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Article

Supplementation with detox juice added with probiotic improves atherogenic parameters in healthy individuals

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Phytochemicals present in detox juices and probiotics have demonstrated protective effects on cardiovascular risk factors. The consumption of these products alone modulate metabolic mechanisms and biomarkers. However, the effects of the combination of detox juice and probiotics have not yet been evaluated on atherogenic parameters. A randomized controlled study was carried out with 40 healthy volunteers (20 men and 20 women), aged between 18 and 50 years old. The volunteers ingested 200mL of juice for 30 days. Before and after supplementation, the anthropometric and lipid profiles and plasma concentrations of TBARS, Myeloperoxidase, Glutathione, Protein and non-protein Thiols and Vitamin C were analyzed. A reduction in LDL-c ($p=0.05$), triglycerides ($p=0.05$) and a significant increase in HDL-c ($p=0.002$) was observed. There was a significant decrease in the concentrations of TBARS ($p=0.01$), myeloperoxidases ($p=0.02$) and a significant increase in the Vitamin C and GSH ($p=0.01$). There wasn`t improvement in anthropometric parameters and total cholesterol. The findings highlight that supplementation with probiotic detox juice improves the lipid and antioxidant profile, suggesting a possible positive effect in reducing the risk of cardiovascular disease in healthy volunteers. Nevertheless, more robust researches with a prolonged treatment period should be conducted.

Keywords: Probiotics. Detox juice. Oxidative stress. Cardiovascular diseases.

INTRODUCTION

Dyslipidemias and cardiovascular diseases are important causes of morbidity and mortality among adults, with the prevalence increasing mainly in countries that have experienced “westernization” of lifestyle (Barreto *et al*., 2005). In general, these diseases are long-term, multiple, require permanent multidisciplinary monitoring and generate large material and human resources (Brasil, 2018). Dyslipidemias can even start in childhood, being more frequent when changes in eating habits are associated with a reduction in the practice of physical activities. There is also the genetic determination to develop dyslipidemia, the so-called primary dyslipidemias, the changes in lipid metabolism being generated by family inheritance, and they are also influenced by environmental factors, such as diet and physical inactivity. It is well established that inappropriate eating practices are associated with the development of dyslipidemia and cardiovascular disease (Ayman *et al*., 2019).

Multiple mechanisms can contribute to the development of comorbidities related to dyslipidemia and

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cardiovascular diseases, including abnormal adipokine production, aberrant oxidative stress and unregulated pro-inflammatory response in tissues such as muscles and liver (Furukawa, 2017). The presence of dyslipidemia can influence the speed of installation of atherosclerosis. Elevated plasma levels of low-density lipoprotein (LDL-c) are a risk factor for the development of atherosclerosis, as they result in increased permeability of the arterial intima layer, favoring the oxidation of lipoprotein (Sniderman, De Graaf, Couture, 2012; Hee Park, 2013).

The interest in a healthy lifestyle emerges as a strategy for the treatment and prevention of cardiovascular events. One of the most important factors to prevent lipid peroxidation and atherosclerosis is the intake of antioxidants, as these nutrients determine the composition of LDL-c and, consequently, its susceptibility to oxidation. Thus, food consumption is important in the generation or not of cardiovascular risk, with the intake of fruits and vegetables (rich in antioxidants) associated with the reduction of this risk. Nutritional therapy is indicated for the prevention and in the treatment of dyslipidemia and must address cultural, regional, social and economic issues (Mirmiran, 2014).

Among these strategies, functional foods and probiotics can be highlighted. Functional foods when consumed regularly in diets have, in addition to their nutritional functions, metabolic and physiological effects on the body and are related to increased intake of functional foods that have numerous phytochemicals that can act pharmacologically as antioxidants, hypoglycemic agents, hypotensors, stimulators of insulin release and metabolism in general (Sousa, Almeida, Silva, 2018).

Experimental studies show that the use of probiotics can contribute to the prevention and treatment of metabolic diseases, possibly through the modulation of the intestinal microbiota, the immune response, synthesis of microbial substances against pathogenic bacteria, competition for nutrients, inhibition of their adhesion to intestinal mucosa, modification of the pH of the intestinal environment, increased secretion of the mucosa, inactivation of toxins and their receptors and the stimulation of phagocytosis and specific immune responses (Sirin, Aziz, 2017), or nonspecific (Kumar *et al*., 2012).

Initially, probiotics were added to yogurt and other fermented dairy products. However, there has been an increased demand for non-dairy probiotic products in recent years for several factors, such as lactose intolerance, cholesterol content and allergy to milk protein, or even for not liking foods that contain milk. In view of these circumstances, the development of studies that seek alternative probiotic products, including products based on fruits and vegetables is conducted (Praepanitchai *et al*., 2019; Zhu *et al*., 2016; Terpou *et al*., 2019).

Thus, the development of functional foods, such as detox juices and foods with the incorporation of probiotics, has increased in the last decade, as a result of consumer awareness that improving quality of life is related to good nutrition. The well-known correlation between diet and physiological effects on the human body has generated great possibilities for the food industry to promote its products together with the health of (Silva *et al*., 2016).

Thus, the development of new functional foods has focused mainly on the nutritional composition of foods and not only on their organoleptic characteristics. In this sense, this study is a branch of a great research with functional foods and probiotics that aimed to verify in vivo, after 30 days of supplementation, the activity of a detox juice made with natural products such as fruits, green leaves, green tea, water and added probitotics *Lactobacillus acidophilus* LA 14, elaborated and standardized by the research team, on parameters of atherogenicity in healthy individuals.

MATERIAL AND METHODS

Study population

The study population consisted of 40 healthy volunteers, 20 men and 20 women, chosen for convenience and randomly, aged between 18 and 50 years old. The participation was voluntary in the study and after receiving detailed explanations of the intervention protocol, the participants signed the Free and Informed Consent Form. The protocol was in accordance Resolutions of the National Health Council (CNS) and the recommendations of the National Research Ethics

Commission (CONEP) and was approved by the Ethics and Research Committee with Human Beings of the University of the West of Santa Catarina (UNOESC - no 219.091).

Volunteers who used continuous medication or dietary supplements, previous use of probiotics, with liver/kidney disease, neoplasms, thyroid disorders, diabetes, angina, alcoholism, smoking and morbid obesity were excluded from the study. The participants who did not use the juice correctly during the protocol period or who acquired flu or infectious processes during the study were excluded.

Experimental design

A randomized controlled research was carried out. The volunteers were supplemented daily with 200 mL of the probiotic detox juice for a period of 30 uninterrupted days and evaluated at the beginning and end of the period. The volunteers were instructed to ingest the 200 mL daily for breakfast and to maintain their usual diet and physical activities, so that the only difference in their lives during the study was the daily consumption of probiotic detox juice.

The probiotic detox juice was produced weekly by the team and distributed to participants in standardized, sterilized glass bottles and packed in coolers. The volunteers were instructed to keep the juice refrigerated in their homes. The necessary ingredients for the preparation of the probiotic detox juice, were obtained in the local market and sent to the Food Technology Laboratory of UNOESC, Campus São Miguel do Oeste. After selecting the fruits and washing in 200 ppm chlorinated water they were rinsed in drinking water. The fruits were manually peeled, cut and crushed with a blender. After crushing, the juice obtained was pasteurized at 65°C for 30 minutes and *Lactobacillus acidophilus* strain LA 14 was added at a concentration of 10 log UFC/mL. In the preparation of the juice, the following formulation (v/v) was used: 25% pineapple juice cv. Pearl, 25% green tea, 5% spinach (made in 1:2 ratio, spinach: water), 15% apple juice cv. Fuji, 10% kale juice (made in the ratio 1:2, kale: water), 15% drinking water, added 0.1% ginger (w/v), 0.1% mint (p/v) and 1% fructose (w/v).

Anthropometric assessment

The techniques used to obtain the anthropometric measurements were according to the Anthropometric Standardization Reference Manual (Lohman, Roche, Martorel, 1988), with three measurements being made and considering the mean between them. All assessments happened at the UNOESC Anthropometry Laboratory. Height was measured in centimeters (cm) on a Professional ES2020 Sanny® wall stadiometer, with a precision of 0.1 cm, with the individual in an orthostatic position, barefoot, with the posterior surfaces of the heel, pelvic waist and scapular and occipital region in contact with the measuring instrument. The weight was verified in kilograms (kg), on a scale G-techC, model Glass 180, platform type, with a maximum capacity of 180 kg and precision of 100 grams, with the individual barefoot, positioned standing in the center of the platform, with arms down.

The abdominal circumference (AC) and the neck circumference (NC) were measured in cm, using a flexible tape measure, with 0.1 cm precision. For AC, the tape was applied above the iliac crest, with the individual standing, with the abdomen relaxed and with the arms along the body and the feet together. For NC, the participants remained in the same position and tape was placed on the middle of the neck over the hyoid bone (Fernández-Real, *et al*., 2004). The percentages of fat and body water, fat weight and lean mass weight were determined by bioimpedance with Biodynamic Model 450 equipment. The Corporal Mass Index (BMI) was calculated using the formula weight/(height)^2 (kg/m^2).

Systolic (SBP) and diastolic (DBP) blood pressures were measured in the seated individual, after 10 minutes of rest. The right arm was supported at the cardiac level and using a pressure device from the manufacturer Becton Dicknson, BD® in accordance with Malachias *et al*., 2016.

Laboratory reviews

Blood samples were collected from the participants after a 12-hour overnight fast. Total cholesterol, HDL-c, triglycerides were measured on the Elecsys 2010 analyzer

(Roche diagnostics®), using a commercial kit (Labtest Diagnostics®-Brazil), according to the manufacturer's instructions. LDL-c was calculated using the Friedewald formula (Friedewald, Levy, Fredrickson, 1972). GSH activity was tested spectrophotometrically at 340 nm with the standard substrate (1-chloro-2,4-dinitrobenzene, CDNB) and co-substrate (reduced glutathione, GSH), using the Sigma Aldrich® kit. Lipid peroxidation was estimated by measuring substances reactive to thiobarbituric acid (TBARS) standardized by Lapenna *et al.* (2001). The levels of protein and non-protein sulfhydryl groups (protein and non-protein thiols) were estimated as previously described by Boyne and Ellman (1972). Vitamin C was measured by Enzyme Linked Immunosorbent Assay (ABCAM - Ascorbic Acid Assay Kit® and Rac Beta - Tocopherol Assay Kit®), expressed in nmol/μl. The plasma activity of the proinflammatory myeloperoxidase enzyme (MPO) was measured spectrophotometrically by an assay system coupled with modified peroxidase involving phenol, 4-aminoanthypyrine and H_2O_2. The results were expressed in μmol/quinoneimine produced at 30 min, a procedure standardized by Alba-Loureiro *et al.*, 2007.

Statistical analysis

The data were analyzed using the Statistica 6.0 software (StatSoft, Tulsa, OK, USA). The data were expressed as mean ± SD. The Kolmogorov-Smirnov test was used to examine the distribution of variables. Comparisons of data among groups were performed using Student's t-test for parametric variables and Mann-Whitney for nonparametric variables. The effect of potential confounding factors was tested in multivariate linear regression models. A value of $p<0.05$ was considered statistically significant.

RESULTS

Anthropometric characteristics of the studied population

The anthropometric characteristics of the study participants are described in Table I. After ingesting the probiotic detox juice, there was an improvement in the anthropometric parameters, however these were not significant.

TABLE I - Anthropometric characteristics of the study population

Determination	Before ingestion	After ingestion	p
Weight (Kg)	68.4±10.2	68.1±10.5	0.95
BMI (Kg/m²)	23.8±2.8	23.2±2.8	0.96
% of fat (%)	24.3±6.4	21.9±6.7	0.10
Fat weight (Kg)	16.6±4.8	16.0±6.7	0.70
Lean weight (Kg)	51.8±8.7	52.1±9.0	0.91
Neck circ (cm)	34.2±3.1	34.0±3.0	0.88
Abdominal circ. (cm)	81.5±9.9	80.8±9.4	0.81
SBP (mmHg)	80.6±12.7	75.5±8.2	0.14
DBP (mmHg)	114.0±10.9	113.5±8.7	0.99

The data were presented as mean ± SD. The data were treated by Student's t-test for parametric variables and Mann-Whitney for non-parametric variables. BMI (Body Mass Index); SBP (Systolic Blood Pressure); DBP (Diastolic Blood Pressure); Circ. (Circumference).

Laboratory analysis of the lipid profile

The concentrations of the lipid profile are shown in Table II. There was a significant reduction in the concentrations of LDL-c ($p = 0.05$), triglycerides ($p = 0.05$), as well as a significant increase in HDL-c ($p > 0.01$). There was also a reduction in total cholesterol concentrations, although it was not significant.

Table II - Lipid profile of the studied population.

Determination	Before ingestion	After ingestion	p
Cholesterol (mg/dL)	176.8±37.2	170.5±24.2	0.87
HDL-cholesterol (mg/dL)	47.3±8.2	56.2±8.7	>0.01
LDL-cholesterol (mg/dL)	110.5±36.1	99.4±21.1	0.05
Triglycerides (mg/dL)	94.6±66.6	91.4±34.2	0.05

The data were presented as mean ± SD. The data were treated by Student's t-test for parametric variables and Mann-Whitney for non-parametric variables.

Oxidative stress markers

The concentrations of oxidative stress markers are shown in Figure 1. After 30 days of juice intake, there was a significant decrease in the concentrations of TBARS ($p = 0.01$) and myeloperoxidases ($p = 0.02$), together with a significant increase in Vitamin C and GSH ($p = 0.01$). There was also an increase in protein and non-protein thiols although not significant.

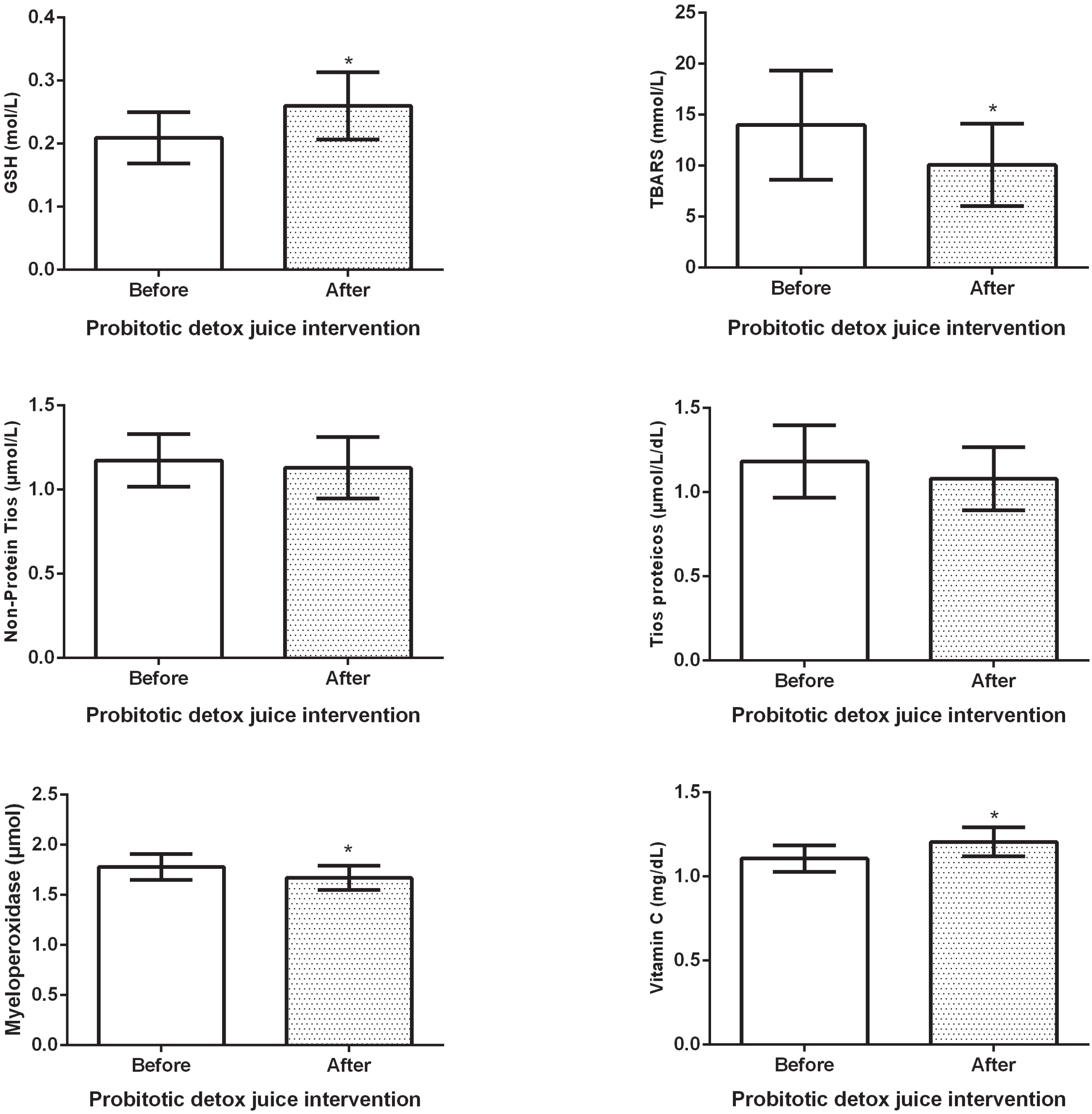

FIGURE 1 - Evaluation of oxidative stress markers in the studied population. A. GSH values (mol/L); B. TBARS values (mmol/L); C. Values of non-protein Tiols (µm/L); D. Protein thiol values (µ/mol/L/dL); E. Myeloperoxidase values (µmol); F. Vitamin C values (mg/dl). The data were presented as mean ± SD. The data were treated by Student›s t-test for parametric variables and Mann-Whitney for non-parametric variables.

DISCUSSION

The present research showed that ingestion of prototype detox juice, during 30 uninterrupted days, improves the lipid profile of consumers with a reduction in serum concentrations of total cholesterol, LDL and triglycerides, an increase in HDL and an efficient improvement of antioxidant parameters, contributing for the reduction of atherogenicity and cardiovascular risk. It is noteworthy that so far there are no reports in the literature between the association of detox juice and probiotics and their activities in the body, so these results are unprecedented and innovative.

Detox juices, by containing fruits and vegetables, they are sources of phytochemicals, with potential for disease prevention and treatment (Rahman, De Camargo, Shahidi, 2017; Toscano, *et al*., 2017; González-Aguilar *et al*., 2009). In addition, probiotics are able to modulate immunological and oxidative responses (Ou *et al*., 2012). However, there are many unanswered questions related to detox juices and probiotics and the health of humans. In this context, we investigated the effect of the association of probiotic detox juice on oxidative stress markers, biochemical cardiovascular parameters in healthy individuals, since these products have been popularly consumed as a health supplement.

There was a significant reduction in LDL-c and triglycerides and an increase in HDL-c, total cholesterol also showed an interesting tendency to decrease. We raised three hypotheses for these effects on the lipid profile. The first one suggests that the decrease in the concentrations of LDL-c, total cholesterol and TG would be due to an improvement in the systemic oxidative state. This could be observed by an increase in the concentrations of serum vitamin C and GSH, accompanied by a reduction lipid peroxidation (TBARS) and myeloperoxidase. A better oxidative state due to the consumption of polyphenols present in the juice can induce the reduction of LDL-c concentrations, decreasing the number of small particles of this lipoprotein (Toscano, *et al*., 2017; Kathiresan *et al*., 2006; Gentile *et al*., 2013). Improvements in the general oxidative state have been associated with better expression and activity of LPL (Yang *et al*., 2006; De Camargo *et al*., 2014), contributing to the decrease in concentrations of TG-rich lipoproteins (eg, VLDL-c) and LDL-c because LPL is the main enzyme involved in the removal of TGs from the blood and has some activity at the LDL-c receptor (Otarod, 2004).

Vitamin C is considered the most important and potent water-soluble nutritional antioxidant (Engler *et al.*, 2003) and the composition of the probiotic detox juice contains fruits with a high concentration of vitamin C. It is believed that the increase in the serum concentration of this vitamin, induced by daily juice intake, exercised a protection against lipid peroxidation directly, by eliminating peroxide radicals before they initiate lipid peroxidation, acting as a reducing agent, donating electrons to various reactive species, and eliminating them before they react with membranes and lipoproteins (Rique, Soares, Meirelles, 2002), and indirectly, regenerating the active form of vitamin E and other antioxidants such as β-carotene, flavonoids and glutathione (Misha *et al*., 2015). In addition, vitamin C improves vascular tissue integrity, vascular tone, lipid metabolism and blood pressure (Rique, Soares, Meirelles, 2002). Meta-analysis of seven controlled, double-blind, randomized studies showed no evidence that antioxidant supplements (vitamin C, E and β-carotene) prevent the progression of arteriosclerosis in adults (Bleys *et al*., 2006).

In the same vein, a significant improvement in glutathione concentrations was observed after 30 days of ingestion. Glutathione has the incredible ability to reduce not only H_2O_2 and synthetic organic peroxides, but also fatty acids and esterified cholesterol hydroperoxides. Thus, it can act on residues of peroxidized fatty acids within membranes and lipoproteins, reducing them to alcohol, as well as, it can also reduce thymine hydroperoxide, a product of the attack of free radicals on DNA (Misha *et al*., 2015).

Associated with this, probiotic detox juice reduced myeloperoxidase, which has long been suggested to collaborate in the oxidation of lipoproteins *in vivo* (Sokolov *et al*, 2014; Malle *et al*., 2006). Oxidative modifications of LDL-c leads to an increase in its reuptake and degradation by macrophages, resulting in cholesterol deposit and formation of foam cells, the cell mark of fatty streaks. Tests of high sensitivity and specificity, showed several stable end products generated by myeloperoxidase in atherosclerotic plaques (Park *et al*., 2012). Sokolov *et al*., 2014 characterized the MPO-H_2O_2-NO_2 system as the preferred route, used by monocytes, to convert LDL-c into atherogenic forms with greater affinity for the CD36 receptor, the main macrophage receptor for oxidized LDL-c directly involved in the formation of foam cells *in vivo*.

More recently, HDL has also been shown to be susceptible to oxidative changes mediated by myeloperoxidase by nitration or halogenation of tyrosine residues in Apolipoprotein AI. These impair the protein's ability to promote ABCA-1-dependent cholesterol reverse transport, contributing to the formation of atherosclerotic lesions (Malle *et al*., 2006; Zheng *et al*., 2004), corroborating with this, the results of this research show that there was an increase in HDL and probably an improvement in its activity by reducing myeloperoxidases.

The second hypothesis considers that flavonoids can also stimulate the increase in the excretion of bile salts in the feces and increase the activity of the hepatic mitochondrial system, with the consequent increase in lipid metabolism (Ayman *et al*., 2019). Ahmed *et al*., (2010) suggested that the reduction in total cholesterol by the action of flavonoids is due to the increase in the activity of LDL-c receptors in hepatocytes, responsible for the increase in endocytosis and the reduction of plasma cholesterol levels.

The third hypothesis is based on the action of probiotics, since Gadelha and Bezerra (2019) has stated that the consumption of fermented milk with *Lactobacillus acidophilus* reduces serum cholesterol in hypercholesterolemic African individuals. Since then, the hypocholesterolemic effect of fermented dairy products has been investigated in studies on food in humans (Kimoto, Ohmomo, Okamoto, 2002) or animal models (Jeong, 2003). These studies suggest that some strains of lactobacilli can lower total cholesterol and LDL-c, with a beneficial effect on serum cholesterol levels, results that were also found in our study.

Park *et al*., (2007) proposed that several mechanisms may be responsible for this decrease in LDL, including assimilation and incorporation of cholesterol in the bacterial cell membrane, binding of cholesterol to cells and disjugation of bile salts. However, the incorporation of cholesterol and the binding to the mechanisms of the cell membrane of probiotics are the most likely mechanisms compared to other mechanisms studied, since the assimilation of cholesterol by growing probiotic cells was significantly greater than cells at rest and dead. On the other hand, the removal of cholesterol by dead and resting cells confirmed that even non-viable probiotics still had the ability to bind cholesterol and, therefore, can be used as cholesterol-lowering agents in the gastrointestinal system. All the Lactobacilli and the Bifidobacteria strains tested were able to dismantle both sodium glycocholate and sodium taurocholate and consequently increased the disjugation of bile salts.

In the same sense, Varjú *et al*. (2020) concluded that most of the cholesterol removed by *Lactobacillus acidophilus* L1 and ATCC 43121 strains was by incorporation, considering the possibility of cholesterol being incorporated into the bacterial cell membrane. Similar results were observed by Jeong *et al*. (2013), who reported that probiotic bacteria are capable of producing enzymes that disjugate bile acids, the so-called bile salt hydrolases, these when disjugated are less reabsorbed when compared to conjugated bile acids, which results in increased excretion of these acids in the feces. The reduction in plasma cholesterol concentrations occurs as the demand for cholesterol for the synthesis of bile acids that needs to be replaced increases.

Also, the hypocholesterolemic effect of *Lactobacillus acidophilus* ATCC 43121 can be attributed mainly to the decjugation and dehydroxylation of bile acids as already mentioned in this study (Park, 2007). According to Begley and colleagues (2006) unconjugated bile salts are less efficient in solubilizing and absorbing lipids from the diet.

In addition, it is noted that probiotics can reduce cholesterol concentrations and increase the inhibition of hepatic cholesterol synthesis and/or increase the redistribution of plasma cholesterol to the liver, through its action on short-chain fatty acids (AGCC) (González-Aguilar *et al*., 2009). Fukushima *et al*., (2005) attributed the reduction in serum cholesterol concentration in rats fed a hypercholesterolemic diet that received the probiotic microorganism *Amylomyce rouxii*, to an increase in AGCC production and to an increase in the expression of LDL-c receptors in the liver. Varjú *et al*. (2020) conducted a study with healthy and lactose-intolerant men, and concluded that chronic consumption of probiotic yogurt increased the concentrations of propionate and butyrate, which may promote long-term improvement in lipid and glucose metabolism.

However, supplementation with probiotic detox juice did not significantly change anthropometric parameters in the research volunteers. It is believed that 30 days was a short period to reduce these parameters, but if juice intake were incorporated into the routine of individuals, promising medium-term results would be observed, especially if juice intake was associated with physical activity.

The results obtained from the study, show for the first time that the use of detox juice added with probiotic can be a viable alternative to help prevent and control dyslipidemia, reducing LDL-c, lipid peroxidation and myeloperoxidase promoting an increase concentration of HDL-c and antioxidants also contributing to a reduction to the risk of cardiovascular disease. The results suggest that probiotic detox juice plays an important role as an antioxidant and lipid-lowering agent. Further studies should still be carried out to confirm these findings and define a recommendation for the use and all its benefits.

CONFLICT OF INTEREST

We declare that there is no conflict of interest.

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