Additive effect of maqui (*Aristotelia chilensis*) and lemon (*Citrus x limon*) juice in the postprandial glycemic responses after the intake of high glycemic index meals in healthy men

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ABSTRACT

Berries have demonstrated potential in the prevention and treatment of metabolic syndrome. In this work, the effect of beverages prepared with maqui (*Aristotelia chilensis*) and/or lemon (*Citrus x limon*) in the modulation of postprandial glycemia was assessed. A type I clinical trial with a crossover design was carried out to determine the effects of beverages prepared with extracts from maqui, lemon and a mixture of maqui and/or lemon juices in the postprandial glycemia, when added to the intake of high glycemic index (GI) meals. Beverages containing the different extracts were formulated and then characterized by total polyphenols content, DPPH antioxidant activity and HPLC-DAD profile analyses. Postprandial glycemia was determined in healthy men (N = 10) at 0 and 15, 30, 45, 60, 90 and 120 min after the intake of meals. The tests meals were: glucose; glucose + maqui; glucose + lemon; glucose + maqui and lemon; rice; rice + maqui and rice + maqui and lemon. The GI determined for glucose and rice were 100 $\pm$ 7.9 and 90.6 $\pm$ 5.5, respectively. GI of maqui + glucose and rice + maqui drink were reduced to 91.4 $\pm$ 5.8 and 83.6 $\pm$ 3.9, respectively. Glucose + lemon juice reduced the GI to 91.4 $\pm$ 8.7. Maqui and lemon blends decreased the GI to 83.9 $\pm$ 5.1 for glucose and to 80.2 $\pm$ 4.5 for rice. Our results are discussed in terms of the mechanisms mediated by these ingredients to induce the additive effects in the glycemic response observed.

1. Introduction

Glucose is the main monosaccharide involved in the bioenergetics processes for most cells of human body [1]. The homeostatic levels of glucose are highly regulated in healthy individuals and a failure to maintain glucose levels outside of the normal range, results in hyper or hypoglycemia [1]. Hyperglycemia in subjects presenting impaired glucose tolerance (glucose range between 140 and 199 mg/dL, 2 h after oral glucose tolerance test) or impaired fasting glucose (100 mg/dL to 125 mg/dL), are considered as pre-diabetic subjects [2]. Chronic hyperglycemia from diabetes results at long term in the development of a wide spectrum of pathologies including cataracts, diabetic retinopathy, cardiovascular diseases, among others [3,4]. On the other hand, acute hyperglycemia in healthy subjects can be induced by the intake of high glycemic index meals. The dietary pattern related based on the intake of high glycemic index foodstuffs, has been proposed as a risk factor for the development of numerous chronic diseases, including:

cardiovascular diseases [5], cataract [6], type 2 diabetes [7,8], among others. Therefore, the development of new foodstuffs that could decrease the postprandial glycemia in healthy subjects constitutes an interesting goal for plant-based foods.

Aristotelia chilensis [Molina] Stuntz, *Elaeocarpaceae* is an evergreen native plant from Central and Southern Chile and Western Argentina [9]. The edible fruit arising from this plant commonly known as Maqui, has been widely assessed for its high phenolic content [10]. The phenolic composition of this fruit has been well characterized and different authors have shown that this berry possesses phenolic acids, pro-anthocyanidins, anthocyanins and other flavonoids [10]. It has been determined that 100g of fresh fruit, contains 137.6 mg of delphinidin 3-glucoside equivalents [11], with delphinidin-3-sambuboside-5-glycoside as the main anthocyanin (34% of the total) [12].

Human and animal studies have been performed with this native berry as the main ingredient, to assess its functional characteristics. Delphinol®, a maqui berry-based nutraceutical, has been used to study

its effects in the decrease of blood glucose induced by high glycemic index meals [13]. The intake of Delphinol® before meals reduced postprandial glycemic and insulinemic peaks after a glucose tolerance tests, as well as after boiled rice intake in human volunteers [13,14]. This effect was attributed to the capacity to inhibit the glucose intestinal transporter SGLT1 [13].

A decrease in postprandial glycemia in humans have been determined in foodstuffs containing a high polyphenolic content, including apple juice which possesses a high content of chlorogenic acid and phlorizin [15], as well as coffee polyphenols, containing mainly chlorogenic acid derivatives and feruloylquinic acids [16].

A synergic effect resulting from the mixture of different phenolic compounds arising from plants, as well as in combination with pure compounds has been reported for: antioxidant activity [17–19]; anticancer activity in squamous cells [20], among others. The formulation of a high phenolic content drink based on maqui (5%) and lemon juice mixtures has been previously assessed [21]. The antioxidant activity of the blends between maqui and lemon juices were higher, when compared with the activity of each component separately [19]. An increase in the antioxidant activity determined by the DPPH assay, as well as an higher efficiency in the inhibition of the pro-oxidative capacity of HOCl, superoxide radical anion and hydroxyl radical have been reported [19]. However, whether a combination of maqui berry and lemon juice can induce a synergic effect in postprandial glycemia has not been previously assessed.

In this work a type 1 clinical trial with a crossover design was performed, to assess the effects of a combination of maqui berry extracts with lemon juice in terms of the postprandial glycemia response, after the intake of high glycemic index meals.

2. Materials and methods

2.1. Chemicals

DPPH (2,2-diphenyl-1-picrylhydrazyl radical), Folin Ciocalteu reagent, gallic acid and catechin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ethanol, formic acid, acetonitrile, sodium carbonate were obtained from Merck (Darmstdat, Germany). Cyanidin-3-arabinoside and Quercetin-3-glucuronide were obtained from Phytolab (Hamburg, Germany).

2.2. Test meals containing maqui berry extracts

Maqui berry (*Aristotelia chilensis*) lyophilized extracts were obtained from Nativ For Life (Santiago, Chile). A volume of 250 mL of the drink containing an amount of total polyphenols of approximately 1000 μmol equivalents of gallic acid was prepared. Lemon fruits (*Citrus x limon*) were purchased in a Talca market. The juice was obtained by pressing at room temperature (25 °C) and stored at − 20 °C, until use (no more than 3 months). Lemon juice was added to achieve a final concentration of 2% w/w. The drink was filtered with the aim to remove the solid residues of the maqui extract and sucralose was used as a sweetener. The drinks were prepared the same day before the intervention with the volunteers.

2.3. Characterization of test meals containing glucose, maqui and/or lemon juice

Total polyphenols, pH, DPPH antioxidant assay were determined for the following test meals: 1) glucose; 2) glucose + maqui; 3) glucose + lemon; 4) glucose + maqui + lemon.

Total polyphenols were determined by means of the Folin Ciocalteu method as described elsewhere [22]. Briefly 200 μL of Folin Ciocalteu reagent were added to 600 μL of the samples together with 600 μL of sodium carbonate 7.5%. The absorbance was measured in a UV–Vis spectrophotometer (Spectroquant ® Pharo 300, Merck, Darmstadt,

Germany) setting the wavelength at 700 nm. Concentrations were interpolated from a standard curve made with gallic acid.

2.3.1. DPPH antioxidant activity

The DPPH antioxidant activity was measured for the following meals: 1) glucose; 2) glucose + maqui; 3) glucose + lemon; 4) glucose + maqui + lemon. The antioxidant activity was performed according to Simirgiotis et al. with modifications [22], 0.1 mM ethanolic DPPH was prepared an aliquot of 195 μL of this solution was mixed with 5 μL of the different samples prepared at different dilutions (1, 10, 100 and 1000) a positive control of gallic acid was prepared at 5 mg/mL and final concentrations of 125, 62.5; 31.25; 15.63; 7.81; 3.91, 1.95; 0.98; 0.49 and 0.24 μg/mL. Microplates (96 wells) were incubated at 37 °C for 30 min and the absorbance was measured in a microplate reader Biotek (model ELX 800, Winooski, Vermont, USA) setting the absorbance at 517 nm. Results were expressed as the scavenging concentration of required to decrease the DPPH absorbance to 50% (SC50).

2.3.2. HPLC-DAD profile and phenolic quantification of drinks containing maqui, lemon, and maqui with lemon

Beverages were homogenized in a vortex and 5 mililiters of each sample were taken into a glass container and freeze-dried (Scanvac Coolsafe 55-15 Pro, Labogene, Allerød, Denmark). The resulting powder was resuspended in methanol and analyzed by HPLC. The analyses were performed using a Shimadzu HPLC (Shimadzu Corp., Kyoto, Japan), consisting of a LC-20AT pump, a SPD-M20A diode array detector, a CTO-20AC column oven, a DGU-20A degasser, and a SIL-20A autosampler. The control of the system and data analysis was achieved using the LabSolution software (Shimadzu). Anthocyanin separation separation was carried out using a MultoHgih C18 column (5 μm, 250 × 4.6 mm) (CS-Chromatographie Service GmbH, Germany). The solvent systems were: A (H_2O-formic acid-ACN, 87:5:3, v/v/v) and B (H_2O-formic acid-ACN; 40:5:50, v/v/v). A flow rate of 0.8 mL/min was used and temperature was set at 30 °C, with an equilibration time of 10 min in the initial conditions before the next injection. Anthocyanins were analyzed using the following gradient only with solvents A and B: t = 0 min, 95% A, 5% B; t = 50 min, 75%A, 25%B; t = 55 min, 95%A, 5%B. The compounds were monitored at 520 nm and UV spectra from 200 to 600 nm were recorded for peak characterization.

The non-anthocyanin polyphenol analysis was carried out using an Inertsil ODS-4 C18 column (5 μm, 250 × 4.6 mm) (GL Sciences, Tokyo, Japan). The solvent systems were: A (H_2O-formic acid, 99.9:0.1, v/v) and acetonitrile as solvent B. A flow rate of 0.4 mL/min was used and temperature was set at 30 °C, with an equilibration time of 10 min in the initial conditions before the next injection. The gradient used was as follows: t = 0 min, 99% A, 1% B; t = 50 min, 84%A, 16%B; t = 65 min, 70%A, 30%B; t = 75 min, 70%A, 30%B; t = 85 min, 99% A, 1%B. The compounds were monitored at 280 and 360 nm. The UV spectrum was recorded from 200 to 600 nm for peak characterization.

Quantification was carried out using external calibration curves with several commercial standards. The analytical parameters were determined using the ICH guidelines (ICH, 2005). Five-point calibration curves were prepared in triplicate using the commercial standards: cyanidin-3-arabinoside (10–500 mg/L, r^2: 0.9989) for anthocyanins, quercetin-3-glucuronide (25–100 mg/L, r^2: 0.9996) for total flavonoids, and catechin (25–200 mg/L, r^2: 0.9996) for total polyphenols. Integrated area under the curve (AUC) was calculated for peaks observed at 520 nm, 360 nm and 280 nm for anthocyanins, flavonol and flavan-3-ols, respectively. Results are expressed as mg/mL of beverage.

2.4. Study design

A randomized type I clinical trial with a crossover design was carried out. A four period, four treatments crossover design was used for the meals involving glucose and a three period, three treatments

crossover design was used for the meals involving rice. The basic design involved administration of a test meal followed by glycemia determination by capillary blood analysis (Gluco Dr. Auto, model AGM- 4000, Beolmal-ro, Republic of Korea.). Each subject has arrived to the laboratory early morning after 12h fasting-period, and randomly ingested one of the six different test meals on six different occasions separated by 1 week wash-out period. Glycemia measurements were carried out in triplicate by each point, following World Health Organization guidelines.

2.5. Bioethical statement

Informed consent was obtained from all the participants involved in this study. This study was approved by the scientific ethics committee of the Health Sciences Faculty of the Universidad de Talca (resolution 202016FÁvila) in line with the World Medical Helsinki Declaration.

2.6. Subjects

Healthy male subjects (N = 11), between 18 and 30 years, were recruited through an advertisement placed on the Nutrition and Dietetics School at the Universidad de Talca, Talca, Chile. The inclusion criteria were male between 18 and 35 years that agree to participate in the study and that have read and signed the informed consent. The exclusion criteria were: 1) pharmacological treatment or medical condition that that would affect carbohydrate digestion and metabolism, these criteria were self-reported; 2) subjects diagnosed with type 2 diabetes, arterial hypertension or dyslipidemias and chronic inflammatory diseases. One subject did not complete the study and was excluded of the analysis (N = 10).

An anthropometric evaluation was carried out, using the following techniques: height and weight (Stadiometer Seca 220, Hamburg, Germany); waist circumference (Seca 201, Hamburg, Germany); skinfold thickness was measured using a Lange caliper (Beta technology, Santa Cruz, CAL, USA) to the nearest 0.1 mm at the biceps, triceps subcapsular and suprailiac sites [23]. Percent of body fat was determined according to the Siri equation [24]. Fasting glycemia was determined in order to characterize and select the volunteers participating in the study.

2.7. Test meals

Subjects were given meals containing food-grade glucose and rice, containing 50 g of carbohydrates by each meal. The test meals were the following: 1) glucose; 2) glucose + maqui (containing aprox. 1000 μmol GAE of polyphenols); 3) glucose + lemon and 4) glucose + maqui and lemon (containing aprox. 1000 μmol GAE of polyphenols).

Glucose was dissolved in 250 mL of hot boiled water and the solutions including maqui, lemon and maqui and lemon, were added once room temperature was reached (22–25 °C).

Glycemic modulation after the intake of rice was assessed preparing a drink with the same total amounts of maqui and lemon in the studies carried out with glucose. Test meals were the following: 1) rice + 250 mL of water; 2) rice + 250 mL of maqui (containing aprox. 1000 μmol GAE of polyphenols) and 3) rice + maqui (containing aprox. 1000 μmol GAE of polyphenols) + lemon (2% w/w, final concentration).

Rice (*Oryza sativa Japonica*), (grade 2, Banquete, Retiro, Chile), was prepared in a rice cooker (NEX model PH – 1200, China) using a ratio H_2O: rice of 2:1. Boiled water was added to rice until the rice cooker automatically stopped, typically after 20 min. The final weight of the portions was calculated determining the mass of the cooked rice and dividing it by the number of portions containing 50g of carbohydrates. The amount of carbohydrates was determined according to information provided in the nutritional food label by the manufacturer. The meals

containing rice were prepared 16–20 h before the intake, and were kept at 4 °C until the next day. Subjects reported to the laboratory in the morning following 12-h overnight fast. The morning of the intervention the subjects ingested one of the preparations randomly.

2.8. Statistical analysis

The results are expressed as mean values ± SD. The area under the curve was calculated using the trapezoidal method from 0 to 120 min. One way ANOVA followed by a Tukey post hoc test was used to determine statistical differences in the DPPH antioxidant activity. Paired Student t-test and one way ANOVA with a Bonferroni post hoc analysis were used to determine statistical significance ($p < 0.05$) between meals in glycemic peak and glycemic index between meals. All statistical analyses were carried out in the SPSS 14.0 for windows (IBM, Armonk, NY, USA).

3. Results

3.1. Phenolic characterization of test meals

A single dose of maqui extract containing approximately 1000 μmol of total polyphenols (expressed in gallic acid equivalents), was prepared, in order to determine its effects in the postprandial glucose modulation induced by high glycemic index meals. This extract was mixed with lemon juice in order to determine whether the combination of these two extracts can induce a synergic effect in terms antioxidant capacity, as well as a decrease in the postprandial glycemic response. Table 1 shows the chemical characterization of test meals used in this study. It can be observed that pH, changed from the lower to the highest values as follows: glucose + lemon juice > glucose + maqui + lemon juice > glucose + maqui > glucose.

Total polyphenolic content are shown in Table 1, indicating that blends composed by glucose + maqui and glucose + maqui + lemon juice, contain similar amounts of phenolic compounds, but significantly higher when compared to glucose or glucose + lemon juice blends. It can be observed that although glucose + lemon possessed a significantly lower amount of total polyphenols compared with maqui blends, it contains approximately 3 times more phenolics when compared to glucose (Table 1). The antioxidant activity assessed by means of the DPPH method could only be determined for blends glucose + maqui and glucose + maqui + lemon juice, which presented significant differences between them. Table 1 depicts that SC_{50} for blends composed by glucose + maqui + lemon juice are 64% more efficient to scavenge the DPPH radical when compared with glucose + maqui mixture.

Phenolic composition of the drinks used to perform the clinical trial using rice as the test meal, was characterized by means of HPLC-DAD analyses. The anthocyanin profile of the beverages showed the presence of seven main peaks with UV max at 520 nm (Fig. 1, Panel A and B). The total content of anthocyanins in maqui drink was 1.27 ± 0.01 mg

Table 1
Chemical and antioxidant capacity characterization of glucose mixtures used in the postprandial study.

Test Meal	pH	Total polyphenols (μmol GAE)*	DPPH SC_{50} (mg/mL)
Glucose	7.39 ± 0.13	22.5 ± 2.19	–
Glucose + lemon juice	2.69 ± 0.23	65.07 ± 5.28	–
Glucose + maqui extract	3.86 ± 0.45	992.02 ± 27.32	15.1 ± 3.8[a]
Glucose + maqui extract + lemon juice	3.01 ± 0.17	1004.16 ± 17.89	9.2 ± 0.6[b]

*Contained in 250 mL.
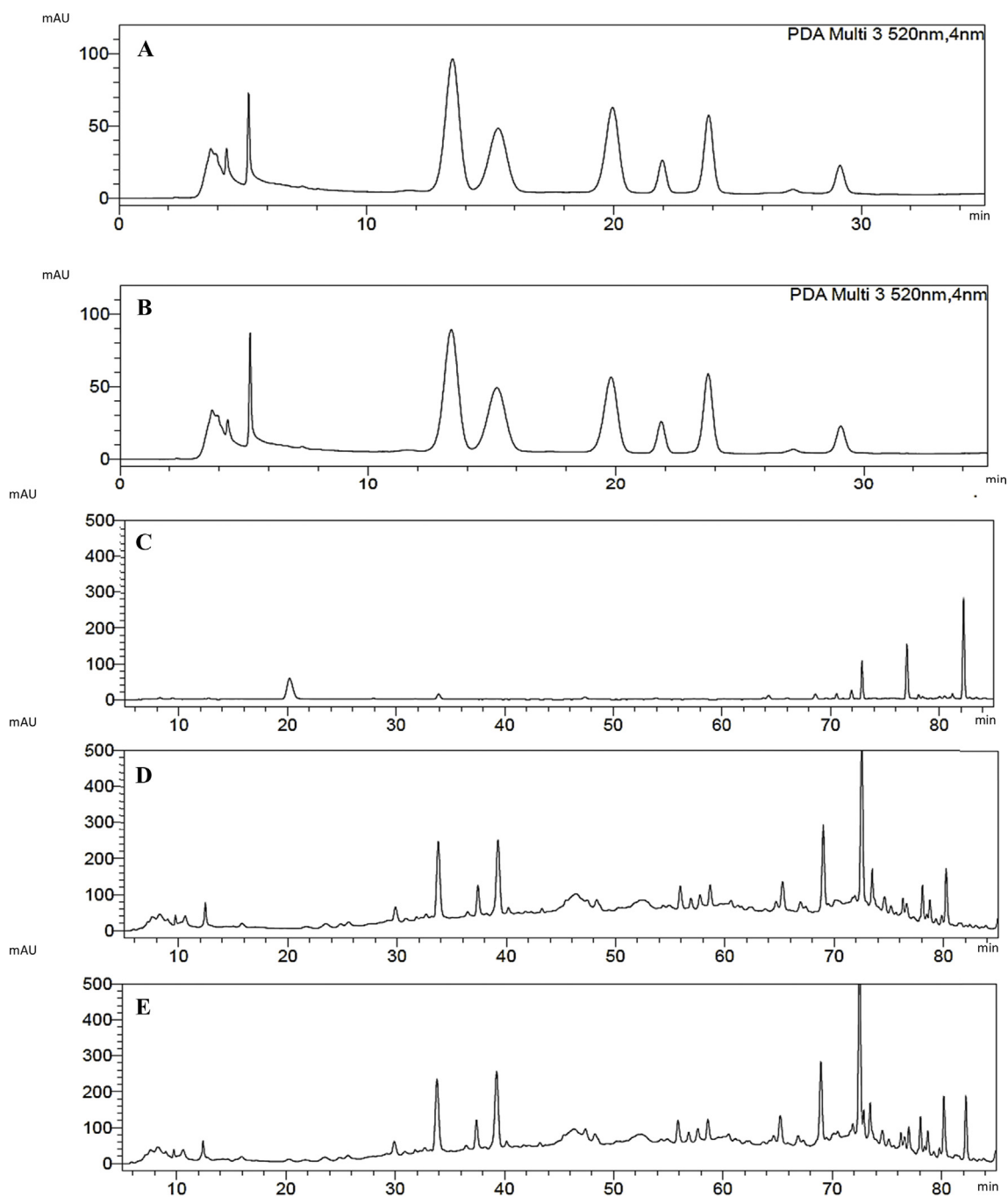[a] Mean values statistically significant P < 0.05.

Fig. 1. HPLC-DAD characterization of the drinks formulated in base to maqui berry and lemon juice. Panel A and B shows the anthocyanin profile determined at 520 nm for maqui drink and maqui + lemon juice drink, respectively. Panel C, D and E shows the phenolic profile determined at 280 nm for drinks composed by lemon juice, maqui and maqui + lemon juice, respectively.

of cyanidin-3-arabinoside equivalents (CyE)/mL of drink, while in the "maqui + lemon" drink was 1.18 ± 0.02 mg CyE/mL of drink.

The profile at 280 nm of phenolic compounds present in the drinks is shown in Fig. 1 (Panels C–E). The total content of polyphenols was determined using the external calibration curve using catechin and the AUC at 280 nm. The polyphenol content of the maqui drink was 3.98 ± 0.78 mg catechin equivalents (CaE)/mL drink. In lemon drink, the content was 0.98 ± 0.08 mg CaE/mL of drink. In the maqui + lemon drink, the total content was 4.15 ± 0.52 mg CaE/mL of drink. As depicted in Fig. 1 (Panels A–E) the main polyphenols of the maqui + lemon drink can be attributed to the maqui sample. Compounds eluting after 75 min showed the characteristic UV profile of

flavonoids. The total flavonoid content was determined using quercetin-3-glucuronide for the calibration curve. For the maqui drink, the content was determined as 0.30 ± 0.01 mg quercetin-3-glucuronide equivalents (QE)/mL of drink. For the lemon drink, the content was 0.10 ± 0.00 mg QE/mL drink. In the maqui + lemon drink, the content was 0.32 ± 0.01 mg QE/mL drink.

3.2. Postprandial glycemic response induced by glucose containing maqui and maqui-lemon mixtures

Human male volunteers were characterized by anthropometric means, as well as by fasting glucose determination (Table 2). Table 2

Table 2
Characterization and inclusion criteria of male volunteers that completed the trial (N = 10).

Parameter	Mean value ± SD	Min	Max	Inclusion criteria	Reference value[a]
Age (years)	23.1 ± 1.9	20	26	18–30	
Weight (kg)	69.4 ± 6.8	59	79.2	–	–
BMI (kg/m^2)	23.4 ± 1.5	20.5	24.7	18.5–24.9	> 30[b]
Fat (%)	18.5 ± 3.2	14.3	23.1	12–24	18.5–24.9[c]
Waist circumference (cm)	81.6 ± 4.8	76	89	< 90	> 102[a]
Fasting glucose	91.1 ± 5.9	73	99	≤125	≤110[a]

[a] Considering the ATPIII cutoff values for metabolic syndrome diagnostic.

[b] Considering the cutoff recommended for the World Health Organization for obesity diagnostic.

[c] Considered as normal range for World Health Organization.

shows that all volunteers meet with the inclusion criteria of this study. With the aim to determine whether maqui and lemon extracts can induce a reduction of postprandial glycemia, a clinical trial with a crossover design was performed. Fig. 2 shows the postprandial glycemic response after the intake of glucose. Fig. 2A shows that glucose induced the highest increase in glycemia, while the blend glucose + maqui + lemon showed the lowest increase. It can be observed that glycemic peak was observed to occur 30 min after intake, for glucose and all blends used in this study. Fig. 2B shows that glycemic peak of glucose was significantly reduced in a 20.5 ± 8.4% for the blend glucose + maqui + lemon (when compared to glucose). This amount represents a reduction of 36.7 ± 15.0 mg/dL in postprandial blood glycemia. When compared to glucose, glycemic peaks were reduced in 12.3 ± 8.9% and 12.1 ± 12.8%, for the blends glucose + maqui and glucose + lemon, respectively. Statistically significant differences were observed between glycemic peaks induced by intake of glucose + maqui and glucose + maqui + lemon blends.

Glycemic index were calculated to be 100 ± 7.9; 91.4 ± 5.8; 91.4 ± 8.7 and 83.9 ± 5.1 for glucose; glucose + maqui; glucose + lemon and glucose + maqui + lemon, respectively (Fig. 2C). Statistically significant differences were observed between glycemic index of glucose + maqui and glucose + maqui + lemon blends.

When the statistical analysis of t-test for paired samples was changed for one way ANOVA with a Bonferroni post-hoc contrast, only the blend glucose + maqui + lemon juice was statistically significant different when compared to glucose (p = 0.001 for glycemic peak and p < 0.001 for glycemic index).

The effect of maqui and maqui + lemon extracts in the postprandial glycemia was assessed using rice as a test meal. Fig. 3 A shows the glycemic response before and after the intake of rice; rice + maqui and rice + maqui + lemon. It can be observed that the mean value of glycemia induced by the intake of rice was higher when compared to rice + maqui and rice + maqui + lemon (Fig. 3A). The mean value of glycemia reached a maximum 30 min after intake (Fig. 3A). Therefore, glycemic peak for rice intake was calculated at 30 min postprandial. A reduction of 10.2 ± 5.4 and 13.1 ± 7.9% in the glycemic peak was observed for rice + maqui and rice + maqui + lemon, respectively (Fig. 3B). Glycemic index were calculated to be 90.6 ± 5.5; 83.6 ± 3.9 and 80.1 ± 4.4 for rice; rice + maqui and rice + maqui + lemon, respectively.

4. Discussion

Chilean native berries have been widely assessed by their high content in phenolic compounds. We have reported that a Chilean native berry concentrate containing a high phenolic content was able to modulate the postprandial oxidative stress when human volunteers ingested a 250 g of a ground turkey burger [25]. In addition, a single dose of 200 mg of Delphinol ®, a Chilean native berry Maqui extract, has

shown to decrease the postprandial glycemia after intake of rice. However, no studies have been performed to determine whether a combination of lemon juice and maqui berry could exert synergic or additive effects to decrease the postprandial glycemia after the intake of high glycemic index meals.

The antioxidant activity determined by the SC_{50} for the DPPH assay decreased from 15.1 ± 3.8 mg/mL for the blend glucose + maqui to 9.2 ± 0.6 mg/mL for glucose + maqui + lemon mixture. This result shows that samples containing lemon possesses a higher antioxidant capacity, when compared to glucose + maqui samples. These results agree with previous studies showing that the combination of different types of polyphenols exert synergic, additive or even antagonic effects, which have been mainly assessed by *in vitro* antioxidant activity assays [26,27]. However, only few studies have reported these effects in physiologic responses. In this regard, it has been reported that the supplementation with 1800 mg of total phenolic (determined by Folin-Ciocalteau analysis) from apple and blackcurrant polyphenol-rich drink has shown to decreased acute postprandial hyperglycemia in humans after the intake of 75 g of glucose [28].

In this study we have used a single dose of approximately 1000 μmol GAE of polyphenols arising from maqui berry extract and lemon juice, to determine its effect in the postprandial glycemic response after the intake of simple (glucose) and complex (rice) carbohydrates. The dose of 1000 μmol GAE of polyphenols was selected considering that similar doses have been successfully used to modulate the postprandial oxidative stress response in healthy male volunteers [25,29]. A similar dose was also effective to reduce postprandial glycemia (induced by the intake of 25 g of glucose) by the intake of coffee containing 2.5 mmol/L of chlorogenic acid (a total amount of 1000 μmol of chlorogenic acid) [30].

Beverages composed by blends between maqui and lemon have been previously formulated. Gironés-Vilaplana et al. have shown that beverages based on maqui extracts (2.5 or 5% w/v), lemon juice and sodium benzoate (200 mg/L) were relatively stable, reducing the levels of anthocyanins in a 37% (for the 2.5% in maqui extract drink), when stored at 4 °C during 70 days [21]. Our mixture was lower in the content of maqui extract (1.4% w/v) compared to the formulation by Gironés-Vilaplana et al. In this sense our results indicate that the doses containing aprox. 1000 μmol GAE of total polyphenols (contained in maqui and lemon juice blends) are enough to induce a significant decrease in the glycemic response induced after the intake of high glycemic index foodstuffs.

The reduction of postprandial glycemia after intake of glucose or rice, mediated by natural compounds, can be exerted at different physiological levels. We have observed that glycemic peak appears 30 min after the intake of glucose or rice and a statistically significant reduction mediated by the blend glucose + maqui + lemon was observed. This fact agrees with the glycemic peak time determined by the intake of cranberry juice sweetened with glucose in type 2 diabetic patients [31] or after rice intake by healthy adults [32]. A reduction in the glycemic peak was also observed in pre-diabetic subjects when different doses of Delphinol® were administered 60 min before 75 g of glucose intake (p for trend = 0.0273) [13]. The reduction in postprandial glycemia induced by Delphinol® was attributed to an inhibition of the SGLT1 glucose transporters activity in the small intestine, as it has been demonstrated to occur in rat duodenum [13,14]. Besides the effect exerted at the digestive tract, endogenous processes that can result in a reduction of post-prandial glycemia such as an: increase in glycosuria, incretin-mediated effects or regulation of gluconeogenesis, among other processes, cannot be discarded [33–35]. These processes could be occurring in our system after the intake of glucose or rice, considering that: 1) cyanidin-3-glucoside, a major component of maqui berry, possesses a non-negligible bioavailability of 12.38 ± 1.38% and 2) the maximum concentration in blood of cyanidin-3-glucoside and phase II conjugates of cyanidin 3-glucoside and cyanidin was observed to appear between 1 and 2 h after intake [36].
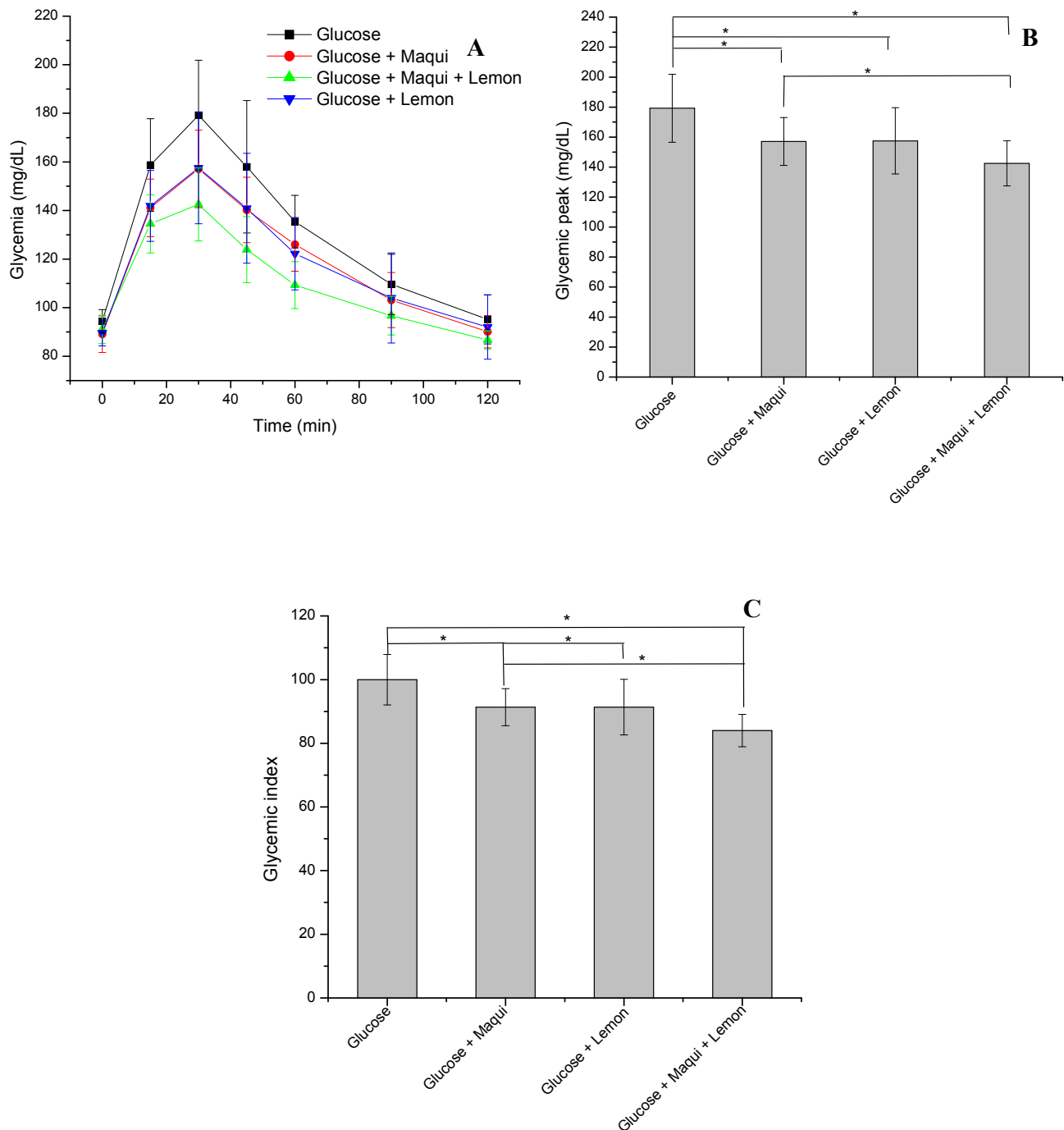
Fig. 2. Effect of maqui and lemon addition on post-prandial glycemic responses in healthy men (n = 10) after the intake of: glucose, glucose + maqui berry; glucose + lemon and glucose + maqui berry + lemon. Panel A shows the changes in glycemia. Panel B shows the changes in the glycemic peak (determined 30 min postprandial). Panel C shows the changes in the glycemic index. Data are expressed as mean ± SD. * indicate the statistically significant differences (p < 0.05).

A reduction in blood glycemia by effects induced in the digestive tract, such as a reduction in the gastric emptying and inhibition of digestive hydrolytic enzymes has been reported [37,38]. Starch requires six digestive enzymes to be hydrolyzed to glucose, salivary amylase, pancreatic amylase, and four intestinal mucosal α-glucosidases, including the N and C terminal subunits of sucrose-isomaltase and maltase-glucoamylase [39]. The inhibition of human salivary α-amylase by lemon juice [40], as well as inhibition of porcine α-amylase and yeast α-glucosidase by maqui fruit [41], have been reported. Therefore, inhibition of digestive enzymes by compounds present in maqui and/or lemon, could not explain the reduction of postprandial glycemia observed for glucose, but could be implied in the glycemic reduction of rice. The glycemic index calculated for rice in this study (90.6 ± 5.5) can lead us to classify this foodstuff as a high glycemic index food, as previous studies have concluded [42]. However, it should be noticed that glycemic index of white rice varies, showing values from 47 to 102 when compared to glucose as a reference food [42]. The wide range observed for the glycemic index of rice was attributed to length of cooking time, water-to-rice ratio, cooling time for the cooked rice, soaking time before cooking or other process variable [42]. This fact emphasizes that comparisons should be carried out under the same experimental procedures, which can decrease the variability of the response. Here, we have determined that the glycemic index of rice decreased to 80.2 ± 4.5 when a beverage containing maqui + lemon
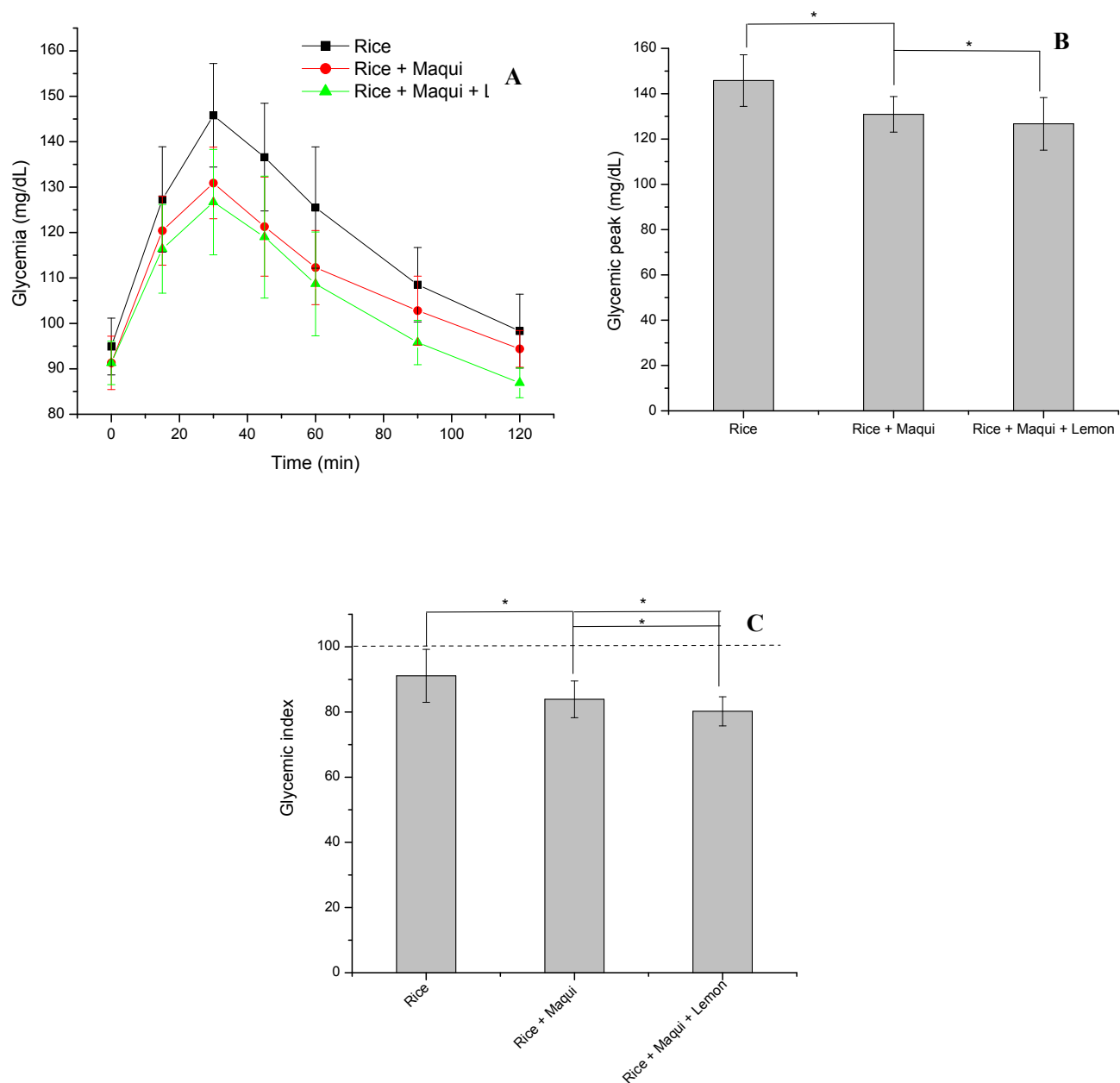
Fig. 3. Effect of maqui and lemon addition on post-prandial glycemic responses in healthy men (n = 10) after the intake of: rice + water; rice + maqui berry juice and rice + maqui berry + lemon juice. Panel A shows the changes in glycemia. Panel B shows the effect in the glycemic peak (determined 30 min postprandial). Panel C shows the effect in the glycemic index. Dashed line indicates the glycemic index of glucose Data are expressed as mean ± SD. * indicate the statistically significant differences (p < 0.05).

was administered to the volunteers.

Lemon juice has shown to possess numerous antioxidants such as ascorbic acid, diosmin glycosides, hesperidin, eriocitrin, vinenin-2, diosmin, quercetin, myricetin and hydroxycinamic acids [43–45]. Beneficial effects of lemon juice intake to human health, has been mainly assessed in terms of hypertension [46]. However, to the best of our knowledge the effects of lemon juice in the glycemic response in human volunteers have not been previously assessed. A reduction of postprandial glycemia of other citric fruits has been mainly assessed in terms of dietary fiber addition [47,48]. Furthermore, the addition of acidic foodstuffs such as vinegar has also shown to decrease the gly-cemic postprandial response [49]. Therefore, the effects observed in the reduction of the glycemic peak and glycemic index produced by the blend composed by maqui + lemon juice, can be explained by additive effects mediated by different types of phenolic compounds (with

mechanisms of action described above), but also by effects mediated by phenolic compounds combined with a reduction of the pH produced by lemon juice.

5. Conclusions

The present study demonstrate that additive effects in humans can be achieved by a rationale combination of plant foods, presenting re-levance for decreasing the glycemic index, a parameter widely asso-ciated with the etiology of numerous chronic diseases. However, more studies are necessaries to elucidate which are the responsible me-chanisms for these additive effects observed in the glycemic response elicited after glucose and rice intake.

Declaration of competing interest

The authors declare that there are no competing interests.

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