

Clinical Trial Protocol

NCT Number: NCT07043829

Protocol ID: PCSAEPF29-2021

Official Title: Effect of Consuming a Cimarrón Bean Extrudate Solution on Platelet Function and Postprandial Biochemical Parameters.

Sponsor: University of Talca, Chile.

Principal Investigator: PhD. Iván Francisco Palomo González

Ethics Approval: Folio 29-2021 (Approved)

Version Date: June 19th, 2025

1. Background

The common bean (*Phaseolus vulgaris*), a legume with high nutritional value, has been shown to have beneficial effects on cardiovascular health. Its bioactive components, including proteins, polyphenols, and fiber, contribute to the modulation of platelet activity. Evidence from previous studies indicates that regular bean consumption may reduce inflammatory markers, oxidative stress, and overall cardiovascular risk, positioning it as a key functional food for the prevention and management of chronic diseases. Incorporating beans into the regular diet could represent a practical strategy to improve cardiovascular health and promote healthy eating habits in the general population. The primary objective of this trial is to obtain preliminary evidence on the acute capacity of the extrudate to reduce thrombotic activity under controlled conditions, minimizing potential confounding factors that could happen when using a delivery vehicle.

2. Objectives

General Objective

To analyze the acute effect of consuming a *Phaseolus vulgaris* L. var. Cimarrón extrudate solution on platelet function, postprandial blood glucose, and total plasma polyphenol levels.

Specific Objectives

1. To evaluate the effect of consuming SPVCE (Solution *Phaseolus vulgaris* L var. Cimarrón extrudate) on platelet function, assessed by PFA and platelet reactivity.
2. To determine the change in postprandial blood glucose after consuming SPVCE.

3. To quantify the effect of consuming SPVCE in postprandial total plasma polyphenol levels.

3. Study Design

Model Description: A single-arm, open-label, acute, non-randomized interventional study in which all participants consumed a single dose of a functional solution containing 10 grams of *Phaseolus vulgaris* L var. Cimarrón extrudate dissolved in water. No placebo or comparator group is included. Outcomes are measured before and after the intervention within the same participants to assess acute changes in platelet function, postprandial glycemia, and total plasma polyphenol levels.

4. Participants

Recruitment: Participants were recruited through posters, flyers, and announcements distributed in various university facilities, inviting the community to participate. Individuals expressing interest received a supplementary information sheet about the study and completed an initial digital survey for preliminary data collection. After reviewing the information, any questions regarding the study procedures were addressed. Written informed consent was obtained from each participant, and a copy was provided for their records.

Inclusion Criteria

1. Adults 20-59 years old, male or female.
2. Not taking medications affecting platelet function.
3. Voluntary participant, willingness to participate, and provide written informed consent

Exclusion Criteria:

1. Documented food allergies, especially to legumes or beans
2. Diagnosed with liver, kidney, autoimmune diseases, or severe illnesses such as cancer.
3. Diagnosed gastrointestinal diseases, including inflammatory bowel disease (e.g., Crohn's disease, ulcerative colitis), celiac disease, or any condition causing chronic gastrointestinal symptoms such as malabsorption, persistent diarrhea, or gastrointestinal bleeding

4. Use medication known to alter platelet function like antiplatelet agents (aspirin, clopidogrel); non-steroidal anti-inflammatory drugs (NSAIDs, ibuprofen, naproxen); Anticoagulants (Warfarin, heparin, direct oral anticoagulants like rivaroxaban or apixaban); Selective serotonin reuptake inhibitors (SSRIs) and other drugs known to impact platelet aggregation or hemostasis

5. Intervention

Phaseolus vulgaris L. var. *Cimarrón* Extrudate composition

Phaseolus vulgaris L. var. *Cimarrón* was processed using a hammer mill extruder equipped with a 6 mm screen, operating at an extrusion temperature of 120 °C, with a product exit temperature of 110 °C. Moisture content of the extruded flour was determined with a TK100 moisture sensor and measured at 9.5%. Processing was conducted at the University of Concepción, Chile. The proximate physicochemical characterization and an organoleptic evaluation performed by ANALAB S.A. (Santiago, Chile), indicated a beige to light brown coloration, an aroma reminiscent of nuts with a toasted note, a mild flavor with slight natural sweetness, and a fine, uniform, floury texture, smooth to the touch. The nutritional profile of the extruded flour is summarized in Table 1 (proximate and physicochemical analysis) and Table 2 (nutrition facts).

Table 1. Nutritional analysis carried out on *Phaseolus vulgaris* L. var. “Cimarrón”

Bioactive Compound	
Total Protein (g/100 g DM-Dumas)	29.1
Soluble Protein (mg/mL)	3.8
Amino acids (mg/g Protein)	
Gly.His	55.3
Thr	34.3
Val	36.9
Met	7.4
Ile	31.5
Leu	58.4
Phe	45.3
Lys	54.4
EAA s	323.4
Asp	78.1
Glu	115.0
Ser	48.1

Arg	58.7
Ala	34.4
Pro	34.0
Tyr	24.8
NEAAs	394.3
Macro elements (g/10 g DM)	
P	4.6
K	15.8
Ca	1.1
Mg	1.9
Micro elements (mg/10g DM)	
Mn	14.0
Zn	45.3
Cu	10.3
Fe	105.0
B	18.0
Sugar	
RFO (mmol/100g DM)	5.0
Sucrose (g/ 100g DM)	2.3
Antioxidant capacity	
TPC (mg GA/ 100g DM)	74.5
ORAC (umol TE /100g DM)	9474.9

Table 2. Nutrition facts- *Phaseolus vulgaris* L. var. Cimarrón Extrudate

Nutrition Facts	100 g	Portion (10 g)
Calories (kcal)	227.9	22.79
Total Fat (g)	0.4	0.04
Sodium (mg)	0.62	0.062
Total Carbohydrate (g)	44.29	4.29
Dietary Fiber (g)	17.83	1.78
Total Sugars (g)	6.82	0.68
Includes 0g Added Sugars	0	0
Protein (g)	29.1	2.5

Time line

Participants were scheduled in advance and instructed to arrive after an overnight fast of 10–12 hours. Upon arrival, they completed a digital basic health assessment questionnaire covering medical history, current health status, dietary and nutritional habits, tobacco and alcohol consumption, education level, and socioeconomic status. A phlebotomist inserted an intravenous line to maintain venous access for blood collection, and participants remained at rest throughout the intervention.

1. At time 0 (baseline), blood samples were collected in two tubes containing 3.2% sodium citrate (Ez MedLab Co., Zhejiang, China) for baseline platelet function analysis (platelet reactivity and PFA) and quantification of total plasma polyphenols, and in one serum separator tube (Ez MedLab Co., Zhejiang, China) for baseline glucose measurement.
2. Two hours after SPVCE consumption, an additional serum separator tube was collected for postprandial glucose measurement and quantification of total plasma polyphenols.
3. Six hours after SPVCE consumption, blood samples were again collected in two sodium citrate tubes for postprandial platelet function analysis (platelet reactivity and PFA) and quantification of total plasma polyphenols.

Preparation and administration details

1. **Fasting state:** about 10 to 12 hours of nocturnal fasting; the participant can drink water while in the fasting state.
2. **Administration of SPVCE:** The participant has 10 to 15 minutes to drink the SPVCE. The research team made the SPVCE, and the self-team supervised the administration.

7. Outcomes

Primary endpoint

- **Platelet function measured by flow cytometry assay:** binding fluorescein isothiocyanate–conjugated anti-fibrinogen antibody (FITC) and PE-conjugated anti-P-selectin (PE) antibody after stimulation with ADP (0.03–30 μ M), collagen-related peptide (CRP; 0.003–3 μ g/mL), and thrombin receptor activator peptide 6 (TRAP-6; 0.05–15 μ M). Measurements performed using a FACSLytic flow

cytometer (BD, San Diego, USA). Analysis performed by Thrombosis Center Research, Talca, Chile.

Secondary endpoints

- 1. Platelet Function (Closure Time):** Measured by PFA-200 (Collagen+ADP cartridge, Siemens Healthcare, Marburg, Germany) at 0 h and 6 h on whole blood. Analysis performed by Thrombosis Center Research, Talca, Chile.
- 2. Baseline and postprandial Glucose:** Serum glucose measured by GOD-POD (glucose oxidase-peroxidase) enzymatic colorimetric method using BS300 Mindray (Shenzhen, China) at time 0h (baseline) and 2 h postprandial. Analysis performed by Clinic laboratory Talca, Chile.
- 3. Plasma total polyphenols concentration:** Prussian blue colorimetric method, measured in plasma at 0 h, 2 h, and 6 h. Analysis performed by Thrombosis Center Research, Talca, Chile.

Safety endpoints: Adverse events, vital signs, and routine clinical laboratory safety tests. Any clinically relevant abnormality will be recorded and reported per the ethics committee's requirements.

Sample handling: specify tubes and processing:

- 1. Flow cytometry platelet reactivity:** Whole blood anticoagulated with 3.2% sodium citrate processed according to Thrombosis Center SOPs, centrifuged at 1200 rpm for 12 minutes at T° ambient, obtaining platelet-rich plasma (PRP). Agonist time-to-stimulation: 10 minutes at ambient temperature and staining 20 minutes at ambient temperature
- 2. PFA-200:** Whole blood anticoagulated with 3.2% sodium citrate processed to Thrombosis Center SOPs in recommended cartridge tube tested within manufacturer's timeframe: 30 minutes after sampling at 15-25 °C.
- 3. Glucose:** Serum separator tubes, centrifuge at 3000 rpm for 8 minutes at ambient temperature, and analyze per Clinic Lab. Talca SOP.
- 4. Total polyphenols:** Whole blood anticoagulated with 3.2% sodium citrate processed to Thrombosis Center SOPs, centrifuged at 3000 rpm for 8 minutes at ambient temperature, obtaining platelet-poor plasma (PPP), aliquot and store at -80°C until assay if not run immediately.

Laboratories: Thrombosis Center Research, University of Talca (Platelet function assays and total polyphenols), and Clinic Laboratory Talca (glucose).

Visit schedule & sample timing

- 1. Day 0 (Treatment):** Participants arrived in fasting conditions (≥ 8 hours).
- 2. Baseline samples at T0h:** PFA-200, flow cytometry (reactivity), serum glucose, plasma total polyphenols. Administer 10 g extrudate in 500 ml water (supervised).
- 3. Post-dose samples:** T2h-serum glucose, plasma total polyphenols; T6h-PFA-200 Flow cytometry, plasma total polyphenols. Record AEs and vital signs throughout.

Discharge/follow-up: The participant has signed a request for sample storage and subsequent use of the samples in future research. Depending on the option selected (Acceptance or Rejection), the samples will be stored accordingly, labeled with the corresponding code, or discarded following the biological sample disposal protocol of the University of Talca, Chile.

8. Sample size and Statistical considerations

Planned enrollment: 30 participants

Statistical software: GraphPad Prism 10.1.1.

Statistical Plan: Paired analysis comparing pre- and post-dose measures, two-sided $p < 0.05$. More details in the SAP appendix.

9. Criteria for Withholding and Stopping

Withholding Criteria (no intervention on study day):

The investigational product will **not be administered**, and the participant's study visit will be suspended for the remainder of that scheduled 8-hour session if any of the following are present at baseline (pre-dose) or detected during the immediate pre-administration checks:

1. Acute illness or fever on the study day.
2. Fasting blood glucose ≥ 126 mg/dL or ≤ 60 mg/dL at baseline measurement.
3. Blood pressure $\geq 160/100$ mmHg at baseline assessment.

4. Self-reported moderate/severe gastrointestinal symptoms within 24 hours before the visit (e.g., vomiting, severe diarrhea, uncontrolled nausea).
5. Any acute condition deemed by the investigator to increase participant risk or compromise data integrity.

Action: If withholding criteria are met, the product will **not** be given during that 8-hour session. The study physician documents the reason, provides appropriate care/advice, and notifies the principal investigator. Rescheduling of the visit may occur only according to the trial's rescheduling policy (if allowed by the protocol); otherwise, the visit is recorded as not completed.

Stopping Criteria (Immediate cessation during session):

The study intervention will be **stopped immediately** for an individual participant during the 8-hour session if any of the following occur:

1. Any **serious adverse event (SAE)** possibly, probably, or related to the product (e.g., anaphylaxis, respiratory distress, hospitalization).
2. Acute clinically significant change in monitored clinical parameters judged to be related to the product (for example: new onset chest pain, severe hypotension or hypertension not responsive to initial measures, severe arrhythmia).
3. New laboratory or point-of-care result during the visit indicating acute organ injury (e.g., markedly abnormal glucose) that the investigator deems unsafe to continue.
4. Severe or worsening gastrointestinal reaction during the session (e.g., persistent vomiting, dehydration) that requires medical intervention.
5. Participant requests to stop or withdraw consent at any time.

Action: On stopping, immediate medical evaluation is provided, the event is documented in the case report form, the principal investigator is notified, and appropriate medical referral (including emergency care) is arranged if needed. The participant's further participation in subsequent visits will be decided according to the protocol and safety assessment.

Additional operational details

1. Who decides: the study physician and/or principal investigator make withholding/stopping decisions. Site staff follow their instructions.

2. Monitoring during the 8-hour visit: vital signs and symptom checks per protocol schedule; any trigger must be recorded with time and action taken.
3. Reporting: all SAEs and stopping events are reported per the trial safety reporting plan and institutional requirements.
4. Re-enrollment/rescheduling: any decision to allow the participant to return for another 8-hour session must follow the protocol's eligibility and rescheduling rules and occur only after medical clearance.

10. Data management and Ethics

Records will be kept confidential by the responsible researcher. Participant names or identifiers will not be used. Blood samples will be labeled with codes and will not be linked to personal data. Identity and health information will not be disclosed and will remain confidential. Study approved by the University of Talca Scientific Ethic Committee (Folio 29-2021).

Statistical Analysis Plan (SAP)

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1. Introduction: This Statistical Analysis Plan (SAP) outlines the methods and procedures for analyzing the data from the clinical trial investigating the acute effects of a Cimarrón Bean Extrudate solution on platelet function and postprandial biochemical parameters.

2. Analysis Populations

- **Per-protocol (pp) population:** Includes all participants who complete the 8-hour observation period and have valid measurements for the outcomes of interest without major protocol deviations (e.g., incomplete fasting, incomplete product ingestion, prohibited medication use). Sensitivity analyses will be conducted in the PP population to verify robustness.
- **Available Case Analysis:** An analysis based on all available data pairs for each outcome; participants with missing paired data will be excluded from that specific analysis.

3. Software: All statistical analyses will be performed using *GraphPad Prism version 10*.

4. General Analysis Design

- The planned sample size is **n = 30**.
- Measurements were obtained at baseline (0 h, pre-ingestion) and at **2 h** and **6 h** after consumption of the extrudate solution.

- Continuous variables will be expressed as mean \pm standard deviation (SD) when approximately normally distributed, or median [interquartile range, IQR] when non-normal.
- Categorical variables will be presented as frequency and percentage. For each time point, the mean/median and change from baseline will be reported.

5. Assessment of Normality and Homogeneity

Normality of data will be evaluated using the Shapiro–Wilk test, and homogeneity of variances will be assessed using Levene’s test where applicable. All statistical tests will be two-sided with the significance level set at $p < 0.05$.

6. Primary Endpoint

- Platelet Function (Flow Cytometry – Dose–Response Analysis): For each activation marker (P-selectin and fibrinogen) and agonist (ADP, TRAP-6, CRP), dose–response curves will be generated using non-linear regression to determine EC₅₀, maximum response, minimum response, and response span per participant. Comparisons of these curve-derived parameters between baseline (0 h) and 6 h post-ingestion will be performed.

The analysis will proceed as follows: For curve-derived parameters (EC₅₀, maximum, minimum, and response span), normality of paired differences between baseline and 6 h will be assessed using the Shapiro–Wilk test. If data are normally distributed, paired t-tests will be used for comparisons; if non-normal, Wilcoxon signed-rank tests will be applied. For the dose–response curves themselves, a two-way ANOVA will be performed to evaluate the effects of treatment (baseline vs. 6 h) and agonist concentration. If assumptions for parametric analysis are met, Sidak’s multiple comparisons post hoc test will be applied. If assumptions are not met and the analysis is non-parametric, a corresponding non-parametric alternative to two-way ANOVA will be used, followed by Dunn’s multiple comparisons post hoc test. Data will be presented as mean \pm SD for normally distributed data and median [IQR] for non-normal data

7. Secondary Endpoints

1. **Platelet Function (Closure Time – PFA-200):** Paired differences between baseline (0 h) and 6 h will be analyzed after testing for normality with the

Shapiro–Wilk test. Normally distributed data will be compared using a paired t-test, and non-normal data using the Wilcoxon signed-rank test.

2. **Baseline and Postprandial Glucose:** Differences between 0 h and 2 h will be analyzed using the same normality testing and paired statistical approach (t-test or Wilcoxon).
3. **Plasma Total Polyphenols Concentration:** Repeated measures at 0 h, 2 h, and 6 h will be analyzed using one-way repeated measures ANOVA if normality assumptions are met, followed by an appropriate post hoc test (e.g., Tukey's or Sidak's) for parametric data. If non-parametric, the Friedman test will be used, followed by Dunn's multiple comparisons post hoc.

8. Missing Data Handling

- The primary analysis will be conducted as an available-case analysis: only complete pairs of baseline and post-dose measurements will be included for paired comparisons, excluding any missing pairs. The number of pairs included will be reported for each analysis.
- If missing data in the primary variable is $\leq 5\%$, only available-case analysis will be performed without imputation.
- If missing data exceeds 5% or is suspected not to be missing completely at random, sensitivity analyses will be conducted using appropriate methods such as:
 - Linear mixed models for repeated measures (assuming Missing At Random), or
 - Multiple imputation (e.g., predictive mean matching) and comparison with available-case results.
- Participants lacking estimable parameters for a specific variable will be excluded from analyses involving that parameter; actual sample sizes will be reported accordingly.

9. Multiple Comparisons Correction

No formal correction for multiple comparisons will be applied. The primary endpoint analyses are limited to a small set of predefined parameters (EC_{50} , max, min, span) for key activation markers and agonists. Secondary endpoint analyses will be considered exploratory. Given the focused nature of the primary analyses and the sample size, results will be interpreted with caution regarding multiplicity. Where applicable, post hoc tests controlling type I error (e.g., Sidak's or Dunn's tests) will be used following ANOVA or non-parametric analyses.

10. Safety and Adverse Events

Gastrointestinal symptoms and any adverse events will be summarized as frequencies and percentages. Where applicable, events will be reported by post-ingestion time point.

11. Sample Size and Power

The planned sample size of **n = 30** is based on feasibility and prior experience in acute postprandial studies. With n = 30 for paired comparisons, the study has approximately **80% power** to detect a medium effect size (Cohen's $d \approx 0.5$) at $\alpha = 0.05$ (paired t-test).

12. Presentation of Results

Results will be presented in tables and figures. Tables will include descriptive statistics per time point, changes from baseline with 95% confidence intervals, and adjusted p-values where applicable. Figures will present time–response profiles, dose–response curves, and bar plots of key comparisons.