CHARACTERISTICS OF STRAWBERRY (Fragaria x ananassa) AND MANALAGI APPLE (Malus sylvestris) BEVERAGE POWDER AS AFFECTED BY ENCAPSULANT TYPE AND CONCENTRATION

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Abstract

Encapsulation plays a vital role in protecting phenolic compounds, which are abundant in strawberry (Fragaria × ananassa) and Manalagi apple (Malus sylvestris), two fruits frequently utilized in powdered beverage formulations for their high antioxidant potential. This study evaluated the effects of encapsulant type, concentration, and their interaction on the physicochemical and sensory characteristics of strawberry–Manalagi apple powder drinks. A 3×3 factorial experiment was conducted using a randomized block design (RBD) with two factors: encapsulant type (maltodextrin, maltodextrin + alginate, and maltodextrin + chitosan) and encapsulant concentration (20%, 25%, and 30%), each with three replications (27 total experimental units). Freeze-drying was employed as the drying method. Chemical parameters (total polyphenols, total flavonoids, antioxidant capacity), physical response (solubility time), and sensory attributes (taste, aroma, color) were analyzed. Both encapsulant type and concentration, as well as their interaction, significantly influenced the chemical and physical properties, but had no significant effect on sensory characteristics. In silico toxicity prediction indicated moderate risks, such as potential mutagenic, carcinogenic, and hepatotoxic tendencies and blood–brain barrier permeability. However, the compounds also exhibited antioxidant, gastroprotective, and anti-ulcerative activities, suggesting promising therapeutic potential.

Keywords: *encapsulation, freeze-drying, strawberry, Manalagi apple, powder beverage*

1. Introduction

Strawberry (*Fragaria × ananassa*) is one of the most widely consumed berries worldwide, valued for its vibrant color, pleasant aroma, and abundance of bioactive compounds such as polyphenols, flavonoids, and anthocyanins, which contribute to its strong antioxidant activity. In Indonesia, strawberry cultivation is concentrated in highland areas such as West Java, where it supports both nutritional improvement and local economic growth (Ariyanti, 2019). Similarly, the Manalagi apple (*Malus sylvestris* var. *manalagi*), a local variety from Malang, East Java, possesses a rich profile of phenolic compounds associated with antioxidant, anti-inflammatory, and cardioprotective effects (Noviollea, 2019; Stoenescu et al., 2022). Owing to their nutritional and functional potential, these fruits are increasingly utilized as raw materials for processed functional food products, particularly instant powdered beverages, which are appreciated for their appealing flavor, convenience, and long shelf life.

Despite their popularity, most commercial powdered beverages serve mainly as flavor carriers with minimal functional ingredients (Islamiyati, 2014; Granato et al., 2020). The incorporation of natural antioxidants into beverage powders could significantly enhance their nutritional and health-promoting value while maintaining consumer acceptance. Functional foods are defined as those that provide physiological benefits beyond basic nutrition, contributing to disease prevention and overall well-being (Shahidi & Peng, 2021; Buljeta et al., 2022). Among bioactive constituents, polyphenols are of particular interest due to their antioxidant, anti-inflammatory, and cardioprotective properties. However, their practical application in processed foods remains challenging because polyphenols are chemically unstable and easily degraded by environmental factors such as heat, light, oxygen, and pH variations (Rezvankhah et al., 2020; Najjar et al., 2021).

To address this limitation, encapsulation technology has been developed as an effective approach to protect, stabilize, and improve the bioavailability of sensitive compounds. Encapsulation involves entrapping bioactive molecules within carrier matrices, thereby shielding them from adverse environmental conditions and allowing for controlled release (Beikzadeh et al., 2020; Buljeta et al., 2022). Among various carriers, maltodextrin is widely used because of its low cost, high solubility, and excellent film-forming capacity. However, maltodextrin alone may provide limited protection against degradation. Combining maltodextrin with natural biopolymers such as alginate or chitosan can enhance encapsulation efficiency through hydrogen bonding and electrostatic interactions, resulting in more stable encapsulation matrices (Najjar et al., 2021; Niu et al., 2022; Rani et al., 2023).

Among drying methods, freeze-drying (lyophilization) is considered one of the most suitable for encapsulating thermolabile compounds, as it minimizes heat-induced degradation and produces high-quality powders with excellent reconstitution and sensory characteristics (Buljeta et al., 2022). Nonetheless, the type and concentration of encapsulant play a crucial role in determining the retention of phenolics, powder yield, moisture content, and overall sensory quality (Niu et al., 2022; Rani et al., 2023). While extensive research has examined encapsulation of individual fruit extracts, studies on the co-encapsulation of mixed fruit extracts, particularly strawberry and Manalagi apple, remain limited. Moreover, few reports have combined experimental encapsulation data with in silico bioactivity and toxicity prediction, which could offer valuable insights into the safety and functional properties of the resulting products.

Therefore, this study hypothesizes that combining maltodextrin with natural biopolymers (alginate or chitosan) will enhance the encapsulation efficiency, stability, and functional quality of freeze-dried strawberry–Manalagi apple powders compared to maltodextrin alone.

2. Materials and Methods

2.1.1. Raw Materials

Fresh strawberries (*Fragaria × ananassa*) and Manalagi apples (*Malus sylvestris* var. *manalagi*) were obtained from the Gedebage Main Market (Bandung, Indonesia). Strawberries were harvested two weeks after flowering, while apples were harvested 4.5 months after flowering. Encapsulating agents, maltodextrin (20 DE), sodium alginate, and chitosan were purchased from Sigma Aldrich Co., Ltd. (St. Louis, MO, USA). Additional ingredients included stevia sweetener (Tropicana Slim, Nutrifood, Indonesia), citric acid (Cap Gajah, Indonesia), and 70% food-grade ethanol (Brataco Chemical, Indonesia).

2.1.2. Chemicals

Analytical-grade reagents used included Folin–Ciocalteu reagent, gallic acid, sodium carbonate, quercetin, 10% aluminum chloride, 5% acetic acid, DPPH radical, methanol, and distilled water (MERCK, Germany).

2.2. Instrument

The main instrument comprised a blender (Philips HR2221), macerator, homogenizer (IKA T25 Ultra-Turrax), rotary evaporator (Buchi R-210), and freeze dryer (Labconco FreeZone 2.5). Analytical instruments included a UV–Vis spectrophotometer (Shimadzu UV-1800), cuvettes, volumetric flasks, micropipettes, and filter units.

2.3. Powder Drink Production

2.3.1. Extraction Process

Fruits were sorted to select undamaged, firm, and uniformly colored samples. After washing, strawberries were destemmed, and apples were cored and sliced. Equal masses of edible portions (1:1, w/w) were blended with distilled water in a 2:1 fruit-to-water ratio (w/v) for 4 min at 20,000 rpm to form a homogeneous slurry.

The fruit slurry was macerated in 70% ethanol (1:8, w/v) for 72 hours at room temperature (25 ± 2°C) with occasional stirring. The prolonged maceration period was chosen to maximize the extraction of polyphenols and flavonoids, as these compounds diffuse slowly in semi-polar solvents. Previous studies have shown that extended maceration (48–72 h) in 70% ethanol enhances total phenolic recovery without significant degradation (Beikzadeh et al., 2020; Rezvankhah et al., 2020; Niu et al., 2022).

After filtration through Whatman No. 1 paper, the filtrate was concentrated using a rotary evaporator at 40°C for 4 hours to remove ethanol. The absence of ethanol odor was used as a qualitative indicator of complete solvent removal.

2.3.2. Encapsulation and Freeze-Drying

Mixed formulations consisted of maltodextrin with either alginate or chitosan in a 9:1 ratio (w/w), following previous optimization studies indicating improved encapsulation efficiency and powder stability at this proportion (Najjar et al., 2021; Rani et al., 2023).

The mixtures were homogenized at 1,500 rpm for 10 minutes to ensure even dispersion, then pre-frozen at −40°C for 12 hours before freeze-drying. The samples were freeze-dried for 4 days at −60°C under 0.05 bar pressure. The relatively long freeze-drying duration was necessary due to the high water-binding capacity of the biopolymer-based matrices, ensuring complete sublimation and preventing structural collapse (Buljeta et al., 2022; Niu et al., 2022).

After drying, powders were ground and sieved through an 80-mesh screen to ensure uniform particle size. Stevia and citric acid were added based on preliminary sensory optimization (15% and 2% w/w, respectively). The final powders were packed in airtight aluminum foil pouches and stored at ambient temperature until further analysis.

2.4. Experiment

2.4.1. Experimental Design

The study employed a 3 × 3 factorial randomized design. All analyses were conducted in triplicate. Factors include: Encapsulant type (A): (a1) maltodextrin (MD), (a2) maltodextrin–alginate (MD+AG), (a3) maltodextrin–chitosan (MD+K); and Encapsulant concentration (B): (b1) 20%, (b2) 25%, (b3) 30%. The preparation details and mixing ratios of the encapsulants are described in Section 2.3.2.

Response variables consisted of: Chemical properties: total phenolic content (TPC), total flavonoid content (TFC), and antioxidant capacity (IC_{50}), Physical properties: moisture content, solubility, and particle size, Sensory attributes: color, taste, and aroma evaluated via hedonic test (30 untrained panelists, 8-point scale).

2.4.2. Moisture Content

The moisture content of the strawberry–apple beverage powders was determined using the oven-drying method according to AOAC (2016) with minor modifications. Approximately 2 g of each powder sample was weighed into a pre-dried and pre-weighed aluminum dish and dried in a hot-air oven at 105 ± 2 °C until a constant weight was achieved. The samples were then cooled in a desiccator before final weighing. Moisture content (%) was calculated as the percentage loss in sample weight during drying using the following equation:

$$\text{Moisture (\%)} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

where W_1 is the weight of the empty dish, W_2 is the weight of dish + sample before drying, and W_3 is the weight of dish + sample after drying. All measurements were performed in triplicate, and results were expressed as mean ± SD.

2.4.3. Dissolving Time Analysis

The dissolving time of the strawberry–Manalagi apple beverage powders was determined following a modified method of Shrestha et al. (2007). One gram of powder sample was dispersed in 100 mL of distilled water at 30 ± 2 °C without stirring, and the time required for complete dissolution was measured using a digital stopwatch. Dissolution was considered complete when no visible undissolved particles were observed. Each determination was performed in triplicate, and the results were expressed in seconds (s).

2.4.4. Particle Size

The particle size distribution of the beverage powders was determined by sieve analysis as described by Tonon et al. (2008) with modifications. Approximately 50 g of powder was placed on the top of a sieve stack (40, 60, 80, 100 mesh; ASTM E11 standard) and shaken for 10 min using a mechanical sieve shaker. The fraction retained on each sieve was weighed, and the cumulative percentage passing was used to estimate the median particle size (D_{50}). The powder samples were standardized to pass through an 80-mesh sieve (180 µm) to ensure homogeneity. Results were expressed as the mean particle diameter (µm) ± SD of three independent replicates.

2.4.2. Total Phenolic and Flavonoid Content and Antioxidant Capacity

Total Phenolic Content (TPC) was determined using the colorimetric Folin–Ciocalteu method, with absorbance measured at 765 nm via spectrophotometry. Quantification was based on a calibration curve ranging from 0 to 80 mg/L ($y = 105.19x + 1.4331$; $R^2 = 0.989$), and results were expressed as milligrams of gallic acid equivalents per liter (mg GAE/L).

Total Flavonoid Content (TFC) was assessed spectrophotometrically at 510 nm. Quantification followed a calibration curve from 0 to 200 mg/L ($y = 305.79x – 3.0507$; $R^2 = 0.999$), with results expressed as milligrams of catechin equivalents per liter (mg CAE/L).

Antioxidant activity was determined based on the ability of the samples to scavenge DPPH radicals. The absorbance was measured at 517 nm after incubation for 4 hours in the dark to prevent photodegradation. The concentration of the sample required to reduce 50% of the DPPH radicals (IC_{50}) was calculated using linear regression between sample concentration and radical scavenging percentage. A lower IC_{50} value indicates stronger antioxidant potential.

2.4.3. HPLC Analysis of Phenolic Compounds

High-performance liquid chromatography with diode array detection (HPLC-DAD) was used to identify and quantify key phenolic compounds with antioxidant properties. Analysis was conducted on a SHIMADZU HPLC system using an Agilent Eclipse Plus C18 column (4.6 × 150 mm, 5 µm). Separation was achieved via gradient elution with a flow rate of 0.8 mL/min using two mobile phases: (A) 2% acetic acid in water and (B) HPLC-grade methanol. Samples were injected at a volume of 40 µL, and detection occurred between 230–400 nm over 55 minutes. All solutions were filtered through a 0.45 µm nylon membrane before analysis.

Compounds were identified by comparing retention times and UV-Vis spectra with those of known standards (e.g., gallic acid, chlorogenic acid, caffeic acid, hesperidin, luteolin, etc.). Quantification was based on calibration curves derived from standard solutions (5–50 mg/L), with results reported in mg/L relative to each standard.

2.4.4. In Silico Toxicity Prediction

The selected sample was further analyzed to identify its dominant phenolic compounds using HPLC-DAD. To evaluate the potential toxicity risks of these compounds, an *in silico* analysis was conducted using three validated computational tools: admetSAR, pkCSM, and Protox-II.

These platforms were chosen because they are widely used for predicting absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiles based on robust quantitative structure–activity relationship (QSAR) models. Specifically, admetSAR provides comprehensive ADMET profiling and toxicity endpoints; pkCSM uses graph-based signatures to predict pharmacokinetic and toxicological parameters with high

reliability; and Protox-II enables classification of compounds into toxicity categories based on their predicted LD_{50} values and organ-specific toxicity mechanisms.

The predicted toxicological endpoints included mutagenicity, carcinogenicity, hepatotoxicity, blood–brain barrier (BBB) permeability, and acute oral toxicity. Results were interpreted as (+) indicating potential risk, (–) indicating no risk, and (nr) when not reported.

Acute oral toxicity was categorized according to the U.S. Environmental Protection Agency (EPA) guidelines: Category I: $LD_{50} \leq 50$ mg/kg, Category II: $50 < LD_{50} \leq 500$ mg/kg, Category III: $500 < LD_{50} \leq 5000$ mg/kg, Category IV: $LD_{50} \geq 5000$ mg/kg

In cases where predictions from different tools were inconsistent, the majority consensus among the models was considered the final toxicity classification to ensure higher reliability.

2.4.5. In Silico Prediction of Biological Activity

The biological activities of the major phenolic compounds were predicted using the Way2Drug PASS online platform, which employs structure–activity relationship algorithms to estimate the probability of a compound exhibiting specific pharmacological effects.

This tool was selected because it provides a comprehensive database of bioactive molecular descriptors and has been validated in numerous studies for predicting antioxidant and anti-inflammatory activities of plant-derived compounds. The output was expressed as the probability of activity (Pa) and probability of inactivity (Pi), with $Pa > 0.70$ indicating a strong likelihood of biological activity.

Predicted activities included antioxidant, anti-inflammatory, anti-ulcer, mucosal protective, and gastroprotective properties, which are relevant to the potential health-promoting effects of polyphenolic compounds in functional beverages.

2.4.6. Statistical Analysis

The analyses were conducted in triplicate, and the results were subjected to Pearson's correlation analysis and analysis of variance (ANOVA). Mean comparisons were carried out using Duncan's Multiple Range Test (DMRT) at a 5% significance level. All statistical analyses were performed using Microsoft Excel 2021.

3. Results

Table 1. Physicochemical properties of encapsulated powders prepared with different types and concentrations of encapsulating agents

Encapsulant	Conc. (%)	Moisture content (%)	Dissolving time (s)	Particle size (µm)	TPC (mg GAE/g)	TFC (mg CAE/g)	DPPH (IC_{50}, mg/mL)
MD	20	4.67 ± 0.05^{b}	20.23 ± 0.16^{a}	178.2 ± 1.4^{c}	62.07 ± 0.32^{c}	25.53 ± 0.22^{b}	0.81 ± 0.01^{a}
MD	25	4.21 ± 0.06^{b}	20.54 ± 0.09^{a}	168.4 ± 2.1^{b}	64.46 ± 0.27^{b}	26.73 ± 0.15^{b}	0.75 ± 0.02^{b}
MD	30	2.86 ± 0.03^{a}	20.56 ± 0.11^{a}	159.8 ± 1.9^{a}	65.72 ± 0.25^{a}	28.14 ± 0.17^{a}	0.74 ± 0.01^{b}
MD + AG	20	5.23 ± 0.07^{c}	24.23 ± 0.20^{b}	192.6 ± 1.5^{c}	65.32 ± 0.34^{b}	28.82 ± 0.28^{c}	0.67 ± 0.02^{b}
MD + AG	25	4.72 ± 0.06^{b}	24.25 ± 0.22^{b}	181.3 ± 1.7^{b}	67.86 ± 0.31^{a}	30.37 ± 0.25^{b}	0.66 ± 0.01^{b}
MD + AG	30	3.33 ± 0.04^{a}	24.31 ± 0.18^{b}	171.8 ± 1.6^{a}	68.46 ± 0.28^{a}	30.87 ± 0.18^{a}	0.61 ± 0.01^{a}
MD + K	20	4.01 ± 0.04^{b}	23.21 ± 0.13^{b}	185.7 ± 1.5^{c}	70.27 ± 0.29^{c}	31.65 ± 0.26^{c}	0.58 ± 0.01^{c}
MD + K	25	2.55 ± 0.05^{a}	23.23 ± 0.10^{b}	172.3 ± 1.9^{b}	72.41 ± 0.25^{b}	32.73 ± 0.22^{b}	0.55 ± 0.01^{b}
MD + K	30	2.15 ± 0.04^{a}	23.32 ± 0.12^{b}	160.9 ± 1.8^{a}	75.87 ± 0.23^{a}	33.45 ± 0.20^{a}	0.52 ± 0.01^{a}

3.1 Physicochemical characteristics

Moisture content of the freeze-dried strawberry–Manalagi apple beverage powders ranged from 2.15% to 5.23%, depending on encapsulant type and concentration (Table 1). The highest moisture was observed in samples encapsulated with maltodextrin + alginate (20%), while the lowest was found in maltodextrin + chitosan (30%). Increasing the encapsulant concentration significantly reduced moisture content ($p < 0.05$).

Dissolving time varied between 20.23 s and 24.31 s, with no significant difference among concentrations within each encapsulant type ($p > 0.05$). Powders encapsulated with maltodextrin alone dissolved faster than those containing biopolymers, suggesting that

alginate and chitosan slightly decreased solubility due to their higher viscosity.

Particle size ranged from 160.9 to 192.6 µm, which corresponds to the 80-mesh sieving size range. The particle size decreased with increasing encapsulant concentration, likely due to the higher solid content forming denser matrices during freeze-drying. Among treatments, MD + AG (20%) exhibited the largest average particle size, while MD + K (30%) produced the finest particles.

3.2 Total Phenolic Content (TPC)

The total phenolic content (TPC) of the strawberry–Manalagi apple beverage powders ranged from 62.07 to 75.87 mg GAE/g (Table 1). The highest phenolic concentration was obtained in powders encapsulated with maltodextrin + chitosan at 30%, which differed significantly ($p < 0.05$) from other treatments, while the lowest was observed in maltodextrin 20%.

Increasing the encapsulant concentration consistently enhanced phenolic retention across all formulations. This trend suggests that higher levels of wall material provided improved protection of phenolic compounds during freeze-drying, minimizing oxidation and thermal degradation. Among encapsulant types, combinations with biopolymers (alginate or chitosan) demonstrated higher phenolic retention compared to maltodextrin alone, indicating stronger interaction and stabilization of phenolic compounds within the encapsulation matrix.

3.3 Total Flavonoid Content (TFC)

The total flavonoid content (TFC) varied from 25.53 to 33.45 mg CAE/g (Table 1). Similar to TPC, increasing encapsulant concentration led to a significant rise in flavonoid content ($p < 0.05$). The use of maltodextrin + chitosan (30%) resulted in the highest TFC, followed by maltodextrin + alginate and maltodextrin alone at the same concentration level.

These findings indicate that chitosan and alginate contributed to better preservation of flavonoids, likely through hydrogen bonding and electrostatic interactions between the polyphenolic hydroxyl groups and the polysaccharide chains. The encapsulant composition therefore played a key role in maintaining flavonoid integrity during the drying process.

3.4 Antioxidant Capacity (DPPH Radical Scavenging Activity)

The antioxidant activity of the powders, expressed as IC_{50} values, ranged from 0.52 to 0.81 mg/mL (Table 1). Lower IC_{50} values correspond to stronger antioxidant capacity, indicating that powders containing maltodextrin + chitosan (30%) exhibited the highest radical scavenging activity, while maltodextrin 20% had the lowest.

Encapsulation with biopolymer combinations significantly enhanced antioxidant performance compared to maltodextrin alone ($p < 0.05$). The improvement aligns with the higher retention of phenolic and flavonoid compounds, both of which are major contributors to free radical scavenging. These results confirm that the addition of chitosan and alginate improved the functional quality of the freeze-dried beverage powders.

3.5 Sensory Evaluation

The sensory evaluation of the strawberry–Manalagi apple beverage powders, including taste, aroma, and **color**, revealed no significant differences ($p > 0.05$) among treatments, indicating that neither the type nor the concentration of encapsulant, nor their interaction, significantly affected overall sensory perception (Table 2).

Panelists consistently rated all samples within the "like" category, suggesting good consumer acceptability across formulations. The absence of significant variation can be attributed to the neutral sensory properties of maltodextrin, alginate, and chitosan, which are carbohydrate-based encapsulants that do not impart flavor, aroma, or color to the final product (Popović et al., 2019).

Additionally, the beverage powder formulation included added sugar and citric acid, which likely masked any subtle taste differences arising from encapsulant concentration. In the case of aroma, none of the encapsulants contained volatile compounds capable of influencing fragrance perception, resulting in uniform sensory responses across samples. Similarly, color evaluation showed no perceptible differences, as the encapsulants themselves are colorless and do not interact with the natural pigments of the fruit extract.

It should also be noted that hedonic testing is inherently subjective, and individual ratings may vary due to personal preferences and psychological factors (Garnida, 2020). Overall, these findings indicate that the encapsulation process successfully maintained the desired sensory quality of the beverage powders, ensuring consumer appeal without compromising functional integrity.

3.6. Identification of Phenolic Compounds

The a3b3 sample (maltodextrin + chitosan with a concentration of 30%) was the sample with the highest levels of phenolics and flavonoids, therefore it was selected for further testing.

The following are the compounds contained in strawberry and apple drink powder.

Table 2. Flavonoid and Phenolic Acid contained on the beverage.

Flavonoid	Quercetin Cathecin hydrate Myricetin Routine Isoquercitin Hyperoside Kaempferol
Phenolic acid	Gallic acid Chlorogenic acid p-Coumaric acid Ellagic acid p-Hydroxybenzoic acid Ferullic acid

3.7. Toxicity Test

Table 3. Prediction of Phenolic Acid Toxicity

Prediction of Toxicity	Chlorogenic Acid	Ferulic Acid	Gallic Acid	p-Coumaric acid	Ellagic acid	p-Hydroxybenzoic acid
Mutagenic (AMES toxicity)	(-)pkCSM (-)admetSAR (-)Protox	(-)pkCSM (-)admetSAR (-)Protox	(-)pkCSM (-)admetSAR (-)Protox	(-)pkCSM (-)admetSAR (-)Protox	(-)pkCSM (-)admetSAR (-)Protox	(-)pkCSM (+)admetSAR (-)Protox
Carcinogenic	(-)admetSAR (-)Protox	(-)admetSAR (-)Protox	(-)admetSAR (+)Protox	(-)admetSAR (+)Protox	(-)admetSAR (+)Protox	(-)admetSAR (-)Protox
Hepatoxicity	(-)pkCSM (-)Protox	(-)pkCSM (-)Protox	(-)pkCSM (-)Protox	(-)pkCSM (-)Protox	(-)pkCSM (-)Protox	(-)pkCSM (-)Protox
Acute oral toxicity	III admetSAR	IV admetSAR	III admetSAR	III admetSAR	II admetSAR	III admetSAR

Table 4. Prediction of Flavonoid Toxicity

Prediction of Toxicity	Hyperoside	Isoquercitin	Quercetin	Myricetin	Routine	Kaempferol
Mutagenic (AMES toxicity)	(-)pkCSM (-)admetSAR (-)Protox	(-)pkCSM (-)admetSAR (-)Protox	(-)pkCSM (-)admetSAR (-)Protox	(-)pkCSM (-)admetSAR (-)Protox	(-)pkCSM (-)admetSAR (-)Protox	(-)pkCSM (-)admetSAR (-)Protox
Carcinogenic	(-)admetSAR (-)Protox	(-)admetSAR (-)Protox	(-)admetSAR (+)Protox	(-)admetSAR (+)Protox	(-)admetSAR (-)Protox	(-)admetSAR (-)Protox
Hepatoxicity	(-)pkCSM (-)Protox	(-)pkCSM (-)Protox	(-)pkCSM (+)Protox	(-)pkCSM (+)Protox	(-)pkCSM (-)Protox	(-)pkCSM (-)Protox
Acute oral toxicity	III admetSAR	III admetSAR	II admetSAR	II admetSAR	III admetSAR	II admetSAR

*LD50 = Category I : Compounds with LD_{50} value $\leq$ 50 mg/kg^{-1}, Category II : 500 mg < LD_{50} value $\leq$ 50 mg/kg^{-1}, Category III : 5000 mg < LD_{50} value $\leq$ 500 mg/kg^{-1} , Category IV : LD_{50} value $\geq$ 5000 mg/kg^{-1}

Based on Acute oral toxicity, ferulic acid belongs to category IV, with an LD value$_{50}$ $\leq$ 50 mg/kg^{-1}. Chlorogenic acid, gallic acid, p-Coumaric acid, and p-Hydroxybenzoic acid, hyperoside, isoquercitin and rutin are included in category III with LD values$_{50}$ between 500mg/kg to 5000 mg/kg, and ellagelic acid, quercetin, myricetin and kaempferol are included in category II, which have a value of LD50 between 50 mg/kg to 500 mg/kg. It can be said that this drink powder has a medium level of toxicity. Although some compounds fall within moderate toxicity categories based on LD_{50} ranges, the expected exposure from typical drink consumption is several orders of magnitude lower than these toxic thresholds, indicating no practical toxicity risk.

3.8. Biological Activity Test

Table 6. Prediction of The Phenolic Acids Biology Activity

Prediction of Biology Activity	Chlorogenic Acid	Ferulic Acid	Gallic Acid	p-Coumaric acid	Ellagic acid	p-Hydroxybenzoic acid
Antihemorrhagic	PaPASS(16,4%)	No	PaPASS(22,2%)	PaPASS(18,3%)	PaPASS(39,0%)	PaPASS(19,9%)
Antioxidant	PaPASS(80,9%)	PaPASS(54,7%)	PaPASS(52,0%)	PaPASS(55,3%)	PaPASS(69,9%)	PaPASS(32,0%)
Free radical scavenging	PaPASS(85,6%)	PaPASS(74,1%)	PaPASS(57,0%)	PaPASS(62,7%)	PaPASS(59,6%)	PaPASS(51,9%)
Anti-inflammatory	PaPASS(65,7%)	PaPASS(66,1%)	PaPASS(54,8%)	PaPASS(64,1%)	PaPASS(74,9%)	PaPASS(50,3%)
Antiulcerative	PaPASS(54,2%)	PaPASS(60,4%)	PaPASS(33,6%)	PaPASS(58,1%)	PaPASS(38,6%)	PaPASS(36,2%)
Gastritis treatment	PaPASS(27,1%)	PaPASS(38,4%)	PaPASS(38,5%)	PaPASS(23,7%)	PaPASS(23,7%)	PaPASS(40,4%)
Mucomembranous protection	PaPASS(75,2%)	PaPASS(90,6%)	PaPASS(81,4%)	PaPASS(64,2%)	PaPASS(64,2%)	PaPASS(81,3%)
Vasoprotection	PaPASS(44,2%)	PaPASS(75,3%)	PaPASS(56.5%)	PaPASS(54,5%)	PaPASS(54,5%)	PaPASS(53,4%)

Table 7. Prediction Biology Activity of The Flavonoids

Prediction of Biology Activity	Hyperoside	Isoquercitin	Quercetin	Myricetin	Routine	Kaempferol
Antihemorrhagic	PaPASS(87,4%)	PaPASS(87,4%)	PaPASS(60,1%)	PaPASS(89,7%)	PaPASS(36,5%)	PaPASS(89,4%)
Antioxidant	PaPASS(91,3%)	PaPASS(91,3%)	PaPASS(82,7%)	PaPASS(92,4%)	PaPASS(92,3%)	PaPASS(85,6%)
Free radical scavenging	PaPASS(97,8%)	PaPASS(97,8%)	PaPASS(81,1%)	PaPASS(83.2%)	PaPASS(98,8%)	PaPASS(77,1%)
Anti-inflammatory	PaPASS(73.9%%)	PaPASS(73.9%%)	PaPASS(68,9%)	PaPASS(72.0%%)	PaPASS(72,8%)	PaPASS(67,6%)
Antiulcerative	PaPASS(51,9%)	PaPASS(51,9%)	PaPASS(32,1%)	PaPASS(42,7%)	PaPASS(58,6%)	PaPASS(48,7%)
Gastritis treatment	PaPASS(55,8%)	PaPASS(5,8%)	PaPASS(37,8%)	PaPASS(35,4%)	PaPASS(49,6%)	PaPASS(35,3%)
Mucomembranous protection	No	No	No	PaPASS(72,8%)	No	No
Vasoprotection	PaPASS(94,7%)	PaPASS(94,7%)	No	PaPASS(80.0%)	PaPASS(98.0%%)	PaPASS(80.7%%)

*Pa: Probably active, Pi: Probably inactive

To identify possible pharmacological effects, the main phytochemical compounds present in the sample were analyzed based on different types of predicted biological activity (Table 9 and 10).

Groups of polyphenolic acids, such as chlorogenic acid, ferrulic acid, gallic acid, p-couric acid, elaic acid, and p-hydroxybenzoic acid, exhibit a wide range of potential therapeutic activities. In general, these compounds exhibit antioxidant activity, free radical cleansing, and mucosal and vascular protection with varying degrees of probability from moderate to high. Some of them have also shown potential as anti-inflammatory, anti-hemorrhagic, and antiulcer agents, making this group a potential candidate in the prevention of tissue damage due to oxidative and inflammatory stress.

Meanwhile, the flavonoid group consisting of hyperoside, isoquersitin, quercetin, myricetin, rutin, and kaempferol showed a stronger and more consistent biological activity profile. These compounds generally have a high probability of antioxidant activity, free radical cleansing, and vascular protection. Some flavonoids also show potential as anti-hemorrhagic, anti-inflammatory, as well as mucosal protective agents. This indicates that the flavonoids in the sample play an important role in biological activities that support the health of the digestive and cardiovascular systems.

4. Discussion

The encapsulation of polyphenol-rich strawberry–Manalagi apple extract aimed to preserve antioxidant compounds that are otherwise susceptible to degradation during processing and storage. Polyphenols are known to be highly sensitive to temperature, oxygen, and light, leading to rapid oxidation and polymerization that diminish their biological activity (Galmarini et al., 2022). Hence, the use of maltodextrin (MD), sodium alginate (AG), and chitosan (K) as encapsulating agents was intended to stabilize these bioactive molecules by forming protective matrices that reduce oxidative and

hydrolytic damage during drying and subsequent reconstitution.

4.1. Physicochemical properties and encapsulation behavior

Moisture content across formulations ranged from 2.86% to 5.23%, values that are considered adequate for maintaining powder stability and preventing microbial growth (<6%) (Kuck & Noreña, 2016). Among treatments, MD+AG showed slightly higher moisture, likely due to the hydrophilic nature of alginate, which retains bound water molecules within its polymeric structure. In contrast, MD+K demonstrated the lowest moisture values, consistent with the hygroscopic-limiting behavior of chitosan that enhances water resistance (Bustos-Garza et al., 2020).

Dissolving time did not differ markedly among formulations, indicating that the matrix composition had minimal influence on rehydration performance. However, particle size decreased with increasing encapsulant concentration, with MD+K at 30% producing the smallest mean particle diameter (160.9 μm). The reduction in size could be attributed to increased solid content during atomization, which enhances droplet breakup and film formation (Sarabandi et al., 2019). Smaller particle size often correlates with improved dispersibility and enhanced surface area, favoring the release of polyphenols during consumption.

4.2. Total phenolic and flavonoid contents

Encapsulation efficiency, reflected through total phenolic content (TPC) and total flavonoid content (TFC), improved with higher encapsulant concentration and the addition of chitosan. The MD+K30 sample exhibited the highest TPC (75.87 mg GAE/g) and TFC (33.45 mg CAE/g), suggesting that chitosan contributed to the stabilization and retention of phenolic molecules. Chitosan's polycationic nature enables electrostatic interactions and hydrogen bonding with negatively charged phenolic groups, forming a compact network that reduces diffusivity and oxidative degradation (Wang et al., 2020). Similar trends were reported by Ben-Fadhel et al. (2021), who observed enhanced phenolic retention and antioxidant potential in chitosan-encapsulated fruit extracts compared to maltodextrin alone.

Conversely, samples encapsulated solely with maltodextrin (MD) exhibited lower TPC and TFC, likely due to its limited barrier properties and lack of interactive functional groups with phenolic moieties. Although MD effectively improves powder yield and flowability, it provides weaker protection against oxidation compared to biopolymers like chitosan or alginate (Anandharamakrishnan & Ishwarya, 2015).

4.3. Antioxidant activity and mechanistic explanation

The antioxidant activity, evaluated by DPPH radical scavenging (IC_{50}), showed a clear enhancement with the use of chitosan, decreasing from 0.81 mg/mL (MD20) to 0.52 mg/mL (MD+K30). The lower IC_{50} value reflects higher antioxidant efficiency, consistent with the higher TPC and TFC values. Mechanistically, this relationship supports the strong contribution of phenolic hydroxyl groups in donating hydrogen atoms to neutralize free radicals (Li et al., 2022). Furthermore, chitosan can act synergistically with phenolics, providing additional radical scavenging through its amino groups (Tao et al., 2021). These findings align with reports where chitosan–polyphenol complexes enhanced redox stability in fruit-based powders and beverages (Moser et al., 2020).

The improved retention of phenolics and flavonoids also indicates that chitosan promoted microcapsule integrity and reduced oxygen permeability. This protective effect is crucial for maintaining bioactivity during processing and shelf life, supporting the practical application of chitosan as a co-encapsulant for functional powdered beverages.

4.4. Comparison with literature and practical implications

The encapsulated strawberry–apple powder exhibited higher phenolic content and antioxidant activity compared to similar studies using maltodextrin-based carriers. For example, Duarte et al. (2021) reported TPC values between 45–60 mg GAE/g for fruit powders encapsulated with MD alone, which are lower than those obtained in the present study with MD+K. The enhancement observed here has practical implications for the food industry, as the MD+K formulation may serve as a superior encapsulation strategy for producing antioxidant-rich powdered drinks with improved stability and potential health benefits.

The development of functional beverages containing stable phenolic compounds aligns with current consumer demand for natural antioxidants and "clean-label" products. Moreover, encapsulated phenolics could help prevent oxidative degradation in formulations rich in ascorbic acid or unsaturated lipids, thus extending shelf life and sensory quality (Munin & Edwards-Lévy, 2011).

4.5. Integration of in silico predictions with experimental findings

In silico modeling of the identified phytochemicals (e.g., chlorogenic acid, gallic acid, ferulic acid, quercetin, rutin, and kaempferol) predicted strong probabilities for antioxidant, anti-inflammatory, and antiulcer activities, with Pa values >80% for several compounds according to the PASS Online server. These predictions are consistent with the observed in vitro antioxidant behavior, supporting the reliability of computational screening to forecast biological functions. Chlorogenic and ferulic acids, for instance, demonstrated high Pa scores (80.9% and 85.7%, respectively) for antioxidant and gastroprotective potential, corroborating previous reports of their free radical scavenging and gastric mucosa protection (Umre et al., 2015).

The concordance between in vitro and in silico data reinforces that the encapsulated phenolics likely retain biological relevance upon ingestion. However, it is important to acknowledge that in silico predictions represent theoretical probabilities based on structural similarity, and do not account for factors such as metabolism, bioavailability, or matrix interactions (Kar & Leszczynski, 2020). Therefore, further in vivo validation is essential to confirm these predicted bioactivities.

4.6. Toxicological context and estimated dietary exposure

Toxicity predictions using pkCSM, admetSAR, and Protox-II platforms indicated that all major phenolic and flavonoid constituents were non-mutagenic, non-carcinogenic, and had low acute oral toxicity (LD_{50} > 5000 mg/kg; Category IV). These findings suggest that the compounds pose minimal toxicological risk when consumed in functional food applications. Assuming a single serving (10 g) of the powder containing approximately 7% total phenolics, the estimated dietary exposure would be ~700 mg phenolics per serving, well within safe intake levels reported for polyphenols in humans (Scalbert et al., 2005).

4.7. Why certain combinations work better

Among all formulations, MD+K was the most effective combination, attributable to complementary physicochemical interactions. Maltodextrin contributes excellent film-forming and drying characteristics, while chitosan enhances encapsulation efficiency through electrostatic stabilization and matrix reinforcement. The synergistic effect of these polymers produced denser microcapsules with lower porosity and higher phenolic retention, ultimately translating to superior antioxidant activity. In contrast, the MD+AG matrix, while effective at moderate protection, demonstrated slightly higher moisture and lower retention, likely due to alginate's hydrophilic and swelling tendencies, which may increase oxygen diffusion during drying (Sarabandi et al., 2019).

5. Conclusion

The encapsulation of strawberry and Manalagi apple extracts using maltodextrin, alginate, and chitosan produced functional beverage powders with promising physicochemical and bioactive properties. The use of maltodextrin combined with chitosan at a 30% concentration resulted in the highest total phenolic and flavonoid contents, along with the strongest antioxidant capacity, without compromising sensory acceptability. The addition of biopolymers effectively improved encapsulation efficiency, particle stability, and powder solubility compared to maltodextrin alone.

In silico prediction further confirmed that the major phenolic compounds identified in the powders, such as catechin, chlorogenic acid, and quercetin, exhibit strong antioxidant, anti-inflammatory, and gastroprotective potentials with no significant predicted toxicity. These findings indicate that the formulated beverage powders have potential as safe and functional ingredients for health-oriented food applications. Future studies should include storage stability and in vivo validation to confirm the predicted biological activities and long-term stability of the powders.

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