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Scientific Article

Development of a supplement containing cocoa, mesquite, agavins, pea protein, vitamins and minerals: effects on bone density and body composition

Desarrollo de suplemento con cocoa, mezquite, agavinas, proteína de chícharo, vitaminas y minerales: efectos en densidad ósea y composición corporal

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Abstract

The development of supplements is a strategy to promote population health. This study aimed to develop a powdered supplement containing mesquite flour, pea protein, cocoa, agavins, xanthan gum, vitamins, and minerals; characterize its phytochemical and nutritional composition; and evaluate its glycemic index, glycemic load, and effects on bone mineral density and body composition. The impact of particle size and mixing time on wettability, solubility, fluidity, and foam stability was assessed through physicochemical analysis; formulations with 600 µm particles exhibited greater stability, water absorption, solubility, and fluidity. The nutritional profile was established in accordance with NOM-051-SCFI/SSA1-2010, yielding an energy value of 366.03 kcal/100 g, with 29.53 g of carbohydrates, 58.59 g of proteins, and 1.15 g of lipids. The phytochemical profile identified by LC-MS/MS revealed a polyphenol content of 6608.57 μg/100 g, highlighting hydroxycinnamic acids (2597.12 μg/100 g), flavanols (1606.77 μg/100 g), and flavonols (1526.47 μg/100 g). In an adult intervention study (n=32, 18–40 years, 30 g/day for two months), the supplement showed a glycemic index of 11.18 and a glycemic load of 5.59. Consumption improved bone health in both sexes, particularly in the radial and femoral regions, and reduced body fat, especially visceral fat.

Keywords: supplement, functional food, bone health, body composition, health

Resumen

El desarrollo de suplementos es una estrategia para favorecer la salud poblacional. Este estudio buscó elaborar un suplemento en polvo con harina de mezquite, proteína de chícharo, cocoa, agavinas, goma xantana, vitaminas y minerales; caracterizar su composición fitoquímica y nutricional; y evaluar índice glucémico, carga glucémica y efecto sobre densidad mineral ósea y composición corporal. Se analizó fisicoquímicamente el impacto del tamaño de partícula y tiempo de agitación sobre humectabilidad, solubilidad, fluidez y estabilidad de la espuma; las formulaciones con partículas de 600 μm mostraron mayor estabilidad, absorción de agua, solubilidad y fluidez. El perfil nutricional se estableció conforme a la NOM-051-SCFI/SSA1-2010, obteniéndose un valor energético de 366.03 kcal/100 g, con 29.53 g de carbohidratos, 58.59 g de proteínas y 1.15 g de lípidos. El perfil fitoquímico identificado por LC-MS/MS mostró un contenido de 6608.57 μg/100 g, destacando ácidos hidroxicinámicos (2597.12 μg/100 g), flavanoles (1606.77 μg/100 g) y flavonoles (1526.47 μg/100 g). En el modelo de intervención en adultos (n=32, 18-40 años, dosis de 30 g/día por dos meses), el suplemento presentó un índice glucémico de 11.18 y carga glucémica de 5.59. Su consumo mejoró la salud ósea en ambos sexos, especialmente en regiones radial y femoral, y redujo grasa corporal sobre todo visceral.

Palabras clave: suplemento, alimentos funcionales, salud ósea, composición corporal, salud

1. Introduction

The development of nutritional supplements with functional ingredients represents a promising strategy for supporting metabolic and bone health, thereby contributing to body recomposition and musculoskeletal integrity in adults. An appropriate combination of macronutrients (proteins, carbohydrates, and lipids, as primary sources of energy and structural substrates) and micronutrients (vitamins and minerals, required cofactors for enzymatic catalysis and regulators of metabolic homeostasis), together with glycemic index and caloric content, can promote body recomposition and maintenance of musculoskeletal tissue through thermogenesis and bone regeneration processes (Martiniakova et al., 2022).

Cocoa-derived compounds contribute to the prevention of cardiovascular, neurodegenerative, metabolic, and bone-related conditions (Fideles et al., 2023). Xanthan gum, employed as a stabilizing agent in cocoa-based beverages, enhances viscosity and stability without affecting antioxidant activity (Muhammad et al., 2021). In addition, agave, mesquite, and pea protein provide dietary fiber, polyphenols, and proteins with anabolic, hypoglycemic, hypolipidemic, and prebiotic properties, which promote the balance of the gut microbiota and metabolic homeostasis (Jaimes-Morales et al., 2022; Medina-Larqué et al., 2022; Wang et al., 2022).

Considering these physiological benefits, it is relevant to assess the use of functional supplements in clinical settings where body composition alterations (such as visceral and subcutaneous fat accumulation and decreased bone mineral density) are increasingly observed, even in adults under 45 years old. These conditions may compromise quality of life and predispose to long-term health difficulties (Herath et al., 2022). Diagnostic assessment is typically performed via dual-energy X-ray absorptiometry (DXA), the gold-standard imaging modality for estimating T-scores (-1.0 to -2.5) and accurately measuring fat, lean, and bone mass, as well as body fat distribution.

Based on this evidence, a supplement was formulated with functional ingredients (mesquite flour, pea protein, non-alkalized cocoa, agavins, xanthan gum as a stabilizer, and a premix of vitamins and minerals) to characterize its physicochemical and nutritional properties, determine its glycemic index and glycemic load, and evaluate its effects on bone mineral density and body composition in a longitudinal study involving adult men and women.

2. Materials and methods

2.1 Materials

2.1.1 Chemical reagents

Catechin, epicatechin, epigallocatechin gallate, procyanidin B1, quercetin, quercetin-3-O-glucoside, naringenin, apigenin, rutin, hesperidin, taxifolin, phloretin, phloridzin, kaempferol, quinic acid, protocatechuic acid, syringic acid, chlorogenic acid, caffeic acid, p-coumaric acid, shikimic acid, gallic acid, salicylic acid, ferulic acid, sinapic acid, trans-cinnamic acid, and benzoic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Acetonitrile, formic acid, and ethyl acetate were obtained from J.T. Baker Inc. (Phillipsburg, NJ, USA). The vitamin and mineral premix (A, B_1 (thiamine), B_2 (riboflavin), B_5 (pantothenic acid), B_6 (pyridoxine), B_7 (biotin/H), B_9 (folic acid), B_{12} (cobalamin), C (ascorbic acid), D_3 (cholecalciferol), K, calcium, phosphorus, potassium, magnesium, iron, zinc, copper, manganese) was purchased from Alimentos América S.A. de C.V. (Guadalajara, Jalisco, Mexico).

2.1.2 Experimental materials

Mesquite pods were collected in Durango, Dgo., Mexico (24° 02′ N, 104° 40′ W). The pods were disinfected using a 1 % sodium hypochlorite solution, dried at 32 °C for 36 hours, pulverized, and homogenized through a 2 mm mesh sieve. The resulting flour was vacuum-sealed and stored at ambient temperature until use. Pea protein isolate (90 % protein content) was obtained from a local commercial supplier. Agavins were acquired from Nutriagaves de México (Guadalajara, Jalisco, Mexico). Cocoa powder and xanthan gum were sourced from a local retail outlet in Durango, Dgo., Mexico.

2.1.3 Formulation and mixing procedure

The supplement was formulated by mixing powders at different proportions, including hydrolysed pea protein, mesquite flour (*Prosopis juliflora*), agave fructans, non-alkalized cocoa powder, xanthan gum, and a premix of vitamins and minerals (Table 1). All powders were homogenized to ensure a uniform mixture and transferred to a 250 mL beaker, where they were stirred using an Ika Eurostar Power digi-visc homogeniser (Staufen, Germany) equipped with helical turbine geometry, at 50 rpm for 0, 180, and 240 seconds.

Table 1. Distribution of powders in the supplement blend
Tabla 1. Distribución de los polvos en la mezcla del suplemento

Ingredients	Proportion (% w/w)
Pea protein	72
Mesquite powder	16
Agave fructans	6
Non-alkalized cocoa powder	5
Xanthan gum	0.50
Vitamins	0.25
Minerals	0.25

This formulation was used as the foundational matrix for all assays conducted in this study

2.2 Methods

2.2.1 Physicochemical analysis of powder attributes

2.2.1.1 Bulk and compacted density

Bulk density and compacted density of the powder mixture were evaluated according to the methodology described by Camargo et al. (2019). To determine Bulk density, 10 g of the sample was weighed and transferred to a 25 mL graduated glass cylinder (2.5 cm in diameter). The volume occupied by the powder was recorded directly from the cylinder. Compacted density was obtained using the same cylinder and sample mass. The cylinder was tapped 30 times against a stainless steel surface covered with a sheet of ethylene vinyl acetate, and the final volume was recorded. Both densities were calculated as the ratio of sample mass to the volume occupied in the cylinder (Goula and Adamopoulos, 2012).

2.2.1.2 Wettability

Wettability was determined using a previously established method (Ji et al., 2016). A 6 g portion of each powder sample was carefully sprinkled into a 400 mL beaker (70 mm diameter) containing 100 mL of distilled water at 25 °C. The time required to achieve complete wetting was recorded.

2.2.1.3 Solubility

Solubility was determined following the procedure proposed by Liaotrakoon et al. (2022). One gram of powdered sample was weighed into a centrifuge tube, mixed with 10 mL of distilled water, and vigorously homogenized. The mixture was incubated at 37 °C for 30 min, followed by centrifugation at 3000 rpm for 10 min. The insoluble residue was dried in a convection oven at 105 °C for 3 h. Solubility was expressed as the percentage of soluble solids, calculated using Eq. (1).

$$Solubility = \left(\frac{(g\ of\ residue)}{(g\ de\ sample)}\right) \times\ 100$$ **Eq. (1)**

2.2.1.4 Carr and Hausner indices

The Carr and Hausner indices were calculated using the method proposed by Macías-Cortés et al. (2022). In summary, the Carr index was determined using Eq. (2) and the Hausner index using Eq. (3).

$$Carr\ Index = \left(\frac{(Bulk\ density - tapped\ density)}{tapped\ density}\right) \times\ 100$$ **Eq. (2)**

$$Hausner\ Index\ = \frac{Bulk\ density}{Tapped\ density}$$ **Eq. (3)**

2.2.1.5 Foam properties

Foam formation in reconstituted powders was considered a fundamental quality parameter, closely linked to consumer acceptance. Foams were produced by vigorously shaking a sealed cylindrical container (130 mL internal volume, graduated to 100 mL in 1 mL increments) filled with 40 mL of solution and 90 mL of air (Marinova et al., 2017). Foam stability was evaluated across three time points (0, 180, and 600 s) during a 600-second observation. The total volume of foam and underlying liquid (VF+L, upper limit) and the volume of drained liquid (VDrL, lower limit) were registered. Calculation of the liquid fraction within the foam (ε) was performed based on Eq. (4), and the gas fraction (ε^{-1}) was determined using Eq. (5) (Marinova et al., 2017).

$$\varepsilon\ = \frac{(40 - \mathrm{VDrL})}{(\mathrm{VF} + \mathrm{L} - \mathrm{VDrL})}$$ **Eq. (4)**

$$\varepsilon^{-1} = \frac{Vtotal}{Vgas}$$ **Eq. (5)**

2.2.2 Characterization of the supplement

2.2.2.1 Determination of caloric content

The energy content of the supplement was estimated based on its nutritional composition, in accordance with Mexican Official Standard NOM-051-SCFI/SSA1-2010. Quantification of the total macronutrient content (proteins, lipids, and carbohydrates) was performed. Subsequently, Atwater's general conversion factors were applied, assigning 4 kcal per gram to proteins and carbohydrates, and 9 kcal per gram to lipids (Sánchez-Peña et al., 2017). Results were represented as kilocalories per 100 grams of supplement (kcal/100 g).

2.2.2.2 Phytochemical characterization

Triplicate portions of 100 mg were prepared for phytochemical analysis. The samples dissolved in distilled water at a 1:10 (w/v) ratio and homogenized for 1 minute using an Ultra-Turrax (Ika Eurostar Power Digi-visc homogenizer, Staufen, Germany) for 1 minute. The homogenates were centrifuged, and the supernatant was recovered. Subsequently, liquid-liquid microextraction was performed with ethyl acetate (2:1, v/v). Vortex agitation was applied for 1 minute, and the sample was then centrifuged to recover the organic phase. The washing was repeated a second time. Both supernatants were combined in a single tube and concentrated using a Labconco CentriVap centrifugal vacuum concentrator (Thermo Fisher Scientific, US). The samples were dissolved in 200 µL of methanol and filtered using 0.45 µm PTFE membranes before analysis.

The phytochemical profile was defined according to the methodology described by De Santiago-Romero et al. (2024). Chromatographic separations were performed using LC-MS/MS, employing 7.5 mM formic acid in Milli-Q water (solvent A) and acetonitrile (solvent B). A concentration gradient of solvent B was applied at 0, 1.2, 3.8, 11.4, and 13.2 minutes, with concentrations of 3 %, 9 %, 16 %, 50 % and 3 %, respectively. An Acquity BEH C18 column (1.7 µm, 2.1 × 50 mm) was used at 35 °C, with a constant flow rate of 0.250 mL/min^{-1} and a stabilisation time of 2.8 minutes at 3 % solvent B. Electrospray ionisation (ESI) was performed in negative mode, using nitrogen as the nebulising gas. ESI source included a capillary voltage of 2.25 kV, cone voltage of 30 V, and a collision gas flow of 0.15 mL/min^{-1}. Collision energies were adjusted to 5.0 eV for MS and 20.0 eV for MS/MS. Desolvation and source temperatures were maintained at 400 °C and 150 °C, respectively. Compounds were identified and quantified through multiple reaction monitoring (MRM), ensuring selective detection.

2.2.3 Biological methods (human intervention model)

The intervention model was approved by the Research Ethics Committee of Comité de Ética en Investigación (CEITecNM/I.T. Durango) (registration number CEI-003-2022-0301-003). A non-probabilistic convenience sampling technique was applied, as the participants were volunteers who met the inclusion criteria and provided written informed consent. This approach is both ethically acceptable and methodologically consistent for exploratory nutritional interventions in healthy populations. It enabled the determination of the glycemic index and glycemic load of the supplement through a cross-sectional assessment, in accordance with International Standard ISO 26642:2010(E). The effect of supplement consumption on body indicators and bone density was also analyzed through a longitudinal assessment.

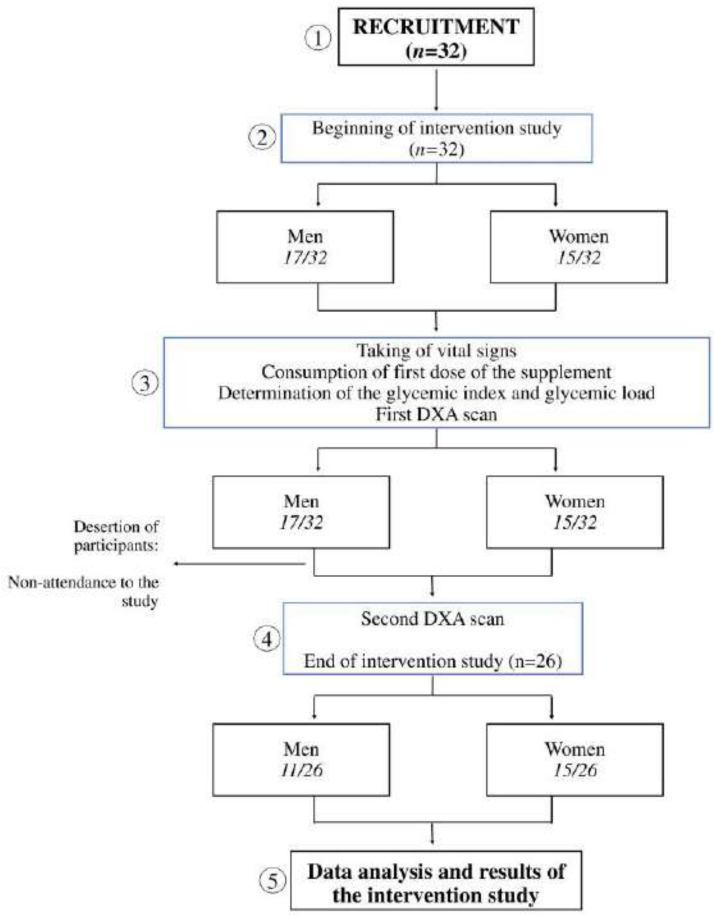

Figure 1. Flow of participants through the study stages. (1) recruitment (n = 32); (2) intervention start (17 men, 15 women); (3–4) supplement intake, glycemic index and load assessment, DXA scans (baseline and after 2 months), with dropout of 6 men; (5) final data collection and analysis

Figura 1. Se muestra el flujo de los participantes a través de las etapas de (1) reclutamiento (n = 32); (2) inicio de intervención (17 hombres, 15 mujeres); (3)-(4) consumo del suplemento, medición de índice y carga glucémica, escaneos DXA (inicio y 2 meses), registrando diserción por abandono del estudio de 6 hombres; (5) obtención de resultados y análisis final

A total of 32 participants (15 women and 17 men) were initially recruited. Eligibility required an age range of 18 to 40 years and the absence of any metabolic disease diagnosis, which was verified through the clinical record of each participant. Evaluations were conducted at the facilities of the

Laboratorio Nacional CONAHCYT de Apoyo a la Evaluación de Productos Bióticos (LaNAEPBi), part of the Tecnológico Nacional de México /I.T. Durango. After signing the informed consent form, clinical records were initiated, including the recording of vital signs such as blood pressure, respiratory rate, heart rate, and oxygen saturation. Body composition was assessed via DXA, providing data on weight (kg), body mass index (kg/m^2), fat, lean and bone mass (%), subcutaneous adipose tissue area (cm^3), and bone mineral density in various anatomical regions (radius, femur, and spine). Participants were directed to consume a daily dose of 30 g of the supplement dissolved in water, a portion defined in the study design as practical, safe, and consistent with ranges reported in nutritional interventions (20–40 g/day) and commercial powdered supplements. Following the initial dose, a glucose tolerance test was performed to determine the glycemic index and glycemic load of the supplement. Daily supplementation continued for two months. At the end of the intervention, the DXA scan was repeated to assess post-intervention changes in body composition (Fig. 1).

2.2.4 Experimental design and data analysis

Experimental data of physicochemical properties were expressed as mean ± standard deviation, and the data of biological model were expressed as mean ± standard error due to the nature of the data. A one-way analysis of variance (ANOVA) was performed, followed by Tukey's post hoc test for multiple comparisons (p < 0.05 was considered statistically significant), using Statistica v12.1 software (StatSoft, Tulsa, OK, USA). Mixture homogeneity was assessed through analysis of skewness and kurtosis. T-scores and body composition variables were analyzed using the relative change method (Fold Change) via the MetaboAnalyst v6.0 platform.

3. Results and discussion

3.1 Physical properties of the supplement

The analyses were performed on the formulated supplement, and all results reflect the properties of the complete mixture (Table 1). Based on the data presented in Table 2, both mixing time and particle size had statistically significant effects (p < 0.05) on the specific evaluated responses. However, the analysis of variance showed that the interaction between mixing time and particle size did not significantly influence the results (p > 0.05), suggesting that each variable independently impacts the physical properties of the supplement. Regarding wettability, a higher water absorption capacity was observed in treatments with 600 µm particles, probably attributable to a lower specific surface area and increased porosity, potentially facilitating water penetration into the particle matrix. This property is essential, as powder reconstitution represents the initial stage of the process (Gaudel et al., 2023). Conversely, the decrease in wettability with increasing mixing time could be due to physical redistribution of the particles; in this case, the resulting values may represent a weighted average of the entire mixture (Ong et al., 2020).

Table 2. Rehydration and flow properties of the powders used to obtain the supplement
Tabla 2. Rehidratación y propiedades de fluidez de los polvos utilizados para obtener el suplemento

Time (s)	Particle size (mm)	Bulk density (g/cm³)	Compact density (g/cm³)	Carr index	Hausner index	Wettability (s)	Solubility index (%)
0	0.600	0.302±0.08	0.397±0.00	23.90±1.50	1.31±0.02	33.0±0.50	25.46±0.40
0	0.250	0.299±0.02	0.423±0.01	24.22±1.10	1.42±0.12	26.5±0.50	26.83±0.05
0	0.145	0.285±0.01	0.401±0.00	29.05±1.12	1.41±0.02	25.5±0.50	21.13±1.10
180	0.600	0.294±0.00	0.411±0.00	28.46±0.00	1.40±0.00	35.0±0.50	26.70±0.50
180	0.250	0.295±0.01	0.401±0.00	26.68±1.25	1.36±0.02	25.5±0.50	21.50±2.70
180	0.145	0.302±0.01	0.401±0.00	25.68±2.24	1.31±0.04	25.5±0.50	20.30±1.63
240	0.600	0.294±0.00	0.397±0.00	25.93±0.74	1.34±0.01	36.5±0.50	24.81±0.40
240	0.250	0.295±0.01	0.409±0.01	28.11±0.20	1.38±0.00	25.5±0.50	23.23±0.60
240	0.145	0.292±0.01	0.406±0.01	28.20±0.30	1.38±0.00	25.5±0.50	21.53±0.05

The results are expressed as mean ± standard deviation

In terms of solubility, the 600 µm particle size showed greater solubility compared to smaller sizes (25 % vs. 21 %) ($p < 0.05$). This behaviour can be attributed to the presence of mesquite flour, which is rich in carbohydrates that enhance molecular interactions, favouring system solubility. Although mixing time did not exert a statistically significant effect on this parameter, its indirect influence may be related to the dispersion of soluble ingredients. These values were consistent with results reported by Lai et al. (2024) in alfalfa leaf powder mixtures. In particular, the results for bulk and compact density did not indicate statistically significant differences with respect to mixing time or particle size, indicating that these properties are more influenced by powder composition than by processing conditions.

To evaluate fluidity, the intermediate mixing time condition was selected to calculate the Carr and Hausner indices. The values obtained were favorable (Table 2), indicating that the presence of pea protein decreases the cohesion of the particles present in the supplement, promoting its handling and transport; therefore, the addition of ingredients that could enhance fluidity is not necessary. The Hausner index and the Carr index are two empirical parameters, closely related to each other, that are used to estimate the flowability of a powder. Despite the above, it is essential to mention that the Carr index was developed based on the concept that the more compressed a material is, the less flowable it will be. In contrast, the Hausner index was conceived as an indicator of the friction conditions between powders (Saker et al., 2019). Therefore, based on the data shown and the description above, the addition of an ingredient is not necessary.

Table 3. Foaming properties of reconstituted powder mixtures
Tabla 3. Propiedades espumantes de las mezclas de polvos reconstituidos

Time (s)	Particle size (mm)	ε0 s	ε180 s	ε600 s	(ε-1)0 s	(ε-1)180 s	(ε-1)600 s
0	0.600	0.76±0.02	0.77±0.02	0.77±0.02	0.24±0.02	0.23±0.02	0.23±0.02
0	0.250	0.77±0.03	0.79±0.02	0.79±0.02	0.23±0.03	0.21±0.02	0.21±0.02
0	0.145	0.80±0.02	0.81±0.01	0.81±0.03	0.20±0.02	0.19±0.03	0.19±0.03
180	0.600	0.81±0.03	0.81±0.01	0.81±0.01	0.19±0.03	0.19±0.01	0.19±0.01
180	0.250	0.78±0.03	0.78±0.02	0.78±0.02	0.22±0.03	0.22±0.02	0.22±0.02
180	0.145	0.80±0.03	0.80±0.03	0.80±0.03	0.20±0.03	0.20±0.03	0.20±0.03
240	0.600	0.80±0.02	0.79±0.02	0.80±0.01	0.20±0.02	0.21±0.02	0.20±0.01
240	0.250	0.77±0.02	0.78±0.02	0.77±0.02	0.23±0.02	0.22±0.02	0.23±0.02
240	0.145	0.76±0.02	0.78±0.01	0.78±0.01	0.24±0.02	0.22±0.01	0.22±0.01

The results are expressed as mean ± standard deviation

The results in Table 3 show the foaming values at different times. In the case of foaming at time zero, it can be seen that mixing time has an influence, as a higher liquid fraction value (ε) was observed in the powders that were mixed (0.81 vs. 0.76, approximately), regardless of the foaming time applied. Although this behavior becomes insignificant over time (ε at 0 s vs. ε at 600 s), there is a tendency for foam stability to improve in treatments with longer mixing times, as the liquid fraction remains constant.

Regarding particle size, no significant influence was observed on the liquid or gas fraction of the foam, indicating that this variable does not directly impact the foam formation capacity or stability under the conditions evaluated. The drainage volume, calculated as the difference between the liquid fraction at 600 s and at 0 s, showed that the agitation time had a positive influence on foam stability (Fig. 2). No significant change was recorded in the agitated powders. In contrast, an increase in drainage volume was observed in the non-agitated powders. The homogenization of the powder can explain this behavior during agitation, which allows for a more uniform distribution of the protein in the system, favoring the formation of more stable bubbles (Narsimhan and Xiang, 2018). In addition, the inclusion of xanthan gum in the mixture may have contributed to the observed stability, as this polysaccharide supplies structural support to the foam formed by the protein, preventing premature collapse of the foam matrix (Sheng et al., 2021).

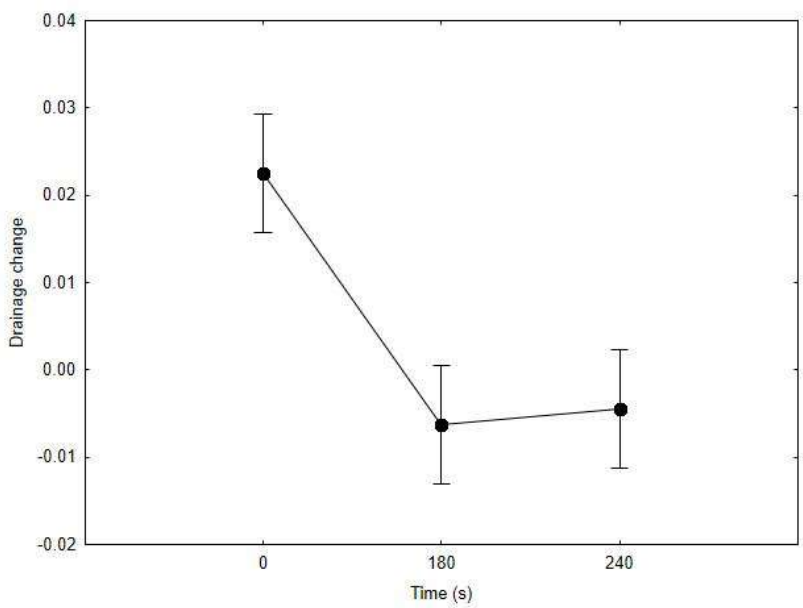

Figure 2. Effect of agitation time on the liquid drainage of the foam in the reconstituted supplement. The bars indicate the standard deviation, while the dots are the average values
Figura 2. Efecto del tiempo de agitación sobre el drenaje líquido de la espuma en el suplemento reconstituido. Las barras indican la desviación estándar, mientras que los puntos son los valores medios

3.2 Supplement formulation

The supplement formulation had a functional and phytochemical profile, as detailed in Table 4. The total caloric content was 366.03 kcal/100 g, with a macronutrient distribution of 29.53 g of carbohydrates, 58.59 g of protein, and 1.15 g of lipids. This composition primarily consists of a protein supplement, which may promote muscle protein synthesis processes and help regulate energy metabolism (Lim *et al.,* 2024). Micronutrient analysis revealed that the vitamin and mineral premix contained 430.93 mg/100 g, reinforcing its functional potential to contribute to physiological functions, including bone mineralization processes and electrolyte balance (Schiefermeier-Mach *et al.*, 2020).

As a complement to the formulation, the intervention study helped determine the glycemic index and glycemic load, with values of 11.18 and 5.59, respectively. This indicates a low glycemic response, which is proper for preventing metabolic sensitivity or the risk of insulin resistance (Yu *et al.,* 2025). These parameters are vital for preventing postprandial glucose and insulin spikes, especially in preventive contexts and in metabolic disorders such as type 2 diabetes mellitus or insulin resistance (Peres *et al.,* 2023).

Table 4. Nutritional statement for the dietary supplement developed from mesquite flour, agave fructans, agavins, cocoa, pea protein, and a blend of vitamins and minerals
Tabla 4. Declaración nutricional del suplemento dietético elaborado a partir de harina de mezquite, fructanos de agave, agavinas, cacao, proteína de chícharo y una mezcla de vitaminas y minerales

Macronutrients	**Amount per 100 g**
Energy content	366.03 kcal
Total protein (g)	58.59
Pea protein	42.18
Total lipids (g)	1.51
Total carbohydrates (g)	29.53
Agave fructans	4.72
Micronutrients	**Amount per 100 g**
Total vitamins (mg/100 g)	430.993
Thiamine (B_1)	2.33
Riboflavin (B_2)	2.64
Pantothenic acid (B_5)	10.88
Retinol (A)	1.55
Folic acid	311.11
Pyridoxine (B_6)	3.11
Ascorbic acid (C)	91.33
Cholecalciferol (D_3)	7.77
Biotin (H)	0.150
Vitamin K	0.120
Cyanocobalamin (B_{12})	0.003

Total minerals (mg/100 g)	1834.68
Calcium	871.27
Phosphorus	566.57
Potassium	244.70
Magnesium	137.25
Iron	5.88
Zinc	5.88
Copper	1.17
Manganese	1.96
Additional information	**Amount per 100 g**
Non-alkaline cocoa (g/100 g)	5.00
Total polyphenols (μg/100 g)	6608.57
Glycemic index (GI)	11.18
Glycemic load (GL)	5.59

The data correspond to the nutritional profile of the supplement used in the experimental trials described in this study

Chemical characterization identified 35 polyphenolic compounds, with a total abundance of 6608.57 ± 168.60 μg/100 g, predominantly hydroxycinnamic acids (2597.12 ± 106.65 μg/100 g), flavanols (1606.77 ± 65.85 μg/100 g), and flavonols (1526.47 ± 94.96 μg/100 g), identified for their antioxidant activity and modulating effects on inflammatory and metabolic pathways (Rudrapal et al., 2024). Flavanones (9.84 ± 0.73 μg/100 g) and flavones (3.82 ± 1.75 μg/100 g) were found in lower abundance, completing the bioactive profile of the supplement (Table 5). These compounds, together with the functional synergy of the base ingredients (pea protein, mesquite flour, agave fructans, cocoa, and the vitamin and mineral premix), support their potential impact in promoting advances in bone health and body recomposition (Bogdanis et al., 2023).

Table 5. Phytochemical profile of the supplement determined by LC-MS/MS
Tabla 5. Perfil fitoquímico del suplemento determinado por LC-MS/MS

No.	Compound	Nominal mass (g/mol)	Main transitions	RT (min)	λmax	Abundance (µg/100g)
1.	Shikimate	173.18	111.07, 93.06	0.598	297.53	378.14±34.04
2.	Gallic acid	169.15	125.05, 79.07	1.095	269.15	143.58±8.54
3.	Protocatechuic acid	153.15	108.92, 81.09	2.159	293.53	98.82±14.05
4.	p-salicylic acid	137.04	93.05, 65.09	3.221	254.53	20.18±1.06
5.	Syringic acid	197.21	182.05, 153.11	4.152	273.53	4.42±0.60
6.	Salicylic acid	137.04	93.05, 65.09	6.410	254.53	10.05±0.67
7.	Benzoic acid	121.10	77.06	6.453	272.53	21.34±2.56
8.	Quinic acid	191.20	93.06, 85.06	0.580	320.00	974.61±44.35
9.	Neochlorogenic acid*	353.34	191.04, 85.05	2.616	324.53	9.15±1.14
10.	Caffeic acid	179.19	135.08, 89.09	3.975	322.53	12.02±1.08
11.	Chlorogenic acid	353.34	191.04, 85.05	3.565	324.53	1551.77±81.60
12.	Cryptochlorogenic acid*	-	-	-	-	22.96±2.35
13.	*p*-coumaric acid	163.24	119.08, 98.07	5.111	308.53	32.69±2.51
14.	Ferulic acid	193.24	178.07, 134.04	5.720	322.53	18.17±0.61
15.	Sinapic acid	223.24	164.06, 149.04	5.785	323.53	6.81±0.88
16.	3,4-dicaffeoylquinic acid*	515.43	353.20, 179.06	6.638	323.53	0.35±0.11
17.	t-cinnamic acid	147.17	103.08, 77.07	8.574	275.53	8.55±2.79
18.	Gallocatechin*	305.33	125.02	2.646	273.53	1.54±0.16
19.	Procyanidin B1	577.44	407.21, 289.18	3.253	278.53	82.26±10.50

20.	Catechin	289.24	245.12, 123.03	3.608	278.53	664.73±40.39
21.	*(epi)*- catechin	289.24	245.12, 123.03	4.474	278.53	855.64±25.65
22.	*(epi)*- catechin gallate	441.27	169.04	6.080	276.53	2.58±0.15
23.	Rutin	609.28	300.24, 271.13	5.940	353.53	117.98±18.24
24.	Miquelianin	477.26	301.10, 151.03	6.059	353.53	1166.73±86.03
25.	Isoquercetin*	463.35	300.42, 271.11	6.086	353.53	78.15±8.57
26.	Astragalin*	447.34	284.24, 255.12	6.667	347.53	10.23±0.86
27.	Quercetin	301.20	179.04, 151.02	8.320	370.53	125.78±5.01
28.	Kaempferol	285.22	185.02, 151.02	9.345	363.53	27.58±8.46
29.	Apigenin	269.27	177.04, 148.58	9.151	335.53	3.82±1.75
30.	Hesperidin	609.40	301.15, 164.02	7.080	352.53	0.17±0.04
31.	Neohesperidin*	609.40	301.15, 164.02	7.080	352.53	0.10±0.01
32.	Naringenin	271.28	151.04, 119.06	9.135	288.53	9.57±0.75
33.	Taxifolin	303.03	285.00, 125.02	6.041	303.00	47.05±8.14
34.	Phloridzin	471.34	435.16, 273.15	7.43	283.53	9.86±1.05
35.	Phloretin	273.23	167.03, 123.08	9.233	284.53	118.69±1.00

Retention time; *Compounds identified based on their major transitions and peak spectra, abundance data are mean ± standard deviation (n=3)

3.3 The impact of supplement consumption on bone mineral density and body composition

The intervention model began with 32 participants (17 men and 15 women) and concluded with 26 active participants (15 women and 11 men). Despite this decrease, the final sample was statistically valid. The supplement showed different effects in women and men, especially in bone mineral density and visceral fat redistribution. Both groups maintained vital signs within normal ranges (Table 6).

Table 6. Health indicators and body composition of participants before and after the intervention
Tabla 6. Indicadores de salud y composición corporal de los participantes antes y después de la intervención

	Women		**Men**	
Parameter	**Beginning**	**End**	**Beginning**	**End**
n=	15	15	17	11
Age (years)	28.26±1.94	28.26±1.94	24.45±1.08	24.45±1.08
Height (m)	1.62±0.01	1.62±0.01	1.71±0.01	1.71±0.01
Body weight (kg)	68.81±3.30	68.69±3.26	98.32±5.61	97.75±5.52
Body Mass Index (kg/m^2)	25.55±1.03	25.92±1.17	33.51±1.64	33.10±1.64
Heart rate	72.53±2.27	72.53±2.27	69.00±1.36	69.00±1.36
Respiratory frequency	17.6±0.17	17.6±0.17	18.18±0.07	18.18±0.07
Systolic pressure	111.93±2.20	111.93±2.20	129.27±2.60	129.27±2.60
Diastolic pressure	72.33±1.21	72.33±1.21	77.54±2.37	77.54±2.37
Oxygen saturation	97.66±0.12	97.66±0.12	97.63±0.13	97.63±0.13

Data showed are the mean ± standard error (SE).

DXA analysis showed improvements in body and radial T-scores after two months of intervention. In women, an increase in radial bone density was observed (−1.81 ± 0.17 to −1.50 ± 0.17), while in men, improvements were observed in radial (from −1.90 ± 0.17 to −1.45 ± 0.16) and femoral (from −0.07 ± 0.15 to 0.17 ± 0.11) densitometry (Table 7). These changes may be associated with the synergistic action of the supplement's ingredients. Cocoa has been reported to have effects on the femoral and lumbar regions by increasing levels of insulin-like growth factor 1 (IGF-I), which reduced bone turnover in rats (Sarmadi et al., 2020). On the other hand, agave fructans improved femoral calcium content and bone mineral density in calcium-deficient rats (Topolska et al., 2020). Additionally, peas and mesquite provide polyphenols such as quercetin, rutin, catechin, and epicatechin, and micronutrients such as calcium, phosphorus, zinc, and vitamin D, which help modulate physiological pathways implicated in osteoblastogenesis and bone remodeling (Shaghaghian et al., 2022; Tomczyk-Warunek et al., 2024).

With regard to body composition, a reduction in visceral fat was observed in both groups (Table 7). Specifically, in the women's group, visceral fat decreased from 656.04 ± 96.32 g to 603.07 ± 88.31 g, and in men, from 1310.59 ± 174.11 g to 1253.13 ± 154.55 g. This result can be explained by the plant proteins, dietary fiber, and antioxidant compounds present in mesquite, peas, and cocoa, which promote lipid oxidation and thermogenesis processes (Jean-Marie et al., 2021; Oliveira et al., 2021). On the other hand, agave fructans may have contributed to the observed effect by modulating the gut microbiota and enabling the production of microbial metabolites, such as butyrate, which is involved in bone and lipid metabolism (Ocampo et al., 2023).

Table 7. Body composition and mineral density of participants at beggining and at the end of the intervention study
Tabla 7. Composición corporal y densidad mineral de los participantes al inicio y al final del estudio de intervención

	Women		**Men**	
Parameter	**Beginning**	**End**	**Beginning**	**End**
Fat mass (%)	39.66±1.28	40.05±1.35	38.09±1.27	38.35±1.21
Lean mass (%)	57.36±1.19	56.80±1.23	59.23±1.16	58.99±1.10
Bone mass (%)	2.96±0.10	2.95±0.10	2.70±0.11	2.66±0.11
Visceral adipose tissue area (cm^3)	135.30±13.13	128.87±12.87	208.40±22.66	203.96±19.99
Visceral adipose tissue mass (g)	656.04±96.32	603.07±88.31	1310.59±174.11	1253.13±154.55
Visceral adipose tissue volume (cm^3)	110.26±11.53	119.10±10.79	185.49±17.60	188.25±18.75
Body bone mineral density (T-score)	-1.22±0.15	-1.19±0.15	-1.72±0.07	-1.76±0.06
Radial mineral density (T-score)	-1.81±0.17	-1.50±0.17	-1.9±0.17	-1.45±0.16
Femoral mineral density (T-score)	-0.26±0.17	-0.23±0.15	-0.07±0.15	0.17±0.11
Spinal mineral density (T-score)	0.73±0.14	0.78±0.14	0.59±0.06	0.60±0.05

Data showed are the mean ± standard error (SE).

Likewise, multivariate Fold Change analysis confirmed and expanded upon the previous findings, showing significant variations in body and radial bone density, as well as in the redistribution of adipose tissue, particularly in the visceral area (Fig. 3ab). Positive values show patterns of tissue redistribution and improvement in bone mineral density, which may reflect the physiological impact of the supplement.

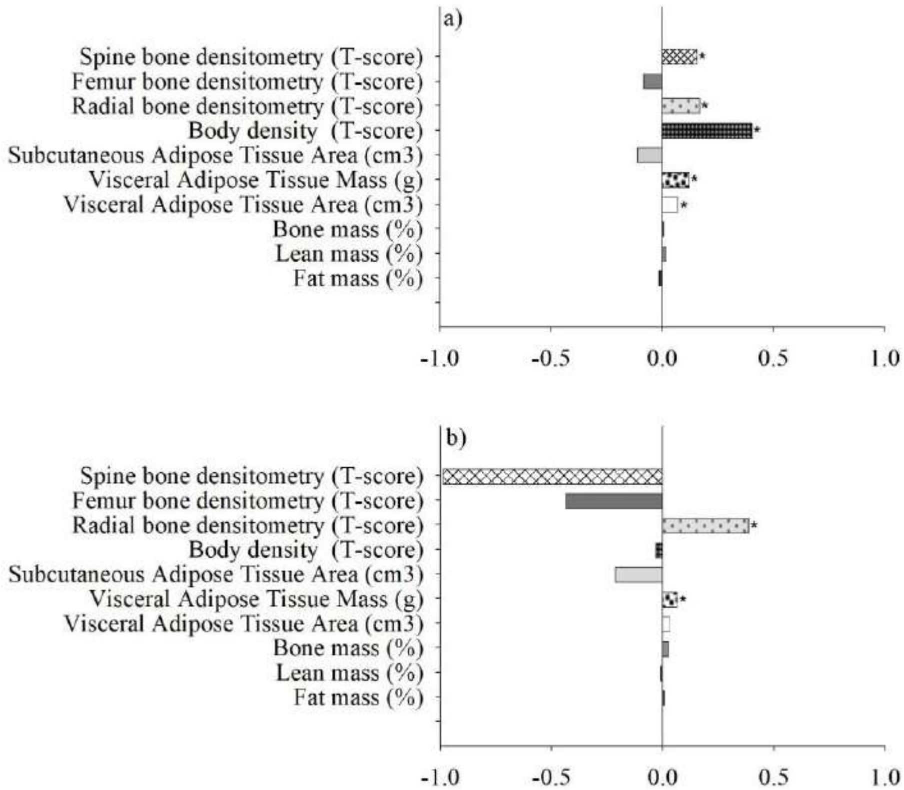

Figure 3. Fold Change analysis in anthropometric parameters and bone densitometry in women (a) and men (b). The bars represent the magnitude of change in each variable between comparative groups. The asterisk (*) indicates the variables with the greatest positive changes.
Figura 3. Análisis del cambio relativo en los parámetros antropométricos y la densitometría ósea en mujeres (a) y hombres (b). Las barras representan la magnitud del cambio en cada variable entre los grupos comparativos. El asterisco (*) indica las variables con los mayores cambios positivos.

4. Conclusions

The combination of functional ingredients enabled the development of a powdered supplement with favorable physical properties, including a particle size of 600 μm that ensured stability, solubility, and fluidity. Its nutritional composition provided sustained energy intake of 366.03 kcal/100 g, with 29.53 g/100 g of carbohydrates, 1.15 g/100 g of lipids, and a high protein content of 58.59 g/100 g, accompanied by an optimal glycemic index of 11.18 and a glycemic load of 5.59. The phytochemical profile revealed a polyphenol concentration of 6608.57 μg/100 g, with hydroxycinnamic acids (2597.12 μg/100 g), flavonols (1606.77 μg/100 g), and flavanols (1526.47 μg/100 g) as the most abundant subfamilies.

Consumption improved bone mineral density in both sexes. In women, radial bone density increased from −1.81 to −1.50, while in men improvements were observed both in radial (−1.90 to −1.45) and femoral regions (−0.07 to 0.17). Supplement intake also promoted body recomposition, as evidenced by a reduction in visceral fat, with values in women decreasing from 656.04 g to 603.07 g, and in men from 1310.59 g to 1253.13 g.

Overall, these findings may suggest the potential of the supplement with implications for population health, particularly in supporting bone health and reducing visceral adiposity. Its innovative formulation and synergistic effects highlight its relevance for nutritional interventions and future research.

Contributions of the authors

Conceptualization, J.A.G.I. and K.M.H.R.; methodology, D.D.J.S.; software, K.M.H.R., J.A.G.I., N.E.R.G.; validation, K.M.H.R., J.A.G.I., N.E.R.G., M.R.M.J., and S.M.G.H.; formal analysis, J.A.G.I. and K.M.H.R.; research, D.D.J.S. and J.N.D.G.; resources, J.A.G.I.; data preservation, K.M.H.R. and J.A.G.I.; writing-drafting of the original manuscript, K.H.M.R., J.A.G.I., and N.E.R.G.; writing-revision and editing, K.M.H.R., J.A.G.I, N.E.R.G, M.R.M.J. and S.M.G.H; visualization, K.M.H.R, J.A.G.I and D.D.J.S; supervision, J.A.G.I.; project management, K.M.H.R., J.A.G.I, N.E.R.G, M.R.M.J., and S.M.G.H.; funding acquisition, J.A.G.I, N.E.R.G, M.R.M.J., S.M.G.H and K.M.H.R. All authors have read and accepted the published version of the manuscript.

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Conflict of interest

The authors declare that they have no conflict of interest related to this research.

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