Journal of Food and Nutrition Research, 2018, Vol. 6, No. 10, 621-625
Available online at http://pubs.sciepub.com/jfnr/6/10/2

DOI:10.12691/jfnr-6-10-2

In-vitro Prebiotic Activity of Grape Seed Flour Highly Rich in Flavonoid and Dietary Fiber

Ji-Hye Kwon[1], Min Young Kim[2], Kun-Ho Seo[3], Hyeon Gyu Lee[1,*], Hyunsook Kim[1,*]

[1]Department of Food and nutrition, Hanyang University, Seoul, South Korea
[2]Department of Food Science and Technology, Chungbuk National University, Chungju, South Korea
[3]KU Center for Food Safety, College of Veterinary Medicine, Konkuk University, Seoul, South Korea
*Corresponding author: hyunsk15@hanyang.ac.kr, hyeonlee@hanyang.ac.kr

Received August 12, 2018; Revised September 17, 2018; Accepted October 25, 2018

Abstract Interactions between lactic acid bacteria (LAB) and dietary polyphenols are not fully understood. This study determined the prebiotic effects of grape seed flour (GSF, a byproduct of wine making) and their polyphenol extracts (GSE) on the growth of 10 strains of probiotic LAB recently isolated from the kefir and pathogenic *Clostridium perfringens*. Growth rate of the LAB, *Leuconostoc mesenteroides* DH 1608, was the highest among 10 strains of LAB when cultivated with GSF. GSE, containing an equivalent amount of polyphenol in GSF, also promoted the growth of *L. mesenteroides* DH 1608 but at a lower rate compared to GSF. Growth of *C. perfringens* decreased significantly at 10 mg/mL of GSF, but GSE did not affect the growth. In conclusion, GSF may be a useful prebiotic dietary supplement due to its selective antimicrobial capacity via stimulation of probiotic bacteria and inhibition of pathogenic bacteria. A symbiotic combination of selected lactic acid bacteria and GSF may improve gut health.

Keywords: *grape seed flour, grape seed extracts, lactic acid bacteria, kefir, prebiotics, antimicrobial activity*

Cite This Article: Ji-Hye Kwon, Min Young Kim, Kun-Ho Seo, Hyeon Gyu Lee, and Hyunsook Kim, "*In-vitro* Prebiotic Activity of Grape Seed Flour Highly Rich in Flavonoid and Dietary Fiber." *Journal of Food and Nutrition Research*, vol. 6, no. 10 (2018): 621-625. doi: 10.12691/jfnr-6-10-2.

1. Introduction

In recent years, there has been growing interest in wine grape seed flour (GSF) which is a by-product of wine making due to its potential health beneficial effects on obesity, cardiovascular disease, nonalcoholic fatty liver disease (NAFLD) [1,2]. Interestingly, these effects were closely associated with regulation of intestinal microbiota suggesting a possible prebiotic role of GSF [2]. GSF contains two-thirds of the extractable flavonoids of grape with high amounts of flavonoids, flavan-3-ols (flavanols) such as catechin, epicatechin, their 3-O-gallates, and (epi)catechin dimers, oligomers, and polymers [3]. The oligo- and polymeric catchins are not readily absorbed by the small intestine and reach the colon where they are metabolized by gut bacteria [4]. Intensive studies have recently explored interactions between aqueous or alcoholic extractable polyphenols and intestinal microbiota. Extractable polyphenols from grape seed pomace stimulated growth of *Lactobacillus acidophilus* and *Bifidobacterium adolescentis* and *B. bifidum* [5] and inhibited the growth of pathogenic bacteria such as *Clostridia* [6,7,8]. However, these studies have not evaluated the effects of whole wine grape seed that is high in dietary fiber and other bioactive components as well as polyphenols, on microbial growth selectively.

Kefir, a probiotic, is a traditional fermented milk of Caucasus Mountains where it is famous for increasing longevity. In addition to lactic acid bacteria, it consists of yeast and acetic acid bacteria that comprise together approximately 10^9 CFU/mL. It has shown potential health benefits including anti-obesity, anti-diabetic, anti-stress, hypocholesterolemic, and antimicrobial activity [9,10]. Previously, we isolated and characterized major lactic acid bacteria from Kefir. These lactic acid bacteria were shown to have a high *in-vitro* survival rate at low pH, were able to grow in an artificial intestinal environment, were bile acid tolerant and had an anti-obesity effect indicating roles as a probiotic [11,12].

To the best of our knowledge, there has been no study reporting the selective modulation of growth of lactic acid bacteria and pathogenic bacteria by whole wine grape flour. This study aimed to determine whether polyphenol-rich whole GSF stimulates the growth of lactic acid bacteria isolated from probiotic Kefir and inhibits the growth of toxigenic bacteria (*Clostridium perfringens*) in *in-vitro* system and compares these effects with GSE.

2. Materials and Methods

2.1. Materials

Lactococcus lactics subsp. Lactics (DH 1601~1603, 1605), *Leuconostoc mesenteroides* (DH 1604, 1606~1609) and *Lactobacillus kefiri* DH5 isolated from kefir fermented milk (Konkuk University, Korea) were used. *Clostridium*

perfringens (KCTC 3269) were cultured in Reinforced Clostridial Broth (MBcell, Seoul Korea). Skimmed milk powder for the lactic acid bacteria stock was purchased from Sigma-Aldrich Chemical Co. Ltd (St. Louis, MO, USA). Lactic acid bacteria were incubated in Man-Rogosa-Sharpe (MRS) broth (Difco, Detroit, MI, USA). MRS agar plates were made by adding agar powder. Chardonnay grape seed flour (GSF) was purchased from Sonomaceuticals, LLC/WholeVine Products (Santa Rosa, CA, USA). Dimethyl sulfoxide (DMSO) was purchased Daejung, Korea.

2.2. Preparation of Methanol and Water Extracts of Grape Seed Flour

Wine grape seed flour (0.3 g) was mixed with 18 mL of 80% methanol or water, sonicated (SD-350H, Seong Dong, Seoul, Korea) for 30 min. The suspension was filtered through filter paper (Whatman No. 1, Whatman International Ltd., Maidstone, England), and the residue was re-extracted with 80% methanol or water for 30 min. The filtrate was freeze dried (Freeze dryer, FD5508, Ilshin Lab Co., Ltd., Dongducheon, Korea). The dried methanol extract (SGME) and water extract (SGWE) were stored at -20°C for further analysis.

2.3. Growth of Lactic Acid Bacteria and *C. perfringens* with Grape Seed Flour and Grape Seed Extracts

GSF (5 and 10 mg), SGME, SGWE, or catechins were individually added into 3 mL of MRS broth containing 0.1% dimethyl sulfoxide (DMSO) and sonicated for a total of 5 min; 50 sec operate time and 10 sec interval time, repeated 5 times. Then 10^7 CFU of lactic acid bacteria were inoculated and anaerobically cultured at 30°C for 48 hrs. Number of *L. lactics* subsp. Lactics and *L. mesenteroides* were counted after 24 h, 48 h. *L. kefiri* DH5, was enumerated after 96 h. *C. perfringens* was anaerobically incubated in Reinforced Clostridial broth at 37°C for 12 h to determine CFU.

The microbial growth was determined as the growth with GSF compared to without GSF by calculation [number of bacteria (log CFU/ml) in the presence of 5 mg GSF – number of bacteria (log CFU/ml) in 0 mg of GSF] or [number of bacteria (log CFU/ml) in the presence of 10 mg GSF – number of bacteria in 0 mg of GSF].

To apply the same amount of catechins across the samples, the catechin content of SGWE and SGME was determined in an equal initial amount of GSF based on extraction yield (SGWE 8.2% and SGME 5.7%). SGWE1 and SGME1 contain same amount of extract as 5 mg of GSF before extraction. SGWE2 and SGME2 contain same amount of extract as 10 mg of GSF before extraction.

2.4. Extractable Flavonoid Content

0.2 g of GSF was extracted with 20 mL methanol for 30 min with shaking. After 10 min of sonication, the mixture was centrifuged and supernatant was analyzed for flavonoids by HPLC and total phenolics. Total phenolic content was determined using the Folin-Ciocalteu method [13] and flavonoid contents were analyzed using a standard HPLC method [14].

2.5. Statistical Analysis

All data were expressed as mean ± SEM and statistical analysis was carried out using SPSS (Version 21.0, SPSS INC., Chicago, IL, USA)

3. Results and Discussion

3.1. Selective Growth of Lactic Acid Bacteria by GSF

Ten strains of lactic acid bacteria (*Lactococcus lactics* subsp. Lactics, *Leuconostoc. mesenteroides*, and *Lactobacillus kefiri*) that we isolated recently from kefir were cultured with GSF for 48 hrs and the strains that grew best were selected for further study. *L. mesenteroides* DH 1608 showed the best growth in the presence of both 5 mg and 10 mg of GSF (Table 1). The relative growth of most of the kefir derived lactic acid bacteria was greater in 5 and 10 mg of GSF than in broth without GSF (Table 1).

Table 1. Growth of kefir derived lactic acid bacteria during fermentation in the presence of 5 mg and 10 mg of GSF compared to growth without GSF

	Growth stimulation	
	5mg*	10mg**
	(log CFU/mL)	
***Lactococcus lactics* subsp. *Lactics* (DH 1601)**	0.266	0.297
***Lactococcus lactics* subsp. *Lactics* (DH 1602)**	0.528	0.528
***Lactococcus lactics* subsp. *Lactics* (DH 1603)**	-0.561	-0.055
***Lactococcus lactics* subsp. *Lactics* (DH 1605)**	-0.226	0.169
***Leuconostoc mesenteorides* (DH 1604)**	0.051	-0.050
***Leuconostoc mesenteorides* (DH 1606)**	1.204	1.255
***Leuconostoc mesenteorides* (DH 1607)**	1.041	1.079
***Leuconostoc mesenteorides* (DH 1608)**	1.447	1.301
***Leuconostoc mesenteorides* (DH 1609)**	0.285	0.285
***Lactobacillus kefiri* DH5**	0.735	0.738

GSF, grape seed flour; (-), growth with 0mg of GSF is greater than 5 or 10 mg of GSF
*; Difference between growth with 0 mg and 5 mg of GSF
**; Difference between growth with 0mg and 10mg of GSF.

Growth stimulation by GSF on lactic acid bacteria may be due to substrate availability since lactic acid bacteria are able to utilize phenolic compounds (high in GSF) [6]. GSF also contains about 60% dietary fiber that are fermentable. Several components of polyphenols (especially p-coumaric acid, epicatechin and catechin) improved the growth of *Lactobacillus rhamnosus* [7]. Consistently, increased growth of lactic acid bacteria was associated with the amount of catechins in GSF 5 and 10 mg/mL, 35 and 70 μg, respectively. Enzyme activities in lactic acid bacteria played a critical role in utilization of polyphenol components in grape seed extract [15]. Therefore, *L. mesenteroides* DH1608 may have enzymes able to efficiently metabolize polyphenols such as catechins. Further research is necessary to determine if polyphenol metabolism is the limiting factor for growth.

Table 2. The content of (+)-Catechins in grape seed flour and grape seed extracts

Sample Code	Contents of phenolic compound (mg/g)
	(+)-Catechins
GSF	7.01
SGWE	10.1 ± 0.18
SGME	8.70 ± 0.25

GSF, grape seed flour; SGWE, sonicated water extract; SGME, sonicated methanol extract.

3.2. The Growth of *Leuconostoc mesenteroides* DH 1608 in Presence of Extracts of Grape Seed Flour and Catechins

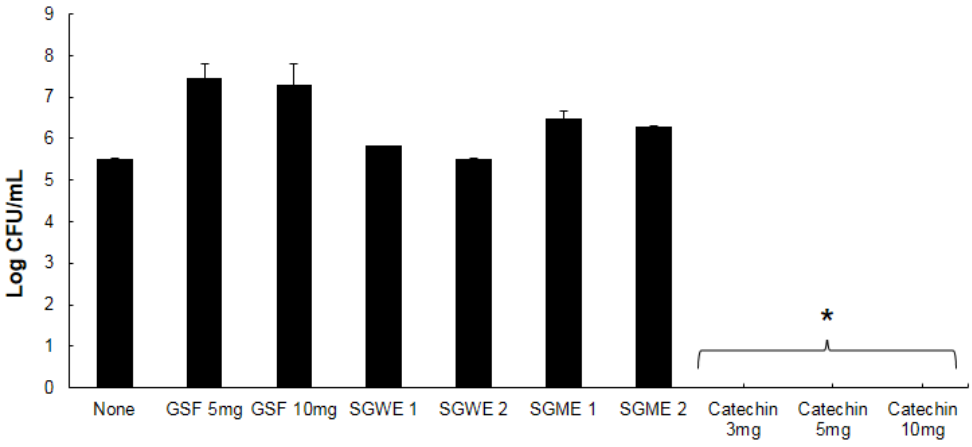
Because *Leuconostoc mesenteroides* DH 1608 grew best in the presence of GSF, its growth in grape seed flour extracts (SGWE, SGME) and catechins were examined. After 48 h incubation, growth of *L. mesenteroides* was greatest with 5 and 10 mg of GSF compared to groups containing GSF extracts, SGWE and SGME (Figure 1). 5 mg of GSF increased the growth by 28% and 15% as compared to SGWE1 and SGME1, respectively. 10 mg of GSF increased the growth by 32% and 16%, in comparison with SGWE2 and SGME2, respectively (Figure 1). These results suggest that growth of *L. mesenteroides* DH 1608 is optimized by the presence of insoluble components, likely dietary fiber, of grape seed flour compared to the grape seed extracts. Previously, growth stimulation of *Lactobacillus acidophilus* was observed with grape pomace extracts [6]. However, the present study demonstrated that the growth of lactic acid bacteria derived from kefir was greater with whole grape seed flour than with grape seed extracts. This result indicates that whole GSF may have additional bioactive components (such as dietary fiber) that contribute to growth stimulation. Soluble dietary fiber has been reported to stimulate growth of lactic acid bacteria from kefir [16]. The growth of *L. mesenteroides* was not observed with catechins 3, 5, 10 mg. However, 2 mg of catechins greatly stimulated its growth and may have contributed to growth of some lactic acid bacteria in this study (data not shown). A previous study [17] also reported that greater than 3 mg of catechins is likely to suppress the growth of lactic acid bacteria.

3.3. The Growth of *Clostridium perfringens* at GSF, SGWE and SGME

After 12 h incubation anaerobically, the growth of *C. perfringens* was inhibited only by 10 mg of GSF (Figure 2); 25% in comparison with SGWE2 and 25% compared to 5 mg GSF. Polyphenols such as catechin and epigallocatechin gallate have been reported to control biofilm formation of pathogenic bacteria [18]. Gram-positive pathogens are 8~16 times more sensitive to epigallocatechin gallate (EGCG) than gram-negative pathogens [19]. Interestingly, the current study showed that *C. perfringens*, gram-positive enteropathogen was more sensitive to GSF than GSE (extracts), indicating whole GSF may have bioactive components in addition to polyphenolics that are more effective for inhibition of *C. perfringens* growth. Furthermore, 35 μg or 70 μg of catechins in both SGWE1&2 and SGME1&2 was not enough to inhibit the growth of *C. perfringens*. This study shows that Chardonnay grape seed flour selectively inhibits a harmful pathogenic bacteria more effectively than grape seed flour extracts.

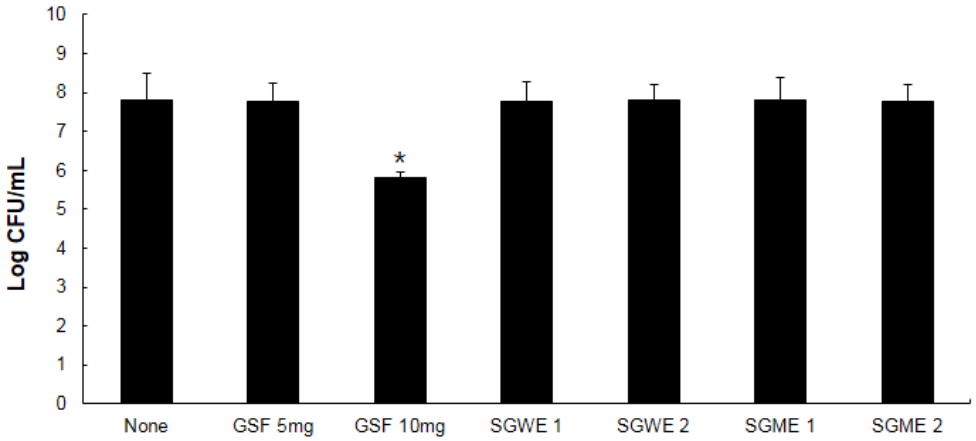
3.4. Influence of GSF and Its Extracts on the Final pH Value of *L. mesenteroides* and *C. perfringens*

Change in pH is indicator of growth of bacteria. The pH of the culture medium was determined at 48 hours after incubation with *L. mesenteroides* DH 1608. The pH was 4.35 and similar to control, GSF 5 mg and 10 mg, SGWE1, SGWE2, SGME1, and SGME2 (Fig 3A). However, the pH of *L. mesenteroides* DH 1608 cultured with catechins was higher than GSF and GSE. Lactic acid fermentation lowers the pH. The lower pH indicates active lactic acid fermentation in the presence of GSF and GSE groups. The higher pH in catechins 3, 5, 10 mg indicates growth inhibition of *L. mesenteroides* DH 1608 by catechins.



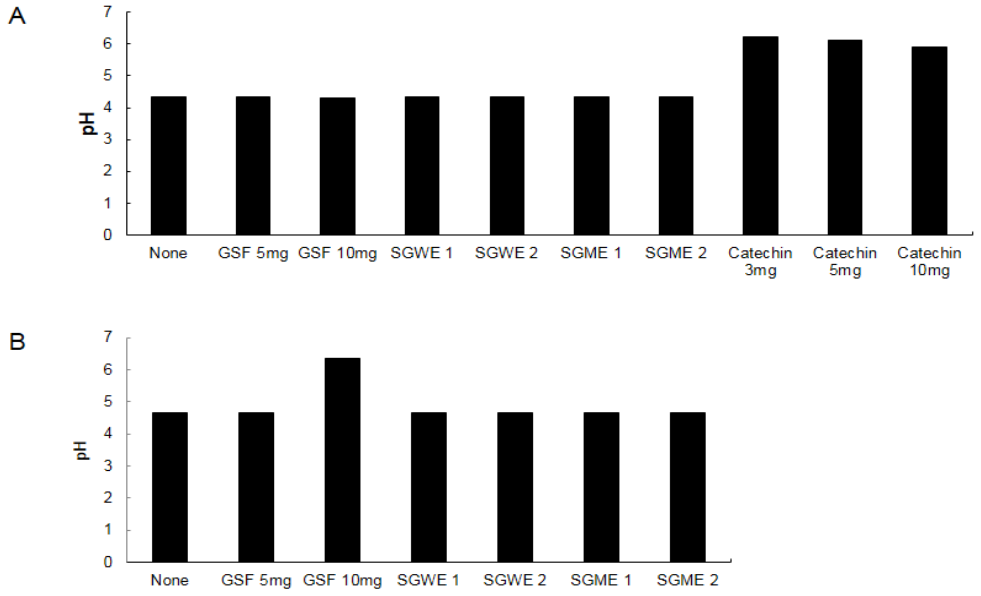
The number of *L. mesenteroides* was observed after 48 h incubation. SGWE1 and SGME1 contain same amount of initial 5 mg of GSF before extraction. SGWE2 and SGME2 contain same amount of initial 10 mg of GSF before extraction. * ; No colonies observed. GSF, grape seed flour; SGME, sonicated grape seed methanol extract; SGWE, sonicated grape seed water extract

Figure 1. Growth of *Leuconostoc mesenteroides* DH 1608 in the presence of GSF, SGWE, SGME and catechins



SGWE1 and SGME1 contain same amount of initial 5 mg of GSF before extraction. SGWE2 and SGME2 contain same amount of initial 10 mg of GSF before extraction. GSF, grape seed flour; SGME, sonicated grape seed methanol extract; SGWE, sonicated grape seed water extract. The number of *C. perfringens* was observed after 12 h incubation.

Figure 2. Growth of *C. perfringens* in the presence of GSF, SGWE, SGME and catechins



SGWE1 and SGME1 contain same amount of initial 5 mg of GSF before extraction. SGWE2 and SGME2 contain same amount of initial 10 mg of GSF before extraction. GSF, grape seed flour; SGME, sonicated grape seed methanol extract; SGWE, sonicated grape seed water extract. The value of pH was measured when the number of bacteria was observed.

Figure 3. Influence of GSF, SGWE, SGME, and catechin at different concentrations (mg/mL) on the final pH value in the medium with *L. mesenteroides* (A) and *C. perfringens* (B)

The pH of media 12 h after incubation with *C. perfringens* was highest with GSF 10 mg (pH 6.35) among all groups (pH is about 4.65) (Figure 3B), indicating growth of *C. perfringens* was inhibited by GSF 10 mg.

In summary, GSF stimulated growth of most lactic acid bacteria isolated from kefir. Growth stimulation of *L. mesenteroides* DH 1608 was highest among the lactic acid bacteria isolated from kefir. When compared to GSE (SGWE and SGME), GSF revealed greater capacity to stimulate the growth of *L. mesenteroides* DH 1608. In contrast, GSF inhibited the growth of *C. perfringens* whereas GSE did not affect growth of this toxigenic bacteria.

3.5. Conclusion

We demonstrated that selective growth stimulation of probiotic lactic acid bacteria derived from kefir and growth inhibition of pathogenic *C. perfringens* by Chardonnay grape seed flour was greater than grape seed extracts. The symbiotic combination of selected lactic acid bacteria from kefir and GSF may provide health promotion via synergism of probiotic growth in the gut.

Acknowledgments

This study was supported by National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (No. 2018R1A2B6001097).

Conflict of Interest

The authors have no conflicts of interest to declare.

References

[1] Seo, K.-H. et al. (2016). Chardonnay Grape Seed Flour Ameliorates Hepatic Steatosis and Insulin Resistance via Altered Hepatic Gene Expression for Oxidative Stress, Inflammation, and Lipid and Ceramide Synthesis in Diet-Induced Obese Mice. PloS one 11 (12), e0167680.

[2] Seo, K.H. et al. (2017). Chardonnay grape seed flour supplemented diets alter intestinal microbiota in diet-induced obese mice. Journal of Food Biochemistry.

[3] Kim, H. et al. (2014). Dietary supplementation of chardonnay grape seed flour reduces plasma cholesterol concentration, hepatic steatosis, and abdominal fat content in high-fat diet-induced obese hamsters. Journal of agricultural and food chemistry 62 (8), 1919-1925.

[4] Ozdal, T. et al. (2016). The reciprocal interactions between polyphenols and gut microbiota and effects on bioaccessibility. Nutrients 8 (2), 78.

[5] Gwiazdowska, D. et al. (2015). The impact of polyphenols on Bifidobacterium growth. Acta Biochim Pol 62 (4), 895-901.

[6] Hervert-Hernández, D. et al. (2009). Stimulatory role of grape pomace polyphenols on Lactobacillus acidophilus growth. International journal of food microbiology 136 (1), 119-122.

[7] Parkar, S.G. et al. (2008). The potential influence of fruit polyphenols on colonic microflora and human gut health. International journal of food microbiology 124 (3), 295-298.

[8] Cueva, C. et al. (2013). In vitro fermentation of grape seed flavan-3-ol fractions by human faecal microbiota: changes in microbial groups and phenolic metabolites. FEMS microbiology ecology 83 (3), 792-805.

[9] Kim, D.H. et al. (2017) Kefir alleviates obesity and hepatic steatosis in high-fat diet-fed mice by modulation of gut microbiota and mycobiota: targeted and untargeted community analysis with correlation of biomarkers. J Nutr Biochem 44, 35-43.

[10] Ulusoy, B.H. et al. (2007). An in vitro study on the antibacterial effect of kefir against some food-borne pathogens. Turk Mikrobiyol Cem Derg 37 (2), 103-107.

[11] Jeong, D. et al. (2017) Modulation of gut microbiota and increase in fecal water content in mice induced by administration of Lactobacillus kefiranofaciens DN1. Food & function 8 (2), 680-686.

[12] Kim, D.H. et al. (2017). Dual function of Lactobacillus kefiri DH5 in preventing high-fat-diet-induced obesity: direct reduction of cholesterol and upregulation of PPAR-alpha in adipose tissue. Mol Nutr Food Res 61 (11).

[13] Singleton, V.L. et al. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in enzymology 299, 152-178.

[14] Lamuela-Raventós, R.M. and Waterhouse, A.L. (1994). A direct HPLC separation of wine phenolics. American Journal of Enology and Viticulture 45 (1), 1-5.

[15] Tabasco, R. et al. (2011). Effect of grape polyphenols on lactic acid bacteria and bifidobacteria growth: resistance and metabolism. Food Microbiol 28 (7), 1345-52.

[16] Mehri HadiNezhad, C.D., Nam Fong Han and Farah Hosseinian. (2013). Flaxseed Soluble Dietary Fibre Enhances Lactic Acid Bacterial Survival and Growth in Kefir and Possesses High Antioxidant Capacity. Journal of Food Research 2 (5), 152-163.

[17] Kottol K., S.P., A. Reni. (2014). Studies on growth effects of catechin on probiotic bacteria. International Journal of Scientific Research 3 (4), 1-3.

[18] Huber, B. et al. (2003). Influence of polyphenols on bacterial biofilm formation and quorum-sensing. Zeitschrift für Naturforschung C 58 (11-12), 879-884.

[19] Yoda, Y. et al. (2004). Different susceptibilities of Staphylococcus and Gram-negative rods to epigallocatechin gallate. Journal of Infection and Chemotherapy 10 (1), 55-58.