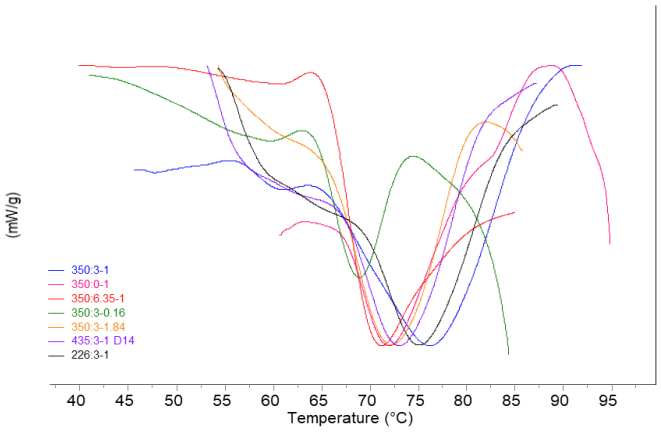


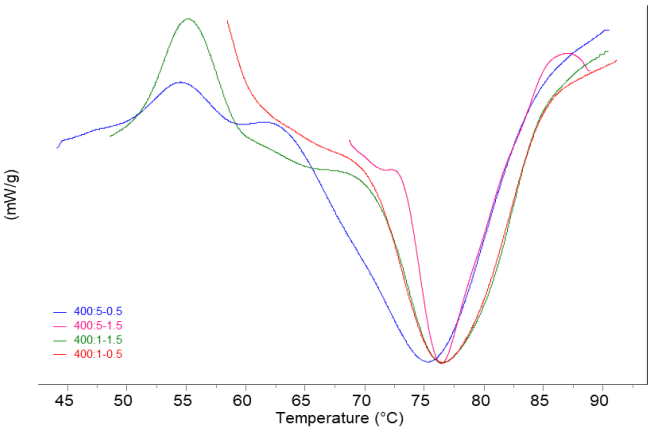


Thermograms of HHP treated liquid egg yolk with nisin and lysozyme during storage

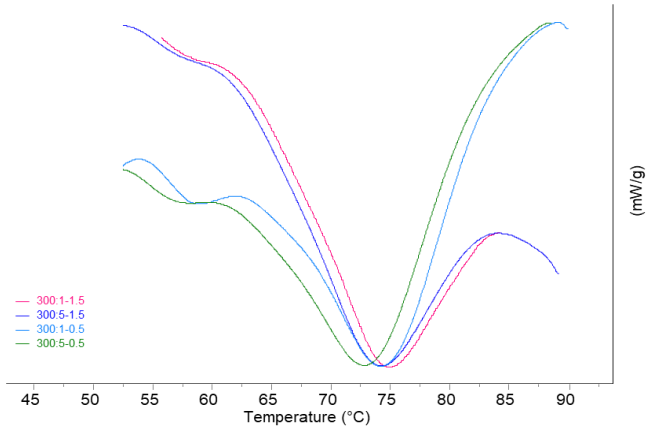
Day 0

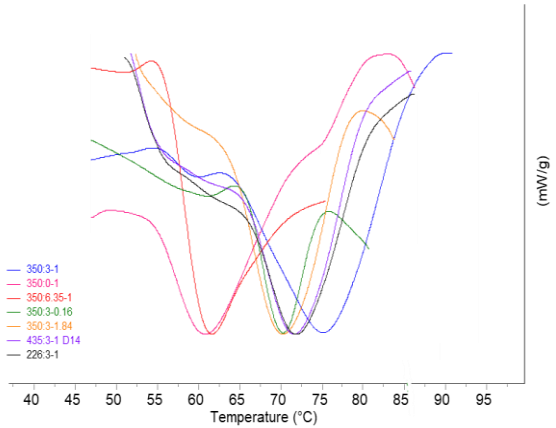


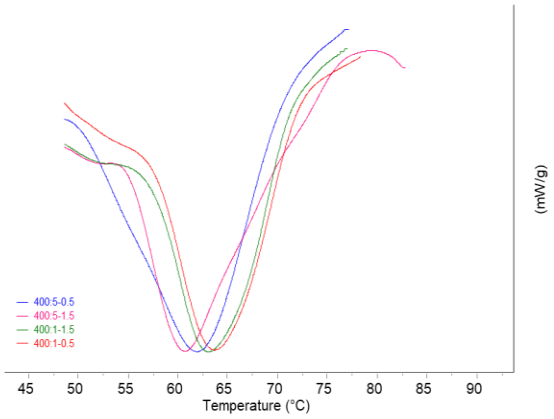




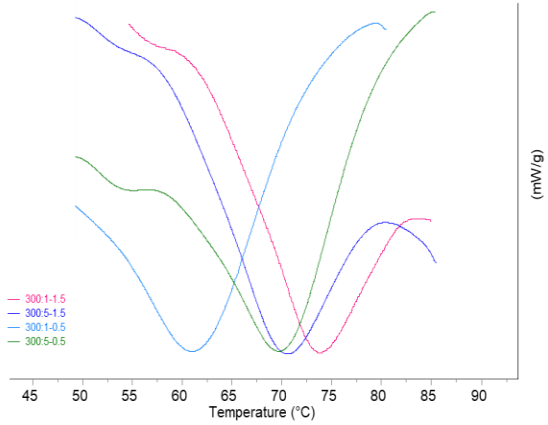
Day 14

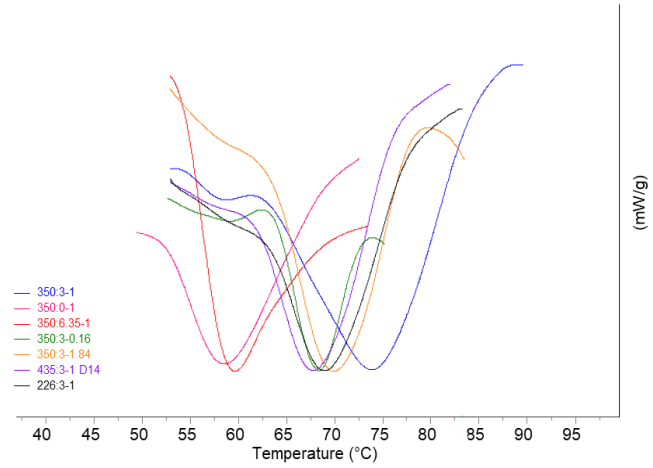

300:1-1.5
300:5-1.5
300:1-0.5
300:5-0.5
45 50 55 60 65 70 75 80 85 90
Temperature (°C)
(mW/g)

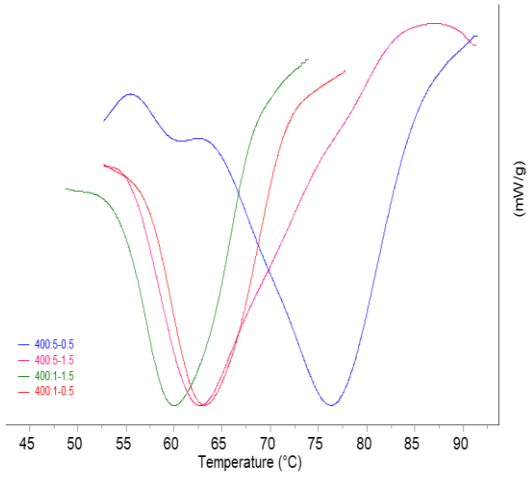

350:3-1
350:0-1
350:6.35-1
350:3-0.16
350:3-1.84
435:3-1 D14
226:3-1
40 45 50 55 60 65 70 75 80 85 90 95
Temperature (°C)
(mW/g)


400:5-0.5
400:5-1.5
400:1-1.5
400:1-0.5
45 50 55 60 65 70 75 80 85 90
Temperature (°C)
(mW/g)

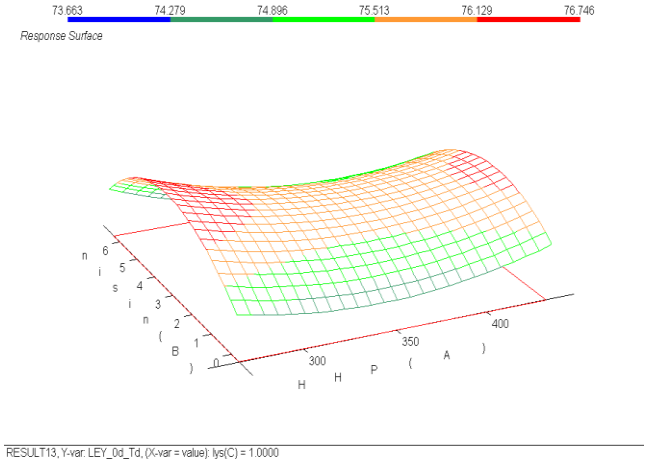
Day 21

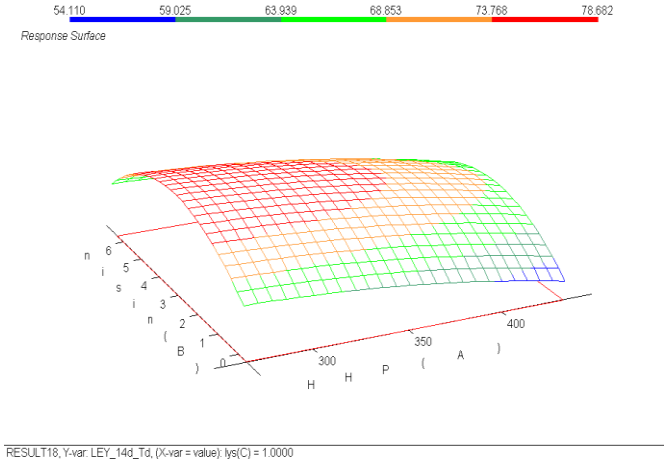

300:1-1.5
300:5-1.5
300:1-0.5
300:5-0.5
45 50 55 60 65 70 75 80 85 90
Temperature (°C)
(mW/g)

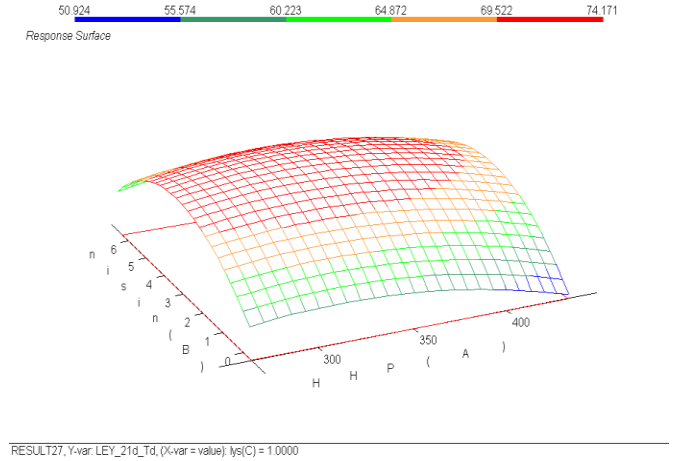

350:3-1
350:0-1
350:6.35-1
350:3-0.16
350:3-1.84
435:3-1 D14
226:3-1
40 45 50 55 60 65 70 75 80 85 90 95
Temperature (°C)
(mW/g)


400:5-0.5
400:5-1.5
400:1-1.5
400:1-0.5
45 50 55 60 65 70 75 80 85 90
Temperature (°C)
(mW/g)

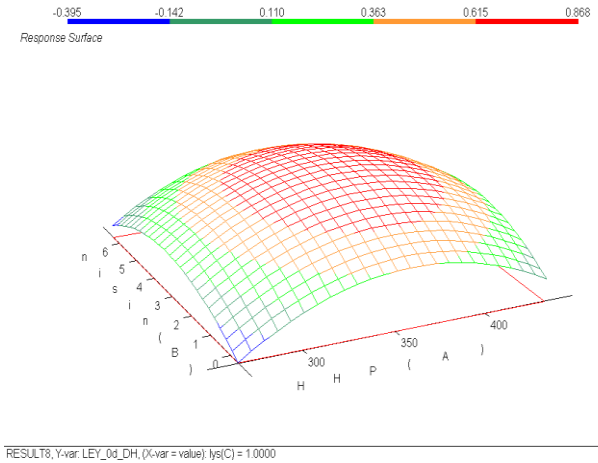
Response surface for denaturation temperature of HHP treated liquid egg yolk with nisin and lysozyme during storage

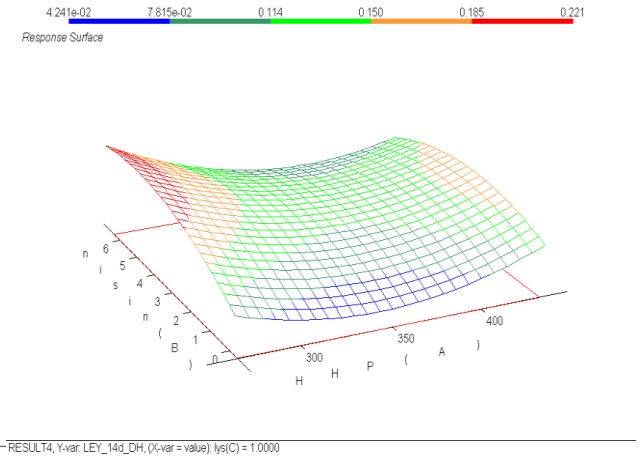


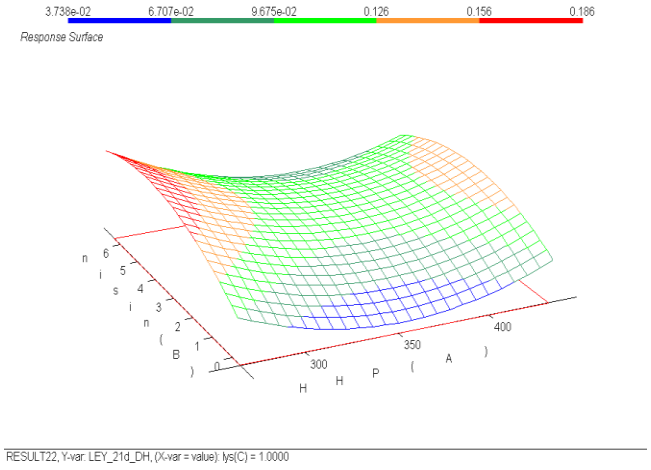




Response surface for denaturation enthalpy of HHP treated liquid egg yolk with nisin and lysozyme during storage

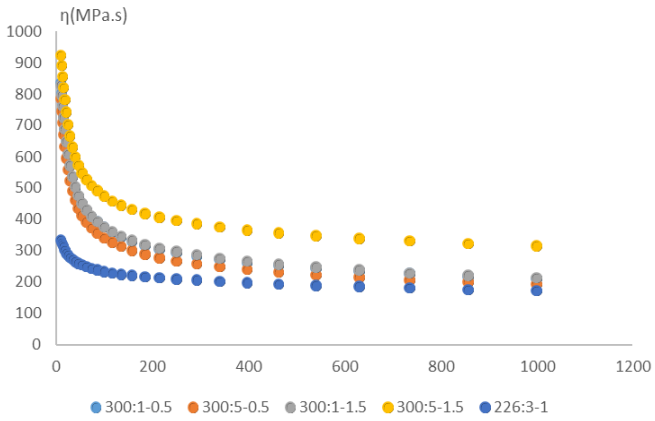


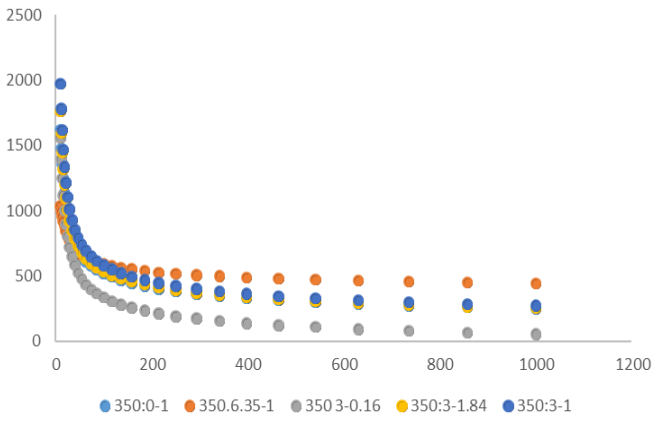


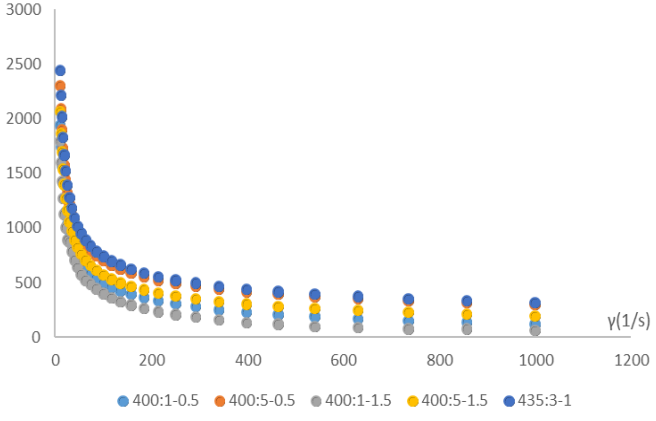


Apparent viscosity of HHP treated liquid egg yolk with nisin and lysozyme during storage

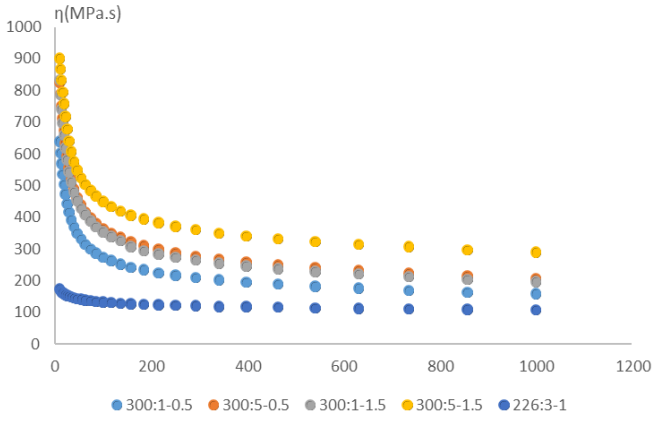
Day 0

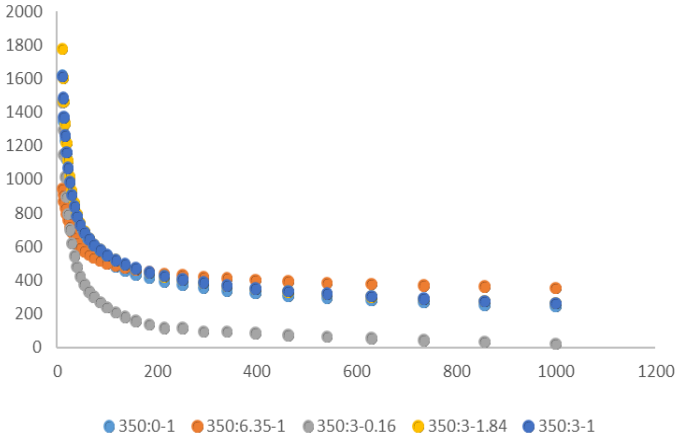


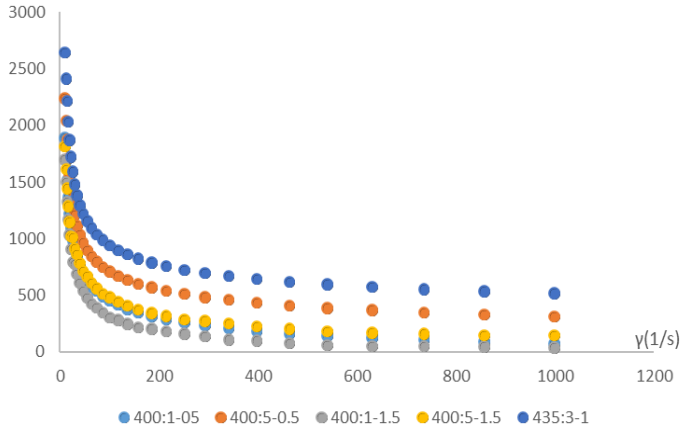




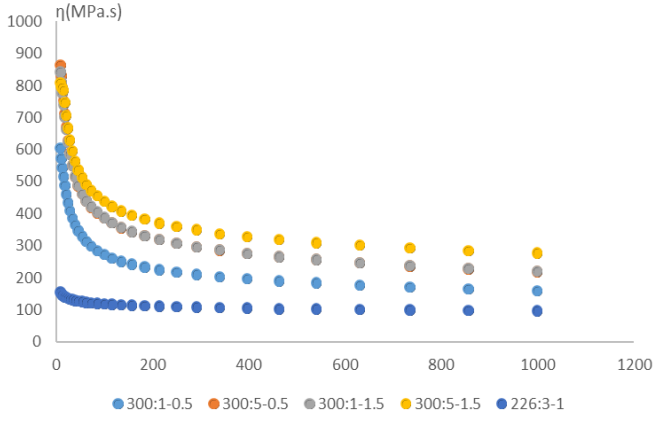
Day 14

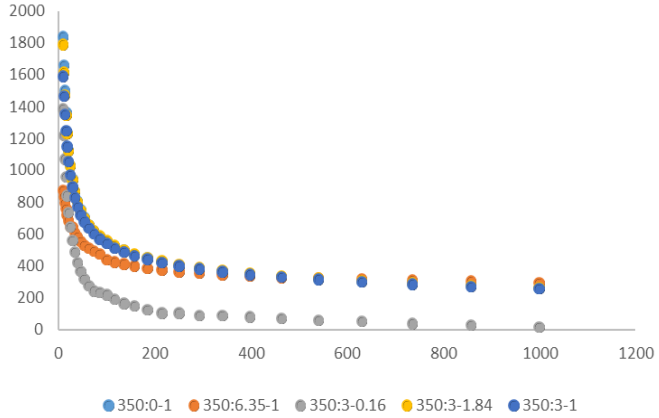






Day 21


η(MPa.s)
1000
900
800
700
600
500
400
300
200
100
0
0
200
400
600
800
1000
1200
300:1-0.5
300:5-0.5
300:1-1.5
300:5-1.5
226:3-1


2000
1800
1600
1400
1200
1000
800
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400
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0
0
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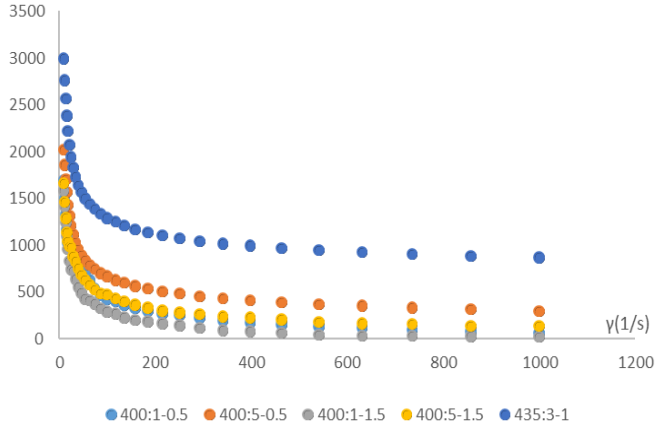
400
600
800
1000
1200
350:0-1
350:6.35-1
350:3-0.16
350:3-1.84
350:3-1


3500
3000
2500
2000
1500
1000
500
0
0
200
400
600
800
1000
1200
γ(1/s)
400:1-0.5
400:5-0.5
400:1-1.5
400:5-1.5
435:3-1

Acknowledgment

Glory and praise be to Allah Almighty for His immense blessings and help provided to me throughout my life including granting me the health, strength, and time to fulfil my desire to complete the Ph.D. degree. I express my gratitude to all who have contributed to this thesis.

First, and with deep gratitude and sincerity, I would like to thank my supervisors Dr. László Friedrich and Dr. Németh Csaba for offering me the precious opportunity to achieve my Ph.D. I am very grateful to them for their invaluable and continuous guidance, knowledge, encouragement, and patience during my study.

I also thank head of committee, committee members and reviewers for their valuable feedback, comments, and suggestions to improve the dissertation work. I want to thank all the faculty, staff members, and students in the Department of Livestock Products and Food Preservation Hungarian University of Agriculture and Life Sciences-Hungary for their help and kindness. I express my warm thanks to Ph.D. students and friends Dr. Adrienn Tóth, Karina Hidas, Dr. Noori Khabat, Meltem boylu, Reem Mourad and Majd Elayan in Hungary for their support and encouragement.

I am highly indebted to my parents Sadok Ayari and Hayette Youssfi who never had the opportunity to receive a formal education, but they grew the love of education in their children and dedicated their entire life for them. This work would not have been accomplished without their prayers, enormous love, and continuous support. I would like to thank my sisters Khaoula, Amira, and Eya for their support and encouragement during my study without their unconditional love, inspiration, and encouragement this journey would have been very difficult. Very special thanks to my Fiancé Adham Almadhoun who joined me during this journey for his support and assistance in every step.

I am grateful to the opportunity provided to me by the Tempus Public Foundation (Stipendium Hungaricum Scholarship), which made it possible for me to achieve my dream of doing and finishing my doctoral degree.

Thank you all

عيشك

Ayari Emna