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Development of a Fermented Probiotic Beverage Inoculated with Kefir Grain

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ABSTRACT

Background: Probiotics are live microorganisms that confer to have health benefits when consumed directly or processed. Regular administration of Probiotics would help in improving the gut microflora that has gained importance as bacteriotherapy which prevents the gastro-intestinal disease caused by pathogenic microorganisms. Vegans who are allergic or intolerant to dairy products can have the benefits of organic acids that are produced through fermentation. The present study was carried out to know the benefits of fermented products using kefir grains and fruits.

Methods: The fermented product was tested for the evaluation of flavours, sensory, and shelf life which seemed to be very promising. In context to biomolecules, the product had a high concentration of absorbable protein, carbohydrate, antioxidant, and antibacterial properties.

Result: The production of fermented beverages of certain fruits on a large scale, and its consumption may promote additional health benefits. The kefir beverage that is produced in this work may help to fill the gap between actual and innovative consumption of the fruits and kefir that may be recommended in the human diet with concern for their health.

Key words: Antibacterial, Antioxidant, Fermentation, Kefir grains, Probiotics.

INTRODUCTION

Probiotics have been defined in several ways, depending on our understanding of the mechanisms of action of their effects on the health and well-being of humans. The term probiotic was coined by Lilly and Stillwell. Parker subsequently defined probiotics as organisms and substances that contribute to intestinal balance. At present, the most commonly used definition is that of Fuller: probiotics are living microbial-fed supplements which show a beneficial effect on the host animal by improving its intestinal microbial balance. Recently a European expert group widened the definition: Probiotics are live microbial food ingredients that have a beneficial effect on human health (Alok *et al*., 2017).

In recent time, bacteriotherapy is gaining importance due to their health benefits in humans. The regular administration of harmless and beneficial microorganisms would help in improving the gut microflora, which in turn preventing the gastro-intestinal disease caused by pathogenic microorganisms (Caglar *et al*., 2005). These beneficial microorganisms play a vital role in activating innate and adaptive immune responses in host, by modulating the functions of macrophages, dendritic cells and along by T and B lymphocytes (Yan and Polk, 2011).

Regular consumption of these probiotic organisms would greatly influence, gut health, prevent infections, enhance mental health, reduces the risk of allergies and food sensitivity, help in weight loss, hypocholesterolemic effect, reduce the incidence of diabetics, skin and oral health, improves sleeps and mood (Gaware *et al*., 2011).

The human gut harbors a complex of 100 trillion microbial cells, belonging to more than 1000 different bacterial species (Guinane and Cotter, 2013) which play a significant role in human health. The load of the microorganisms in the gut is frequently altered by administration of drugs like antibiotics, or the presence of toxins from contaminated food and even due to imbalance in the gastric juice/ acid secretions, malnutrition, low fiber content, stress, chronic diseases, travel, change in dietary lifestyle, lack of physical activities and even by the consumption of alcohol (Alok *et al*., 2017).

The losses that occurred due to the above-mentioned activities should be rejuvenated in order to maintain a healthy lifestyle. This can be achieved by regular consumption of probiotics, which would replace the lost microbiota.

The scientific reports suggest that the probiotics drinks would help in resolving several gastrointestinal and other disorders such as:

Irritable bowel syndrome (IBS)

About 8% of urban people are prone to IBS world-wide, though it's not a critical illness, but affects the lifestyle and work-life. Symptoms involve belly aches, stomach cramping

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and bloating. Most IBS problems can be healed effectively by restoring gut health, so a probiotic drink can play a major role in ensuring the gut flora is optimal. (Aragon *et al*., 2010).

Diarrhoea

Induced by Rotovirus, or pathogenic microorganisms and or by antibiotics would be treated effectively by the consumption of probiotic drinks which are rich in *Lactobacillus GG*, *Bifidiobacterium lactis, Streptococcus thermophilus, Lactobacillus reuteri, Lactobacillus rhamnosus* (not GG) and *Lactobacillus acidophilus.* The clinical trials have also suggested that the *Lactobacillus GG* and *Streptococcus boulardii* are found to be effective in treating the acute watery diarrhoea (Guandalini *et al*., 2011).

Sleep

The presence of good bacteria in the gut would induce good sleep by helping the synthesis of neurotransmitters and hormones such as: serotonin and melatonin (Marotta, *et al*., 2019).

Kefir

The nutritional attributes of this self-carbonated beverage are due to the presence of vital nutrients, minerals, vitamins and some nutraceutical components. Antimicrobial activity, better gut health, anticarcinogenic activity, control of serum glucose and cholesterol, control of lactose intolerance and a better immune system can be achieved through its regular consumption (Ahmed *et al*., 2013).

Water kefir

Water kefir is a beverage with relatively low sugar content, providing an interesting alternative to sugary soft drinks. The microorganisms involved in water kefir fermentation comprise Yeast, Lactic acid bacteria, Bifidobacterium and Acetic acid bacteria. This microbial species diversity was similar in the water kefir liquor and in the water, kefir grains and remained stable during the whole fermentation process. Some strains of these species, such as *Lactobacillus casei* and *Bifidobacterium* spp., might possess probiotic activities. Sucrose, the major substrate of the fermentation, was completely converted after 24 h of fermentation, which coincided with the production of the water kefir grain polysaccharide. The main metabolites of the fermentation were ethanol and lactic acid, whereas glycerol, acetic acid and mannitol were produced in low concentrations (Laureys *et al*., 2014).

Nowadays, water kefir consumption has increased, supported by many scientific studies which have documented that water kefir is a source of probiotic microorganisms and metabolites with potential health benefits that it offers to vegan consumers and those who are intolerant to residual lactose in milk kefir. During the COVID-19 pandemic, the consumption of natural probiotic-containing foods was suggested as beneficial for improving gut health and consequently, overall health. Market studies correlate increased water kefir consumption with increased consumer awareness of the benefits of fermentation and with the knowledge that probiotics may be included in a much wider variety of foods. (Moretti *et al*., 2022).

MATERIALS AND METHODS

Water kefir beverage is a fermented carbonated drink that is produced by using water kefir grains. This fermented probiotic beverage has wide health benefits as it strengthens the immune system, activates digestion, boosts energy, improves gut health, acts as an anti-inflammatory and is also as a great source of antioxidants along with its antimicrobial property. Thus, it is always necessary to produce kefir in order to improve our health (Nor Farahin Azizi *et al*., 2021; Marta Calatayud *et al*., 2021; Fernanda Assumpção Fiorda *et al*., 2017).

Activation of water kefir

The water kefir grains were activated by transferring the stored grains into 10:1 ratio of sterile warm water in organic jaggery powder and incubated for 24-48 hours for the activation.

Preparation of probiotic drink

The activated water kefir grains were filtered and was transferred into a clean double layered cotton cloth (kefir culture bag), in order to prevent the loss of grains during the filtration process. The cotton cloth containing kefir grains was used as an inoculum to ferment the further batches of jaggery water (Darvishzadeh *et al*., 2021; Ozcelik *et al*., 2021; Hampton *et al*., 2021; Cai *et al*., 2020).
The fermentation process was carried out in two stages.

First fermentation

The kefir grains were activated for 24 hours and later incubated for 24 hours at room temperature to ferment the sugar. The pH was recorded before and after the fermentation process. After the incubation period, the kefir culture bag was removed and the fermented jaggery water was used as a probiotic drink. Furthermore the fermented probiotic drinks was utilized for sensory evaluation and rest for other experimental studies such as to estimate protein, carbohydrate, alcohol, bile and acid tolerance and also for antioxidant and antibacterial studies.

Second fermentation

The probiotic drink obtained after the first fermentation, was subjected to the second fermentation for 24 hours at room temperature without inoculum, to enhance the nutrition, flavor and health benefits, for the complete utilization of the sugar source present in it without the formation of alcohol. After the second fermentation with the flavors, it was utilized for sensory studies and for other experimental studies as mentioned above.

Flavor enhancement and shelf-life evaluation

The first fermented kefir drink was biofortified by adding flavors such as star fruit, pomegranate, apple, dragon fruit, guava, orange, pineapple, mango and masala soda.

After second fermentation the product was filtered to separate the insoluble particles and sensory evaluation was carried out. The end fermented probiotic product was evaluated for shelf life in two different parameters *i.e*., at room temperature and in the refrigerator.

Addition of fruit flavour

The different fruits like star fruit, pomegranate, apple, dragon fruit, guava, pineapple, mango and orange juice were extracted by mashing the ripened fruit that was procured from the local markets and mixed with the water kefir after first fermentation in 1:1 ration with 30 g of organic jaggery powder and crushed papaya. The flavor was also enhanced by addition of 15 g of mango ginger and 2.6 g of crushed mint leaves, crushed pepper for 1 L of kefir and was left for fermentation for next 24 hours at room temperature.

Masala soda flavour

100 ml of kindly branded carbonated soda was added to first fermented product along with 10 g of organic jaggery powder, 2 g of chilly, 2 ml of lemon juice, 0.2 g of salt, 5 g of sabja seeds, 1 g of pepper powder, 20 g of mango ginger and 2 g of mint leaves. All these additives were crushed before adding it to the first fermented product. And it was subjected for second fermentation for 24 hours at room temperature.

Evaluation of the fermented beverages

The Fermented product was evaluated for pH estimation, protein estimation, carbohydrate estimation, acid estimation, Bile and acid tolerance, anti-bacterial and anti-oxidant activity (Laureys *et al*., 2018; Cai *et al*., 2020; Ozcelik *et al*., 2021; Hampton *et al*., 2021; Martínez Torres *et al*.,2017; Succi *et al*., 2005; Astuti *et al*., 2021; Alsayadi *et al*., 2013).

pH estimation

"Potential of hydrogen" is a scale to measure the specific acidity or basicity of any aqueous or liquid solution by measuring the hydrogen ion concentration. The mouth feel of any beverages is important to receive its commercial important. The presence of acids in the fermented beverages alters the pH of the drink. Hence in the present study the pH of the beverages before and after fermentation was tested. And pH value is determined for all fermented beverages before and after fermenting it with water kefir grains using calibrated pH meter and the values are tabulated.

Protein estimation

The Lowry's assay was carried out to estimate the amount of protein present in the jaggery water (before fermentation), fermented jaggery water (after first fermentation) and fermented star fruit flavoured kefir beverage (after second fermentation). The standard graph was plotted. By the standard graph, the amount of protein present in the given sample was estimated.

Carbohydrate estimation

Three different carbohydrates *i.e*., sucrose and fructose were used as standards to determine the presence of these carbohydrates in our fermented star fruit-flavored kefir sample by Anthrone's method. The standard graph was plotted to determine the concentration of carbohydrates present and was tabulated.

Acid estimation

As Kefir grains consist of yeast which will be embedded in the symbiotic matrix along with other microorganisms. The sugar, present in the water will be converted into alcohol and acids along with the liberation of carbon dioxide, this process influence kefir fermented sugary water to have a characteristic feature of sour taste. The acidic content in the fermented beverage was evaluated using acid base titration method.

Bile and acid tolerance test

Under normal physiological conditions, our intestine holds bile salt concentration of 40 mM to less than 1 mM-equivalence range being 0.05%-2% concentration.

So, the bile salt tolerance test carried out by adding 2% of bile salt into 100ml of MRS broth and sterilized by autoclaving and then inoculated with the culture and subjected to incubation at 37°C for about 5 hours, after the incubation to check the viability of strains it was assayed on MRS agar and then incubated at 37°C for 2-5 days.

MRS broth was freshly prepared and acidified to pH 2.0 and 3.0 using 1.0N HCl and then it was sterilised. After the sterilisation culture was added into the MRS broth and incubated at 37°C for 1-4 hour, later ascertained in MRS agar at 37°C for 2-5 days.

Antioxidant activity measured by DPPH method

Antioxidant activity is the limitation or the inhibition of nutrient oxidation especially proteins and lipids by oxidative chain reactions. The antioxidant potential is measured by the 2,2-diphenyl- 1picrylhydrazyl (DPPH) method for fermented kefir beverages because DPPH free radical scavenging is an accepted mechanism for scavenging the antioxidant activity. The result was evaluated as the percentage of the scavenging effect.

RESULTS AND DISCUSSION

Evaluation of flavour

All nine flavors, *i.e*., star fruit, pomegranate, apple, dragon fruit, guava, pineapple, mango, orange and masala soda, were fermented with kefir water. Flavored kefir water was subjected to sensory evaluation by volunteers. Among 9 different flavors, star fruit and masala soda kefir were preferred by most of the volunteers.

Evaluation of shelf life

The shelf life of all 9 flavored water kefir was also evaluated up to 21 days. From our experimental study we could come

to the conclusion that eight flavors except star fruit would last not more than 2 days under room temperature, where whereas in the case of refrigerated condition conditions it would last for 4-5 days. The kefir fortified with the star fruit, exhibited a longer shelf life for more than 21 days at room temperature and more than 30 days under refrigeration, without exhibiting any change in the color, taste and mouth feel of the beverage as depicted through graph in Fig 1a and b. Hence, star fruit was chosen for further studies.

pH estimation

The pH of the fermented beverage was estimated before and after the fermentation. Our results clearly revealed that the pH of the fermented beverage decreased from 5.0 to 3.5 during the first fermentation and did not change even after the second fermentation. As in the case of flavored water, kefir-star fruit exhibited variation in the pH from 3.5-3.0 after the second fermentation, which might be because of the presence of additional nutrients for the microbes to feed, which in turn helped in reducing the pH of the beverage tabulated in Table 1.

Protein estimation by lowry method

As we have used Lowry's method for protein estimation, the amount of protein present in fermented jaggery kefir and fermented star fruit kefir was determined. The amount of protein present in fermented jaggery kefir was 0.5 g per 100 ml and the amount of protein present in fermented star fruit flavored kefir was 1 g in 100 ml. The increase in the protein content in the flavored beverages might be due to the proteins released from the star fruit or extracellular protein released by the probiotic organisms tabulated in Table 2.

Carbohydrate estimation by anthrone's method

The carbohydrate content in the fermented beverage was estimated by Anthrone's method. The fermented star fruit kefir contains 4.35 g of carbohydrate whereas the fermented jaggery kefir contains 2.78 g of carbohydrate in every 100ml of the sample tabulated in Table 2.

Bile and acid tolerance

Organisms were subjected to lower pH and bile salts to know whether the organisms can survive these conditions. To carry out this 2% of bile salt containing MRS broth was prepared and sterilized to this fresh culture was added and incubated at 37°C up to 5 hours (1 hour time interval), after the specific incubation time viability of organisms was checked by streaking on MRS agar and kept for incubation, after the incubation period each petri-dish of the different timeline was assessed with the growth. Number of colonies was decreased as the incubation time with bile salts was increased. Least growth was observed on the plates which were incubated for 5 hours with bile salts.

Microorganisms present in kefir grains were subjected to acid tolerant test by treating with HCl. At regular interval of time the cultures were streaked on to MRS agar and checked for the survival of the organisms. The number of

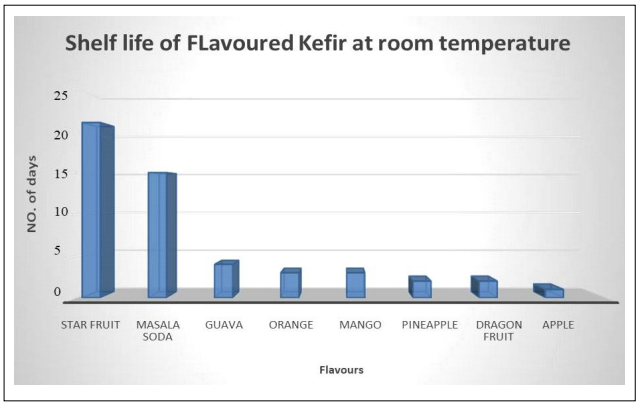

Fig 1a: Shelf-life study of the flavoured water kefir under room temperature.

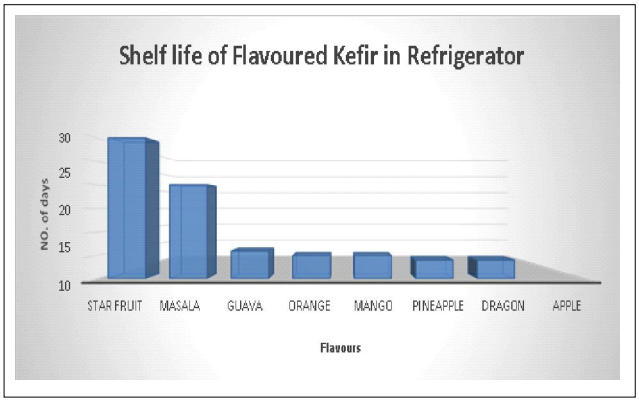

Fig 1b: Shelf-life study of the flavoured water kefir under refrigeration.

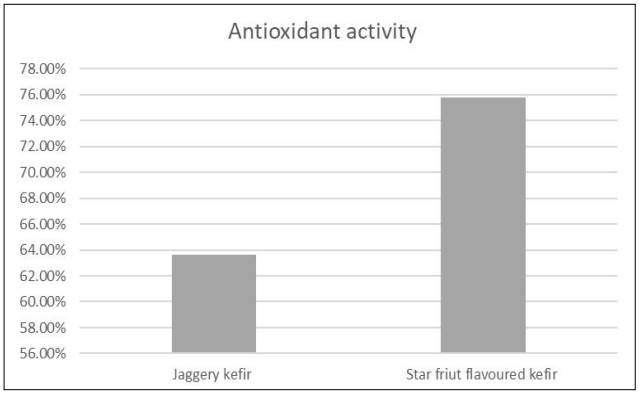

Fig 2: Antioxidant DPPH assay.

Table 1: Acidity of the fermented beverages.

Sample	Before fermentation	First fermentation	Second fermentation
Jaggery kefir	5.0	3.5	3.5
Star fruit kefir	3.5		3

Table 2: Protein and carbohydrate estimation of fermented beverages.

Sample	Protein concentration (per 100 ml)	Carbohydrate concentration (per 100 ml)
Before fermentation	0.5 g	43 g
Jaggery kefir	0.6 g	2.78 g
Star fruit kefir	1 g	4.35 g

organisms present in the culture broth maintained at pH 3.0 for 4 hours did not show any negative effect on the survival of the organisms. Whereas, at pH 2.0 after 4 hours of incubation, the number of colonies on MRS agar drastically reduced indicating the most of the organisms were not able survive at lower pH.

Antioxidant activity

As antioxidant activity by DPPH assay reveals the scavenging ability of the jaggery kefir and star fruit-flavoured kefir. Where the total percentage of the scavenging effect of jaggery kefir is 63.6% and star fruit-flavoured kefir is 75.75%. As expected, the scavenging effect of star fruit-flavoured kefir is significantly high when compared to jaggery kefir graphically represented in Fig 2.

Jaggery kefir upon second fermentation with star fruit juice shows a notable increase in its antioxidant activity by 12%. The release of glutathione, organic acids and phenolic compounds by the kefir grains and the natural antioxidants like vitamin C, β-carotene and gallic acid present in the star fruit, resulted in the increase of the anti-oxidant property (Bourrie *et al*., 2016; Sabokbar and Khodaiyan, 2015).

CONCULSION

By taking a consideration of an increase in complexity of the requirements by consumers including vegans, individuals who are intolerant or allergic to dairy products keeping this in our mind we produced a fermented kefir beverage. This water kefir grain is association of *Lactic acid bacteria, Yeast* and *Acetic acid bacteria*. For the fermented kefir beverages, the analysis was performed to evaluate the flavours, sensory, shelf life, protein, carbohydrate, bile and acid tolerance, antioxidant and antibacterial analysis. Product was appreciated by potential consumers. And these characteristics may have an additional benefit to the consumers, which was determined by testing the product. The production in large scale and consumption by administration of certain fruit that may promote additional health benefits. The kefir beverage that is produced in this work may help to fill the gap between actual and innovative consumption of the fruits and kefir that may be recommended in human diet with concern to their health. The fermented new water kefir product might represent the important foods that provide the live and good microorganisms to the people with the limited availability of fermented products.

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Conflict of interest

We declare that we have no conflict of interest.

REFERENCES

Ahmed, Z., Wang, Y., Ahmad, A., Khan, S.T., Nisa, M., Ahmad, H. and Afreen, A. (2013). Kefir and health: A contemporary perspective. Critical Reviews in Food Science and Nutrition. 53(5): 422-434.

Alok, A., Singh, I.D., Singh, S., Kishore, M., Jha, P.C. and Iqubal, M.A. (2017). Probiotics: A new era of biotherapy. Advanced Biomedical Research. 6(31): doi: 10.4103/2277-9175.192625.

Alsayadi, M.S.M., Al-jawfi, Y., Belarbi, M. and Sabri, F.Z. (2013). Antioxidant potency of water kefir. Journal of Microbiology, Biotechnology and Food Sciences. 2(6): 2444–2447.

Ardiansyah, Nada, A., Rahmawati, N.T.I., Oktriani, A., David, W., Astuti, R.M. and Shirakawa, H. (2021). Volatile compounds, sensory profile and phenolic compounds in fermented rice bran. Plants. 10(6): 1073. doi: https://doi.org/10.3390/plants10061073.

Aragon, G., Graham, D.B., Borum, M. and Doman, D.B. (2010). Probiotic therapy for irritable bowel syndrome. Gastroenterology and Hepatology. 6(1): 39-44.

Azizi, N.F., Kumar, M.R., Yeap, S.K., Abdullah, J.O., Khalid, M., Omar, A.R. and Alitheen, N.B. (2021). Kefir and its biological activities. Foods. 10(6): 1210. doi: https://doi.org/10.3390/foods10061210.

Bourrie, B.C., Willing, B.P. and Cotter, P.D. (2016). The microbiota and health promoting characteristics of the fermented beverage kefir. Frontiers in Microbiology. 7: 647. https://doi.org/10.3389/fmicb.2016.00647.

Caglar, E., Kargul, B. and Tanboga, I. (2005). Bacteriotherapy and probiotics' role on oral health. Oral Diseases. 11(3): 131-137.

Calatayud, M., Börner, R.A., Ghyselinck, J., Verstrepen, L., Medts, J.D., Abbeele, P.V.D. and Damak, S. (2021). Water kefir and derived pasteurized beverages modulate gut microbiota, intestinal permeability and cytokine production *in vitro*. Nutrients. 13(11): 3897. doi: https://doi.org/10.3390/nu13113897.

Cai, Y., Sounderrajan, A. and Serventi, L. (2020). Water kefir: A review of its microbiological profile, antioxidant potential and sensory quality. Acta Scientifci Nutritional Health. 4(6): 10-17.

Darvishzadeh, P., Orsat, V. and Martinez, J.L. (2021). Process optimization for the development of a novel water kefir drink with high antioxidant activity and potential probiotic properties from Russian olive fruit (*Elaeagnus angustifolia*). Food and Bioprocess Technology. 14(2): 248-260.

Fiorda, F.A., de Melo Pereira, G.V., Thomaz-Soccol, V., Rakshit, S.K., Pagnoncelli, M.G.B., de Souza Vandenberghe, L.P. and Soccol, C.R. (2017). Microbiological, biochemical and functional aspects of sugary kefir fermentation-A review. Food Microbiology. 66: 86-95.

Gaware, V., Kotade, K., Dolas, R., Dhamak, K., Somwanshi, S., Nikam, V. and Kashid, V. (2011). The magic of kefir: A review. Pharmacology. 1: 376-386.

Guandalini, S. (2011). Probiotics for prevention and treatment of diarrhea. Journal of Clinical Gastroenterology. 45: S149-S153.

Guinane, C.M. and Cotter, P.D. (2013). Role of the gut microbiota in health and cronic gastrointestinal disease: Understanding a hidden metabolic organ. Therapeutic Advances in Gastroenterology. 6(4): 295-308.

Hampton, J., Tang, C., Jayasree Subhash, A. and Serventi, L. (2021). Assessment of pear juice and puree as a fermentation matrix for water kefir. Journal of Food Processing and Preservation. 45(3): e15223. doi: https://doi.org/10.1111/jfpp.15223.

Laureys, D., Aerts, M., Vandamme, P. and De Vuyst, L. (2018). Oxygen and diverse nutrients influence the water kefir fermentation process. Food Microbiology. 73: 351-361.

Laureys, D. and De Vuyst, L. (2014). Water kefir as a promising low-sugar probiotic fermented beverage. Archives of Public Health. 72(Suppl 1): P1. doi: 10.1186/2049-3258-72-S1-P1.

Marotta, A., Sarno, E., Del Casale, A., Pane, M., Mogna, L., Amoruso, A. and Fiorio, M. (2019). Effects of probiotics on cognitive reactivity, mood and sleep quality. Frontiers in Psychiatry. 10: 164. doi: https://doi.org/10.3389/fpsyt.2019.00164.

Moretti, A.F., Moure, M.C., Quiñoy, F., Esposito, F., Simonelli, N., Medrano, M. and León-Peláez, Á. (2022). Water kefir, a fermented beverage containing probiotic microorganisms: From ancient and artisanal manufacture to industrialized and regulated commercialization. Future Foods. 5: 100123. doi: https://doi.org/10.1016/j.fufo.2022.100123.

Martínez Torres, A., Gutiérrez Ambrocio, S., Heredia del Orbe, P., Villa Tanaca, L. and Hernández Rodríguez, C. (2017). Inferring the role of microorganisms in water kefir fermentations. International Journal of Food Science and Technology. 52(2): 559-571.

Ozcelik, F., Akan, E. and Kinik, O. (2021). Use of Cornelian cherry, hawthorn, red plum, roseship and pomegranate juices in the production of water kefir beverages. Food Bioscience. 42(1): 101219. doi: 10.1016/j.fbio.2021.101219.

Sabokbar, N. and Khodaiyan, F. (2015). Characterization of pomegranate juice and whey based novel beverage fermented by kefir grains. Journal of Food Science and Technology. 52: 3711-3718.

Succi, M., Tremonte, P., Reale, A., Sorrentino, E., Grazia, L., Pacifico, S. and Coppola, R. (2005). Bile salt and acid tolerance of *Lactobacillus rhamnosus* strains isolated from parmigiano reggiano cheese. FEMS Microbiology Letters. 244(1): 129-137.

Yan, F. and Polk, D.B. (2011). Probiotics and immune health. Current Opinion in Gastroenterology. 27(6): 496-501.