EVALUATION OF INTERACTION OF PHENOLIC COMPOUNDS PRESENT IN EUROPEAN CRANBERRYBUSH JUICE WITH LACTIC ACID BACTERIA

by
Gizem Özan

Submitted to Graduate School of Natural and Applied Sciences
in Partial Fulfillment of the Requirements
for the Degree of Master of Science in
Biotechnology

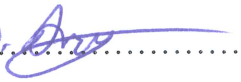
Yeditepe University
2016

EVALUATION OF INTERACTION OF PHENOLIC COMPOUNDS PRESENT IN EUROPEAN CRANBERRYBUSH JUICE WITH LACTIC ACID BACTERIA

APPROVED BY:

Assoc. Prof. F. Yeşim Ekinci ..... 
(Thesis Supervisor)

Prof. Gülgün Tınaz ..... 

Assist. Prof. Ali Özhan Aytekin ..... 

DATE OF APPROVAL: ..../..../2016

ACKNOWLEDGEMENTS

First and foremost, I would like to express my sincere thanks to my advisor Assoc. Prof F. Yesim Ekinci for her guidance, patience and continuous encouragement throughout my study. I also appreciate to her for giving me great opportunity to visit Jean Mayer USDA Human Nutrition Research Center on Aging in Boston, USA. It has been a great experience and honor to work in Antioxidant Research Laboratory and I also would like to thank to Dr. Jeffrey Blumberg and Dr. Oliver Chen my sincere appreciation for accepting me their Antioxidant Research Laboratory.

I am also grateful to work with Research Assistants, and special thanks to Neslihan Kayra, Bahar Hazal Yalcınkaya for their help and motivation throughout during my study.

I would like to acknowledge TUBITAK 2211 National Scholarship to support me during this thesis.

Last but not least, I would like to thank to my family who are Fikret Özan, Nurcan Özan and Sinan Özan for their endless love and support, which makes everything more beautiful.

ABSTRACT

EVALUATION OF INTERACTION OF PHENOLIC COMPOUNDS PRESENT IN EUROPEAN CRANBERRYBUSH JUICE WITH LACTIC ACID BACTERIA

European cranberrybush (ECB) (*Viburnum opulus* L.) fruit species contain high amount of phenolic compounds (PC). The influence of these compounds on lactic acid bacteria (LAB), present naturally in raw fruits and vegetables microbiota, remains unclear. The aim of this study was to investigate the effect of PC in ECB on different LAB. Firstly, the effect of chlorogenic acid (CA), main phenolic compound in ECB, on *Lactobacillus plantarum* ATCC 8014 growth was studied to understand the isolated role of CA present in ECB juice. Also, the growth effect of total ECB phenolics on *L. plantarum* ATCC 8014 and two other LAB strains, *L. plantarum* Lp-115 and *L. brevis* ATCC 8287 were evaluated by addition of different concentration of fresh ECB juice in growth media. The growth kinetic parameters of LAB strains were calculated at different concentrations of CA (2.12-0.71-0.53-0.35-0.1-0.05-0.025 mg/ml) and (100-50-20-10-5 %) of fresh ECB juice added MRS medium in microplates and static batch cultures in tubes. CA showed inhibitory effects at high concentrations (2.12 mg/ml), whereas cell density of *L. plantarum* 8014 increased at low concentrations (0.71-0.53-0.35-0.01mg/ml). Similar to CA, ECB juice showed inhibitory growth effects at high concentrations (100-50%) to all LAB strains while strains was able to grow in ECB juice at lower concentrations (20-10 and 5%). The PC composition analysis of freshly squeezed ECB juice by HPLC-ECD used in growth experiments showed that catechin (4.16±0.48 mg/ml) was the main compound following CA (3.42±0.04 mg/ml). The addition of 0.35 mg/ml CA and 5 % ECB juice increased the cell density (ΔOD) of the cultures whereas there were no significant change in the μ_{max}, log cfu/ml, dry weight values compared to control cultures in scale up experiments. This study was provided detailed valuable information about the interaction ECB phenolics and *Lactobacillus* strains *in vitro*, the growth mechanism and the effect of other PC in ECB juice on LAB is still not clear and needs to be studied in future studies.

ÖZET

EUROPEAN CRANBERRYBUSH SUYU İÇERİSİNDE BULUNAN FENOLİK MADDELERİN LAKTİK ASİT BAKTERİLER İLE ETKİLEŞİMİNİN DEĞERLENDİRİLMESİ

European cranberrybush (ECB) (*Viburnum opulus* L.) meyve türleri yüksek miktarda fenolik madde içermektedir. Bu maddelerin, ham meyve sebzelerin doğal mikrobiyotasında bulunan laktik asit bakteriler (LAB) üzerinde etkisi belirsizdir. Bu çalışmanın amacı, ECB suyunda bulunan fenolik maddelerin farklı LAB suşları üzerindeki etkilerini araştırmaktır. İlk olarak, ECB içerisinde başlıca bulunan fenolik maddelerden olan klorojenik asitin (CA) *Lactobacillus plantarum* ATCC 8014 üzerindeki büyüme etkisi ECB suyu içerisindeki CA nın izole rolunu anlamak için çalışılmıştır. Ayrıca, farklı konsantrasyonlarda taze ECB suyu büyüme ortamına eklenerek toplam ECB fenoliklerin *L. plantarum* ATCC 8014 ve diğer iki LAB suşları üzerinde büyüme etkisi değerlendirilmiştir. Farklı konsantrasyonlarda MRS besiyeri içerisinde mikroplakalara ve tüplerin içerisinde statik kesikli kültürlere eklenmiş CA (2.12-0.71-0.53-0.35-0.1-0.05-0.025 mg/ml) ve taze ECB suyunun (100-50-20-10-5 %) LAB suşları üzerinde büyüme kinetik parametreleri hesaplanmıştır. Yüksek konsantrasyonlarda CA (2.12 mg/ml) büyüme engelleyici etki gösterirken *L. plantarum* 8014 hücre yoğunluğu düşük konsantrasyonlarda (0.71-0.53-0.35-0.01 mg/ml) artmıştır. CA ya benzer olarak, ECB suyu da yüksek konsantrasyonlarda (100-50 %) LAB suşlarının hepsi üzerinde büyüme engelleyici etki gösterirken, düşük konsantrasyonlarda (20-10 ve 5 %) suşlar büyüme göstermişlerdir. büyüme deneylerinde kullanılan taze sıkılmış ECB suyunun fenolik madde kompozisyon analizi HPLC-ECD ile başlıca bulunan madde kateşin (4.16±0.48) ve takibinde CA (3.42±0.04) olarak belirlenmiştir. 0.35 mg/ml CA ve % 5 ECB suyu kültürlere eklendiğinde hücre yoğunluğu (ΔOD) artarken, μ_{max}, log cfu/ml ve kuru ağırlık değerlerinde kontrole kıyasla anlamlı değişim görülmemiştir. Bu çalışma ECB fenolikleri ve *Lactobacillus* türlerinin etkileşimi hakkında ayrıntılı değerli bilgi sağlamaktadır.

TABLE OF CONTENT

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ABTS	2,2’-azonobis(3-etilbenzothiazoline-6-sulfonat
ATCC	American Type of Culture Collection
CA	Chlorogenic acid
CFU	Colony forming unit
BM	Basal Medium
ECB	European cranberrybush
ECD	Electrochemical detector
DPPH	2,2-diphenyl-1-picrylhydrazyl
FRAP	Ferric reducing antioxidant power
GAE	Gallic acid equivalent
HAT	Hydrogen atom transfer
HPLC	High Performance Liquid Chromatography
LAB	Lactic acid bacteria
MRS	Man, Rogosa and Sharp
OD	Optical density
ORAC	Oxygen radical capacity
PAD	Phenolic acid decarboxylase
PB	Phospate buffer
PC	Phenolic compounds
PDA	Photodiode array
RC	Regenerated cellulose
TE	Trolox equivalent
μ	Specific growth rate
ΔOD	Change in optical density
λ	Lag time
t_d	Doubling time

1. INTRODUCTION

Berry fruits have high amount dietary phenolic compounds sources that are natural compounds present in many foods are responsible for giving some sensorial and nutritional characteristics of the food products and also they may play an important role in the maintenance of human health and prevention of several diseases such as cardiovascular diseases, diabetes, cancer, and obesity. European cranberrybush (*Viburnum opulus* L.) fruits are rich in PC such as chlorogenic acid, flavonoids and anthocyanins.

Raw fruits and vegetables have Lactic acid bacteria (LAB) in their autochtonous microbiota. *Lactobacillus plantarum* and *Lactobacillus brevis* are the most frequently used cultures in the food products fermentation. Fermented food products have high amount of PC. There are interactions between LAB and PC however, the effect of these compounds on growth and viability of LAB remains unclear. While some PC stimulate LAB growth and activity, others have inhibitory effect depends on types of microbial strain, different structure and applied concentration of PC.

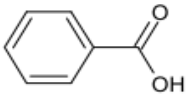
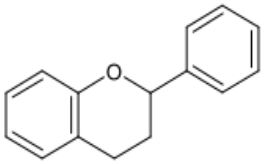
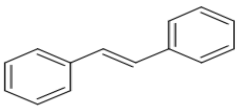
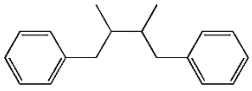
In this study, the interaction between PC present in fresh European cranberrybush (ECB) juice on LAB strains. First, the effect of chlorogenic acid, main phenolic compound in ECB, on *Lactobacillus plantarum* ATCC 8014 growth to understand the isolated role of CA present in ECB juice. Then, the growth effect of total ECB phenolics on *Lactobacillus plantarum* ATCC 8014, *L. plantarum* Lp-115 and *L. brevis* ATCC 8287 were evaluated by addition of different concentration of fresh ECB juice in growth media. After microplate analysis, optimized ECB juice and CA concentrations added LAB cultures growth were evaluated in static batch culture system in tubes to evaluate the changes in the number of LAB, OD_{600}, pH, dry weight, sugar utilization and lactic acid production during incubation period. Compositional analysis of ECB juice used in fermentation was also done to determine sugar, organic and phenolic contents. This study provides growth effect of different concentration of ECB juice and CA on *Lactobacillus* species.

2. LITERATURE REVIEW

2.1. PHENOLIC COMPOUNDS

Phenolic compounds (PC), naturally found in many foods, are responsible for giving some sensorial and nutritional characteristics of the food products such as fruits, vegetables, cereals, tea, coffee and wine [1, 2, 3]. PC are chemically constituted by hydroxylated phenyl moieties [4, 5]. Based on their carbon skeleton, number of phenolic rings and substituting groups, PC can be classified into four major groups; phenolic acids, flavonoids, stilbenes and lignans [6].

Table 2.1. Phenolic compounds classification based on chemical structure

Classification of Phenolic Compounds	Carbon Skeleton	General Chemical Structure	Example
Phenolic acids			
Hydroxybenzoic acid	C_6-C_1		Gallic acid
Hydroxycinnamic acid	C_6-C_3		Chlorogenic acid
Flavonoids			
Flavonols	C_6-C_3-C_6		Quercetin
Flavones	C_6-C_3-C_6		Apigenin
Isoflavones	C_6-C_3-C_6		Genistein
Flavanones	C_6-C_3-C_6		Naringenin
Anthocyanidins	C_6-C_3-C_6		Cyanidin
Flavanols	$(C_6$-C_3-$C_6)_n$		Catechin
Stilbenes	C_6-C_2-C_6		Resveratrol
Lignans	$(C_6$-$C_3)_2$		Secoisolariciresinol

Phenolic acids and flavonoids are present higher amounts than other PC compounds in diet [6]. While phenolic acids are formed by two classes of hydroxybenzoic and hydroxycinnamic acid,

flavonoids are consisted of many subclasses including flavanones, flavones, flavonols, flavan-3-ols, dihydroflavonols, isoflavones, anthocyanidins and proanthocyanidins according to their structural differences [7]. Hydrolysable tannins, gallotannins and ellagitannins are example of complex structures of hydroxybenzoic acids.

Hydroxycinnamic acids such as *p*-coumaric, caffeic and ferulic acids, are more common than hydroxybenzoic acids. In foods, hydroxycinnamic acid, rarely found in free form, are generally esterified with carbohydrates and organic acids. For example, chlorogenic acid is formed with the combination of caffeic and quinic acids that is mainly found in many fruits and vegetables and particularly abundant in coffee [8].

Epidemiological studies have showed that the PC, especially from small colorful fruits have potential in the maintenance of human health and prevention of several diseases such as cardiovascular diseases, diabetes, cancer, and obesity due to exhibiting anti-inflammatory, anti-viral, anti-allergic, anti-carcinogenic, antioxidative and antimicrobial activities [4, 9, 10].

Biological properties of PC depend on amount of consumed and their bioavailability in human body [11]. Absorption of PC varies due to their different structures such as molecular weight, glycosylation and esterification which mostly affect intestinal absorptivity. PC may be readily absorbed in the small intestine due to the their low molecular weight (MW) or reach the colon unchanged due to high MW as close to 40.000 Da [5, 12]. The high MW of tea theaflavins (MW 568) and proanthocyanidins, flavonoid polymers with varying degrees of polymerization and MW of 578 or above, are almost not absorbed in the gut [13]. Apart from MW of PC, glycosylation has impact factor on intestinal absorption. For example, absorption of quercetin glucosides present in onions, a rich source of quercetin-4-O-glucoside and quercetin-3,4’-O-diglucoside, was higher (52%) than that of quercetin aglycone (24%) [13, 14]. The study of Morand et. al. [15] showed that 3-*O*-β-glucoside of quercetin was better absorbed than quercetin in rats. However, the absorbtion of quercetin rhamnosides were poor under the same conditions because rhamnosides needs deglycosylation by the colonic microbiota and this leads to delay absorption when compared to the glycosides [16]. Finally, esterification is also affected on

absorption of polyphenols. Galloylated catechin and chlorogenic acid can be shown an example of esterified phenolic compounds. In the study of Warden et al. [17], after black tea consumption, the recovery of non galloylated catechins was about 10 fold higher than the galloylated catechins in human urine. Caffeic acid, which is hydroxycinnamic acids, is much better absorbed than chlorogenic acid, its ester with quinic acid. Absorption rates in human were 95% and 33% for caffeic acid and chlorogenic acid respectively [18].

The biological activities of the PC also depend on the interaction of PC with colonic microbiota. Many different microbial species inhabit the gastrointestinal tract up to 10^{12} cells per gram. Microbial metabolism of phenolic compounds forms new metabolites with different bioconversion pathways depend on phenolic structure and microbial strain [22]. The remaining unabsorbed polyphenols may accumulate in the large intestine and microbial enzymes takes place to break down of polyphenols into low molecular weight of phenolic metabolites for being absorbable bioactive compounds with beneficial health effect [5]. For example, major part of chlorogenic acid escapes absorption in small intestine and reaches the colon. Colonic microbiota is only site for chlorogenic acid metabolism [10]. Absorption and bioavailability of chlorogenic acid and its metabolites changes depend on microbiota composition and activity [19]. Microbial metabolism of chlorogenic acid occur first hydrolysis to quinic acid and caffeic acid, then able to form metabolites including m-coumaric and derivatives of phenylpropionic, benzoic and hippuric acids [20].

2.2. LACTIC ACID BACTERIA

Certain species of *Lactobacillus*, *Lactococcus* (*Streptococcus*), *Enterococcus*, *Leuconostoc*, *Bifidobacterium* and *Pediococcus* referred as Lactic acid bacteria (LAB) has potential in food fermentation and human health [23, 24]. Fermentation is a simple, natural, valuable and one of the oldest biotechnological processes. LAB can maintain and improve the safety, nutritional, sensory and shelf life properties of many food products. LAB, the main microorganism responsible for the fermentation, are important constituents of dairy and non-dairy fermented food products and are used as starter cultures during fermentation. They are also natural part of

the human gastrointestinal microflora to achieve beneficial role in colonic microbiota [3, 11, 25]. In food industry, the production of many fermented foods and beverages was done with a spontaneous fermentation due to presence of LAB as natural microbiota on raw fruits and vegetables. Direct addition of selected starter cultures to the food matrix is also preferred due to the need for the standardization of final product and improvement of the nutritional, organoleptic and shelf-life properties of food [25, 26]. *Lactobacillus plantarum, Lactobacillus brevis, Lactobacillus acidophilus, Lactobacillus johnsonii, Lactobacillus hilgardii* and *Oenococcus oeni* are the important species of LAB due to their presence in high amount in many foods [3].

2.3. INTERACTION BETWEEN PHENOLIC COMPOUNDS AND LACTIC ACID BACTERIA

Lots of studies showed that there are interactions between LAB and PC present in foods [8, 27, 28, 30, 31, 32, 33]. Epidemiological and experimental studies showed that interaction between phenolic compounds and LAB has potential effect on health [3]. PC may act as either stimulating or promoting substrates on growth of LAB whereas interestingly inhibiting pathogens or LAB can metabolize phenolics during their growth phase to obtain energy while creating new bioactive metabolites [29]. The influence of PC on bacterial growth varies depending on types of microbial strain, different structure of PC and applied concentration of phenolics [29, 34, 35].

2.3.1. Metabolism of PC by LAB

Apart from stimulatory effects of phenolic compounds, several authors have reported metabolism of PC by LAB [3, 8, 30, 31, 32, 44, 45, 46, 47]. LAB that are able to metabolize phenolic compounds, isolated from fermented foods containing high amount of PC such as wine, whisky, sorghum and olives [35, 48, 44, 48, 49, 50]. In fact, metabolism of phenolic compounds by LAB may lead to obtain energy during their growth phase, besides can enhance consumption of nutrients to LAB [11]. The ability to metabolize PC and the tolerance of LAB are strain specific [46, 47, 49, 50]. Degradation of phenolic compounds occur when microorganisms has

related enzymes that able to degrade such as reductase, decarboxylase, and esterase as presented in Table 2.2. Enzymes take place to break down of polyphenols into low molecular weight of phenolic metabolites for being absorbable bioactive compounds with beneficial health effect [5]. It has been reported that *L. plantarum* and *L. brevis* are able to decarboxylate phenolic acids to vinyl derivatives with the presence of phenolic acid decarboxylase (PAD) enzyme [32, 46]. These strains are able to degrade hydroxycinnamic acids which are *p*-coumaric and caffeic acids [8]. In addition, *L. plantarum* also showed an uncharacterized inducible acid phenol reductase activity, able to reduce vinyl derivatives into ethyl derivatives, and to metabolize *p*-coumaric acid into phloretic acid [5, 51]. *L. plantarum* is also able to degrade hydrolsable tannins (tannic acid) [30]. Degradation of several commercial tannic acids evaluated and found that tannic acid was hydrolyzed to gallic acid, which was then decarboxylated producing pyrogallol through the action of tannase and decarboxylase enzymes. Similarly, Landete et al. [44] analyzed the *L. plantarum* metabolic activities against phenolic compounds found in olive products, and found that oleuropein was metabolized to hydroxytyrosol by using β- glucosidase and esterase, while protocatechuic acid was decarboxylated to catechol by using decarboxylase enzyme [8]. Similarly, *L. hilgardii* had ability to degrade gallic acid and catechin, producing catechol, pyrogallol, protocatechuic acid and trace amounts of *p*-hydroxybenzyl alcohol, *p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde, acetovanillone and homovanillic acid [33]. Sanchez-Maldonado et al. [47] analyzed chlorogenic, caffeic, *p*-coumaric, ferulic and protocatechuic acid metabolism by *L. plantarum* TMW 1.460, *L. hammesii* DSM 16381, *L. fermentum* FUA 3165 and *L. reuteri* FUA 3168. The results of the same study showed that *L. plantarum* degraded all phenolic acids except chlorogenic acid, only 4% chlorogenic acid degraded into caffeic acid whereas *L. reuteri* degraded only chlorogenic acid into caffeic acid, metabolites of chlorogenic acid *L. plantarum*, *L. hammesii* and *L. fermentum* decarboxylated caffeic acid to vinylcatechol. Decarboxylation of protecatechuic acid by *L. plantarum* and *L. hammesii* formed catechol. In another study of Bel-Rhlid et al. [52] investigated the potential of *L. johnsonii* to metabolize of chlorogenic acid from green coffee extract and observed that *L. johnsonii* able to transform chlorogenic acid into caffeic and quinic acid by cinnamoly esterase enzymes, then decarboxylation of caffeic acid into 4-vinylcatechol with hydroxycinnamic decarboxylase activity.

Table 2.2. Enzymes involved in phenolic compounds degradation

Microorganisms	Enzyme involved	Phenolic Compound	Compound produced	Reference
L. plantarum	Phenolic acid decarboxylase (PAD)	*p*-coumaric acid caffeic acid	vinyl phenol vinyl catechol	Rodriguez et al. [32]
		ferulic acid	vinyl guaiacol	Rodriguez et al. [8]
	reductase	*p*-coumaric acid	ethyl phenol	Rodriguez et al. [8]
		Ferulic acid	ethyl guaiacol	
	uncharacterized reductase	*p*-coumaric acid	phloretic acid	Barthelmebs et al. [51]
	tannase	Tannic acid	gallic acid	Rodriguez et al. [30]
	decarboxylase	Tannic acid	pyrogallol	Rodriguez et al. [31]
	decarboxylase	protocatechuic acid	catechol	Rodriguez et al. [8]
	Gallate decarboxylase	Gallic acid	pyrogallol	Rodriguez et al. [8]
	β- glucosidase esterase	oleuropein	hydroxytyrosol	Landete et al. [44]
	Benzyl alcohol dehydrogenase	Benzyl alcohol	benzaldehyde	Landete et al. [45]
		Cinnamyl alcohol	cinnamyl aldehhyde	
L.brevis	PAD	*p*-coumaric acid	vinyl phenol	Curiel et al. [46]
	Gallate decarboxylase	Gallic acid	pyrogallol	
L. fermentum	decarboxylase	caffeic acid	vinylcatechol	Sanchez-Maldonado et al. [47]
L. johnsonii	cinnamoyl esterase	chlorogenic acid	Caffeic and quinic acid	Bel-Rhlid et al. [52]
	hydroxycinnamic decarboxylase	caffeic acid	4-vinylcatechol	

2.3.2. Effect of PC on LAB Growth

LAB performs an important role in the production of fermented foods and shows a beneficial role being part of colonic microbiota. Several studies were done related to effect of phenolics on growth of LAB and suggest that phenolic compounds may act as promoting effect of growth, proliferation or survival for beneficial bacteria. Rozès and Peres, [36] reported the effect of caffeic and ferulic acid on the *L. plantarum* viability at different concentrations (0.1, 0.2, 0.5 and 1 g/l) and found that viability of *L. plantarum* was concentration dependent. In the same study it was also found that while low tannin concentrations (0.1 or 0.2 g/l) did not inhibit *L. plantarum* growth, high amounts of tannin (1 g/l) delayed bacterial growth. Salih et al. [37] analyzed the effect of hydroxycinnamic acids, their quinic esters and quinic acid, a non-phenolic acid, on the growth of *L. plantarum* and showed that bacterial growth was only affected by hydroxycinnamic acid at the concentrations up to 3 mM. Alberto et al. [33] studied the effects of different concentrations of gallic acid (100 mg/l) and catechin (200 mg/l) present in wine on the growth of *L. hilgardii* and showed that phenolic compounds not only stimulated growth rate, but also increased slightly final biomass. Similarly, the study of Hervert-Hernandez et al. [29] showed that phenolic extracts from grape seed and grape pomace and various phenolic standards selected based on phenolic content of grape pomace; caffeic acid, gallic acid, tannic acid, catechin, epicatechin and quercetin had no inhibition. Both grape extracts tended to show stimulatory effect on growth of *L. acidophilus* and reported that grape pomace extract (1 mg/ml) increased *L. acidophilus* biomass. The influence of catechin, chlorogenic acid and quercetin on *L. casei* was evaluated by Duda-Chodak et al. [38] and results showed that while catechin (100-400 μM) and chlorogenic acid (400 μM) had a stimulatory effect on *L. casei* growth, quercetin (25-50 μM) showed an inhibitory effect when incubation time longer than 6 h. The *L. plantarum* was also demonstrated growth promoter effect in the presence of flavanol and flavanol rich extracts [39, 40]. Lopez de Felipe et al. [39] evaluated the effect of catechin (100 and 200 mg/l) on growth of *L. plantarum* strain RM71, isolated from red wine, and they found that catechin stimulated the growth of *L. plantarum* strain RM71 by promoting quicker sugar consumption, increasing the extension sugar utilization and stimulating malic acid decarboxylation. Lag period of *L. plantarum* during growth was shorter when the catechin was added into medium

and catechin increased cell density compared to controls because of increased extension of sugar utilization. Likewise, Curiel et al. [40] reported that quercetin promoted quicker growth of *L. plantarum* strain RM71. They found that there was dose dependent effect; lag phase in cultures supplemented with quercetin at 10 mg/l was shorter than in cultures grown on 5 mg/l quercetin. The time course of sugar consumption and lactic acid production data showed that quercetin promoted quicker sugar consumption and lactic acid production compared to control.

2.3.2.1. Effect of PC on growth kinetics of LAB

Bacteria need to liquid nutritious medium and optimum environmental conditions to grow. Increase in the number or mass of bacteria measure as a function of time to draw a growth curve if growth medium has all sufficient nutrients and environmental parameters such as temperature, aerobic or anaerobic conditions are optimum for growth. Typical growth curve of bacteria has four phases in batch cultures including "lag phase, exponential or log phase, stationary phase and death phase" as shown in Figure 2.1.

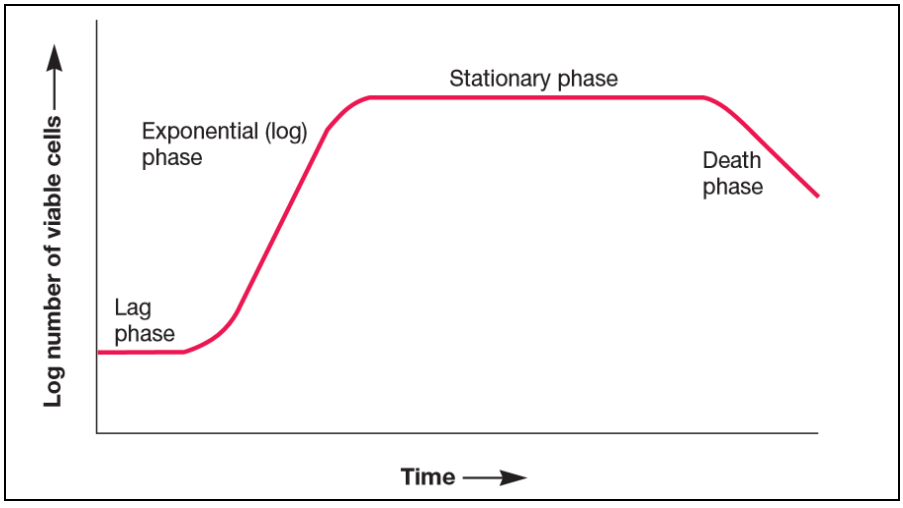


Figure 2.1. Bacterial growth curve in batch system

Lag phase is the transition to the exponential phase where cells are growing with a constant rate, result in an exponential increase in the number of cells present. The number of cells increases

as 2^n, n is the number of division. There are main parameters to characterize the bacterial growth curve which are lag phase duration (λ), specific growth rate (μ) and doubling time (td). The λ is duration of the lag phase. During exponential phase, growth rate is not constant over the growth period, increases until a constant growth rate is reached and the change in the number of cells per time gives growth rate. Doubling time (td) that is the time taken for population to double in number during exponential phase is calculated from the linear region of growth curve and calculated as,

$$\mu = \frac{ln2}{td} \tag{2.1}$$

where μ is the specific growth rate (1/time) and td is the doubling time.

Stationary phase is the third phase of growth and can be defined as there is no net growth in this phase due to mostly depletion of nutrients and formation of waste caused to inhibit cell growth or are toxic to cells. The final phase is the death phase. Growth rate starts to decrease and microbial death rate described as,

$$\frac{dX}{dt} = -k_d X \tag{2.2}$$

where k_d is the specific death rate.

The maximum specific growth rate (μmax) is an important parameter to evaluate microbial growth under batch conditions [111]. Growth rate can be calculated with data obtained from the cell density (colony forming unit (cfu)/ml) at a series of time points or the number of cells per ml of culture is estimated from turbidity of culture that is measured by spectrophotometer and is estimated as optical density (OD) [21, 42, 95,112]. Bacterial culture is a suspension of bacterial cell particles and measure absorbance that is proportional to the number of cells per ml. Absorbance measurements are rapid, non-destructive, inexpensive and relatively easy to do

compare to classical viable count technique for monitoring growth of microbial cultures [21, 112].

Numbers of different growth models have been proposed and used in many studies to calculate bacterial growth rate such as Gompertz [105], Richards [113], Stannard et al. [106], Schnute [107] and logistic model [108]. Almost all growth models have different numbers of parameters, therefore Zwietering et al. [41] re-parameterized all models in terms of growth rate (μ), lag time (λ) and asymptote (A) is the difference in cell density, which are parameters with biological meaning. Zwietering et al. [41] also compared all models statistically and found that the modified Gompertz model was statistically sufficient to describe the growth curve of *L. plantarum.* Modified Gombertz equation has been commonly used to fit the logarithm of cell concentration to calculate the maximum specific growth rate of bacteria. The equation is described as,

$$\mathrm{y} = A \times \exp\left\{-\exp\left[\frac{\mu_{max} \times e}{A}(\lambda - t) + 1\right]\right\} \tag{2.3}$$

where N is the cfu/ml at any time t, N_0 is the initial cfu/ml, A is the log increase in cell population, μ_{max} is the maximum specific growth rate of cell population and λ is lag phase.

Hall et al [42] designed a program that calculates growth rate of microorganisms while growing in microplate titer. The equation used to calculate growth rate:

$$\frac{dN}{dt} = \mu N \tag{2.4}$$

where N is the number of cell at time *t* and μ is the first-order growth rate constant that is growth rate μ is the reciprocal time units, t is in hours and the growth rate is the μ h^{-1}.

$$\frac{dN}{N} = \mu t \tag{2.5}$$

When the equation was integrated from *t*=0 to *t*=*t*, the new equation formed as:

$$\ln \frac{N_t}{N_0} = \mu(t - t_0) \tag{2.6}$$

and μ is the slope of ln *N* versus t.

The PC and LAB interaction studies calculated growth rate of LAB to evaluate the influence of different compound at different concentrations on different LAB strains. PC affect the growth of LAB into two ways, can be caused to either increase or decrease in growth rate. Alberto et al. [48] studied with gallic acid and catechin to understand the effect on *L. hilgardii* growth at different PC concentrations and different medium. The growth rate (μ_{max}) was higher in gallic acid added medium (0.40, 0.46 and 0.41 /h at 50, 100, 200 mg/l, respectively) than in basal medium (0.36 /h). In catechin added medium, growth rate of *L. hilgardii* was higher compared to control (basal medium). Growth rate increased up to 200 mg/l catechin concentration, μmax were 0.46 and 0.52 at 100 and 200 mg/l, then decreased at 400 mg/l catechin (0.44 /h). The highest growth rate was seen in basal medium added with 100 mg/ml gallic acid and 200 mg/ml catechin. Then, the best concentrations also tested in FT80 and MRS with tomato juice added medium, in both medium PC added growth rate were higher than medium without added phenolic compounds. Growth rate were 0.36, 0.45 and 0.52 /h at control, gallic acid and catechin added MRS medium, respectively. The effect of PC naturally found in wine on growth and inactivation of *L. hilgardii* and *O. oeni* examined by Campos et al. [35]. The *p*-coumaric, caffeic, ferulic, gallic and protocatechuic acids showed inhibitory effect on *O. oeni.* The *L. hilgardii* grew in presence 100 mg/l PC medium. However, when the concentration increased growth rate decreased and lag phase increased at 500 mg/l PC. Figueiredo et al. [109] investigated effect of various phenolic aldehydes and flavonoids on wine lactic acid bacteria, *L. hilgardii* and *O. oeni*. Different concentrations tested and the effect on LAB was dose dependent. All tested PC

showed inhibitory effect on *O. oeni*. The *L. hilgardii* grew in PC added medium, but the growth rate decreased in presence of coniferaldehyde at 500 mg/L. Myricetin did not affect the growth of *L. hilgardii* at tested concentration (10, 20, 40 mg/L), quercetin and kaempferol caused to decrease growth rate at 40 mg/L. The effect of quercetin on *L. plantarum* reported in the study of Curiel et al. [40]. Quercetin (5 mg/l and 10 mg/l) tested in both pH 5.5 and 6.5 medium. Quercetin promoted *L. plantarum* growth faster in a pH and dose dependent. The presence of 10 mg/l quercetin the lag phase shortened with respect to control and cultures grown in 5 mg/l quercetin. Medium was added with 100 and 200 mg/l catechin to investigate the interaction between *L. plantarum* and catechin and found that lag phase shortened in presence of catechin [110]. Tabasco et al. [27] evaluated the effect of flavan 3-ol compounds on growth of LAB and bifidobacteria. Growth responses of various LAB and bifidobacteria strains were isolated from dairy products and milk of healthy mothers, to grape seed extracts at 0.25, 0.5 and 1 mg/ml investigated. The *L. plantarum* and *L. casei*, depending on the strain showed the maximum growth rate in presence of grape seed extract compared to their control. Various LAB strains grew in final concentration 2 mM caffeic, *p*-coumaric or ferulic acid supplemented in chemically defined basal medium [111]. Growth of most strains did not significantly affected in presence of PC compared to the growth of control. *Weissella cibaria* and *L. rossiae* showed the highest growth rate during growth in medium.

2.4. BERRY FRUITS

Berry fruits such as blackberry (*Rubus sp*.), bilberry (*Vaccinum myrtillus*), blackcurrant (*Ribes rugrum*), blueberry (*Vaccinum corymbosum*), chokeberry (*Aronia melanocarpa*), cranberry (*Vaccinium macrocarpon*), European cranberry (*Viburnum opulus*), bayberry (*Myrica sp*.), raspberry (*Rubus ideaus*), black raspberry (*Rubus occidentalis*) and strawberry (*Fragaria ananassa*) have high amount of PC and are usually consumed as fresh or processed in human diet [53]. The World Health Organization indicates the importance of antioxidant activity of PC, especially high content in small colorful fruits, for prevention of the most important health problems such as cardiovascular diseases, obesity, diabetes and cancer [9, 10]. Berry phenolics include phenolic acids, flavonoids, stilbenes and tannins [10, 54]. The concentration of PC in

berries varies depend on species, variety, geographic region, storage conditions, climate and ripeness [10].

2.4.1. American Cranberry, *Vaccinium macrocarpon*

American cranberry species, *Vaccinum macrocarpon*, belongs to the *Ericaceae* family are commonly consumed berry fruits as fruit juice and have been considered as healthy food for centuries due to the presence of various PC including anthocyanins, proanthocyanidins (PACs), flavonols and flavan 3-ols [55]. Total flavonol content of cranberries range from 200-400 mg/kg, anthocyanins content 136–1710 mg/kg and PACs content 4188 mg/kg in fresh fruits [55]. Anthocyanin content of ripe cranberry fruit is higher than flavonol concentrations [67], however flavonol content is about three fold higher than anthocyanins present in cranberry juice [68, 69]. The reasons of low anthocyanin levels in juice could be incomplete anthocyanin extraction or susceptibile to oxidative degradation [55]. Anthocyanins are responsible for the red color of cranberry. The major anthocyanins were found as 3-monogalactosides, 3-monoarabinosides, and 3-monoglucosides of cyanidin and peonidin [56, 57, 58]. In addition to these anthocyanins, malvidin, pelargonidin, delphinidin and petunidin were also identified [59]. The level of anthocyanins varies from 13.6 to 140 mg/100 g in whole raw cranberries depend on size of fruit, ripeness, variety and other factors [55, 60, 61, 62, 63]. Cranberry juice cocktails contain lower amounts of anthocyanins, ranging between 1.2 to 2.5 mg/100ml, comparing with cranberry fruit [55]. PACs that is also called as condensed tannins are responsible giving astringent or bitter tastes to the cranberry, wine, cocoa and other PAC rich foods [55, 64]. Gu et al. [65] reported 23.1 mg/100 mL total PAC in cranberry juice cocktail and 418 mg/100 g in fresh cranberries. Flavonols in cranberries were present, mostly in glycosylayed forms of quercetin, myricetin and kaempferol. Flavan-3-ols is another phenolic compound present in cranberry fruit and juice, but there was contradict data. Cunningham et al. [67] determined that epicatechin is the primary flavanol in cranberry juice and Chen et al. [69] found that catechin is only flavanol PC presents in commercial cocktail of cranbery juice and cranberry juice.

2.4.2. European Cranberrybush, *Viburnum Opulus*

European cranberrybush (ECB) species, (*Viburnum opulus L.*), belonging to the *Adoxaceae* family, (formerly *Caprifoliaceae*) is grown in Europe, North Africa, North Asia and also in the central zone of Russia [70, 72]. They are red edible berries that contain one seed, ripened in August- September and remained through winter. There are several names of *V. opulus* known and used in different literature sources such as European cranberrybush, snowball tree, guelder rose, crampbard and gilaburu [71, 72, 73, 74, 75].

The berries have bitter and highly astringent taste and some aroma compounds which are not liked by the consumers. The 3-methyl- and 2-methly-butanoic acids and 2-octanone were reported as main aroma compounds present in ECB fruits [74]. Because of the astringent taste, they generally have been used as sauces, jellies, and drinks which prepared after ECB fruits kept in water containing jar for couple of months [73, 72, 75, 77]. In Turkey, ECB is mostly grown in Kayseri region, where the fruits are consumed as a beverage locally called as gilaburu juice [78]. Gilaburu juice is prepared traditionally by keeping fresh fruits in jars filled with water then holding at room temperature for three to five months. Subsequently, fruits kept in water are squeezed and some water or sugar is added to the juice to suppress its astringent taste before consumption. Sagdic et al [77] stated that while keeping the fruits in water, fermentation takes place spontaneously by the LAB present in ECB fruit. The aroma compounds of gilaburu juice kept in water for 4 months were investigated during this period and shown that aroma compounds were the highest amount in the first month of duration and decreased gradually in the following months [78]. The 3-methyl-butanoic acid was found as a main aroma compound in both raw and ECB fruits kept in water similar to study of Kraujalyte et al. [74].

The compositional analysis of ECB fruits of some *Viburnum* species has been reported to contain high amount of PC [74, 76, 80, 81, 82, 83, 88], including phenolic acids, flavonols, anthocyanins [72, 84, 85, 86, 87, 88, 89] and proanthocyanidins [89, 90]. Ascorbic, L-malic, oxalic and citric acids were detected as organic acids present in ECB fruits [75, 79, 81, 84]. Likewise American cranberry, ECB species contain high amount of phenolic compounds.

Phenolic composition of ECB shows both similarities (anhocyanins, flavanol and flavan-3-ol) and differences (proanthocyanins present in cranberry, phenolic acid present in ECB) with American cranberry (*Vaccinium macrocarpon)* [55, 91]. The phenolic compositions of ECB fruits change depend on differences in genotype, geographic region, cultivation practices, storage conditions, and ripeness. Phenolic composition of ECB species have been reported in previous studies by using HPLC equipped with different detector as shown in Table 2.3.

Table 2.3. Phenolic compounds present in ECB species by using different separation systems

Phenolic composition analysis	**Identified and quantified phenolic compounds**	**References**
HPLC-PDA	chlorogenic acid (2037.0 mg/kg) catechin (290.4 mg/kg) epicatechin (26.9 mg/kg) quercetin glucosides (52.1 mg/kg) cyanidin-3-glucoside (72.3 mg/kg) cyanidin-3-rutinoside (36.9 mg/kg)	Velioglu et al. [72]
LC-MS	chlorogenic acid (0.54-6.93 mg/ml) catechin (0.02-0.09 mg/ml) epicatechin (0.012-0.19 mg/ml)	Kraujalyte et al. [87]
UPLC/ESI-QTOF-MS	catechin, chlorogenic acid, neochlorogenic acid	Kraujalyte et al. [87]
HPLC-UV	chlorogenic acid (718 mg/l)	Oral et al. [88]
HPLC-UV-ESI-MS	chlorogenic acid, catechin, epicatechin, rutin, procyanidin B2, procyanidin trimer, quercetin-deoxyhexose, proanthocyanidin dimer monoglycoside	Karacelik et al. [89]
HPLC-UV	cyanidin 3-glucoside (42%), cyanidin 3-(*O*-(6”- *O*-arabinosylglucoside) (37%) cyanidin 3-*O*-(2”-*O*-xylosyl-6”- *O*-rhamnosyglucoside) (21%)	Jordheim et al [85]

Table 2.4. Some chemical properties of ECB fruits

	Velioglu et al [72]	**Akbulut et al [80]**	**Cam and Hısıl [79]**	**Cam et al [84]**	**Cesoniene et al [73]**	**Cesoniene et al [82]**	**Moldovan et al [86]**	**Rop et al [75]**	**Kraujalyte et al [87]**
Total phenolics	5.90 mg GAE/ml	3.3 mg GAE/g	3.5 mg GAE/ml	3.6 mg GAE/ml	1.11 mg GAE/g	11.68-8.0 mg GAE/g	-	6.80 - 8.25 mg GAE/ml	5.47-10.61 mg GAE/g
Total Anthocyanin	-	0.7 mg/g	-	0.11 mg/ml	0.4 mg/g	0.51-0.24 mg/g	356 mg//l	0.36 mg/ml	-
Organic acid	-	Ascorbic acid (0.6 mg/kg)	L-ascorbic acid (0.4 mg/ ml) L-malic acid (8.6 mg/ml) Oxalic acid (0.6 mg/ ml)	malic (10.8 mg/ml) oxalic (0.82 mg/ml), ascorbic (0.53 mg/ml) citric acids (0.39 mg/ml)	Ascorbic acid (0.3 mg/g))	-	-	Ascorbic acid (1.01-1.64) mg/ml	-
Sugar	-	Reducing Sugar (6.34 %)	Reducing Sugar (6.72 %) Total sugar 8.41 g/100ml	-	Reducing Sugar (7.6 %)	-	-	-	-
pH	-	3.04	2.95	2.95	-	-	2.0-3.0	-	-
Acidity	-	1.79 %	1.50 mg/100g	1712 mg/100g	3.2 g/100g	-	-	-	-

Many studies analyzed chemical properties of ECB fruits. All results were summarized in Table 2.4. Total phenolic content of ECB fruits expressed as gallic acid equivalent (GAE) and total anthocyanin expressed as cyanidin 3-glucoside.

Antioxidant and antimicrobial activities of ECB species with different cultivars or their extracts have also been reported to exhibit biological activities in *in vitro* studies [73, 76, 82, 83, 87, 89, 92]. Studies have confirmed that ECB fruits are a potential source of natural antioxidant [83, 84, 87, 89]. Antioxidant activity of ECB shows different activity depend on differences in genotype, geographic region, cultivation practices, storage conditions, and ripeness of fruits like difference in phenolic composition. The antioxidant activities of ECB fruits which harvested from Turkey in 2005, seed and fruit flesh were found as 2.35 mg /mg DPPH and 24.56 mg/mg 2,2-Diphenyl-1-picrylhydrazyl (DPPH), respectively where the antioxidant capacity of fruit seed was higher than fruit flesh [84]. The antioxidant activity of methanol extracts of ECB fruits found 315.50 mg/g [77]. Altun et al [83]. also measured the antioxidant capacity of ECB fruits harvested from Turkey in 2005, found that DPPH with inhibitory concentration 50 % (IC_{50}) value of 0.057 mg/ml. Same antioxidant activity methods used in Cesoniene et al. [82] study to measure different cultivars of ECB collected from Russia in 2009. Results ranged from 52.2% to 56.7% of cultivars expressed as radical scavenging activity. Antioxidant activity determined by DPPH and 2,2'-azonobis(3-etilbenzothiazoline-6-sulfonat (ABTS) methods on ECB fruits collected from Czech Republic in 2007-2009 and ranged between 8.55 to 9.79 g of ascorbic acid equivalents (AAE)/kg determined in DPPH and between 9.10 to 11.12g AAE /kg in ABTS [75]. Moldovan et al [67] found 7.05 g AAE/kg with ABTS in ECB fruits harvested Romania in 2010. Kraujalyte et al. [87] analyzed antioxidant activity of different cultivars with different methods and ABTS, Ferric reducing antioxidant power assay (FRAP) and Oxygen radical absorbance (ORAC) were in range of 31.9-109.8, 32.3-61.8 and 141.6-260.4 µmol trolox equivalents (TE)/g, respectively.

Antimicrobial activity of ECB juice and extracts were also investigated in *in-vitro* conditions [76, 82, 93]. The antimicrobial activity of dried methanol extract of ECB at different concentrations (2, 5, 10 and 15 %) was tested on both pathogenic and spoilage microorganisms [76]. The results showed that 15% methanol extracts found as the most

effective concentration completely inhibited the growth of *Aeromonas hydrophila, Bacillus cereus, Enterobacter aerogenes, Escherichia coli, Klebsiella pneumonia, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhimurium, Staphylococcus aureus* and *Yersinia enterocolitica.* While 2% concentration of the extracts did not inhibit the growth of any bacteria, 5% concentration of the extracts only had an inhibitory effect against *A. hydrophila* and *S. aureus*. Hızlısoy [93] examined the effect of different extracts (methanol and aqueous) of ECB fruits against *S. aureus, E. coli, P. aeruginosa, Candida albicans* and both extracts showed antibacterial effect on pathogenic bacteria. Different concentration of ECB fruits prepared with methanol were tested on microorganisms, and showed antibacterial effect concentration and strain dependent. Aqueous extracts of ECB fruits had bactericidal effect on *S. aureus, E.coli* and *P. aeruginosa* at 200, 200 and 100 mg/ml concentrations, respectively. In this study, both extracts did not show fungicidal effect on *C. albicans*. Besides, antimicrobial activity of different cultivars of fresh ECB juice was evaluated and found that while *S. typhimurium* was the most sensitive (25.3-27.7 mm) among all tested pathogenic bacteria [82] ECB juice was effective on fungi including *Trichosporon cutaneum, Kluyveromyces marxianus* var. *lactis,* and *Saccharomyces cerevisiae*.

ECB has been commonly used in traditional folk medicine for the treatment of some diseases and disorders such as, high blood pressure, duodenal ulcers, kidney, stomach, and menstrual pain, coughs and colds [72, 73, 86]. However, health studies related with ECB juice is limited [91, 95]. Animal studies with ECB juice revealed that ECB might have a potent to decrease gastrointestinal mucosal damage [90]. Another health study investigated the effect of ECB juice on colon tumorogenesis [94] and the result showed that ECB juice would be used for prevention of colon cancer at the initiation phase.

Microflora analysis of ECB fruits kept in water for 3 to 5 months was investigated to characterize LAB strains and to determine which strains contribute to produce gilaburu juice [77]. Total of 332 isolates belonging to *Lactobacillus* and *Leuconostoc* species characterized from traditionally produced ECB juice. The major LAB strains were *L. plantarum* (173 isolates), *L. casei* (52 isolates) and *L. brevis* (24 isolates), while *L. buchneri, L. parabuchneri, L. pantheris, Leuconostoc pseudomesenteroides* and *L. harbinensis* were the least in isolated LAB strains. LAB counts of the traditional produced ECB juice were in the

range of 3.92–8.30 log cfu/g. In same study, also probiotic potential LAB stains were checked and *L. plantarum* identified as predominant LAB species was found to have high hydrophobicity, antimicrobial activity and bile salt resistance properties suggesting that traditionally produced ECB juice may also be considered as a functional food.

2.5. AIM OF THE STUDY

The aim of this study was to investigate the effect of chlorogenic acid, main phenolic compound in ECB, on *Lactobacillus plantarum* ATCC 8014 growth to understand the isolated role of CA present in ECB juice. Also, the growth effect of total ECB phenolics on *L. plantarum* ATCC 8014 and two other LAB strains, *L. plantarum* Lp-115 and *L. brevis* ATCC 8287 were evaluated by addition of different concentration of fresh ECB juice in growth media.

3. MATERIALS AND METHODS

3.1. PLANT MATERIAL AND SAMPLE PREPARATION

The fresh European cranberrybush (ECB) fruits were supplied from Kayseri, Turkey in the August 2014. Fruits were removed from its stalks and seeds, squeezed and stored at -80 ^{o}C (Sanyo, Japan) until further analysis.

3.2. BACTERIAL STRAINS AND GROWTH CONDITIONS

Lactobacillus plantarum ATCC 8014 and *Lactobacillus brevis* ATCC 8287 were supplied from Yeditepe University, Food Microbiology Laboratory Collection, Istanbul, Turkey. *Lactobacillus plantarum* Lp-115 was kindly provided by Danisco, USA. The identity of the all strains were confirmed by gram staining (Salubris Gram Staining Kit) and biochemical assays. The both *L. plantarum* species were cultured at 37 °C for 48 h under flowing CO_2 (5%) (New Brunswick, UK) and *L. brevis* was cultured at 30°C (Binder, UK) for 48 h in Man Rogosa Sharp (MRS) broth and agar (MRSA) (Merck, Germany). The pH of MRS medium was adjusted to 5.5 by using 0.1 N HCI (Sigma-Aldrich, Germany) before sterilization. All cultures were stored in MRS medium containing 30 % glycerol (Panreac, Spain) at -80 °C.

3.3. GROWTH OF *L. PLANTARUM* ATCC 8014 IN MRS CONTAINING DIFFERENT CONCENTRATION OF CHLOROGENIC ACID

3.3.1. Growth in Microplates

Chlorogenic acid (CA) was found to be one of the main phenolic compounds present in ECB juice. Therefore, the potential growth effect of pure CA to the *L. plantarum* ATCC 8014 was investigated in presence of different concentrations 2.12- 0.71-0.53-0.35-0.1-0.05-0.025 mg/ml of CA (Sigma-Aldrich, USA) in MRS medium. Before addition into MRS, the CA was dissolved in phosphate buffer (pH 7.2) solution, and then filter sterilized using 0.22 µm regenerated cellulose (RC) syringe filter (Corning, Germany). The overnight cultures (10^9-

10^8 cfu/ml) of each *Lactobacillus* strains (1.6 %) were inoculated in 96-well microplates (Nunc, Thermo Scientific, USA) containing 300 µl of MRS medium (pH 5.5) with or without presence of ECB and incubated at 37 °C for 48 h. Readings were obtained with 30 min intervals by assessing optical density at 600 nm (OD_{600}) using automated microplate reader (Thermo Scientific, MultiskanGo, Finland). Non-inoculated MRS medium supplemented with different concentrations of ECB and MRS inoculated with culture were used as controls.

After determination of optimum concentration for CA, *L. plantarum* ATCC 8014 was also grown in basal medium (BM) with galactose (2 g/L) or glucose (2 g/L) as a carbon source in the presence of optimum concentration of CA. The BM was composed of either galactose (2.0 g/l) (Merck, Germany) or glucose (2.0 g/l) as carbon source, trisodium citrate dehydrate (0.5 g/l) (Riedel-de Haen, Germany) D-, L-malic acid (5 g/l) (Sigma, USA), casamino acids (1.0 g/l) (Fluka, Germany) and yeast nitrogen base without amino acids (6.7 g/l) (Sigma, USA) and the pH of the medium was adjusted to 5.5 modified basal medium described previously for *L. plantarum* Rodríguez et al. [8]. Fermentation of the culture in microtiter plate was carried out as described in previous sections using automated microplate reader at 37 °C.

3.3.2. Growth of *L. plantarum* ATCC 8014 in MRS Containing Optimized CA Concentration in Static Batch Culture Tubes

Optimized concentration of CA determined in microplate readings was also used in test tube fermentation of *L. plantarum* ATCC 8014 in higher medium volumes to able to assess the changes in pH, the number of *L. plantarum* (Log cfu/ml) and OD_{600} at the same sampling points (0, 4, 8, 10, 24 and 48 h) under the same conditions. OD measurements were done by using at UV-Vis Spectrophotometer (Evolution 220, Thermo Scientific, USA) at 600 nm. *Lactobacillus* species were enumerated by plating automatic spiral plater (easySpiral Pro, Interscience, USA) after the samples were serially diluted in phosphate buffer (pH 7.2, Fluka, USA) on MRSA. The plates were incubated at 37°C for 48 h under flowing CO_2 (5 %) and the results were expressed as Log cfu/ml. Degradation of CA during fermentation period was also analyzed by using HLPC.

3.3.2.1. Degradation of CA by L. plantarum ATCC 8014

In order to find whether *L. plantarum* has ability to degrade CA, the change in CA amount, antioxidant activity (FRAP, ORAC, DPPH) and total phenolic content of 0.35 mg/ml CA added *L. plantarum* 8014 cultures were analyzed. Firstly, total anthocyanin and total phenolic contents of fresh ECB juice were determined spectrophotometrically. Also, to understand in which concentration of ECB juice has 0.35 mg/ml CA, the phenolic composition of the fresh ECB juices was determined by using HPLC-ECD.

3.3.3. Determination of Total Anthocyanin Content

The total anthocyanin content of fresh ECB juice was measured by the pH-differential method [98] and the total anthocyanin content was expressed as cyanidin 3-glucoside equivalents. Potassium chloride buffer (pH 1.0) and sodium acetate buffer (pH 4.5) were used for dilution of fresh juice (diluted as 1/5) samples. Absorbance measurements were done in duplicate, expressed as cyanidin 3-glucoside equivalents (mg/L) at 520 and 700 nm with UV-Vis spectrophotometer.

3.3.4. Determination of Total Phenolic Content

Total phenolic content of the ECB juice samples and static batch cultures were determined using Folin-Ciocalteu reagent according to method of Singleton et al. [99]. The fresh ECB juice and static batch cultures (diluted as 1/50) were prepared with ultrapure water. Hundred µL of diluted sample was mixed with 200 µL of 10 % Folin-Ciocalteu phenol reagent 2N (Sigma, USA) into microcentrifuge tubes and mixed thoroughly. Eight hundred µL of 700 mM sodium carbonate anhydrous (Na_2CO_3 Sigma, USA) was then added to each tube and the tubes were incubated for 90 minutes in dark. After incubation, solutions were transferred into disposable cuvettes. Water was used as blank and absorbance reading of each sample was measured at 765 nm using a UV-1601 (Shimadzu, Japan) UV-Vis spectrophotometer in duplicate at room temperature. Gallic acid (Sigma, USA) was used as a standard in the concentration range of 0.02–0.2 mg/ml were used for the calibration curve. The results were expressed as mg gallic acid equivalents per ml extract (mg GAE/ml).

3.3.5. Determination of Phenolic Compounds by HPLC

The phenolic compositions of fresh and static batch cultures were determined using HPLC system equipped with an electrochemical detector (ECD) [104] (ESA Coularray System, ESA Inc., USA) with 16 channel system. Fresh ECB juice (diluted as 1/20) and optimized concentration of CA added static batch cultures (diluted as 1/10) were prepared with mobile phase A and all samples were filtered with centrifugal filters (Merck Millipore, USA). Separation was done using an Zorbax SB-C18 column (5µm, 4.6 x 250 mm, Agilant, USA) at 30 ^{0}C. Mobile phase gradient from mobile phase A (75 mM citric acid (Sigma Aldrich, USA) and 25 mM ammonium acetate (Sigma Aldrich, USA) in 90 % water and 10 % acetonitrile (HPLC grade, JT Bakers, USA) to mobile phase B (75 mM citric acid and 25 mM ammonium acetate in 50 % water and 50 % acetonitrile) for 68 min. Mobile phases were filtered over 0.2 µm Nylon membrane filters (GE Healthcare Life Sciences, UK) before analysis. The following mobile phase gradient profile was used (% solvent B): 1 % (0–15 min), 1–10 % (15–25 min), 10–80 % (25–60 min), 80–10 % (60–65 min), and 10–1 % (65–68 min). Flow rate was 0.6 mL/min and 50 µL of the sample was injected. Detection was achieved with potentials applied from 60 to 900 mV with 80 mV increments until channel 8 which it then increase in increments of 40 mV. Gallic acid, catechin, epicatechin, chlorogenic acid, caffeic acid, ferulic acid, *p*-coumaric acid, rutin, quercetin and quercetin 3-glucoside were used as standards (Sigma Aldrich, USA). All standards were prepared in 100 % Methanol (HPLC grade, VWR, Belgium). Identification of the phenolic compounds was done by comparing the retention times of the sample peaks with those of standards. Standard calibration curves were drawn for quantification of each compound. The amounts of phenolic compounds were expressed as mg phenolic compounds per ml sample.

3.3.6. Determination of Antioxidant Activity

Antioxidant activities of ECB juice and optimized concentration of CA added static batch culture samples were determined with three different *in vitro* antioxidant measurement methods.

3.3.6.1. Ferric Reducing Antioxidant Power Assay (FRAP)

Ferric reducing antioxidant power of ECB juice sample and static batch cultures were determined [100]. This assay based on the reduction of ferric ion (Fe3+) to ferrous ion (Fe2+) under acidic (pH 3.6) conditions due to for iron solubility followed by a blue complex formation of Fe2+ and 2,4,6-Tripyridyl-S-Triazine (TPTZ). The fresh ECB juice (diluted as 1/50), juice squeezed from ECB fruits kept in water (diluted as 1/10) and static batch cultures (diluted as 1/50) were prepared with ultrapure water. The 30 µl of diluted samples, blank (water), and Trolox (Sigma, USA) used as a standard were put into disposable cuvettes. Water (90 µl) and 900 µl FRAP reagent (300 mM Acetate Buffer: TPTZ: Iron Chloride in the ratio 10:1:1) were added into all cuvettes and incubated for 60 min at room temperature in dark place. Absorbance readings were taken at 593 nm on the UV-1601 (Shimadzu, Japan) spectrophotometer. FRAP values were calculated from standard curves using Trolox at 31.25–1000 µM Trolox, expressed as mM Trolox equivalent (TE) per ml sample.

3.3.6.2. DPPH Radical Scavenging Activity Assay

The 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging was assessed for ECB juice and *in-vitro* static fermenter culture samples [102]. Same dilutions were used for all samples as in FRAP assay, except that the samples were prepared in 100 % methanol. Before analysis, 100 µM DPPH solution was prepared freshly in 100 % methanol and absorbance of solution adjusted between 0.900-1.100. The 100 µl of samples, or standards were put into cuvettes with 900 µl of 100 µM DPPH solution and mixed well. Following 30 min incubation at room temperature in the dark, absorbance was measured at 517 nm. DPPH values were determined using standard curves of 7.8–350 µM Trolox and results were expressed as mM TE per ml sample.

3.3.6.3. Oxygen Radical Absorbance Capacity (ORAC)

The ORAC values of same samples were measured according to Ou et al. [103]. In ORAC assay, the 2, 2’ – Azobis (2-amidino propane) dihydrochloride (AAPH) (Wako) used for peroxyl radical generator and fluorescein (Sigma, USA) used as a target molecule to detect

the ability of the added antioxidant to inhibit their interaction relative to Trolox standard. Briefly, all samples or standards were diluted in 750 mM phosphate buffer (pH 7.0) (O-buffer). The fresh ECB juice diluted as 1/4000, juice squeezed from ECB fruits kept in water diluted as 1/2500 and static batch cultures diluted as 1/1000 before analyzing. Samples incubated with AAPH, prepared with 750 mM O-buffer and fluorescein (200 nM). The reaction was performed at 37 °C as the reaction was started by thermal decomposition of AAPH for 70 min. due to sensitivity of fluorescein to pH. The measurements were made in 96 Black flat-bottom microplate (Greiner). In each well, 50 µl of sample, blank (O-buffer) or trolox standard and 100 µl fluorescein were placed into wells. Fluorescence was monitored using a FLUOstar OPTIMA plate reader (BMG LABTECH Inc., USA) with fluorescence filters for an excitation wavelength of 485 nm and an emission wavelength of 535 nm. Standard curve of Trolox were prepared as 5 to 50 μM. Total antioxidant capacity was quantified by employing the area under the curve (AUC) of the magnitude and time of inhibition of peroxyl radicals attack against fluorescein. The ORAC values were expressed as mM TE per ml sample.

3.4. GROWTH OF *L. PLANTARUM* ATCC 8014, *L. PLANTARUM* LP-115 AND *L. BREVIS* ATCC 8287 IN DIFFERENT CONCENTRATIONS OF FRESH ECB JUICE ADDED MRS MEDIUM

3.4.1 Growth in Microplate

To understand whether ECB juice stimulates the growth rate of LAB, different concentrations of freshly squeezed ECB juice (100-50-20-10-5 %) was added into MRS medium. The overnight cultures (10^9-10^8 cfu/ml) of each (1.6 %) were inoculated in 96-well microplates containing MRS medium (pH 5.5) with or without presence of ECB and incubated at 37 °C for 48 h. Readings were obtained with 30 min intervals by assessing optical density at 600 nm (OD_{600}) using automated microplate reader. Non-inoculated MRS medium supplemented with different concentrations of ECB and MRS inoculated with culture were used as controls. The *Lactobacillus* strains growth was monitored in microtiter plates as stated previously (section 3.3.1).

3.4.2. Growth in Static Batch Culture Tubes

The preliminary growth studies of LAB stains in different concentrations of ECB juice added MRS medium in a 96-well microtiter plates showed that the maximum growth of *Lactobacillus* species was obtained in 5 % ECB juice added MRS medium. For this reason, static batch cultures of bacterial strains in test tubes were carried out in MRS containing 5 % ECB juice to evaluate the change in pH, the number of microorganisms (Log cfu/ml), optical density (600 nm), dry weight, sugar utilization and organic acid production throughout the fermentation period of 5 % ECB added MRS.

Static batch culture fermentation was performed in falcon tubes filled with sterilized 5 ml MRS medium supplemented with final concentration 5 % ECB juice. Overnight cultures of all strains were inoculated in the same rate (1.6%) as in microtiter plate growth experiments and incubated for 48 h at 37 °C in under flowing CO_2 (5%) incubator. Fermentation was carried out in equal volumes of the cultures for each time interval and samples were collected at 0, 4, 8, 10, 24 and 48 h to evaluate the change in pH, the number of microorganisms (Log cfu/ml), OD_{600}, dry weight, sugar utilization and organic acid production. The control experiment was done under the same conditions by incubating media with microorganism without ECB and incubating media with ECB without microorganisms.

Lactobacillus species were enumerated by plating automatic spiral plater after the samples were serially diluted in phosphate buffer (pH 7.2) on MRSA. The plates were incubated at 37°C for 48 h under flowing CO_2 (5 %) and the results were expressed as Log cfu/ml. The pH of fresh ECB juice and static batch cultures were measured with a pH meter (Hanna Instruments, Italy).

Remained samples in fermentation tubes were centrifuged (Sigma 3-30K, Germany) at 12000 x g for 10 min at 4 ^{o}C and while the pellet formed after centrifugation was used for the determination of dry weight of the samples, the supernatants were kept at -80^{o}C to determine sugar and organic acid content of the samples. A modified method of Bratbak and Dundas [101] was used for the dry weight measurements. The pellet was washed twice with 4 ml saline solution (0.85 % NaCI, Merck, Germany) and centrifuged again at 12000 x g for

10 min at 4 ^{o}C. Supernatant was removed and pellet was dissolved with 3 ml distilled ultrapure water and transferred into metal pans dried formerly to constant weight. Then, the samples in the pans were dried at 105 ^{o}C till the constant weight was obtained, cooled in desiccator and weighed on the analytical balance. The results were expressed as mg dry weight per ml.

Sugar and organic acid analyses of the fermented samples were explained in section 3.7.

3.4.3. Growth of *L. plantarum* ATCC 8014 in ECB Juice as a Growth Media

ECB juice (100 %) and ECB juice diluted with phosphate buffer (PB) (50-20-10-5 %) were also used as growth medium to understand the potential of ECB juice as a growth medium for *L. plantarum* ATCC 8014. Overnight culture of bacterial strain with same inoculation rate (1.6 %) was added into the ECB juice and diluted ECB juice at the same conditions as mentioned in section 3.4.1.

3.5. GROWTH KINETICS BASED ON OD VALUE

The growth kinetics of all strains in ECB added media were calculated by using modified Gompertz model. Equations which were derived from modified Gompertz equation used to determine growth rate (μ) and doubling time (td) of each *Lactobacillus* species by using OD values according to Widdel [95] and Hall et. al [42]. The equation used to calculate growth rate:

$$\frac{dN}{dt} = \mu N \tag{3.1}$$

where N is the number of cell at time *t* and μ is the first-order growth rate constant that is growth rate μ is the reciprocal time units, t is in hours and the growth rate is the μ h^{-1}.

$$\frac{dN}{N} = \mu t \tag{3.2}$$

When the equation was integrated from *t*=0 to *t*=*t*, the new equation formed as:

$$\ln \frac{N_t}{N_0} = \mu(t - t_0) \tag{3.3}$$

and μ is the slope of ln *N* versus t.

Lag time, λ, is the length of the lag phase and expressed as hour and doubling time, t_d is the time it takes for 1 cell to become 2 during exponential phase and the equation used:

$$\mu = \frac{\ln 2}{t_d} \tag{3.4}$$

The cell density (ΔOD) was calculated as the difference in OD values between initial value and the value reached after 48 h fermentation.

3.6. DETERMINATION OF SUGARS AND ORGANIC ACIDS

3.6.1. Sugar Analysis

The sugar content of ECB juice and time course sugar utilization of static batch cultures were determined by using Thermo Accela High Performance Liquid Chromatography (HPLC) (Thermo Scientific, England) equipped with a Refractive Index Detector (Thermo Scientific, England). Andersson and Hedlund (1983) [96] method was used with some modifications for sugar compounds identification and quantification. A HyperREZ XP Carbohydrate Ca++ column (300 x 7.7mm, 8μm; Thermo Scientific, England) was used at 50°C and detector temperature was maintained at 35°C. Mobile phase was 0.005 M H_2SO_4 (Sigma- Aldrich, Germany) with a 0.6 mL/min flow rate. The volume of the injected sample for each run was 10 μl. Samples were filtered through a 0.45 μm RC filter (Minisart

Sartorius, Germany) before injection. External standards of D (+) Glucose, D (-) Fructose, D (+) Mannose, D (+) Xylose, D (-) Arabinose (Supelco, USA) and D (+) Sucrose (Fluka, Germany) were used to detect sugar compounds in ECB and static batch culture samples. Calibration curves of detected sugars in samples were prepared in six different concentrations to calculate amount of sugar within the samples. Results expressed as mg sugar per ml.

3.6.2. Organic Acid Analysis

The organic acid content of ECB juice and static batch cultures was determined by same HPLC equipped with a different detector, PDA detector (Thermo Scientific, England). A Hypersil Gold C18 column (250 x 4.6mm x 5µm; Thermo Scientific, England) was used to detect compounds and column temperature was kept at 25 ^{o}C. Mobile phase was 0.01M KH_2PO_4 (potassium phosphate monobasic, Sigma-Aldrich, Germany) buffer solution and pH was adjusted to 2.60 by adding a few drops of phosphoric acid (Fluka, Germany) and flow rate was used as a 1 ml/min according to method of Scherer at al. [97] with some modifications. All samples was diluted with ultrapure water and filtered over a 0.45 µm RC filter before injection of 20 µl sample volume into the liquid chromatogram.

The presence of oxalic, quinic, D-tartaric, D-malic, benzoic, L-ascorbic, L-(+)-Lactic, citric, formic, fumaric, succinic (-)-shikimic, maleic acids (Supelco, USA), and acetic acid (Fisher Scientific, UK) was analyzed by using organic acid standards to identify peaks according to their retention times with samples. Detection was set to 210 nm for all organic acids other than ascorbic acid which was 250 nm. The stock solution and the corresponding dilutions of organic acid standards were prepared with ultrapure water, except ascorbic acid, prepared with mobile phase to keep stability of the compound. Different concentrations of external standards of L-(+)-Lactic acid (1.57, 3.13, 6.25, 12.5, 25, 50 mg/ml), acetic acid (0.20, 0.41, 0.82, 1.64, 3.28, 6.56 mg/ml), L-ascorbic acid (0.01, 0.02, 0.05, 0.09, 0.19, 0.38 mg/ml), oxalic acid (0.06, 0.12, 0.25, 0.5, 1 mg/ml), quinic acid (0.2, 0.4, 0.6, 0.8, 1.0 mg/ml), D-malic acid (0.31, 0.63, 1.25, 2.5, 5, 10 mg/ml), maleic acid (0.004, 0.008, 0.015, 0.03, 0.06 mg/ml), citric acid (0.06, 0.12, 0.25, 0.5, 1 mg/ml) and fumaric acid (0.001, 0.002, 0.004, 0.008, 0.016 mg/ml) were prepared and concentrations of individual organic acids in samples

were calculated from calibration curves drawn from external standards. Organic acids were detected by absorbance and quantified with external calibration graphs. Results were expressed as mg organic acid compound per ml.

3.7. STATISTICAL ANALYSIS

All growth studies of microplates and static batch culture analyses performed in two independent parallel experiments with two analysis replicate for each measurements. One-way analysis of variance (ANOVA) and Tukey test used to determine significant difference ($p<0.05$) among samples. All statistical analyses were performed with Statistical Package for Social Sciences (SPSS) version 20.

4. RESULTS

4.1. GROWTH OF *L. PLANTARUM* ATCC 8014 IN MRS CONTAINING DIFFERENT CONCENTRATION OF CHLOROGENIC ACID

4.1.1. Growth in Microplates

The growth pattern of the *L. plantarum* 8014 in the presence of CA, which was found as main phenolic compounds present in ECB [72, 74], in MRS medium, was shown in Figure 4.1.

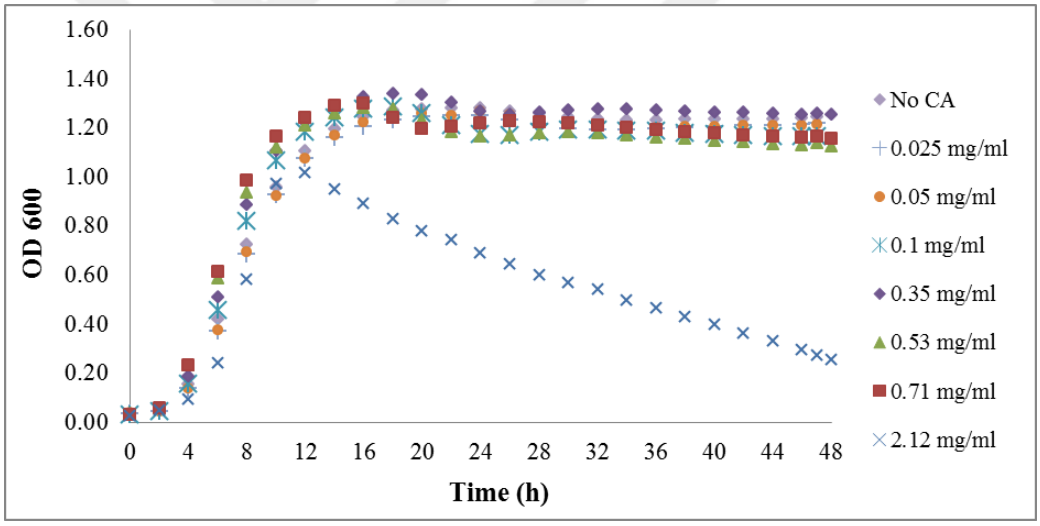


Figure 4.1. Growth effect of different concentrations of CA on *L. plantarum* ATCC 8014 in MRS medium

The *L. plantarum* 8014 grew in the presence of all concentrations of CA in MRS medium (2.12- 0.71-0.53-0.35-0.1-0.05-0.025 mg/ml). The OD values of 0.71, 0.53, 0.35 and 0.1 mg/ml CA added cultures was higher than control cultures in exponential phase. However, in the presence of 2.12 mg/ml CA, *L. plantarum* 8014 grew up to 12 h and then OD values decreased and reached the lower values compared to other concentrations of CA and control

culture until the end of fermentation period. Also, the ΔOD and μ_{max} values of 8014 at this concentration were significantly lower than other concentrations (p<0.05) (Table 4.1).

Although the OD increase of 0.35 mg/ml CA added cultures in exponential phase were higher than control cultures, the ΔOD values of both cultures were similar but ΔOD of 0.35 mg/ml CA added cultures was still significantly higher compared to other CA added cultures (p<0.05). Interestingly, while the cell density was higher, the μ_{max} of the 0.35 mg/ml CA added cultures were comparable with other concentrations.

Table 4.1. Growth parameters of *L. plantarum* ATCC 8014 in MRS containing different concentrations of CA*

CA Concentration (mg/ml)	**ΔOD (600 nm)**	**μ_{max} (/h)**	**λ (h)**	**td (h)**
No CA	1.23±0.03	0.51±0.01	0.55±0.30	1.36±0.02
2.12	0.23±0.05	0.45±0.01	-0.24±0.13	1.56±0.02
0.71	1.12±0.01	0.62±0.01	0.55±0.39	1.12±0.01
0.53	1.09±0.02	0.63±0.00	0.74±0.37	1.19±0.02
0.35	1.22±0.00	0.60±0.01	0.96±0.37	1.16±0.02
0.10	1.13±0.00	0.58±0.01	1.05±0.02	1.24±0.18
0.05	1.10±0.04	0.55±0.06	1.20±0.01	1.27±0.14
0.025	1.18±0.05	0.51±0.07	1.30±0.14	1.36±0.02

* Data expressed as mean values ± standard deviations for each parameter

Since 1mM concentration of PC was generally used both for bacterial growth and metabolism studies of LAB [8, 28, 30], 0.35 mg/ml CA (~1 mM) was chosen to analyze *L. plantarum* 8014 growth in different growth medium other than MRS. The growth curves of 0.35 mg/ml CA added *L. plantarum* 8014 cultures grown in basal media (BM) either containing 2 mg/ml galactose or glucose was shown in Figure 4.2.

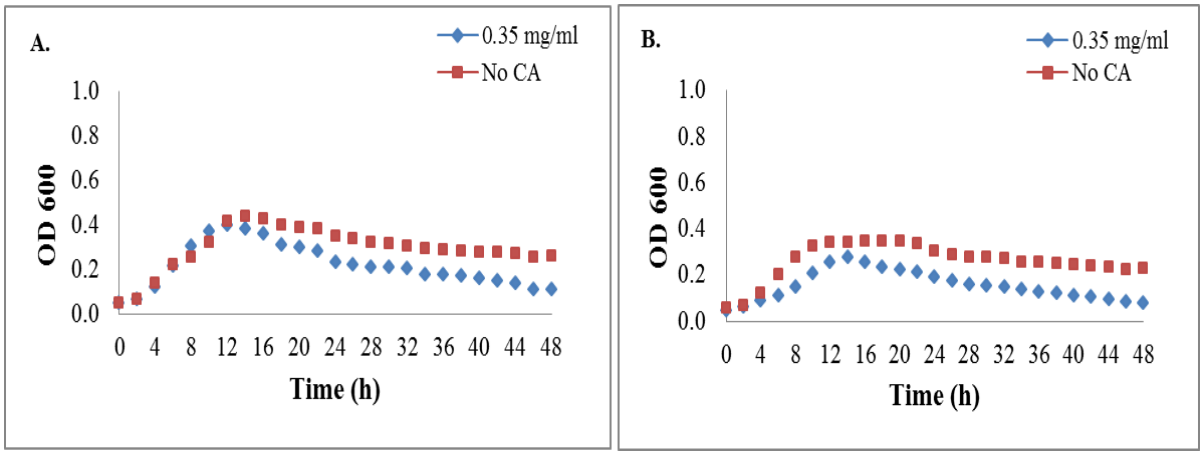


Figure 4.2. Growth effect of 0.35 mg/ml CA on *L. plantarum* ATCC 8014 in basal medium with glucose (2 g/L) (**A**) galactose (2 g/L) (**B**)

The *L. plantarum* 8014 grew in both glucose and galactose added BM even growth of culture was lower than MRS medium (Figure 4.2). However, the OD values of 8014 cultures were higher in glucose added BM than galactose added BM. The addition of CA did not increase the growth in both media. Although the ΔOD values of glucose and galactose added BM in presence of CA was same, the μ_{max} of culture grew in glucose added BM (0.24/h) was 2 fold higher than galactose added BM culture (0.13/h) (Table 4.2). Using galactose as carbon source, extend the adaptation and the td (5.34 h) of the culture got much longer compared to BM added glucose media (2.94 h).

Table 4.2. Growth parameters of *L. plantarum* ATCC 8014 in 0.35 mg/ml CA added BM with glucose and galactose*

Growth medium	**Sugar amount**	**ΔOD (600 nm)**	**μ_{max} (/h)**	**λ (h)**	**td (h)**
BM	2 mg/ml glucose	0.30±0.13	0.32±0.05	1.19±0.13	2.17±0.30
BM added CA		0.08±0.03	0.24±0.06	1.27±1.08	2.94±0.71
BM	2 mg/ml galactose	0.18±0.01	0.26±0.02	1.31±0.11	2.65±0.20
BM added CA		0.08 ±0.06	0.13±0.02	0.86±1.07	5.34±0.98

* Data expressed as mean values ± standard deviations for each parameter

4.2. GROWTH OF *L. PLANTARUM* ATCC 8014 IN MRS CONTAINING OPTIMIZED CA CONCENTRATION IN STATIC BATCH CULTURES IN TUBES

To observe the change in OD_{600}, the number of *L. plantarum* (Log cfu/ml), and pH, the fermentation of 0.35 mg/ml CA added *L. plantarum* 8014 was also examined static batch cultures in tubes (Table 4.3). The results were similar with 0.35 mg/ml CA added microplate cultures. The OD and Log cfu/ml measurements were close in the presence and absence of CA in MRS. While OD_{600} values of CA added medium increased from 0.07 to 2.28 after 48 h of incubation. *L. plantarum* grew from 7.69 to 9.43 Log cfu/ml after 24 h and gradually decreased to 8.61 Log cfu/ml at the end of fermentation (Table 4.3). The pH values of the growth medium containing CA decreased from 5.85 to 3.72 within 48 h while the pH values of control were 5.30 to 3.58.

Table 4.3. The OD_{600}, the number of *L. plantarum* ATCC 8014 (Log cfu/ml) and pH measurements for 48 h in the presence of 0.35 mg/ml CA in MRS medium[*]

Time (h)	Samples	OD (600 nm)	Log (cfu/ml)	pH
0	**CA**	0.07±0.02	7.69±0.17	5.85±0.04
	C[**]	0.09±0.02	7.82±0.02	5.30±0.04
4	**CA**	0.66±0.09	8.45±0.12	5.47±0.10
	C	0.64±0.09	8.34±0.13	5.06±0.07
8	**CA**	2.03±0.04	9.35±0.10	4.32±0.11
	C	1.96±0.02	9.12±0.05	4.28±0.09
10	**CA**	2.15±0.01	9.30±0.35	4.07±0.09
	C	2.10±0.02	9.38±0.10	4.06±0.09
24	**CA**	2.26±0.01	9.43±0.01	3.81±0.04
	C	2.28±0.01	9.43±0.09	3.73±0.04
48	**CA**	2.28±0.01	8.61±0.40	3.72±0.04
	C	2.29±0.01	8.66±0.19	3.58±0.04

[*] Data expressed as mean values ± standard deviations for each parameter.
[**] C was the control samples which had 0.35 mg/ml CA in MRS with *L. plantarum* 8014

4.2.1. Degradation of CA by *L. plantarum* ATCC 8014

In order to find whether *L. plantarum* has ability to degrade CA, the change in CA amount, antioxidant activity (FRAP, ORAC, DPPH) and total phenolic content of 0.35 mg/ml CA added *L. plantarum* 8014 cultures were analyzed. Total anthocyanin and total phenolic contents of fresh ECB were determined with spectrophotometric methods. Beside, to understand in which concentration of ECB juice has 0.35 mg/ml CA, the phenolic composition of the fresh ECB juices was determined by using HPLC-ECD.

While total anthocyanin content of fresh ECB juices was found as 0.03 mg cyanidin 3-glucoside/ml, total phenolic content of fresh ECB juice was 5.34 mg gallic acid equivalent (GAE)/ml. Catechin, chlorogenic acid, caffeic acid and epicatechin (mg/ml) in fresh ECB juices were detected by using HPLC-ECD (Table 4.4) and chromatograms shown in Figure 4.3 and 4.4. Catechin and chlorogenic acid were found the main phenolic compounds in ECB juice. Fresh ECB juice had 4.16 mg/ml catechin, 3.42 mg/ml chlorogenic acid, 0.009 mg/ml caffeic acid and 0.020 mg/ml epicatechin.

Table 4.4. Phenolic compounds (mg/ml) in ECB juices

	Phenolic Compounds (mg compound/ml)			
	Catechin	**Chlorogenic acid**	**Caffeic acid**	**Epicatechin**
Fresh ECB juice	4.16±0.48	3.42±0.04	0.009±0.01	0.020±0.01

* Data expressed as mean values ± standard deviations for each parameter.

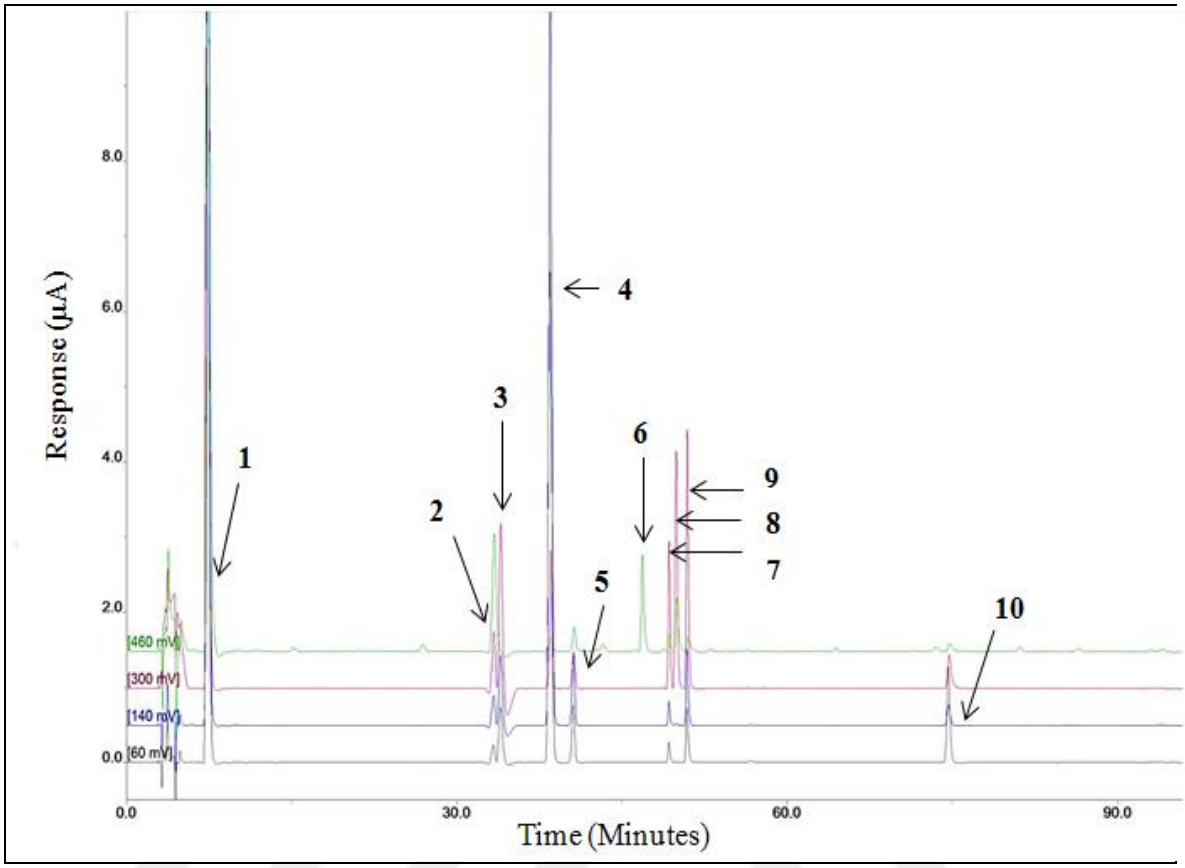


Figure 4.3. Chromatogram of standard phenolic compounds detected by HPLC-ECD. (1-Gallic acid, 2-Catechin, 3-Chlorogenic acid, 4-Caffeic acid, 5-Epicatechin, 6-*p*-coumaric acid, 7-Rutin, 8-Ferulic acid, 9-Quecetin 3-glucoside, 10-Quercetin)

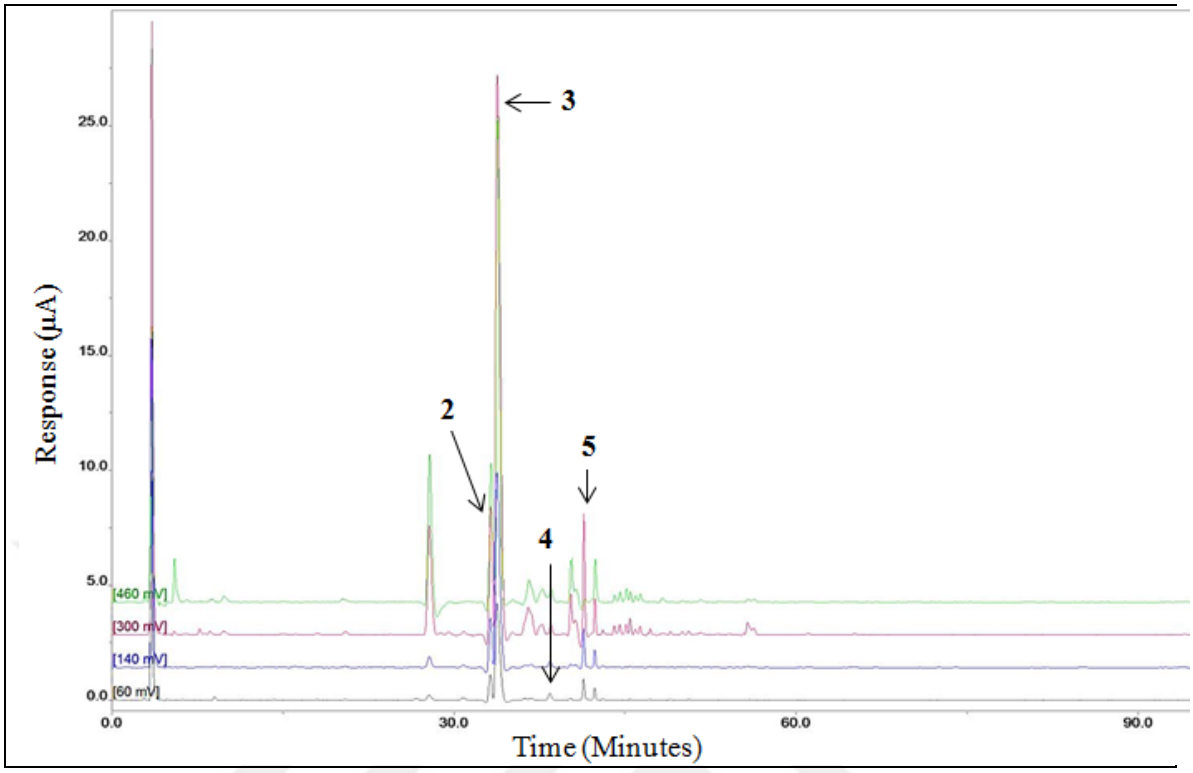


Figure 4.4. Chromatogram of fresh ECB juice (2-Catechin, 3-Chlorogenic acid, 4-Caffeic acid, 5-Epicatechin)

The antioxidant activity of fresh ECB juices was evaluated by FRAP, ORAC and DPPH assays and found as 14.05, 185.09 and 18.46 mM Trolox equivalent (TE)/ml, respectively.

The CA concentration, antioxidant activity (FRAP, ORAC, DPPH) and total phenolic content of 0.35 mg/ml CA added *L. plantarum* 8014 and control cultures assessed during fermentation period were shown in Table 4.5. The CA was not metabolized by *L. plantarum* and the amounts in both CA added cultures and CA added sample without *L. plantarum* were same throughout the fermentation. FRAP, ORAC and DPPH values of static batch cultures with CA were ranged 1.20-1.29, 16.21-24.83, 1.92-2.86 mM TE/ml, respectively and total phenolic content varied in range of 0.80-0.89 mg GAE/ml. The antioxidant activity results of CA added samples did not change significantly compared to CA added samples without *L. plantarum.*

Table 4.5. Antioxidant activity (FRAP, ORAC, DPPH) and Total phenolic content of static batch cultures (0-48 h) of *L. plantarum* ATCC 8014 in 0.35 mg/ml CA added MRS*

Time (h)	Samples	CA (mg/ml)	Antioxidant activity (mM Trolox Eq/mL)			Total Phenol Content (mg GA/ml)
			FRAP	ORAC	DPPH	
0	CA	0.36±0.02	1.20±0.07	17.90±0.18	2.64±0.05	0.80±0.23
	C**	0.37±0.05	1.38±0.04	14.71±3.50	2.48±0.80	0.81±0.27
4	CA	0.34±0.03	1.27±0.10	16.21±0.05	2.86±0.22	0.87±0.12
	C	0.36±0.02	1.38±0.05	19.66±0.39	2.79±0.10	0.95±0.13
8	CA	0.36±0.04	1.19±0.11	20.56±1.81	2.12±0.35	0.86±0.15
	C	0.35±0.00	1.30±0.12	25.17±3.02	2.85±0.19	0.91±0.09
10	CA	0.37±0.01	1.21±0.07	24.83±0.32	2.12±0.30	0.89±0.08
	C	0.24±0.03	1.28±0.09	23.81±1.40	2.95±0.17	0.94±0.16
24	CA	0.36±0.00	1.27±0.07	21.82±1.34	1.92±0.22	0.88±0.10
	C	0.35±0.01	1.31±0.09	22.59±2.70	2.71±0.05	0.78±0.23
48	CA	0.33±0.03	1.29±0.11	19.61±1.05	1.98±0.10	0.86±0.14
	C	0.36±0.01	0.98±0.12	14.91±2.83	2.45±0.36	0.76±0.00

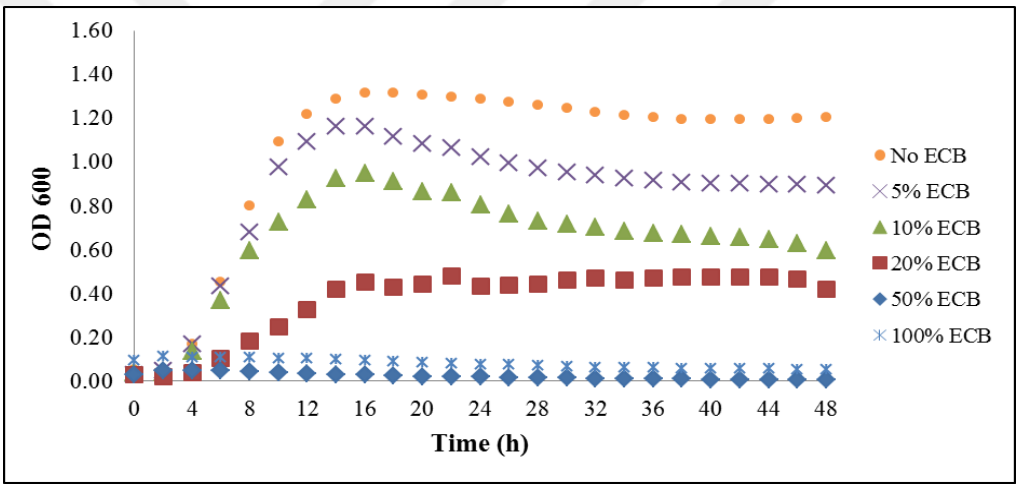
* Data expressed as mean values ± standard deviations for each parameter
** C was the control samples which had 0.35 mg/ml CA in MRS without *L. plantarum* 8014

4.3. GROWTH OF *L. PLANTARUM* ATCC 8014, *L. PLANTARUM* LP-115 AND *L. BREVIS* ATCC 8287 IN ECB JUICE ADDED MRS MEDIUM

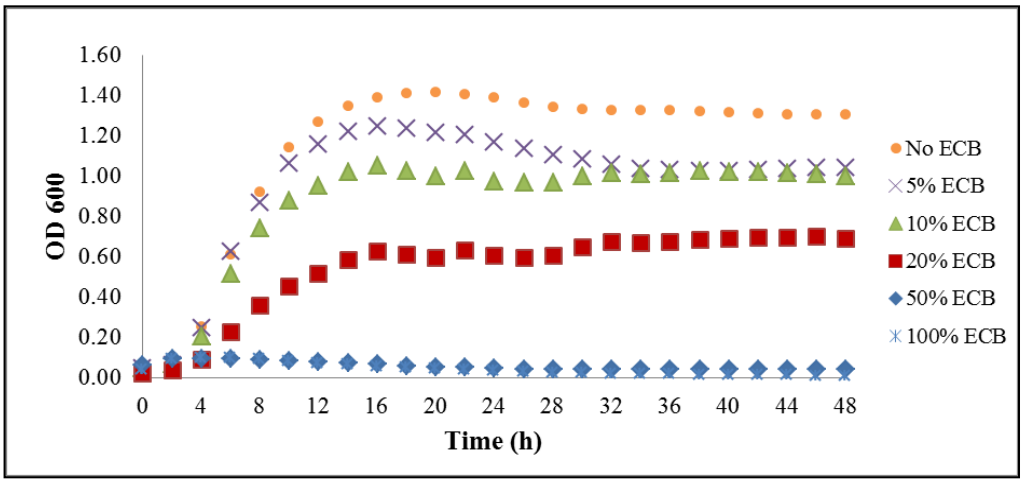
After determination of phenolic composition of fresh ECB juice by using HPLC-ECD, opposite the literature, catechin was found as the main phenolic compound following chlorogenic acid. So, we figured out that it might be better to understand the total effect of ECB phenolics and then test the isolated role of each individual phenolic compound in ECB on LAB. For this reason, the effect of different concentrations (100, 50, 20, 10, 5 %) of fresh ECB juice on *L. plantarum* ATCC 8014 and two other LAB strains, *L. plantarum* Lp-115 and *L. brevis* ATCC 8287, were evaluated.

Lactobacillus species grew slightly in the presence of high concentrations (100-50 %) of ECB juice added media at first 2 to 5 h of fermentation and the OD measurements showed 0.75 to 2.5 fold increase compared to their starting OD values. However, the OD values of bacterial strains decreased thereafter and reached lower values than initial numbers until the end of fermentation while the cultures without ECB in MRS grew (Figure 4.5). When the concentration of ECB juice in MRS decreased from 50 to 20, 10 and 5 %, *Lactobacillus* species started to grow, however growth was still lower compared to control cultures. However, growth curve pattern of *L. brevis* cultures was different than *L. plantarum* species in the presence of ECB juice. Addition of ECB juice (5 -10 %) caused early exponential and the stationary phases of *L. brevis* cultures compared to control cultures.

A.



B.



C.

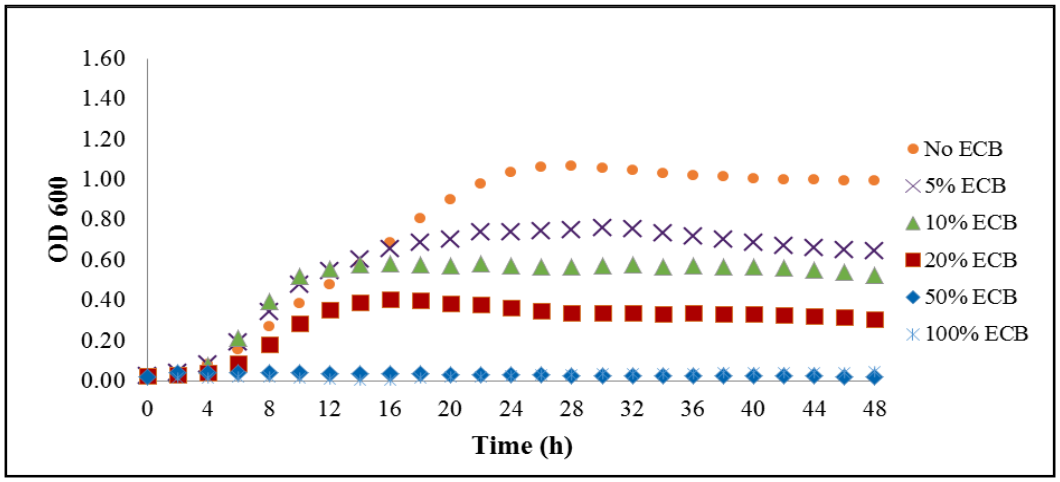


Figure 4.5. Growth curve of (**A**) *L. plantarum* ATCC 8014, (**B**) *L. plantarum* Lp-115 and (**C**) *L. brevis* ATCC 8287 in MRS medium containing different concentrations (100-50-20-10-5 %) of ECB juice at 37 °C during 48 h of incubation

Growth kinetic parameters including the cell density (ΔOD), maximum specific growth rate (μ_{max}), lag time (λ) and doubling time (td) of the cultures grown in 20-10 and 5 % ECB juice added media were analyzed to understand the differences among concentrations in detail (Table 4.6). Addition of ECB juice did not stimulate the growth and the ΔOD values of all strains were higher in control cultures compare to ECB added cultures. Whereas the addition of 5 % ECB juice displayed higher ΔOD values than other all ECB juice added cultures, the increase in ECB juice concentration up to 20 % lower the ΔOD values significantly (p<0.05) compare to control and other ECB concentrations of cultures. On the other hand, the kinetic patterns of μ_{max}, λ and td values of the cultures showed differences depending on the bacterial strain and added ECB juice amount.

The addition of 5 % of ECB juice to media increased the μ_{max} of *L. plantarum* 8014 significantly and adapted to the medium faster (0.65 h) compared to other cultures (p<0.05). When concentration of ECB increased from 5 to 10 % while the μmax and td values were similar, the lag time of 8014 cultures got longer. Further increase of the juice concentration (20 %), declined the μ_{max} significantly and consequently td value escalated and the lag time got longer (3.14 h).

Similar to 8014 cultures, even ΔOD values were lower than control cultures addition of 5 % ECB juice increased the μmax and lower td values (1.20 h) following extension in td values of *L. plantarum* 115 cultures. The lowest μ_{max} was seen when the addition of 20 % ECB juice added.

On the other hand, as stated previously, growth kinetic patterns of *L. brevis* in ECB added media was different than *L. plantarum* cultures. Even the ΔOD values were lower than control cultures, 5 to 10 % ECB juice addition to media increased the μ_{max} values significantly (0.36 to 0. 44/h) and lower the td values compare to control cultures (0.29/h). Interestingly, 10 % ECB juice caused higher growth rate than 5 % ECB juice addition. But when the concentration of ECB juice increased up to 20 %, the growth decreased parallel with μ_{max} values.

Table 4.6. Growth parameters of *Lactobacillus* strains in the absence and the presence of 20-10-5 % ECB juice added MRS medium

***Lactobacillus* strains**	**ECB (%)**	**ΔOD (600 nm)**	**μ_{max} (/h)**	**λ (h)**	**td (h)**
L. plantarum ATCC 8014	No ECB	1.15±0.02	0.52±0.00	1.96±0.25	1.32±0.00
	5	0.87±0.00	0.56±0.00	0.65±0.00	1.26±0.00
	10	0.55±0.02	0.56±0.07	1.93±0.42	1.26±0.15
	20	0.39±0.04	0.37±0.05	3.14±0.50	1.94±0.20
L. plantarum Lp-115	No ECB	1.25±0.04	0.55±0.02	1.37±0.60	1.25±0.04
	5	0.98±0.02	0.58±0.01	1.59±0.43	1.20±0.02
	10	0.83±0.15	0.52±0.05	1.12±0.11	1.33±0.13
	20	0.60±0.15	0.38±0.07	1.72±0.11	1.95±0.19
L. brevis ATCC 8287	No ECB	0.97±0.01	0.29±0.03	-0.45±0.12	2.37±0.22
	5	0.62±0.05	0.36±0.03	0.27±0.01	1.93±0.14
	10	0.50±0.07	0.44±0.03	1.00±0.82	1.57±0.12
	20	0.28±0.06	0.34±0.00	1.87±0.22	2.06±0.01

* Data expressed as mean values ± standard deviations for each parameter

4.4. GROWTH OF *L. PLANTARUM* ATCC 8014 IN ECB JUICE AS A GROWTH MEDIA

To test the metabolic adaptation and response of *L. plantarum* 8014 to ECB juice, the growth and the survival of the culture in 100 % ECB juice and different concentration of ECB juice diluted in phosphate buffer (50-20-10-5 %) was evaluated (Figure 4.2).

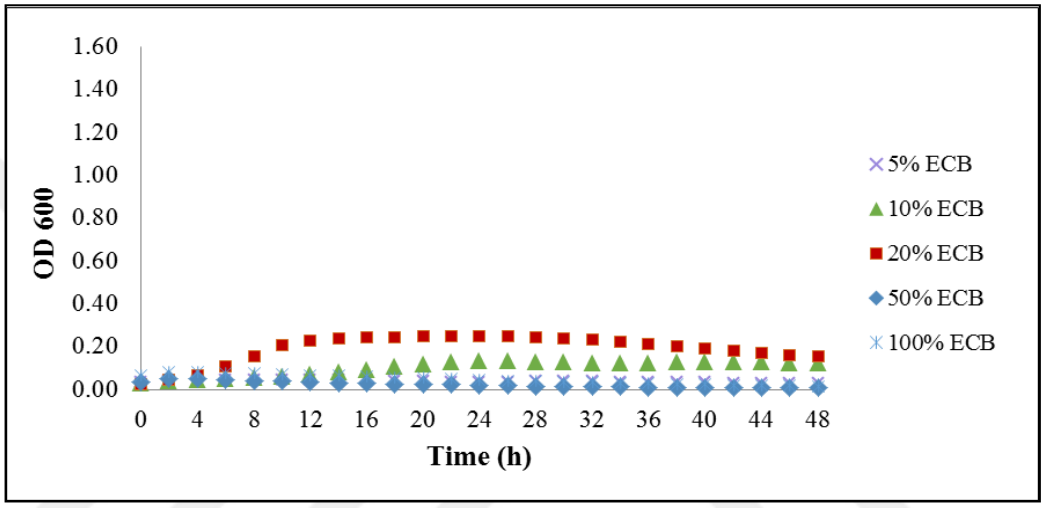


Figure 4.6. Effect of fresh ECB juice (100-50-20-10-5 %) on *L. plantarum* ATCC 8014 in PB over 48 h at 37°C

As seen from Figure 4.6, in the presence of 100 % ECB juice, *L. plantarum* 8014 grew slightly first 1.5 h and the OD values decreased thereafter with respect to initial OD values. When ECB juice diluted with PB (50 %), there was a very small increment at OD values (0.04 to 0.22). Even the growth of the culture was much lower compare to growth media, lowering the ECB amount from 50 to 20 % increased the OD values from 0.03 to 0.25 after 18 h of incubation following decrease until the end of fermentation. However, at concentrations lower than 20 % growth diminished again. The results of the growth parameters (ΔOD, μ_{max}, λ and td) were parallel with OD values (Table 4.7).

Table 4.7. Growth kinetic parameters of *L. plantarum* ATCC 8014 in ECB juice diluted in PB*

Media		ΔOD (600 nm)	μ_{max} (/h)	λ (h)	td (h)
ECB %	100	-0.04±0.01	-	-	-
	50	0.18±0.15	0.09±0.02	-0.91±0.02	7.71±1.44
	20	0.12±0.02	0.21±0.00	-0.76±0.03	3.27±0.05
	10	0.10±0.01	0.06±0.01	-0.58±0.02	11.17±1.87
	5	-0.16±0.03	0.02±0.00	-	28.13±2.65

* Data expressed as mean values ± standard deviations for each parameter
- Not detected

4.5. GROWTH OF *L. PLANTARUM* ATCC 8014, *L. PLANTARUM* LP-115 AND *L. BREVIS* ATCC 8287 IN MRS CONTAINING 5% ECB JUICE IN STATIC BATCH CULTURES

Increase in the fermentation volume of the ECB juice added cultures was carried out using 5 % ECB juice addition since it resulted with higher growth for all strains compared to other concentrations in microplate assay. The OD_{600}, dry weight, the number of microorganisms (Log cfu/ml), pH, sugar utilization and organic acid production of *Lactobacillus* species were measured to evaluate the effect of ECB juice on their growth in more detail.

There were slight changes between growth parameters of ECB added and control cultures of *L. plantarum* species but the difference was not significant during 48 h fermentation. OD and dry weight values of both ECB added cultures and control cultures of *L. plantarum* species increased significantly (p<0.05) compare to initial values throughout fermentation (Table 4.8).

Table 4.8. The change in OD_{600}, dry weight (mg/ml), the number of *L. plantarum* ATCC 8014 and Lp-115 (Log cfu/ml) and pH in absence and presence of 5% ECB juice added medium for 48 h*

Samples		Time (h)	OD (600 nm)	Dry weight (mg/ml)	Log (cfu/ml)	pH
L. plantarum 8014	ECB	0	0.11±0.01	0.18±0.09	7.79±0.03	5.09±0.01
	C**		0.09±0.02	0.16±0.00	7.81±0.01	5.42±0.02
	ECB	4	0.69±0.02	0.18±0.10	8.37±0.05	4.99±0.01
	C		0.68±0.00	0.35±0.01	8.29±0.01	5.16±0.00
	ECB	8	1.64±0.08	0.61±0.03	9.29±0.13	4.32±0.01
	C		1.69±0.05	0.37±0.03	9.34±0.03	4.38±0.00
	ECB	10	1.62±0.03	0.62±0.09	9.56±0.04	4.11±0.01
	C		1.68±0.09	0.60±0.02	9.54±0.04	4.15±0.01
	ECB	24	1.77±0.03	0.82±0.02	9.69±0.32	3.73±0.00
	C		1.72±0.12	0.82±0.04	9.58±0.02	3.73±0.00
	ECB	48	1.80±0.03	0.47±0.15	9.01±0.02	3.63±0.01
	C		1.75±0.11	0.55±0.10	8.80±0.09	3.68±0.01
L. plantarum 115	ECB	0	0.17±0.00	0.33±0.00	7.82±0.07	5.10±0.04
	C		0.21±0.00	0.29±0.13	7.84±0.05	5.41±0.00
	ECB	4	1.03±0.02	0.50±0.13	8.74±0.01	4.94±0.01
	C		1.07±0.01	0.34±0.04	8.76±0.01	5.05±0.01
	ECB	8	1.69±0.13	0.71±0.10	9.74±0.02	4.12±0.00
	C		1.80±0.04	0.58±0.11	9.74±0.09	4.17±0.01
	ECB	10	1.73±0.11	0.71±0.03	9.82±0.04	4.02±0.00
	C		1.76±0.17	0.68±0.02	9.77±0.03	4.05±0.01
	ECB	24	1.83±0.24	0.75±0.03	9.69±0.03	3.70±0.00
	C		1.73±0.17	0.80±0.05	9.67±0.02	3.74±0.00
	ECB	48	1.77±0.04	0.71±0.10	9.12±0.20	3.64±0.01
	C		1.73±0.15	0.76±0.03	8.52±0.14	3.72±0.00

* Data expressed as mean values ± standard deviations for each parameter
** C was the control cultures with ECB added into MRS medium

The number of 5 % ECB juice added cultures of *L. plantarum* species ranged between 7.79 to 9.69 log cfu/ml while the numbers in the control of these cultures were between 7.81 to

9.77 log cfu/ml. As expected, the starting pH values of ECB added cultures were lower than control cultures because fresh ECB had low pH value (3.0). The pH values of both ECB added and control cultures decreased significantly after 4 h of incubation (p<0.05).

As in microplate readings, the growth of *L. brevis* was slower than *L. plantarum* strains. The addition of ECB juice to media increased the OD higher values (0.03 to 0.81) after 8 h of incubation compare to control cultures OD values (0.03 to 0.58) of *L. brevis* (Table 4.9). However, the OD values reached the close numbers for both cultures after 24 h of fermentation. The obtained values for dry weight and the log cfu/ml were similar in the presence and absence of ECB. The pH of medium decreased during fermentation similar to *L. plantarum* strains.

Table 4.9. The change in $OD_{600,}$ dry weight (mg/ml), the number of *L. brevis* ATCC 8287 (Log cfu/ml) and pH for 48 h in absence and presence of 5% ECB juice*

Samples		Time (h)	OD (600 nm)	Dry weight (mg/ml)	Log (cfu/ml)	pH
***L. brevis* 8287**	**ECB***	**0**	0.03±0.00	0.08±0.00	7.20±0.14	5.12±0.01
	C		0.03±0.00	0.08±0.00	7.11±0.04	5.43±0.01
	ECB	**4**	0.13±0.00	0.08±0.00	7.48±0.07	5.09±0.01
	C		0.12±0.02	0.08±0.00	7.59±0.01	5.38±0.01
	ECB	**8**	0.81±0.02	0.08±0.00	8.80±0.04	4.97±0.01
	C		0.58±0.03	0.08±0.00	8.82±0.06	5.33±0.02
	ECB	**10**	1.25±0.01	0.16±0.00	9.17±0.02	4.85±0.01
	C		0.86±0.05	0.16±0.00	9.02±0.06	5.23±0.04
	ECB	**24**	1.80±0.01	0.38±0.00	9.34±0.01	4.34±0.00
	C		1.84±0.02	0.31±0.00	9.62±0.06	4.47±0.04
	ECB	**48**	1.77±0.00	0.49±0.05	8.96±0.04	4.20±0.01
	C		1.73±0.00	0.41±0.05	8.45±0.57	4.32±0.01

* Data expressed as mean values ± standard deviations for each parameter

The change in sugar utilization versus organic acid production was analyzed in both ECB added static batch and control cultures throughout the fermentation period (Figure 4.9).

Before analyzing fermentation cultures, sugar content and organic acid content of fresh ECB juice were checked by using HPLC-RI and HPLC-PDA, respectively (Figure 4.7 and Figure 4.8). The fresh ECB juice had two main sugars which were found as glucose (28.59 mg/ml) and fructose (38.74 mg/ml). Organic acid analysis of fresh ECB juice with HPLC-PDA showed that eight different organic acid compounds were present in fresh ECB juice including D-malic acid (19.79 mg/ml), acetic acid (3.11 mg/ml), quinic acid (1.81 mg/ml), oxalic acid (0.82 mg/ml), L-ascorbic acid (0.44 mg/ml), maleic acid (0.04 mg/ml), citric acid (0.64 mg/ml) and fumaric acid (0.004 mg/ml) (Figure 4.8).

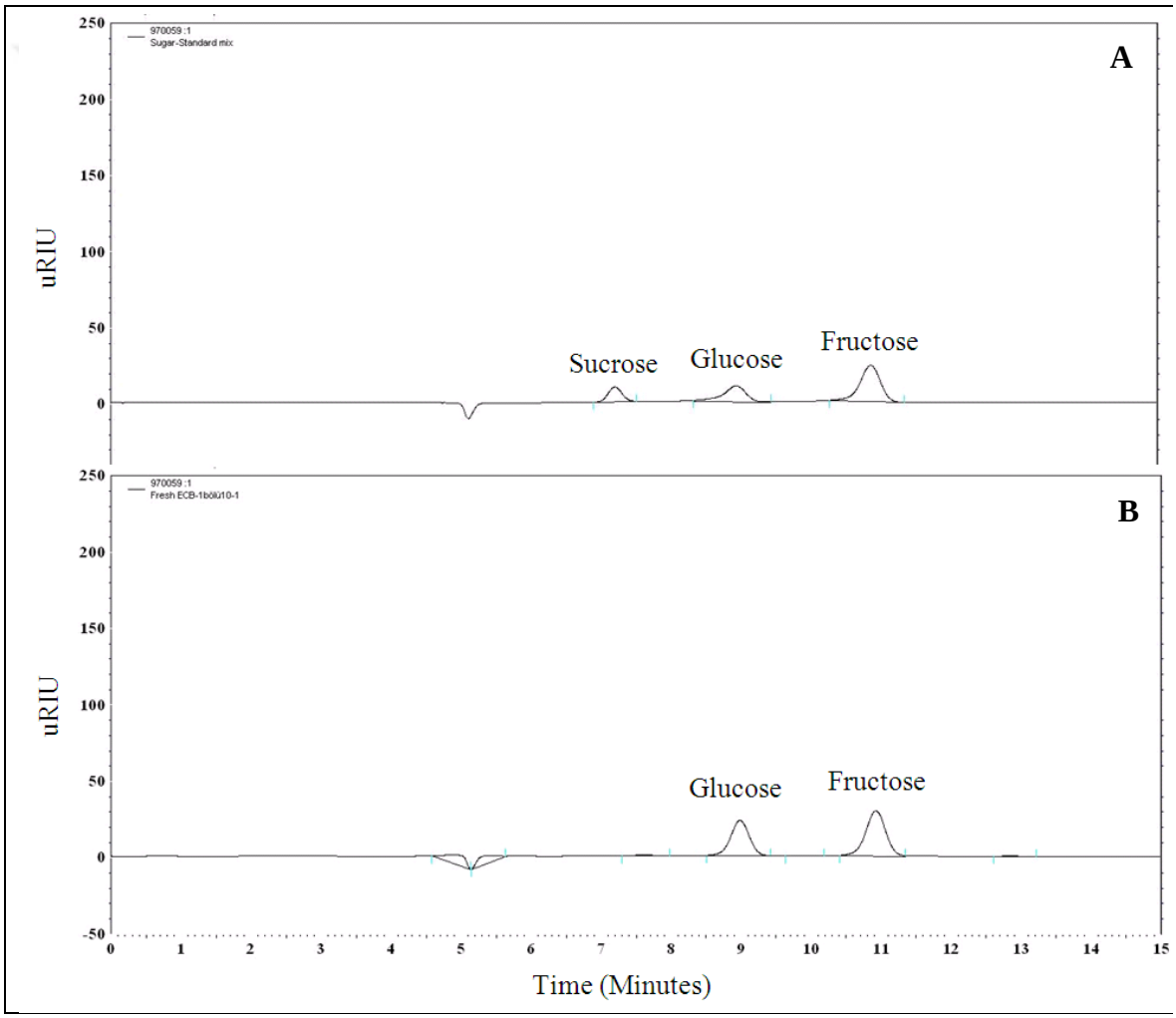


Figure 4.7. HPLC chromatogram of standard compounds of sugar compounds (**A**) and ECB juice of sugar compounds (**B**)

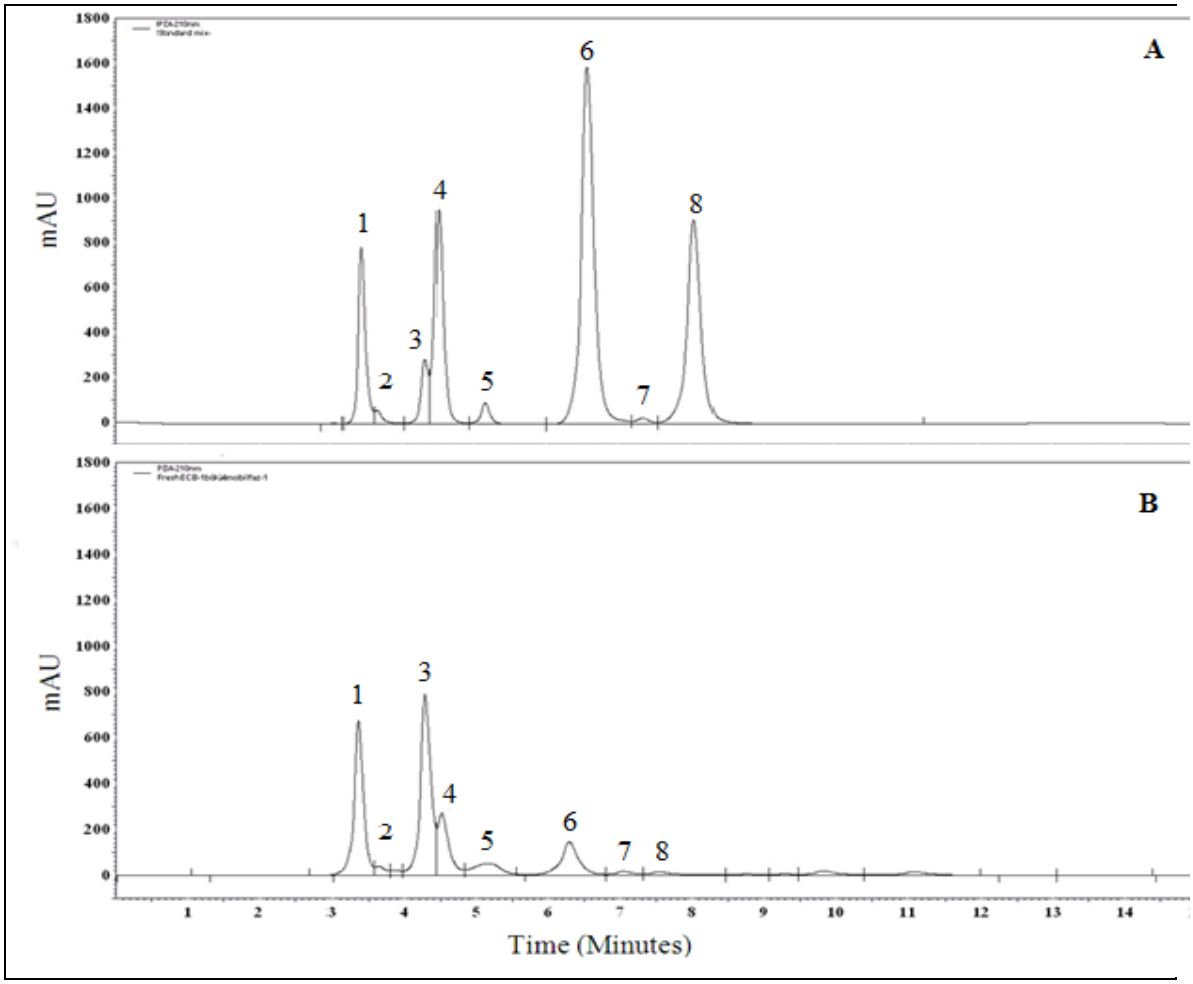


Figure 4.8. Chromatogram (**A)** of standard mixture of oxalic acid (**1**), quinic acid (**2**), D-malic acid (**3**), L-ascorbic acid (**4**), acetic acid (**5**), maleic acid (**6**), citric acid (**7**) and fumaric acid (**8**) and organic acid compounds present in ECB juice (**B**)

While sugar consumed into the medium, all *Lactobacillus* strains produced only lactic acid as an organic acid (Figure 4.9).

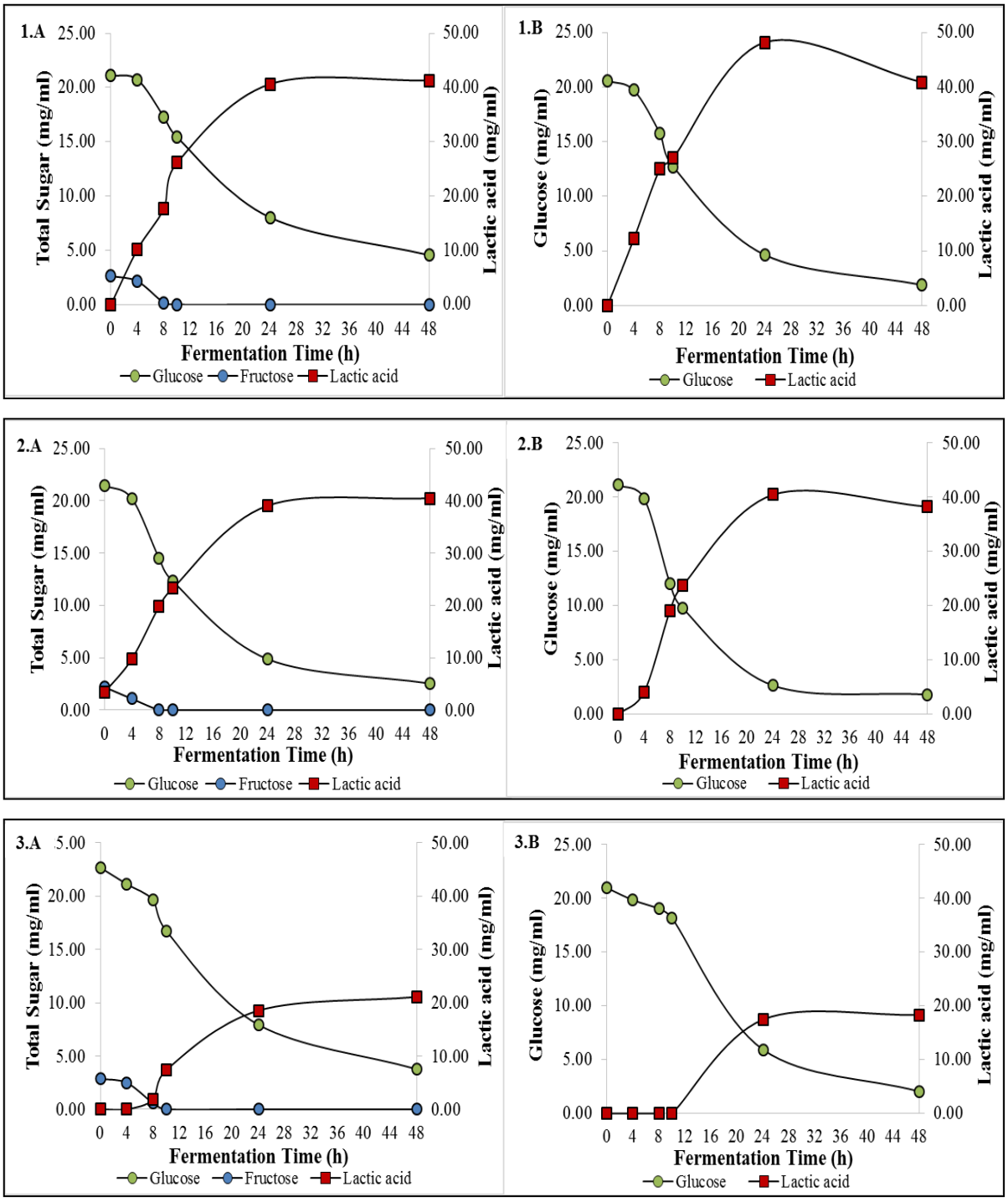


Figure 4.9. Time course for the utilization of sugar and production of lactic acid by *L. plantarum* ATCC 8014 (**1**), *L. plantarum* Lp-115 (**2**) and *L. brevis* ATCC 8287 (**3**) in presence of 5% ECB juice (**A**) and absence of 5% ECB juice (**B**) into the MRS medium

Even it was stated that commercially produced MRS medium, has 20 mg/ml glucose in its composition, the glucose content of MRS used in fermentation was found as 21.27 mg/ml with HPLC-RI. As seen from Figure 4.9, while the sugar content declined gradually, the amount of lactic acid increased for all cultures. Both glucose and fructose were simultaneously consumed. The addition of ECB juice decreased the glucose utilization in ECB added cultures compare to control cultures. The residual glucose amounts of 5 % ECB juice added cultures at the end of fermentation were 21.55 %, 11.77 % and 16.61 % for *L. plantarum* 8014, 115 and *L. brevis*, respectively while the values were 9.19 %, 8.59 % and 9.72 % for control cultures of *L. plantarum* 8014, 115 and *L. brevis,* respectively.

The change in lactic acid production of *L. plantarum* 8014 and 115 were same in presence and absence of ECB in the medium. Amount of lactic acid increased significantly up to 24 h and then slight increase was observed at 48 h in 5 % ECB juice ($p<0.05$) while the amount of lactic acid of control cultures of 8014 and 115 increased up to 24 h and then decreased. On the other hand, lactic acid production profile of *L. brevis* cultures was different between ECB added and control cultures. In ECB added cultures, while lactic acid production (1.85 mg/ml lactic acid) started after 4 h of incubation, in control cultures even there was no lactic acid production at 8th h, the amount of lactic acid sharply increased and reached the 17.48 mg/ml lactic acid after 10 h of incubation. Interestingly, produced lactic acid amounts were similar at the end of fermentation in both ECB added (21.09 mg/ml lactic acid) and control cultures (18.34 mg/ml lactic acid).

5. DISCUSSION

Many studies showed that there are interactions between LAB and PC present in foods [8, 27, 28, 30, 31, 32, 33]. The influence of PC on bacterial growth varies depending on types of microbial strain, different structure of PC and applied concentration of phenolics [29, 34, 35]. The fruits of European cranberrybush have been reported to have high amount of PC. This study aimed to provide information on the effects of PC in fresh ECB juice on growth of different strains of LAB.

CA was previously found as a main phenolic compound present in ECB [72, 87, 88]. That's why, in this study, firstly the growth effect of different concentrations of CA on *L. plantarum* 8014 was investigated in microplates. At high concentration (2.12 mg/ml), CA was inhibitory to *L. plantarum* growth. Inhibitory effects of phenolic compounds at high concentrations have been previously reported in literature [3, 35, 36, 48, 49]. In the study of Campos et al. [35], high concentrations of hydroxybenzoic and hydroxycinnamic (0.5 mg/ml) acids inhibited the growth of *Oenococcus oeni* and *L. hilgardii* isolated from wine. Similarly, gallic acid and catechins higher than 0.2 mg/ml concentrations showed inhibitory effects on *L. hilgardii* [48]. Rozes and Peres [36] showed that the lag phase of *L. plantarum* induced in the presence of 1 mg/ml of tannin and the viable count was reduced. In another study [116], the maximal OD_{600} and μ_{max} values of *L. plantarum* IFPL935 decreased together with an increase in lag time during growth in presence of catechin, epicatechin, epicatechin-3-*O*-gallate and B and A type procyanidins at concentration form 0.25 to 1.0 mM. Similar results were observed in the study of Duda-Chodak et al. [38], quercetin showed an inhibitory effect at concentrations of 25-50 μM on *L. casei* growth. The growth of *L. plantarum* 8014 was comparable and even higher than control cultures at concentrations lower than 0.71 mg/ml of CA added cultures suggesting that CA at these concentrations might have stimulatory effect on *L. plantarum* growth. It has been stated that PC may act as either stimulating or promoting substrates on growth of LAB [29, 35, 38]. Catechin at concentration of 100-400 μM and chlorogenic acid (400 μM) had a stimulatory effect on *L. casei* growth [38]. The study of Campos et al. [35] showed that the final cell density of hydroxycinnamic acid (0.1-0.2 mg/ml) added cultures of *L. hilgardii* was higher than control

cultures suggesting that at these concentrations hydroxycinnamic acid could be activating *L. hilgardii* growth. Likewise, the addition of 0.1-0.2 mg/ml of catechin stimulated the growth of *L. hilgardii* in growth media. Figueiredo et al. [109] determined an increase in the final cell density of *L. hilgardii* in the presence of quercetin (0.04 mg/ml) compared to control culture. Since the addition of 0.35 mg/ml CA (1 mM) to media resulted with higher cell density (ΔOD) than other CA concentrations and also the growth rate (μ_{max}) at this concentration was significantly better than control, it was chosen as optimum concentration for scale up experiments. Also, in the studies related with the influence of phenolic compounds on the LAB growth, generally 1mM of phenolic compound was chosen as final concentration because this concentration allows bacterial growth and provides an adequate chromatographic resolution for metabolism studies [8, 28, 30, 31, 32].

The result of 0.35 mg/ml CA added cultures of *L. plantarum* 8014 grown in the presence of glucose or galactose in BM showed that the growth was better in glucose. Glucose was replaced with galactose to avoid a possible glucose carbon catabolite repression on *L. plantarum* 8014 growth [30] since *L. plantarum* preferentially uses glucose as a carbon source [8]. However, no metabolic shift was observed for utilization of carbon source during *L. plantarum* growth in the presence of CA. Log cfu/ml values of 0.35 mg/ml CA added *L. plantarum* cultures in tubes gave comparable results with OD readings of same cultures in microplate. The amount of CA did not change during fermentation period indicating that CA was not metabolized by *L. plantarum* 8014. The reason might be related that *L. plantarum* ATCC 8014 might not have the enzymes to degrade the bound form of hydroxycinnamic acids, which are glycosylated derivatives or esters of quinic, shikimic and tartaric acid. CA, hydroxycinnamic acid, is formed by the combination of caffeic and quinic acid. In the study of Rodriguez et al. [8], the metabolism of *L. plantarum* CECT 748 on different types of free and bound form of hydroxycinnamic acid was analyzed and the results showed that while phenolic acid decarboxylase enzymes was able to degrade *p*-coumaric, caffeic and ferulic acids, CA was not degraded. On the other hand, *L. johnsonii, L. gasseri* and *L. reuteri* metabolized CA present in coffee [44, 52, 115]. While *L. johnsonii* transformed chlorogenic acid into caffeic and quinic acid by cinnamoly esterase enzymes, *L. gasseri* and *L. reuteri* degraded CA through the activity of chlorogenic acid esterase. The antioxidant activity (FRAP, ORAC, DPPH) and total phenolic content of 0.35 mg/ml CA added cultures

remained unchanged during fermentation most probably due to no consumption of CA by the *L. plantarum* ATCC 8014.

The growth effect of different percentage of fresh ECB juice on *L. plantarum* ATCC 8014, *L. plantarum* Lp-115 and *L. brevis* ATCC 8287 showed that all strains can grow in the presence of ECB in concentration dependent manner. Similar to CA added cultures, high concentrations (100-50 %) of ECB juice showed inhibitory effect while the decrease in the concentration of ECB juice (from 20 % to 5 %) in MRS medium increased the cell density (ΔOD) of all *Lactobacillus* strains. The μ_{max} of 10 and 5 % ECB juice added *L. plantarum* and *L. brevis cultures* increased significantly with consequential decrease in lag time of cultures compared to control ones showing that the addition of ECB juice in low concentrations stimulated the growth rate of the strains. The addition of 5-10 % ECB juice caused early exponential phase of *L. brevis* cultures than control cultures by reducing time for lag phase. Unlike microplates cultures, there were no significant differences between the OD, dry weight and Log cfu/ml values of 5 % ECB juice added and control cultures of *L. plantarum* species in tubes while the OD values of 5 % ECB juice added *L. brevis* cultures in tubes were significantly higher in exponential phase at 8^{th} and 10^{th} h than control cultures like in microplate assay. In literature, there are some studies related with the effect of different concentration of extracts on LAB strains growth [27, 116]. The concentration and strain dependent growth effect of commercial riched flavan 3-ol grape see extract (GSE) on *L. plantarum* IFPL935 and *L. casei* 7190 studied by Tabasco et al [27]. Whereas high concentration (1 mg/ml) of GSE had inhibitory effect to all GSE added LAB cultures, the μ_{max} of GSE added *L. plantarum* IFPL935 and *L. casei* 7190 cultures were comparable with control cultures [27]. On the other hand, high concentration (1 mg/ml) of commercial cranberry extract had neither inhibitory or stimulatory effect on the growth of *L. plantarum* IFPL935 based on OD readings of culture in microplates [116]. With regard to the mechanism involved in bacteria inactivation by phenolic compounds, it was stated that PC alter the cell membrane structure producing leakage of bacterial cell constituents such as proteins, nucleic acids and inorganic ions [8].

The cell free extracts of 5 % ECB juice added cultures of both *L. plantarum* and *L. brevis* strains in tubes produced only lactic acid as organic acid after fermentation. The addition of

5 % ECB juice did not induced the rate of sugar consumption of *L. plantarum* strains. Glucose was utilized more in control cultures than 5 % ECB juice added cultures. This might be because of the presence of fructose and high amount of malic acid in ECB beside glucose as energy source. LAB convert malic acid into lactic acid during their growth [119]. So that in this study malic acid might be used by LAB strains during fermentation with glucose and fructose for production of lactic acid. Parallel to our results, *L. plantarum* grown in cucumber juice having both glucose and fructose as main sugars, produced only lactic acid [120]. On the other hand, different than *L. plantarum* strains, the cell density of 5 % ECB juice added *L.brevis* culture was significantly higher than control cultures, together with high glucose consumption at 8^{th} and 10^{th} h of the incubation indicating that addition of ECB juice increased sugar utilization during exponential phase of *L. brevis.* Phenolic compounds might affect the sugar utilization of LAB strain by leading to increase or decrease the sugar consumption rate [40, 48, 110, 118]. Similar to results of 5 % ECB juice added *L. brevis* cultures, the glucose and fructose consumption rates of *L. hilgardii* were higher than in the control cultures due to presence of catechin and gallic acid in the study of Alberto et al. [48]. In study of Lopez de Felipe et al. [110], the addition of 1 and 2 mg/ml catechin to growth media showed stimulatory effect on of *L. plantarum* RM71. Cell density of catechin added cultures was higher than control cultures suggesting that catechin stimulated growth by promoting quicker sugar utilization. By contrast with, the rate of sugar consumption of *O. oeni* reduced in the presence of *p*-coumaric, ferulic, caffeic, gallic acid, catechin and malvidin to the media [118]. In addition of caffeic, ferulic, *p*-coumaric, gallic acid, catechin and malvidine, 3,5-diglucoside compounds reduced the consumption rate of glucose and fructose during growth of *O. oeni* culture.

The initial fermentation pH values of the 5 % ECB juice added static cultures were lower than control cultures. However, the pH decreased in both cultures throughout the fermentation due to production of lactic acid. The results showed that low pH of ECB did not change the survival of *L. plantarum* ATCC 8014 and probiotic Lp-115, and *L. brevis* and the growth were same in 5 % ECB juice added static and control cultures during fermentation. The 20 and 10 % ECB strains grew also in low pH media such as 20 and 10 % ECB juice added MRS medium indicating that the low pH might not affect the growth of these strains. Besides, tested strains might be acid tolerant microorganisms. Similarly, *L.*

acidophilus LA39, *L. plantarum* C3, *L. casei* A4 and *L. delbrueckii* D7 survived in the presence of tomato juice which had low pH value (4.1) [122].

The low growth of *L. plantarum* ATCC 8014 in ECB juice diluted in phosphate buffer (50, 20, 10 and 5 %) and no growth at 100 % ECB juice showed that ECB juice was not suitable for the growth of *L. plantarum* ATCC 8014. It has been shown that natural and acidic conditions in fruit juice media limit the growth of LAB [121]. Parallel to this information, both high phenolic content and low pH of 100 % ECB juice might have a potential to inhibit LAB growth.

6. CONCLUSION

In this study, the interaction between phenolic compounds (PC) and lactic acid bacteria were investigated to provide information on the effect of PC in fresh ECB juice on growth of different *Lactobacillus* strains.

The results of this study showed that all *L. plantarum* ATCC 8014, probiotic *L. plantarum* Lp-115 and *L. brevis* ATCC 8287 strains could grow in the presence of CA and fresh ECB juice added to media in concentration dependent manner. Inhibitory effect of CA and ECB juice on growth of strains were resulted at high concentrations may be related with high content of phenolic compounds. Phenolic composition analysis of fresh ECB juice showed that it had high content of catechin and CA and the rich in antioxidant activity. The increase in the cell density and growth rate of the strains at low concentrations of CA and ECB added cultures indicated that ECB juice might be used as a nutrient for this cultures. However, the growth mechanism and the effect of PC other than CA in ECB juice on tested *Lactobacillus* strains is still not clear and needs to be studied in future studies before ECB juice can be considered as growth media in the production of functional foods or probiotics, for sustaining and developing viability of beneficial microorganisms and for the enrichment of products in terms of phenolic content with high antioxidant activities. Also, interaction of ECB juice with different strains of LAB present in gut flora should be investigated to get more information and to increase the utilization of ECB juice in daily diet since PC and LAB interaction might contribute to the regulation of metabolism by intestinal microflora.

REFERENCES

1. B. Dimitrios. Sources of Natural Phenolic Antioxidants. *Trends in Food Science and Technology,* 17:505–512, 2006.

2. C. Kapur, H.C. Kapoor. Antioxidants in Fruits and Vegetables - The Millenium's Health. *International Journal of Food Science and Technology,* 36:703–725, 2001.

3. H. Rodríguez, J. A. Curiel., J. M. Landete, B. de las Rivas, F. López de Felipe, C. Gómez-Cordovés, J. M. Mancheño, R. Muñoz. Food Phenolics and Lactic Acid Bacteria. *International Journal of Food Microbiology,* 132:79–90. 2009.

4. R. Puupponen-Pimiä, A. M. Aura, K. M Oksman-Caldentey, P. Myllärinen, M. Saarela, T. Mattila-Sandholm, and K. Poutanen, Development of Functional Ingredients for Gut Health. *Trends in Food Science and Technology*, 13: 3–11, 2002.

5. F. Cardona, C. Andrés-Lacueva, S. Tulipani, F. J. Tinahones, and M. I. Queipo-Ortuño. Benefits of Polyphenols on Gut Microbiota and Implications in Human Health. *The Journal of Nutritional Biochemistry*, 24: 1415–22, 2013.

6. A. Scalbert, and G. Williamson. Chocolate: Modern Science Investigates an Ancient Medicine Dietary Intake and Bioavailability of Polyphenols. *Journal of Nutrition*, 130: 2073–2085, 2000.

7. C. Manach, A. Scalbert, C. Morand, C. Rémésy and L. Jime. Polyphenols: Food Sources and Bioavailability. *American Journal of Clinical Nutrition,* 79:727-747, 2004.

8. H. Rodríguez, J. M. Landete, B. de las Rivas, R. Muñoz. Metabolism of Food Phenolic Acids by *Lactobacillus plantarum* CECT 748T. *Food Chemistry,* 107:1393–1398, 2008.

9. A. P. Stapleton, E. M. James, G. A. Goodwill, J. C. Frisbee. Obesity and Vascular Dysfunction. *Pathophysiology* 15:79–89, 2008

10. O. Paredes-López, M. L. Cervantes-Ceja, M. Vigna-Pérez and T. Hernández-Pérez. Berries: Improving Human Health and Healthy Aging, and Promoting Quality Life--a review. *Plant Foods for Human Nutrition (Dordrecht, Netherlands)*, 65: 299–308, 2010.

11. D. Hervert-Hernández and I. Goñi. Dietary Polyphenols and Human Gut Microbiota: a Review. *Food Reviews International*, 27:154–169, 2011.

12. C. Manach, G. Williamson, C. Morand, A. Scalbert, C. Rémésy. Bioavailability and Bioefficacy of Polyphenols in Humans: Review of 97 Bioavailability Studies. *American Journal of Clinical Nutrition*, 81:230-242, 2005.

13. A. Scalbert, C. Morand, Manach, C. and C. Rémésy. Absorption and Metabolism of Polyphenols in the Gut and Impact on Health. *Biomedicine and Pharmacotherapy*, 56:276–282, 2002.

14. P. C. Hollman, J. H. de Vries, S.D. van Leeuwen, M. J. Mengelers and M. B. Katan. Absorption of Dietary Quercetin Glycosides and Quercetin in Healthy Ileostomy Volunteers. *American Journal of Clinical Nutrition*, 62:1276-1282, 1995.

15. C. Morand, C. Manach, V. Crespy, C. Rémésy. Quercetin 3-O-beta- glucoside is Better Absorbed than Other Quercetin Forms and is not present in Rat Plasma. *Free Radical Research*, 33-667, 2000.

16. P. C. H. Hollman. Absorption, Bioavailability, and Metabolism of Flavonoids. *Pharmaceutical Biology*, 42:74–83, 2004.

17. B. A. Warden, L. S. Smith, G. R. Beecher, D. A. Balentine, B. A. Clevidence. Catechins are Bioavailable in Men and Women Drinking Black Tea Throughout the Day. *Journal of Nutrition*, 131:1731-1737, 2001.

18. M. R Olthof, P. C. H. Hollman, M.B. Katan. Chlorogenic Acid and Caffeic Acid are Absorbed in Humans. *Journal of Nutrition*, 131:66-71, 2001.

19. F. Tomas-Barberan, R. Garcia-Villalba, A. Quartieri, S. Raimondi, A. Amaretti, A. Leonardi, M. Rossi. *In vitro* Transformation of Chlorogenic Acid by Human Gut Microbiota, *Molecular Nutrition Food Research*, 58: 1122-1131, 2014.

20. P. M. Gonthier, A. C. Verny, C. Besson, C. Remesy, A. Scalbert. Chlorogenic Acid Bioavailability Largely Depends on Its Metabolism by the Gut Microflora in Rats, *Journal of Nutrition*, 133:1853-1859, 2003.

21. P. Dalgaard and K. Koutsoumanis. Comparison of Maximum Specific Growth Rates and Lag Times Estimated from Absorbance and Viable Count Data by Different Mathematical Model. *Journal of Microbiological Methods*, 43:183-196, 2001.

22. N. T. Huynh, J. Van Camp, G. Smagghe, and K. Raes. Improved Release and Metabolism of Flavonoids by Steered Fermentation Processes. *International Journal of Molecular Sciences*, 15:19369–19388, 2014.

23. G. Klein, A. Pack, C. Bonaparte and G. Reuter. Taxonomy and Physiology of Probiotic Lactic Acid Bacteria. *International Journal of Food Microbiology*. 41:103–125, 1998.

24. M. E. Stiles, W. H. Holzapfel. Lactic Acid Bacteria of Foods and Their Current Taxonomy. *International Journal of Food Microbiology*, 36:1-29, 1997.

25. F. Leroy and L. De Vuyst. Lactic Acid Bacteria as Functional Starter Cultures for the Food Fermentation Industry. *Trends in Food Science and Technology*, 15:67–78, 2004.

26. P. Florou-paneri, E. Christaki and E. Bonos. Lactic Acid Bacteria Lactic Acid Bacteria as Source of Functional Ingredients. In: J. M. Kongo, editor, *Lactic Acid Bacteria- R and D for Food*, pages 589-614, Intech, 2013.

27. R. Tabasco, F. Sánchez-Patán, M. Monagas, B. Bartolomé, M. Victoria Moreno-Arribas, C. Peláez and T. Requena. Effect of Grape Polyphenols on Lactic Acid Bacteria and Bifidobacteria Growth: Resistance and Metabolism. *Food Microbiology*, 28:1345–52, 2011.

28. J. A. Curiel, H. Rodríguez, , J. M. Landete, B. de las Rivas and R. Muñoz. Ability of *Lactobacillus brevis* Strains to Degrade Food Phenolic Acids. *Food Chemistry*, 120: 225-229, 2010.

29. D. Hervert-Hernández, C. Pintado, R. Rotger and I. Goñi. Stimulatory Role of Grape Pomace Polyphenols on *Lactobacillus acidophilus* Growth. *International Journal of Food Microbiology*, 136: 119–22, 2009.

30. H. Rodríguez, B. de las Rivas, C. Gómez-Cordovés, R. Muñoz. Degradation of Tannic Acid by Cell-Free Extracts of *Lactobacillus plantarum*. *Food Chemistry*, 107:664–670, 2008.

31. H. Rodríguez, B. de las Rivas, R. Muñoz, C. Gómez-Cordovés. Characterization of Tannase Activity in Cell-Free Extracts of *Lactobacillus plantarum* CECT 748T. *International Journal of Food Microbiology*, 121:92–98, 2008.

32. H. Rodríguez, J. M Landete, J. A. Curiel, B. de las Rivas, J. M. Mancheño, R. Muñoz. Characterization of the *p*-coumaric Acid Decarboxylase from *Lactobacillus plantarum* CECT 748T. *Journal of Agricultural and Food Chemistry*, 56:3068-307, 2008.

33. M. R. Alberto, C. Gomez-Cordoves, M. C. Manca de Nadra. Metabolism of Gallic Acid and Catechin by *Lactobacillus hilgardii* from Wine. *Journal of Agriculture and Food Chemistry*, 52:6465– 6469, 2004.

34. M. P. Almajano, R. Carbó, J. A. López-Jiménez, M. H. Gordon. Antioxidant and Antimicrobial Activities of Tea Infusions. *Food Chemistry*, 108:55–63, 2008.

35. F. M. Campos, J. A. Couto, T. A. Hogg. Influence of Phenolic Acids on Growth and Inactivation of *Oenococcus oeni* and *Lactobacillus hilgardii*. *Journal of Applied Microbiology*, 94:167–174, 2003.

36. N. Rozès and C. Peres. Effects of Phenolic Compounds on the Growth and the Fatty Acid Composition of *Lactobacillus plantarum*. *Applied Microbiology and Biotechnology*, 49:108–111, 1998.

37. G Salih, J. M. Le Quéré, J. F. Drilleau. Action Des Acides Hydroxycinnamiques Libres et Esterifiés Sur la Croissance Des Bactéries Lactiques. *Science des Aliments*, 20:537–560, 2000.

38. A. Duda-Chodak, T. Tarko and M. Statek. The Effect of Antioxidants on *Lactobacillus casei* Cultures. *Acta Scientiarum Polonorum Technologia Alimentaria*, 7:39-51, 2008.

39. F. López de Felipe, J. A. Curiel, R. Muñoz. Improvement of the Fermentation Performance of *Lactobacillus plantarum* by the Flavanol Catechin is Uncoupled from its Degradation. *Journal of Applied Microbiology***,** 109: 687–697, 2010.

40. J. A. Curiel, R. Muñoz and F. López de Felipe. pH and Dose-Dependent Effects of Quercetin on the Fermentation Capacity of *Lactobacillus plantarum*. *LWT - Food Science and Technology*, 43:926–933, 2010.

41. M. H. Zwietering, I. Jongenburger, F. M. Rombouts and K. van't Riet. Modeling of the Bacterial Growth Curve. *Applied and Environmental Microbiology*, 56:1875–1881, 1990.

42. B. G. Hall, H. Acar, A. Nandipati, M. Barlow. Growth Rates Made Easy. *Molecular Biology and Evolution*, 31:232-238, 2014.

43. S. Gupta, N. Abu-Ghannama, A.G. M. Scannell. Growth and Kinetics of *Lactobacillus plantarum* in the Fermentation of Edible Irish Brown Seaweeds. *Food and Bioproducts Processing*, 89:346-355, 2011.

44. J. M. Landete, J. A. Curiel, H. Rodríguez, B. de las Rivas, R. Muñoz. Study of the Inhibitory Activity of Phenolic Compounds Found in Olive Products and Their Degradation by *Lactobacillus plantarum* Strains. *Food Chemistry*, 107:320–326, 2008.

45. J. M. Landete, H. Rodríguez,, B. de las Rivas, R. Muñoz, Characterization of a Benzyl Alcohol Dehydrogenase From *Lactobacillus plantarum* WCFS1. *Journal of Agricultural and Food Chemistry*, 56: 4497–4503, 2008.

46. J. A. Curiel, H. Rodríguez, , J. M. Landete, B. de las Rivas, R. Muñoz. Ability of *Lactobacillus brevis* Strains to Degrade Food Phenolic Acids. *Food Chemistry*, 12:225–229, 2010.

47. A. F. Sanchez-Maldonado, S. Schieber, M. G. Ganzle. Structure-Function Relationships of the Antibacterial Activity of Phenolic Acids and Their Metabolism by Lactic Acid Bacteria. *Journal of Applied Microbiology*, 111:1176-1184, 2011.

48. M. R. Alberto, M. E. Farias, M. C. Manca de Nadra. Effect of Gallic acid and Catechin *on Lactobacillus hilgardii* 5w Growth and Metabolism of Organic Compounds. *Journal of Agricultural and Food Chemistry*, 49:4359–4363, 2001.

49. L. Svensson, B. Sekwati-Monang, D. Lopes Lutz, A. Schieber and M. G. Ganzle. Phenolic acids and Flavonoids in Nonfermented and Fermented Red Sorghum (*Sorgum bicolor* (L.) Moench). *Journal of Agriculture and Food Chemistry*, 58:9214-9220, 2010.

50. S. Van Beek and F. G. Priest. Decarboxylation of Substituted Cinnamic Acids by Lactic Acid Bacteria Isolated During Malt Whisky Fermentation. *Applied Environmental Microbiology*, 66:5322-5328, 2000.

51. L. Barthelmebs, C. Divies, J. F. Cavin. Knockout of the p-coumarate Decarboxylase Gene From *Lactobacillus plantarum* Reveals the Existence of Two Other Inducible Enzymatic Activities Involved in Phenolic Acid Metabolism. *Applied and Environmental Microbiology*, 66:3368–3375, 2000.

52. R. Bel-Rhlid, D. Thapa, K. Kraehenbuehl, C. E. Hansen and L. Fischer. Biotransformation of Caffeoyl Quinic Acids from Green Coffee Extracts by *Lactobacillus johnsonii* NCC 533. AMB Express, 3:28, 2013.

53. R. Puupponen-Pimiä, L. Nohynek, S. Hartmann-Schmidlin, M. Kähkönen,M. Heinonen, K. Määttä-Riihinen, K. M. Oksman-Caldentey. Berry Phenolics Selectively Inhibit the Growth of Intestinal Pathogens. *Journal of Applied Microbiology*, 98:991–1000, 2005.

54. P.N. Seeram. Berry fruits: Compositional Elements, Biochemical Activities, and the Impact of Their Intake on Human Health, Performance, and Disease, *Journal of Agricultural Food Chemistry* 56:627–629, 2008.

55. E. Pappas and K. M. Schaich.. Phytochemicals of Cranberries and Cranberry Products: Characterization, Potential Health Effects, and Processing Stability. *Critical Reviews in Food Science and Nutrition*, 49:41–81, 2009.

56. S. Sakamura and F. J. Francis. The Anthocyanins of the American Cranberry. *Journal of Food Science.* 26:318–321, 1961.

57. Zapsalis, C. and Francis, F. J. Cranberry Anthocyanins. *Journal of Food Science,* 30:396–399,1965.

58. T. Fuleki and F. J. Francis. The Cooccurrence of Monoglucosides and Monogalactosides of Cyanidin and Peonidin in the American Cranberry, *Vaccinium macrocarpon*. *Phytochemistry,* 6:1705–1708, 1967.

59. X. Wu and R. L. Prior. Identification and Characterization of Anthocyanins by High-Performance Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry in Common Foods in the United States: Vegetables, Nuts and Grains. *Journal of Agricultural and Food Chemistry*, 53:3101-13, 2005.

60. G. M. Sapers, S. B. Jones, and G. T. Maher, Factors Affecting the Recovery of Juice and Anthocyanin From Cranberries. *Journal of American Society for Horticultural Science.* 108:246–249, 1983.

61. G. M. Sapers, J. G. Phillips, H.M. Rudolf, A. M. DiVito. Cranberry Quality: Selection Procedures for Breeding Programs. *Journal of American Society for Horticultural Science.* 108:241–246, 1938.

62. N. Vorsa, and W. V. Welker. Relationship between Fruit Size and Extractable Anthocyanin Content in Cranberry. *Horticultural Science.* 20:402–403, 1985.

63. M. Ozgen, K. M Farag, S. Ozgen and J. P. Palta. Lysophosphatidyl Ethanolamine Accelerates Color Development and Promotes Shelf Life of Cranberries. *Horticultural Science.* 40:127–130, 2005.

64. C. Santos-Buelga and A. Scalbert. Proanthocyanidins and Tannin-like Compounds Nature, Occurrence, Dietary Intake and Effects on Nutrition. *Journal of Science of Food and Agriculture,* 80:1094–1117, 2000.

65. L. Gu, M. A. Kelm, J. F. Hammerstone, G. Beecher, J. D. Holden Haytowitz, S. Gebhardt and R. L. Prior. Concentrations of Proanthocyanidins in Common Foods and Estimations of Normal Consumption. *Journal of Nutrition,* 134:613–617, 2004.

66. B. Moldovan, O. Ghic, D. Luminita, C. Chisbora. The Influence of Storage on the Total Phenols Content and Antioxidant Activity of the Cranberrybush (*Viburnum opulus* L.) Fruits Extract. *Revista de Chimie*, 63:463-464, 2012.

67. D. G. Cunningham, S. A. Vannozzi, R. Turk, R. Roderick, E. O'Shea. Cranberry phytochemicals and Their Health Benefits. In: Nutraceutical Beverages: Chemistry, Nutrition, and Health Effects (ACS Symposium Series 871), pp. 35–50. Shahidi, F and Weerasinghe, D. K., Eds., American Chemical Society, Washington, DC, 2004.

68. W. Mullen, S. C. Marks, A. Crozier. Evaluation of Phenolic Compounds in Commercial Fruit Juices and Fruit Drinks. *Journal of Agricultural and Food Chemistry*, 55:3148–3157, 2007.

69. H. Chen, Y. Zuo and Y. Deng. Separation and Determination of Flavonoids and Other Phenolic Compounds in Cranberry Juice by High Performance Liquid Chromatography. *Journal of Chromatography A*, 913:387–395, 2001.

70. R. Herwig. 350 Trees, Shrubs and Conifers in full color. London: David and Charles Newton Abbot, 200, 1986.

71. R. G Ovodova, V. V. Golovchenko, S. V. Popov, A. S. Shashkov and Y. S. Ovodov. The Isolation, Preliminary Structural Studies, and Physiological Activity of Water-soluble Polysaccharides from the Squeezed Berries of the Snowball Tree Viburnum opulus, *Russian Journal of Bioorganic Chemistry*, 26:54–59, 2000.

72. S. Y. Velioglu, L. Ekici, E. S. Poyrazoglu. Phenolic composition of European cranberrybush (Viburnum opulus L.) Berries and Astringency Removal of its Commercial Juice. *International Journal of Food Science and Technology*, 41:1011-1015, 2006.

73. L. Cesoniene, R. Daubaras, J. Vencloviene and P. Viškelis. Biochemical and Agro-biological Diversity of *Viburnum opulus* Genotypes. Central European. *Journal of Biology*, 6:864–871, 2010.

74. V. Kraujalytė, E. Leitner P. R.. Venskutonis. Chemical and Sensory Characterisation of Aroma of *Viburnum opulus* Fruits by Solid Phase Microextraction-Gas Chromatography–Olfactometry. *Food Chemistry*, 132:717–723, 2012.

75. O. Rop, V. Reznicek, M. Valsikova, T. Jurikova, J. Mlcek, D. Kramarova. Antioxidant Properties of European cranberrybush fruit (*Viburnum opulus* var. *edule*). *Molecules (Basel, Switzerland)*, 15:4467–4477, 2010.

76. O. Sagdıc, A. Aksoy, G. Ozkan. Evaluation of the Antibacterial and Antioxidant Potentials of Cranberry (Gilaburu, *Viburnum opulus* L.) Fruit Juice. *Acta Alimentaria*, 35:487-492, 2006.

77. O. Sagdic, I. Ozturk, N. Yapar, H. Yetim. Diversity and Probiotic Potentials of Lactic Acid Bacteria Isolated From Gilaburu, a Traditional Turkish Fermented European cranberrybush (*Viburnum opulus* L.) Fruit Drink. *Food Research International*, 64:537–545, 2014.

78. M. Yilmaztekin and K. Sislioglu. Changes in Volatile Compounds and Some Physicochemical Properties of European cranberrybush (*Viburnum opulus* L.) During Ripening Through Traditional Termentation. *Journal of Food Science*, 80:687–694, 2015.

79. M. Cam and Y. Hisil. Comparison of Chemical Characteristics of Fresh and Pasteurised Juice of Gilaburu (*Viburnum opulus* L.). *Acta Alimentaria*, 36:381–385, 2007.

80. M. Akbulut, S. Causir, T. Marakoglu, H. Coklar. Chemical and Technological Properties of European cranberrybush. *Asian Journal of Chemistry*, 20:1876-1885, 2008.

81. L. Cesoniene, R. Daubaras, P. Viškelis. Evaluation of Productivity and Biochemical Components in Fruit of Different *Viburnum* accessions. *Biologija*, 54:93–96, 2008.

82. L. Cesonienė, R. Daubaras, P. Viškelis, A. Sarkinas. Determination of the Total Phenolic and Anthocyanin Contents and Antimicrobial Activity of *Viburnum opulus* Fruit Juice. *Plant Foods for Human Nutrition (Dordrecht, Netherlands)*, 67:256–61, 2012.

83. M. L. Altun, G. S. Citoğlu, B. S. Yilmaz, T. Coban. Antioxidant Properties of *Viburnum opulus* and *Viburnum lantana* Growing in Turkey. *International Journal of Food Sciences and Nutrition*, 59:175–80, 2008.

84. M. Cam, Y. Hısıl, A. Kuscu. Organic acid, Phenolic Content and Antioxidant Capacitiy of Fruit Flesh and Seed of *Viburnum opulus*. *Chemistry of Natural Compounds*, 43:460-461, 2007.

85. M. Jordheim, N. H. Giske, O. M. Andersen. Anthocyanins in Caprifoliaceae. *Biochemical Systematics and Ecology*, 35:153–159, 2007.

86. B. Moldovan, L. David, C. Chişbora, C. Cimpoiu. Degradation Kinetics of Anthocyanins From European cranberrybush (*Viburnum opulus* L.) Fruit Extracts. Effects of temperature, pH and Storage Solvent. *Molecules (Basel, Switzerland)*, 17:11655–66, 2012.

87. V. Kraujalytė, P. R. Venskutonis, A. Pukalskas, L. Česonienė, R. Daubaras. Antioxidant Properties and Polyphenolic Compositions of Fruits From Different European cranberrybush (*Viburnum opulus* L.) Genotypes. *Food Chemistry*, 141:3695–702, 2013.

88. R. A. Oral, M. Doğan, K. Sarioğlu. Recovery of Bioactive Phenolic Compounds From Olive Mill Waste Water, Pomegranate Peel, and European cranberrybush (*Viburnum opulus* L.) Juice by Preparative MPLC, *Journal of Liquid Chromatography and Related Technologies*, 37:1827-1836, 2014.

89. A. Karaçelik, M. Küçük, Z. İskefiyeli, S. Aydemir, S. De Smet, B. Miserez, P. Sandra. Antioxidant components of *Viburnum opulus* L. determined by on-line HPLC-UV-ABTS Radical Scavenging and LC-UV-ESI-MS Methods. *Food Chemistry*, 175:106–14, 2015.

90. O. S. Zayachkivska, M. R. Gzhegotsky, O. I. Terletska, D. A. Lutsyk, A. M. Yaschenko, O. R. Dzhura. Influence of *Viburnum opulus* Proanthocyanidins on Stress Induced Gastrointestinal Mucosal Damage. *Journal of Physiology and Pharmacology*, 5:155–167, 2006.

91. J. B. Blumberg, T. A. Camesano, A. Cassidy, P. Kris-Etherton, A. Howell, C. Manach, L. M. Ostertag, H. A. Sies Skulas-Ray, J. A. Vita. Cranberries and Their Bioactive

Constituents in Human Health. *Advances in Nutrition: An International Review Journal*, 4:618-632, 2013.

92. N. Yapar. The possibility of Using Lactic Acid Bacteria Isolated From Traditionally Fermented European Cranberrybush (*Viburnum Opulus* L.) Juice in the Manufacture of Industrial Fruit Juice. Erciyes University, Graduate School of Natural and Applied Sciences Master of Science Thesis, July 2008

93. H. Hızlısoy. The investigation of Antimicrobial Effects of Gilaburu Against Various Microorganisms. Erciyes University, Graduate School of Health Sciences Master of Science Thesis, January 2009.

94. H. Ulger, T. Ertekin, O. Karaca, O. Canoz, M. Nisari, E. Unur, F. Elmalı. Influence of Gilaburu (*Viburnum opulus*) juice on 1, 2-dimethylhydrazine (DMH)-induced Colon Cancer. *Toxicology and Industiral Health*. 29:824–29, 2013.

95. F. Widdel, Theory and Measurement of Bacterial Growth, 1–11. *Grundpraktikum Mikrobiologie, 4. Sem. (B.Sc.) Universität Bremen.* 2010.

96. R. Andersson, and B. Hedlund. HPLC Analysis of Organic Acids in Lactic Acid Fermented Vegetables., *Zeitschrift für Lebensmittel-Untersuchung und -Forschung*, 176:440–443, 1983.

97. R. Scherer, , A. C. Poloni Rybka, C. A. Ballus, , A. Dillenburg Meinhart, , J. Teixeira Filho, H. Teixeira Godoy. Validation of a HPLC Method for Simultaneous Determination of Main Organic Acids in Fruits and Juices. *Food Chemistry*, 135:150-154, 2012.

98. J. Lee, R. W. Durst, R. E. Wrolstad. Determination of Total Monomeric Anthocyanin Pigment Content of Fruit Juices, Beverages, Natural Colorants and Wines by the pH Differential Method: Collabrative study. *Journal of AOAC International*, 88:1269-1278, 2005.

99. V. L. Singleton, R. Orthofer, and R. M. Lamuela-Raventos. Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin– Ciocalteu Reagent. *Methods in Enzymology*, 299:152–178, 1999.

100. I. F. Benzie, and J. J. Strain. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of Antioxidant Power: the FRAP Assay. *Analytical Biochemistry*, 239:70–76, 1996.

101.G. Bratbak and Dundas I. Bacterial Dry Matter Content and Biomass Estimations. *Applied and Environmental Microbiology*, 48:755-757, 1984.

102.W. Brand-Williams, M. E. Cuvelier, C. Berset. Use of Free Radical Method to Evaluate Antioxidant Activity. *Lebensm Wiss Technology,* 28:25-30, 1995.

103. B. Ou, M. Hampsch-Woodill, R. Prior. Development and Validation of an Improved Oxygen Radical Absorbance Capacity Assay Using Fluorescein as the Fluorescent Probe. *Journal of Agricultural and Food Chemistry*, 49:4619–4626, 2001.

104. C. Y. Chen, P. E. Milbury, K. Lapsley, J. B. Blumberg. Flavonoids From Almond Skins are Bioavailable and Act Synergistically With Vitamins C and E to Enhance Hamster and Human LDL Resistance to Oxidation. *Journal of Nutrition*, 135:1366−1373, 2005.

105. B. Gompertz. On the nature of the function expressive of the law of human mortality, and on a new mode of determining the value of life contingencies. *Philosophical Transactions of the Royal Society B,*115:513-585, 1825

106. Stannard, C. J., A. P. Williams, and P. A. Gibbs. Temperature/growth relationship for psychotrophic food-spoilage bacteria. *Food Microbiology*, 2:115-122, 1985.

107. Schnute, J. A versatile growth model with statistically stable parameters. *Canadian Journal of Fisheries and Aquatic Sciences,* 38:1128-1140, 1981.

108. Ricker, W. E. Growth rates and models. *Fish Physiology and Biochemistry*, 8:677-743, 1979.

109. A. R. Figueiredo, F. Campos, V. de Freitas, T. Hogg, A. Couto. Effect of Phenolic Aldehydes and Flavonoids on Growth and Inactivation of *Oenococcus oeni* and *Lactobacillus hilgardii*. *Food Microbiology*, 25:105-112, 2008.

110. F. Lopez de Felipe, J. A. Curiel, R. Munoz. Improvement of the Fermentation Performance of *Lactobacillus plantarum* by the Flavanol Catechin is Uncoupled from its Degradation. *Journal of Applied Microbiology*, 2:687-697, 2010.

111. P. Filannino, M. Gobbetti, M de Angelis and R. Di Cagno. Hydroxycinnamic Acids Used as External Acceptors of Electrons: an Energetic Advantage for Strictly Heterofermentative Lactic Acid Bacteria. *Applied and Environmental Microbiology*, 80:7574-7582, 2014.

112.S. Perni, P. W. Andrew, G. Shama. Estimating the Maximum Growth Rate from Microbial Growth Curves: Definition is Everything. *Food Microbiology*, 22:491-495, 2005.

113.I. Mytilinaios, M. Salih, H. K. Schofield, R. J. W. Lambert. Growth Curve Prediction from Optical Density Data. *International Journal of Food Microbiology*, 154:169-176, 2012

114. F. J. Richards. A Flexible Growth Function for Empirical Use. *Journal of Experimental Botany*. 10:290-300, 1959.

115. D. Coteau, A. L. Mc Cartney, G. R. Gibson, G. Williamson and C. B. Faulds. Isolation and Characterization of Human Colon Bacteria able to Hydrolyse Chlorogenic Acid. *Journal of Applied Microbiology*, 90:873–881, 2001.

116. F. Sánchez-Patán, R. Tabasco, M. Monagas, T. Requena, C. Pelaez, M. V. Moreno-Arribas, B. Bartolomé. Capability of *Lactobacillus plantarum* IFPL935 to Catabolize

Flavan-3-ol Compounds and Complex Phenolic Extracts. *Journal of Agricultural and Food Chemistry*, 60:7142-7151, 2012.

117. J. Dobes, O. Zitka, J. Sochor, B. Ruttkay-Nedecky, P. Babula, M. Beklova3,4, J. Kynicky, J. Hubalek, B. Klejdus, R. Kizek, V. Adam. Electrochemical Tools for Determination of Phenolic Compounds in Plants. A Review. *International Journal of Electrochemical Science,* 8:4520 – 4542, 2013.

118. N. Rozes, L. Arola and A. Bordons. Effect of phenolic compounds on the co-metabolism of citric acid and sugars by *Oenococcus oeni* from wine. *Letters in Applied Microbiology*, 36:337-341, 2003.

119. F. V. Passos, H. P. Fleming, H. M. Hassan and R. F. McFeeters. Effect of Malic Acid on the Growth Kinetics of *Lactobacillus plantarum*. *Applied Microbiology Biotechnology*, 63:207-211, 2003.

120. F. V. Passos, H. P. Fleming, D. F. Ollis, R. M. Felder and R. F. McFeeters. Kinetics and Modelling of Lactic Acid Production by *Lactobacillus plantarum*. *Applied and Environmental Microbiology*. 60:2627-2636, 1994.

121. R. Yanez, S. Marques, F. M. Girio, J. C. Roseiro. The Effect of Acid on Lactate Production and Growth Kinetics in *Lactobacillus rhamnosus* Cultures. *Process Biochemistry*, 43:356-361, 2008.

122. K. Young, E. Woodams, Y. D. Hang. Probiotication of Tomato Juice by Lactic Acid Bacteria. *Journal of Microbiology*, 42:315-318, 2004.