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Grape (*Vitis vinifera*) Pomace as Natural Antimicrobial Agent

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Abstract

Grape (*Vitis vinifera*) is one of the most produced crops worldwide used mainly for wine production. Grape pomace is the solid waste produced after the alcoholic fermentation of wine. Grape and grape pomace content compounds with beneficial biological activities, such as phytosterols and phenolic compounds. Their concentration and profile of these compounds present great differences in different grape pomace, and this fact could be due to the use of different cultivars and varieties of the plants, differences in the edaphoclimatic conditions and differences in the extraction procedures used, for example. Terpenoid and phenolic compounds from vegetables and grape pomace have antibacterial and antifungal activities against several microorganisms. The predominant phytosterols in vegetables were β-sitosterol, campesterol, and stigmasterol. So, it is possible to reuse grape pomace as a natural antimicrobial agent. Even though, that extracting phenolic compounds or phytosterols will not solve totally the grape pomace problem, could be a partially solve by transform a waste to raw material, giving an environment friendly solution.

Keywords: winery residues, grape pomace, valorization, polyphenols, phytosterols, antimicrobial activity.

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1. INTRODUCTION

Grape (*Vitis vinifera*) is one of the main cultivated crops worldwide. In 2017, at global level, the grape production was higher than 77,000 tones [1]. However, the grape is not used in its total for wine making, producing a great quantity of by-products (seeds, skin and pulp). Grape pomace is the most abundant by-product of wine-making.

Approximately, half of the global production is used for wine-making, but this percentage varies among countries. Actually, approximately 20-30% (w/w) of the total grapes production destined to wine-making ends up as this organic residue, which represents about 9 million tons generated per year in the world [2].

The extracts from grape pomace by-products can present great differences on their content in active compounds. These differences can be explained due to the use of different cultivars and varieties of the plants, differences in the edaphoclimatic conditions in which the plantis grown and in the maturation state of the fruits and differences in the extraction procedures used [3].

In the case of a grape skin extract obtained by the same extraction process, from the same species *Vitis vinifera* but from a different cultivar, the catechin content is very different. For the grape cultivar Muscat Alexandria, the catechin con-tent is 628μg/g, but for the extract of the grape cultivar *Ghara Shani,* the catechin content is 945μg/g [3, 4].

Phenolic compounds are secondary metabolites of plants, which are synthesized by plants and play important structural roles in the cell wall, act as a defense against UV light, protect against pathogen ingress and are involved in repair of injury. Many foods and beverages contain high levels of phenolic compounds, which often provide colour, taste, astringency and other sensory characteristics [5].

Chemically, phenolics can be defined as substances possessing an aromatic ring bearing one or more hydroxyl groups, including their functional derivatives [6]. Polyphenols are compounds that have more than one phenolic hydroxyl group attached to one or more benzene rings [7].

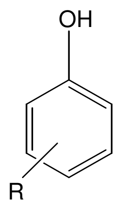

Figure 1. Basical chemical structure of phenolic compounds.

Phenolic compounds may affect growth and metabolism of bacteria [8, 9].

Peixoto et al., [10] tentatively identified twenty eight phenolic compounds (non-anthocyanin and anthocyanin) in the methanol/water (80:20, v/v) extracts prepared from grape pomace mixture, seeds and skins. The authors identified eleven flavon-3-ols (catechinand ephicatechin derivatives and proanthocyanins), seven anthocyanins (malvidin, delphinidin, petunidin and peonidin derivatives), six flavonols (quercetin, laricitrin and syringetin).

Grape skins constitute the major component of grape pomace, containing the highest amounts of anthocyanins and tannins with a high polymerization degree [11, 12]. Grape skins are rich in hydroxycinnamic acids and especially in tartaric esters of these acids, mainly caftaric acid and coutaric acid [13, 14]. Phenolic content of seeds represent about 70% of total extractable polyphenolic compounds from grape pomace. Polyphenolics in grape seeds are mainly flavonoids, including flavan-3-ols and proanthocyanidin oligomers and polymers [15, 16]. The high phenolic content in grape seeds is due to the minimal proportion extracted during pressing in winemaking process.

Rodríguez-Vaquero et al., [18] reported the phenolic compounds concentration in *Malbec, Merlot* and *Cabernet sauvignon* Argentinean wines. The authors also demonstrated that polyphenols present in wines have antibacterial activity against *Proteus mirabilis, Serratia marcescens, Klebsiella pneumoniae, Pseudomonas aeruginosa, S. aureus, listeria monocytogenes* and *E. coli* [17, 18, 19].

Triterpenoids are compounds found in abundance in various plant tissues. Phytosterols comprise a complex class of natural compounds within the triterpene group [19], and are fundamental structural components of all plant membranes [20]. Phytosterols are both structurally and functionally resembling cholesterol, except for a distinctive structural methyl or ethyl group at the C24 carbon atom of their side chain [21]. All phytosterols present one double bond at the C5 position and the saturation of this double bond can succeed either via enzyme reaction in vivo or by hydrogenation [19]. More than 100 types of phytosterols have been reported in plant species, but the most abundant are sitosterol (C-29), stigmasterol (C-29) and campesterol (C-28) [22, 23, 24, 25].

Ruggiero et al., [26] determined the presence and concentrations of *β*-sitosterol, stigmasterol and campesterol in grapes from *Groppello* cultivar. The presence of phytosterols in grape pomace from *Malbec* cultivars was reported by Sosa-Mármol et al. [27].

Phytosterol are well-known for cholesterol-lowering effects, and the structures and forms of phytosterols affect their bioactivity.

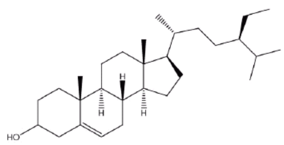

Figure 2. Chemical structure of sitosterol

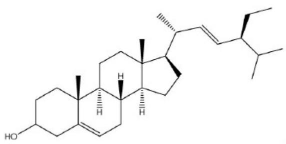

Figure 3. Chemical structure of stigmasterol

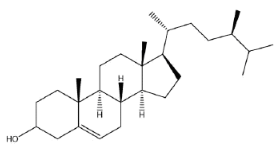

Figure 4. Chemical structure of stigmasterol

Some authors reported several biological activities such as antiinflammatory, antibacterial, antiviral, hepatoprotective, immunomodulatory, hypolipidemic, among others [28, 29, 30, 31, 32]. Sosa-Mármol et al., [27] determined the antibacterial effect of phytosterols from grape pomace against *Xanthomonas citri sp citri.*

Since fruit by-products are considered food waste, their economic value is low. This, combined with their high biological activities, makes them a potential source of bioactive compounds to be included in nutraceutical and pharmaceutical compounds [33].

2. EXPERIMENTAL SETUP, RESULTS AND DISCUSSION.

2.1 Antibacterial and antifungal activities of phenolic compounds and phytosterols from grape pomace

Depending on the concentration, the effect of different phenolic compounds could be beneficial to growth of lactic acid bacteria [34] or inhibitory [35].

The effect of pure phenolic compounds against lactic acid bacteria was reported by several authors. Landete et al., [36], demonstrated that none of the nine phenolic compounds analyzed (cinnamic acid, p-hydroxybenzoic acid, hydroxytyrosol, oleuropein, protocatechuic acid, sinapic acid, syringic acid, tyrosol and vanillic acid) inhibit the growth of *L. plantarum.*

It was reported that the growth and metabolism of *Lactobacillus hilgardii* 5w a wine spoilage lactic acid bacteria, at concentrations normally present in wine of catechin and gallic acid, not only stimulated the growth rate but also resulted in greater cell densities during the stationary growth phase. But higher concentrations of phenolic compounds inhibited bacterial growth [37].

On pathogenic microorganisms the antibacterial activity depends on the phenolic compounds and of the strains tested [17, 38, 39]. Quercetin, rutin and caffeic acid was the most effective compounds as antibacterial agent against *Serratia marcescens Proteus mirabilis, Escherichia coli, Klebsiella pneumoniae, Flavobacterium sp and Listeria monocytogenes* [17, 18, 19] The authors reported a decrease of two, one and half log cycles of *Listeria monocytogenes* in presence of 500 mg/l caffeic acid, rutin and quercetin, respectively.

Some authors reported the antimicrobial activity of grape seed extracts, against several pathogenic and spoilage bacteria, such as *Bacillus cereus, Enterobacter aerogenes, Aeromonas hydrophila, Enterococcus faecalis, Escherichia coli*, including *E. coli* O157:H7, *Klebsiella pneumoniae, Mycobacterium smegmatis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas fluorescens, Salmonella Enteritidis, Salmonella Typhimurium, Staphylococcus aureus* and *Yersinia enterocolitica* [40, 41, 42].

The antifungal and antibacterial activities of olive leaf aqueous extracts against *Bacillus cereus*, *Bacillus* s*ubtilis, Sthaphylococcus. aureus*, *Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella pneumoniae* bacteria, *Candida Albicans* and *Candida neoformans* were reported by Pereira et al., [43].

Some studies showed the potential of grape pomace phenolic compounds to be used as preservatives, by preventing lipid oxidation and suppressing the growth of some bacterial strains, such as *Staphylococcus aureus, Streptococcus mutans* and *Escherichia coli* [44].

The antibacterial activity of methanol/water (80:20, v/v) extracts prepared from grape pomace mixture, seeds and skins was reported against Gram negative (*E. coli, Klebsiella pneumonia, Morganella morganii* and *Pseudomonas aeruginosa*) and Gram positive bacteria (*Enterococcus faecalis*, *Listeria monocytogenes,* methicillin-resistant *Staphylococcus aureus* and methicillin-susceptible *Staphylococcus aureus*) [10]. The authors reported that the lowest minimum inhibitory concentrations (MIC) values were observed in seeds and against Gram-positive bacteria, *Enterococcus faecalis, MRSA* and *MSSA* (2.5, 5 and 5 mg/ml, respectively). For Gram-negative bacteria, seeds also exhibited the lowest MIC values against *K. pneumoniae* (10 mg/ml). The skins showed the poorest antibacterial activity, revealing MIC values > 20 mg/ml for almost all bacteria strains. They demonstrated that the antibacterial activity have a strong correlation with the presence of phenolic compounds.

Anastasiadi et al., [45] tested extracts of *Vitis vinifera* grape pomace (var. Mandilaria, Voidomato, Asyrtiko and Aidani) against *Listeria monocytogenes*, and described that the seed extracts were significantly more antimicrobial than stems.

Antibacterial effect depends on the grape variety. White grape skin extracts presented lower MICs against both Gram-positive (*S. aureus* and *B. cereus*) and Gram-negative bacteria (*E. coli, Salmonella Infantis, Campylobacter coli*) than red ones [46].

Some authors had demonstrated the effect of terpenoids compounds from vegetable extracts as antimicrobial agent against several microorganisms. Burčová et al., [47] reported the antimicrobial activities of β-sitosterol against *Pseudomonas aeruginosa* and *Alternaria alternate;* Kumari et al., [48] demonstrated that β-sitosterol is present in stem of *Nyctanthes arbor-tristis* (Harsingar) and that the chloroform and ethyl acetate fractions possess antibacterial activity against *Xanthomonas citri, Pseudovorax* sp. and *Dickeya zeae*.

The phytosterol profiles in common foods, indicating that the predominant phytosterols were β-sitosterol, campesterol, and stigmasterol, all of which belonged to plant sterols and 4-desmethylsterols [49].

The presence of sitosterol and sitosterol glucoside in *V. vinifera* was informed by Rivero-Cruz et al., [50]. The authors also determined their antimicrobial activity against against two oral pathogens, *Streptococcus mutans* and *Porphyromonas gingivalis.*

The presence of phytosterols, such as B-sistosterol and stigmasterol in grape pomace from *Malbec* cultivars was reported by Sosa-Mármol et al.,

[27]. Later, the authors reported the antibacterial activity against the etiologic agent of lemon canker.

Candidiasis is an infection caused by yeast of the genus Candida. These eukaryotic microorganisms are part of the saprophyte microbiota of humans in the skin, mouth and mucous membranes of the female genitalia. The antifungal agents used for their treatment are imidazole derivatives, however at present a decrease in their effectiveness is observed, mainly due to the emergence of resistant yeasts. Perez-Merello et al., [51] demonstrated the antifungal activity of phytosterols in grape pomace from *Malbec* cultivars, against *Candida albicans,* and the possibility to reutilized natural compounds in this residue as antifungal agents in the treatment of candidiasis.

3. CONCLUSIONS

Phenolic fraction and fatty fraction of grape pomace have antibacterial and antifungal activities. So, it is possible to reuse a winery waste as a natural antimicrobial agent. Even though, that extracting phenolic compounds or phytosterols will not solve totally the grape pomace problem, could be a partially solve by transform a waste to raw material, giving an environment friendly solution.

4. REFERENCES

[1] (FAO, 2018). Food and Agriculture Organization of the United Nations (FAO) (2018). Crop statistics.

[2] Dávila, I., Robles, E., Egüés, I., Labidi, J., Gullón, P. (2017) The Biorefinery Concept for the Industrial Valorization of Grape Processing By-Products. In: Galanakis, C.M. (ed.), Handbook of Grape Processing By-Products, pp. 29-54.

[3] Andrade, A., Lima V..; Sanches Silva A., Vilarinhoa, F., Castilho, M.C., Khwaldia, K., Ramos, F. (2019) Pomegranate and grape by-products and their active compounds: Are they avaluable source for food applications? Trends in food science & Technology, 86 pp.68-84.

[4] Farhadi, K., Esmaeilzadeh F., Hatami M., Forough, M., Molaie R. (2016) Determination of phenolic compounds content and antioxidant activity in skin, pulp, seed, cane and leaf of five native grape cultivars in West Azerbaijan province, Iran. Food Chemistry, 199 pp. 847-855.

[5] Williamson, G., Day, A.J., Plumb, G.W., Couteau, D. (2000) Human metabolic pathways of dietary flavonoids and cinnamates. Biochemical Society Transactions, 28(2) pp. 16-22.

[6] Shahidi, F., Naczk, M. (2004) Phenolics in Food and Nutraceuticals. Pp. 443-482. Boca Raton, FL: CRC Press LLC.

[7] Vermerris, W., Nicholson, R. (2006) Phenolic Compound Biochemistry. Pp. 1-34. West Lafayette, IN: Springer

[8] Reguant, C., Bordons, A., Arola L., Rozés, N. (2000) Influence of phenolic compounds on the physiology of *Oenococcus œni* from wine. J. Appl. Microbiol., 88 pp. 1065-1071.

[9] Campos, F. M., Couto J. A., Hogg, T.A. (2003) Influence of phenolic acids on growth and inactivation of *Oenococcus oeni* and *Lactobacillus hilgardii*. J. Appl. Microbiol., 94 pp. 167-174.

[10] Peixoto, C. M., Dias, M. I., Alves, M. J., Calhelha, R. C., Barros, L., Simão, P., Pinho, I., Ferreira. C. F. R. (2018). Grape pomace as a source of phenolic compounds and diverse bioactive properties. Food Chemistry, 253 pp. 132-138.

[11] Escarpa, A., González, M. C. (1998) High-performance liquid chromatography with diode-array detection for the determination of phenolic compounds in peel and pulp from different apple varieties. Journal of Chromatography. A, 823, pp. 331-337.

[12] Pinelo, M., Arnous, A., Meyer, A. S. (2006) Upgrading of grape skins: Significance of plant cell-wall structural components and extraction techniques for phenol release. Trends in Food Science & Technology, 17(11), pp. 579-590.

[13] Kammerer, D., Claus, A., Carle, R., Schieber, A. (2004) Polyphenol screening of pomace from red and white grape varieties (*Vitis vinifera L.*) by HPLC-DAD-MS/MS. Journal of Agricultural and Food Chemistry, 52 pp. 4360-4367.

[14] Teixeira, A., Baenas, N., Dominguez-Perles, R., Barros, A., Rosa, E., Moreno, D. A., Garcia-Viguera, C. (2014) Natural bioactive compounds from winery by-products as health promoters: a review. International Journal of Molecular Sciences, 15(9) pp. 15638-15678.

[15] da Silva, R., Rigaud, J. M., Cheynier, J., Cheminat, V., Moutounet, M. (1991) Procyanidin dimers and trimers from grape seeds. Phytochemistry, 30(4) pp. 1259-1264.

[16] Prieur, C., Rigaud, J., Cheynier, V., Moutounet, M. (1994) Oligomeric and polymeric procyanidins from grape seeds. Phytochemistry, 36(3) pp. 781-784.

[17] Rodríguez-Vaquero, M.J., Alberto, M.R., Manca de Nadra, M.C. (2007b). Influence of phenolic compounds from wines on the growth of *Listeria monocytogenes*. Food Control, 18 pp. 587-593.

[18] Rodríguez-Vaquero, M.J., Manca de Nadra, M.C. (2008). Growth parameter and viability modifications of *Escherichia coli* by compounds and argentine wine. Applied Biochemistry y Biotechnology, 2 pp. 342-352.

[19] Uddin, M., Sarker, M., Islam, Z., Ferdosh, S., Akanda, M., Haque, J. (2015) Phytosterols and their extraction from various plant matrices using supercritical carbon dioxide: A review. Journal of the Science of Food and Agriculture, 95 pp. 1385-1394.

[20] Suh, H. W., Hyun, S. H., Kim, S. H., Lee, S. Y., Choi, H. K. (2013) Metabolic profilingand enhanced production of phytosterols by elicitation with methyl jasmonate and silver nitrate in whole plant cultures of Lemna paucicostata. Process Biochemistry, 48 pp. 1581-1586.

[21] Miras-Moreno, B., Sabater-Jara, A. B., Pedreno, M. A., Almagro, L. (2016) Bioactivity of phytosterols and their production in plant in vitro cultures. Journal of Agricultural and Food Chemistry, 64 pp. 7049-7058.

[22] Moreau, R.A., Whitaker, B.D., Hicks, K.B. (2002) Phytosterols, phytostanols, and their conjugates in foods: structural diversity, quantitative analysis, and health-promoting uses. Prog. Lipid Res., 41 pp. 457-500.

[23] Berger, A., Jones, P.J.H., Abumweis, S.S. (2004) Plant sterols: factors affecting their efficacy and safety as functional food ingredients. Lipids Health Dis., 3 pp 5-23.

[24] Gylling, H., Simonen, P. (2015) Phytosterols, phytostanols, and lipoprotein metabolism. Nutrients, 7 pp. 7965-7977.

[25] Kritchevsky, D., Chen, S.C. (2005) Phytosterols-health benefits and potential concerns: a review. Nutr. Res., 25 pp. 413-428.

[26] Ruggiero, A., Vitalni, S., Burlini, N., Bernasconi, S. Iriti, M. (2013) Phytosterols in grapes and wine, and effects of agrochemicals on their levels. Food Chemistry, 141 pp. 3473-3479.

[27] Sosa Mármol, S. M., Saguir, F. M., Rodríguez-Vaquero, M.J. (2015) Phytosterols from winery waste as control agent of citris canker. XI Congreso Argentino de Microbiología General.

[28] J□ger, S., Trojan, H., Kopp, T. (2009) Pentacyclic triterpene distribution in various plants-rich sources for a new group of multi-potent plant extracts. Molecules., 14 pp. 2016-2031.

[29] Rezanka, T., Siristova, L., Sigler, K. (2009) Sterols and triterpenoids with antiviral activity. Anti Infect Agents Med Chem., 8 pp. 193-210.

[30] Wolska, K.I., Grudniak, A.M., Fiecek, B. (2010) Antibacterial activity of oleanolic and ursolic acids and their derivatives. Cent Eur J Biol., 5 pp. 543-553.

[31] Bishayee, A, Ahmed, S., Brankov, N. (2011) Triterpenoids as potential agents for the prevention and therapy of breast cancer. Front Biosci., 1(16) pp. 980-996.

[32] Thoppil, R.J., Bishayee, A. (2011) Terpenoids as potential chemopreventive and therapeutic agents in liver cancer. World J Hepatol., 27 pp. 228-249.

[33] Martínez, R., Torres, P., Meneses, M. A., Figueroa, J. G., Pérez-Álvarez, J. A., Viuda Martos, M. (2012) Chemical, technological and in vitro antioxidant properties of mango, guava, pineapple and passion fruit dietary fibre concentrate. Food Chemistry, 135(3) pp. 1520-1526.

[34] Reguant, C., Bordons, A., Arola, L., Rozés, N. (2000) Influence of phenolic compounds on the physiology of *Oenococcus œni* from wine*. J. Appl. Microbiol.,* 88 pp. 1065-1071.

[35] Campos, F. M., Couto, J. A. Hogg, T. A. (2003) Influence of phenolic acids on growth and inactivation of *Oenococcus oeni* and *Lactobacillus hilgardii*. J. Appl. Microbiol., 94 pp. 167-174.

[36] Landete, J. M., Curiel, A., Rodríguez, H., de las Rivas, B., Muñoz, R. (2008) Study of the inhibitory activity of phenolic compounds found in olive products and their degradation by *Lactobacillus plantarum* strains. Food Chemistry, 107 pp. 320-326.

[37] Alberto, M. R., Farías, M. E., Manca de Nadra, M. C. (2001) Effect of gallic acid and catechin on *Lactobacillus hilgardii* 5w growth and metabolism of organic compounds. J. Agric. Food Chem., 49 pp. 4359-4363.

[38] Puupponen-Pimiä, R., Nohynek L., Hartmann-Schmidlin, S., Kähkönen, M., Heinonen, M., Määttä-Riihinen, K. Oksman-Caldentey, K. M. (2005) Berry phenolics selectively inhibit the growth of intestinal pathogens. J. Appl. microbiol., 98 pp. 991-1000.

[39] Wen, A., Delaquis, P., Stanich, K. Toivonen, P. (2003) Antilisterial activity of selected phenolic acids. Food Microbiol., 20 pp. 305-311.

[40] Baydar, N. G., Sagdic, O., Ozkan, G., Cetin, S. (2006) Determination of antibacterial effects and total phenolic contents of grape (*Vitis vinifera L.*) seed extracts. International Journal of Food Science & Technology, 41(7), pp. 799-804.

[41] Rodriguez-Vaquero, M. J., Aredes Fernández, P.,A., Manca de Nadra, M. C., Strasser de Saad, A. M. (2013) Effect of Phenolic Compounds from Argentinean Red Wines on Pathogenic Bacteria in a Meat Model System. Journal of Food Biochemistry, 37(4) pp. 425-431.

[42] Figueiredo, A. R., Campos, F., de Freitas, V., Hogg, T., Couto, J. A. (2008) Effect of phenolic aldehydes and flavonoids on growth and inactivation of *Oenococcus oeni* and *Lactobacillus hilgardii*. Food Microbiology, 25(1), pp. 105-112.

[43] Landete, J.M., Curiel, J.A., Rodríguez, H., de las Rivas, B., Muñoz, R. (2008) Study of the inhibitory activity of phenolic compounds found in olive products and their degradation by *Lactobacillus plantarum* strains. Food Chemistry, 107 pp. 320-326.

[44] Yu, J., Ahmedna, M. (2013) Functional components of grape pomace: their composition, biological properties and potential applications. International Journal of Food Science and Technology, 48 pp. 221-237.

[45] Anastasiadi, M., Pratsinis, H., Kletsas, D., Skaltsounis, A. L., Haroutounian, S. A. (2012) Grape stem extracts: Polyphenolic content and assessment of their in vitro antioxidant properties. LWT - Food Science and Technology, 48(2) pp. 316-322.

[46] Katalinić, V., Možina, S. S., Skroza, D., Generalić, I., Abramovič, H., Miloš, M., Boban, M. (2010) Polyphenolic profile, antioxidant properties and antimicrobial activity of grape skin extracts of 14 *Vitis vinifera* varieties grown in Dalmatia (Croatia). Food Chemistry, 119(2) pp. 715-723.

[47] Burčová, Z., Krepsa, F., Greifováa, M., Jablonskýb, M., Házb, A., Schmidta, Š., Šurinab, I. (2018). Antibacterial and antifungal activity of phytosterols and methyl dehydroabietate of Norway spruce bark extracts. J Biotechnology, 282 pp. 18-24.

[48] Kumari, S., Singh, R., Gurav, N. P., Metha, N. (2017). Isolation and characterization of bioactive compounds from stem of Nyctanthes arbor-tristis linn. and effect of different fractions on phytopathogens. Asian Journal of Chemistry, 29 pp. 787-791.

[49] Wang, W., Huang, Y., Hu, L., Zhang, Y., Shao, M., Wang, F., Zhang, Z., Zhao, X., Mei, T., Li, D. Wang, Y. Liang, J., Li, Y., Huang, L., Zhang, T., Xu, H. (2018) Phytosterol Profiles of Common Foods and Estimated Natural Intake of Different Structures and Forms in China. Mengmeng J. Agric. Food Chem., 66(11) pp. 2669-2676.

[50] Rivero-Cruz, F., Zhu, M., Douglas Kinghorn, A., Wua, C. D. (2008) Antimicrobial constituents of Thompson seedless raisins (*Vitis vinifera*) against selected oral pathogens. Phytochemistry Letters, 1 pp. 151-154

[51] Pérez-Merello, M. M., Vallejo, C. V., Torrés, E. M., Saguir, F. M., Vizoso-Pinto, G., Rodríguez-Vaquero, M. J. (2018) Reutilización de desechos de las industrias vitivinícola y azucarera en el tratamiento de candidiasis. VII Congreso Internacional de Ciencia y Tecnología de Alimentos (CICYTAC 2018), pp. 105-107.