



Microcapsule Formulation of Pumpkin Seeds (*Cucurbita moschata*) as a Potential Supplement to Support Iron and Zinc Adequacy in Stunting Prevention

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ABSTRACT

Purpose of the study: Iron (Fe) and zinc (Zn) deficiencies significantly contribute to childhood stunting. Pumpkin seeds (*Cucurbita moschata*) are rich in these minerals, but poor palatability and rapid gastric degradation limit their direct use as a food ingredient. This study aimed to microencapsulate pumpkin seed powder with gum arabic via solvent evaporation to protect key micronutrients and enhance targeted mineral delivery.

Methodology: The prepared experimental samples were characterized using PSA, FTIR, AAS, and a binocular microscope. We formulated microcapsules with a high-speed homogenizer using the solvent evaporation technique, and statistically analyzed the data using ANOVA in SPSS.

Main Findings: The synthesized pumpkin seed microcapsules were powder-shaped, yellow, aromatic, and tasteless. Formulas ranging from 1.5:5 to 3.5:5 were the most stable through the sixth cycle. The yield (38.8%–77%), particle size (54.68–60.70 µm), and particle uniformity were optimal at a 4.5:5 ratio, exhibiting round morphological shapes. Mineral concentrations reached 18–101 mg/L for Fe and 11–79 mg/L for Zn, with encapsulation efficiency between 2.81% and 30.01%. FTIR spectra showed characteristic peaks from both raw materials, and microbiological testing confirmed safety under official BPOM standards.

Novelty/Originality of this study: Increasing the active ingredient ratio significantly enhanced mineral uptake without altering the fundamental chemical structure. The primary novelty lies in utilizing pumpkin seed waste encapsulated in gum arabic via solvent evaporation. These stable microcapsules represent a safe, effective, and economical local food fortification agent to address stunting.

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1. INTRODUCTION

Stunting remains a serious national child health crisis in Indonesia. In 2024, approximately 19.8% of children across Indonesia were stunted [1]. This figure represents a decrease from 21.5% the previous year, but it is still far from the government's target of reducing the stunting rate to 14% by 2029. Children with stunting

often experience impaired physical growth and delayed brain development, primarily caused by deficiencies in essential nutrients such as iron (Fe) and zinc (Zn) [2]. Zinc plays a crucial role in cell division, while iron is essential for the formation of red blood cells [3]. On the other hand, poor appetite in children poses a critical challenge to the success of supplementation [4].

One local, food based natural ingredient with high potential for addressing this issue is pumpkin seeds (*Cucurbita moschata*). Pumpkin seeds contain carbohydrates, amino acids, vitamins, and are rich in essential minerals [5], [6]. Based on previous studies [7], [8], pumpkin seeds (*Cucurbita moschata*) contain 1.1532 mg/L of zinc and 157.3674 mg/L of iron, as well as various micro and macronutrients that support growth. The use of pumpkin seeds not only supports daily nutritional needs but also maximizes the economic value of a material that is often discarded as waste, while reducing dependence on synthetic supplements that carry the risk of side effects [9].

However, the direct consumption of pumpkin seeds in the form of raw powder is hindered by the low oxidative and thermal stability of the minerals, as well as their limited bioavailability. To overcome these challenges, microencapsulation technology was applied. Encapsulation is the process of trapping a core material within a polymer coating matrix [10]. This technique has been shown to improve the stability, efficiency, and bioavailability of active nutritional ingredients [11], [12]. A particularly effective matrix is gum arabic, a natural biopolymer capable of forming a strong protective layer to enhance encapsulation efficiency and mineral retention [13]. In its preparation, the solvent evaporation technique is used because the process is simple, time efficient, and flexible for various types of core materials [14], [15].

However, to date there has been no study that comprehensively examines the use of gum arabic via the solvent evaporation method to encapsulate (*Cucurbita moschata*) pumpkin seeds, particularly regarding the effect of varying ratios of active ingredients to polymer on Fe/Zn retention, physicochemical stability, and microbiological safety. The development of this formulation is urgently needed to optimize the retention of Fe and Zn minerals and ensure the long term physicochemical stability of natural ingredient based supplements. As a solution, gum arabic was selected as a natural (food grade) biopolymer coating agent using the solvent evaporation method to produce a microencapsulation system that is stable, precise, and microbiologically safe.

Therefore, this study aims to formulate pumpkin seed (*Cucurbita moschata*) microcapsules using gum arabic as a coating via the solvent evaporation technique, as well as to quantitatively evaluate the effect of varying ratios of active ingredient to polymer on encapsulation efficiency (EE), Fe and Zn content retention, physicochemical characteristics (particle size, morphology, FTIR), physical stability over 6 cycles, and microbiological safety in accordance with BPOM standards to obtain an optimal formulation for a locally sourced food fortification supplement.

2. RESEARCH METHOD

This study employed a laboratory experimental design conducted from December 2025 to May 2026 across three research facilities: the Biomedical Laboratory 4 at Adiwangsa Jambi University, the BRMP Legume Testing Laboratory, and the PT DKSH Laboratory. The research examined the effect of varying active ingredient to polymer ratios (independent variable) on the physicochemical characteristics of microcapsules (dependent variable) while controlling environmental conditions during the manufacturing process. The primary samples consisted of high quality pumpkin seeds (*Cucurbita moschata*) selected via simple random sampling to ensure uniformity, along with microcapsule preparations representing ten active ingredient to polymer ratio variations F1 (0.5:5), F2 (1:5), F3 (1.5:5), F4 (2:5), F5 (2.5:5), F6 (3:5), F7 (3.5:5), F8 (4:5), F9 (4.5:5), and F10 (5:5) which served as the experimental units. These ratios were determined based on preliminary assessments of the polymer matrix's coating capacity, with observations conducted on a single experimental unit per formula as an initial optimization study. The collection of physicochemical data was conducted in a quantitative manner using instruments that had been calibrated and validated in accordance with laboratory operating standards.

The test parameters, along with the instruments and reference standards used in this study, are summarized in Table 1. The preformulation stage began with verifying the suitability of the solvents [16], [17] and ensuring that the instruments were calibrated in accordance with laboratory protocols [18], [19], [20], [21]. Next, the fabrication stage and measurement of product parameters including yield, particle size and distribution, surface morphology, coating efficiency, and iron (Fe) and zinc (Zn) mineral content were conducted sequentially for all ten formula variations. Subsequently, the data obtained from the measurements of yield, particle characteristics, coating efficiency, and mineral content were analyzed using SPSS version 20.0 via ANOVA [22] and presented through descriptive analysis to determine the formulation with the most optimal performance.

Tools used : PSA (Particle Size Analyzer), FTIR (Fourier Transform Infra Red), AAS (Atomic Absorption Spectrophotometry), LAF (Laminar Air Flow), Binocular Microscope, incubator, oven, hotplate, autoclave, chiller, homogenizer, grinder, desiccator, analytical scale, sieve (mesh 80), magnetic stirrer, as well as other glassware. Ingredients used: Yellow pumpkin seeds (*Cucurbita moschata*), Ethanol PA, N-Hexane,

Aquadest, Gum arabic, Filter paper, PCA (Plate Count Agar), PDA (Potato Dextrose Agar), SSA (*Salmonella-Shigella* Agar), NaCl 0.9%.

The procedure for preparing yellow pumpkin seed samples which includes the process of washing, drying, refining, sifting, and storage is presented systematically in Figure 1.

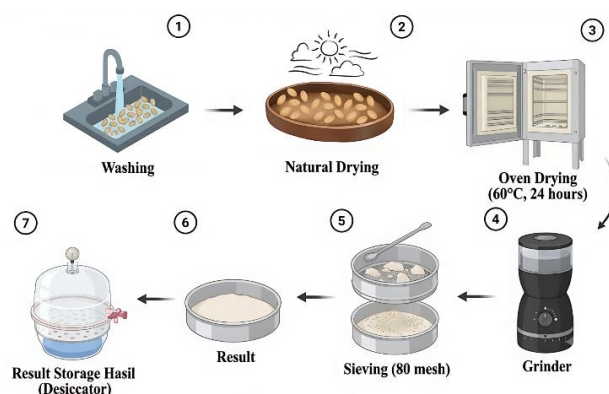


Figure 1. Sample Preparation

The microencapsulation formulations of yellow pumpkin seeds were designed in 10 variations based on the ratio of the weight of the active ingredient powder to the weight of the coating polymer.

Table 1. Yellow Pumpkin Seed Microcapsule Formula

Ratio	Powder Weight (gr)	Polymer Weight (gr)	PA Ethanol Solvent (mL)	Total Sample Weight (gr)
F1 (0.5:5)	4,54	45,46	50	50
F2 (1:5)	8,33	41,67	50	50
F3 (1.5:5)	11,53	38,47	50	50
F4 (2:5)	14,28	35,72	50	50
F5 (2.5:5)	16,67	33,33	50	50
F6 (3:5)	18,75	31,25	50	50
F7 (3.5:5)	20,58	29,42	50	50
F8 (4:5)	22,22	27,78	50	50
F9 (4.5:5)	23,7	26,31	50	50
F10 (5:5)	25	25	50	50

Based on the data in Table 1, the increase in the active ingredient ratio from F1 (0.5:5) to F10 (5:5) is directly proportional to the increase in the weight of the pumpkin seed powder, namely from 4.54 grams to 25 grams. Conversely, Table 1 also shows a proportional decrease in polymer weight from 45.46 grams in F1 to 25 grams in F10 to maintain a total sample weight of 50 grams. The stages of making microcapsules by solvent evaporation method are shown systematically in Figure 2.

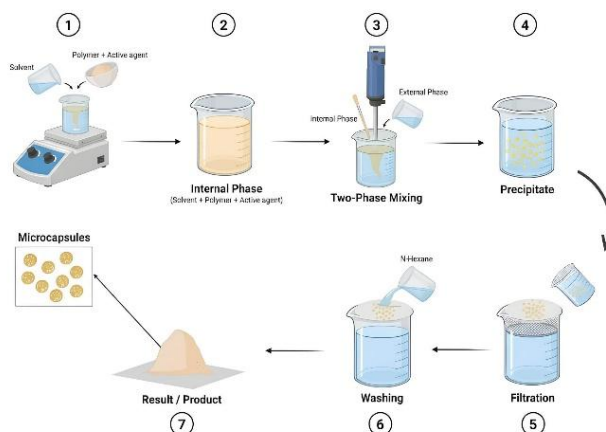


Figure 2. Microcapsule Formulation

Based on the flowchart in Figure 2, the initial stage begins with the dissolution of the polymer and active ingredient in a solvent to form an internal phase. Next, as illustrated in Figure 2, mixing the internal phase and the external phase produces a microcapsule precipitate, which then undergoes filtration and washing until the final product solid microcapsules is obtained.

Microcapsule Characterization

1. Organoleptic Test, Sensory testing was conducted using the five senses to evaluate the shape, odor, color, and taste of the preparation [23].

2. Yield / Yield

$$\text{Yield} = \frac{\text{Final weight (powder simplicia)}}{\text{Initial weight (seed simplicia)}} \times 100\% \dots (1)$$

3. Particle Size Analysis, Particle size was measured using PSA (*Particle Size Analyzer*), the results obtained were expected in the range of 1-5000 μm [24].

4. Sample Morphology, The shape of the sample can be seen using a Binocular Microscope with a magnification of 10x10 [11].

5. Functional Group Analysis, Analysis using FTIR aimed to see the addition of new functional groups present in the sample [25] and [26].

6. Fe and Zn Content, The zinc (Zn) and iron (Fe) content in the sample were measured using an atomic absorption spectrophotometer [27].

7. %EE (*Encapsulation Efficiency*)

$$\%EE = \frac{\text{Total rate trapped in a microcapsule}}{\text{The amount of microcapsules obtained}} \times 100\% \dots (2)$$

8. Microbiology Test, a) Total Plate Count (TPC), A 10 g sample was dissolved in 0.9% NaCl, inoculated into PCA media by the pour plate method. The cup was incubated upside down at 37°C for 24–48 hours, then the colonies were counted and multiplied by the dilution factor [28]. b) Yeast Mold Count (YMC), Samples were diluted to 10^{-3} , inoculated into PDA media by the pour plate method, incubated for 5 days at room temperature, then colonies were counted and multiplied by dilution factors [28]. c) Microbial Contamination Test, A 10 g sample was dissolved in 90 mL of sterile aqueduct. The suspension was inoculated into SSA media by the pour plate method, incubated at 35°C for 24 hours, and then observed a colony of *Salmonella sp.* marked as a transparent colony with a black spot in the middle [29].

9. Stability Test, The microcapsule stability test was carried out by the *Cycling Test* method, which is storage at 4°C for 24 hours and then 40°C for 24 hours (1 cycle = 48 hours = 2 days). This test was carried out in 6 cycles for 12 days, with organoleptic observations (shape, color, smell, and taste) being carried out in cycles 0 to 6 [30].

3. RESULTS AND DISCUSSION

All testing variables were categorized based on the quality evaluation parameters for the microcapsule formulation established in the testing methodology, comprising physicochemical characterization tests (organoleptic properties, yield, PSA, SEM, FTIR, Fe and Zn content via AAS, and %EE), microbiological safety tests (TPC, YMC, and *Salmonella sp.*), and physical stability tests.

3.1. Organoleptic Tests

The results of organoleptic observations including the color, shape, smell, and taste of each yellow pumpkin seed microcapsule formula are presented in Table 2.

Table 2. Organoleptic Test Results of Yellow Pumpkin Seed Microcapsules

Ratio	Color	Shape	Smell	Taste
F1 (0.5:5)	Light Yellow	Powder	Aromatic specialties	No taste
F2 (1:5)	Light Yellow	Powder	Aromatic specialties	No taste
F3 (1.5:5)	Light Yellow	Powder	Aromatic specialties	No taste
F4 (2:5)	Light Yellow	Powder	Aromatic specialties	No taste
F5 (2.5:5)	Light Yellow	Powder	Aromatic specialties	No taste
F6 (3:5)	Light Yellow	Powder	Aromatic specialties	No taste
F7 (3.5:5)	Light Yellow	Powder	Aromatic specialties	No taste
F8 (4:5)	Light Yellow	Powder	Aromatic specialties	No taste
F9 (4.5:5)	Light Yellow	Powder	Aromatic specialties	No taste
F10 (5:5)	Dark Yellow	Powder	Aromatic specialties	No taste

Based on organoleptic observations on the microcapsules of yellow pumpkin seeds with a variation in ratio (0.5:5 to 5:5) overall produced a powdery shape, a distinctive aromatic odor, and no taste, but showed a

change in color from light yellow to dark yellow at the highest ratio (5:5). This color change is in line with the results [31], which say that the yellow pumpkin plant parts including pumpkin seed based materials contain natural pigments of carotenoids that affect the color character of the product, so that the larger the proportion of encapsulated yellow pumpkin seeds, the more concentrated the intensity of the yellow color formed in the microcapsule. Organoleptic examination was carried out to determine the possibility of changes during the storage period [32].

3.2. Yield / Yield

The results of the calculation of the percentage yield of each yellow pumpkin seed microcapsule formula are presented in Table 3.

Table 3. Yield of Yellow Pumpkin Seed Microcapsules

Sample	Starting weight (gr)	Final weight (gr)	Yields (%)
Pumpkin seed powder	250	135	54
F1 (0.5:5)	50	38,50	77
F2 (1:5)	50	20,32	40,64
F3 (1.5:5)	50	25,29	50,58
F4 (2:5)	50	19,50	39
F5 (2.5:5)	50	23,92	47,84
F6 (3:5)	50	30,50	61
F7 (3.5:5)	50	19,4	38,8
F8 (4:5)	50	24,16	48,32
F9 (4.5:5)	50	23,21	46,42
F10 (5:5)	50	28,45	56,9

Yield is a comparison of the dry weight produced by the sample with the initial weight of the sample [33]. The yield values of the yellow pumpkin seed microcapsules varied considerably in the range of 38.8% to 77%, with the highest value in the 0.5:5 formula and the lowest in the 3.5:5 formula. All of these recoveries of less than 100% occurred due to an imperfect emulsification process so that the active substance was not properly coated and discarded into liquid paraffin, less effective stirring methods, and the presence of microscopic potentials left in the mixer, as well as filter paper during the separation and washing process [34].

3.3. Particle Size Analysis

The results of the distribution and measurement of the average particle size (*d mean*) of each microcapsule formula using the *Particle Size Analyzer* are presented in Table 4.

Table 4. Particle Size Test Results using *Particle Size Analyzer*

Ratio	Particle Size Range (µm)	Average Diameter (d)	Quantity (n)	Percentage (%)	Percentage Cumulative (%)	nd	nd ²	mean d (µm)
F1 (0,5:5)	1-10	5	105	9,88	9,88	525	2625	60,09
	10-50	25	310	29,19	39,07	7750	1937	
	50-90	75	647	60,92	100	4852	3639	
			Σ 1062				Σ 3835	
F2 (1:5)	1-10	5	211	14,34	14,34	1055	5275	57,61
	10-50	25	442	30,04	44,39	1105	2762	
	50-90	75	818	55,60	100	6135	4601	
			Σ 1471				Σ 4882	
F3 (1,5:5)	1-10	5	92	7,21	7,21	460	2300	60,70
	10-50	25	392	30,72	37,93	9800	2450	
	50-90	75	792	62,06	100	5940	4455	
			Σ 1276				Σ 4702	
F4 (2:5)	1-10	5	335	20,05	20,05	1675	8375	54,73
	10-50	25	503	30,11	50,17	1257	3143	
	50-90	75	832	49,82	100	6240	4680	
			Σ 1670				Σ 5002	
F5 (2,5:5)	1-10	5	347	18,44	18,44	1735	8675	

Ratio	Particle Size Range (μm)	Average Diameter (d)	Quantity (n)	Percentage (%)	Percentage Cumulative (%)	nd	nd ²	mean d (μm)
	10-50	25	583	30,99	49,44	1457	3643	55,15
	50-90	75	951	50,55	100	7132	5349	
			Σ 1881				Σ 5722	
	1-10	5	114	10,18	10,18	570	2850	
	10-50	25	344	30,74	40,92	8600	2150	
F6 (3:5)	50-90	75	661	59,07	100	4957	3718	59,30
			Σ 1119				Σ 3935	
	1-10	5	146	12,38	12,38	730	3650	
	10-50	25	372	31,55	43,93	9300	2325	
	50-90	75	661	56,06	100	4957	3718	
F7 (3,5:5)			Σ 1179				Σ 3954	57,91
	1-10	5	88,1	8,47	8,47	440,5	2202	
	10-50	25	318	30,57	39,04	7950	1987	
	50-90	75	634	60,95	100	4766	3566	
			Σ 1040,1				Σ 3767	
F8 (4:5)	1-10	5	364	19,34	19,34	1820	9100	54,68
	10-50	25	584	31,03	50,37	1460	3650	
	50-90	75	934	49,62	100	7005	5253	
			Σ 1882				Σ 5627	
	1-10	5	171	14,45	14,45	855	4275	
F9 (4,5:5)	10-50	25	362	30,60	45,05	9050	2262	57,31
	50-90	75	650	54,94	100	4875	3656	
			Σ 1183				Σ 3886	

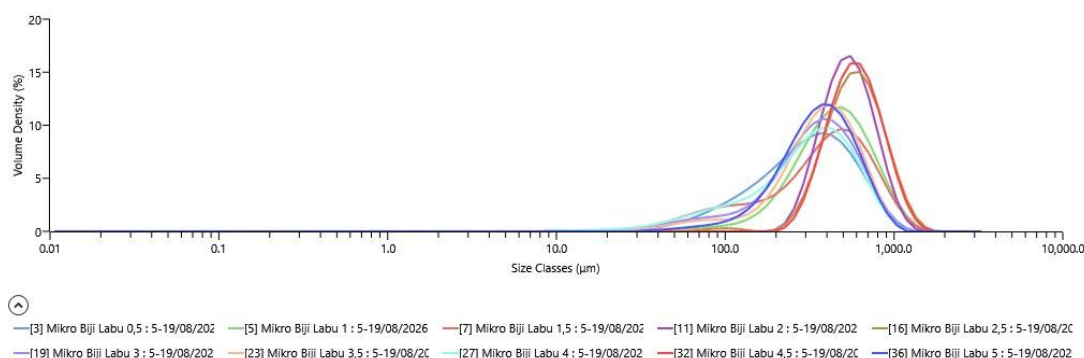


Figure 3. Particle Size Graph on *Particle Size Analyzer*

The particle sizes of all formulations were within the micrometer range, which showed that the microencapsulation process was effective [35]. Particle size testing using Particle Size Analyzer on ten samples of yellow pumpkin seed microcapsules obtained a mean range of d and overall particle size was in a relatively stable range of 54.68 – 60.70 μm where the variation in size and uniformity of this distribution was influenced by the proportion of active ingredients and polymers that coated gum arabic [36]. According to [37] the diverse size of microencapsulation was influenced by several factors, including polymer concentration, distance between pipettes and microcapsule forming solutions. Increasing the concentration of coatings is proven to trigger cohesion and aggregation between particles so that they form a larger size [36], in this study F9 (4.5:5) was successfully obtained as the best formula with the smallest average diameter of 54.68 μm which indicates that the smaller the particle size produced, the better [38], smaller particle sizes are preferable [39].

3.4. Sample Morphology

The morphological test of the yellow pumpkin seed microcapsule sample was carried out using a Binocular Microscope with a magnification of 10x10.

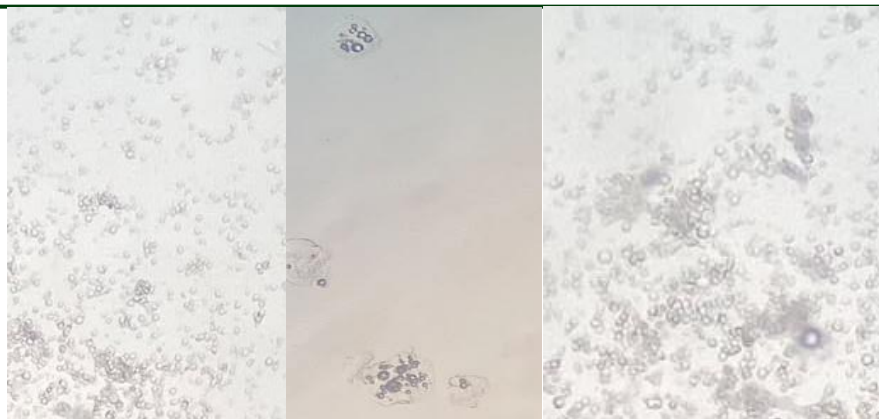


Figure 4. Sample (4.5:5) on a 10x10 magnification binoculars microscope

Morphological observation of the microcapsules of yellow pumpkin seeds in a ratio (4.5:5) using a 10x10 magnification binocular microscope showed that the particles [40] were dispersed separately with a relatively intact surface. The active ingredient encased in a translucent layer of the wall of the gum arabic coating matrix indicated that the encapsulation process was successful to protect the yellow pumpkin seed nucleus from environmental influences such as moisture, mask unpleasant tastes and odors, and maintain its stability from external interactions [11].

3.5. Functional Group Analysis

Analysis of functional groups in the sample was carried out using FTIR (*Fourier Transform Infra Red*).

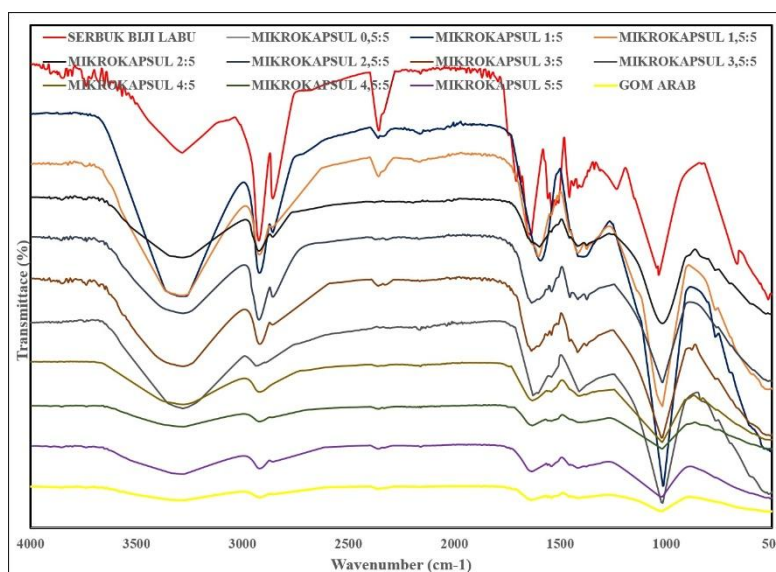


Figure 5. FTIR Spectrum Results

FTIR can identify components in a sample without damaging it, generally used to identify organic bioactive compounds, especially in plants [41]. The results of the FTIR test did not reveal any new peaks. The results of the yellow pumpkin seed powder spectrum with a wide peak of about 3300 cm⁻¹ indicated the presence of an O-H group, peaks of about 2920 cm⁻¹ and 2850 cm⁻¹ indicated the presence of an aliphatic C-H group, peaks of about 1740 cm⁻¹ indicated the presence of a C=O ester group, and at about 1540 cm⁻¹ and about 1000-1100 cm⁻¹ indicated the presence of amide groups I & II of proteins.

The gum arabic peak at about 3271 cm⁻¹ indicates the presence of an O-H group, the peak at about 2899 cm⁻¹ indicates the presence of an aliphatic C-H group, the peak at about 1956-1418 cm⁻¹ indicates the presence of a COO-carboxylate group, the peak at about 1016 cm⁻¹ indicates the presence of an ether group. The FTIR results for the microcapsules at various ratios showed a combination of peaks from the two raw materials, with no new peaks. The absence of new peaks indicates the absence of new functional groups so that there is no chemical interaction. It is suspected that the absence of new compounds proves that the method used has succeeded in maintaining the integrity of the chemical structure of the active ingredient, so that the basic properties of each component are well maintained [42].

3.6. Fe and Zn Content

The data from the Fe and Zn levels of all microcapsule formulas can be seen in Table 5.

Table 5. Results of Iron and Zinc Levels of Yellow Pumpkin Seed Microcapsules

Ratio	Fe Level (mg/L)	Zn level (mg/L)
F1 (0.5:5)	18	11
F2 (1:5)	42	15
F3 (1.5:5)	43	26
F4 (2:5)	41	33
F5 (2.5:5)	65	43
F6 (3:5)	67	51
F7 (3.5:5)	79	55
F8 (4:5)	83	60
F9 (4.5:5)	101	71
F10 (5:5)	98	79

Based on the results, iron (Fe) and zinc (Zn) levels increased as the proportion of yellow pumpkin seeds increased, with Fe values increasing from 18 mg/L to 101 mg/L and Zn increasing from 11 mg/L to 71 mg/L at a ratio of 4.5:5. The 4.5:5 formulation was selected because it produced the highest Fe content and a favorable Zn content. Fe levels higher than Zn have the potential to prevent absorption competition in the body [43] so that it is safe and potentially supports daily nutritional adequacy accordingly [44].

3.7. %EE (Encapsulation Efficiency)

The data from the encapsulation efficiency (%EE) test results of microcapsules can be seen in Table 6.

Table 6. Results %EE Yellow Pumpkin Seed Microcapsules

Ratio	Number of Microcapsules (g)	Trapped Rate (g)	EE (%)
F1 (0.5:5)	1	0,0398	3,98
F2 (1:5)	1	0,0281	2,81
F3 (1.5:5)	1	0,1399	13,99
F4 (2:5)	1	0,1106	11,06
F5 (2.5:5)	1	0,1756	17,56
F6 (3:5)	1	0,1962	19,62
F7 (3.5:5)	1	0,2085	20,85
F8 (4:5)	1	0,2905	29,05
F9 (4.5:5)	1	0,3001	30,01
F10 (5:5)	1	0,1795	17,95

The encapsulation efficiency (EE) indicates the ability of the coating material (the matrix) to wrap the active ingredient. Based on the results of the value (%EE) obtained, the encapsulation efficiency of the yellow pumpkin seed microcapsules increased as the ingredient ratio increased until it reached its peak at a ratio of 4.5:5 with a value of 30.01%, before finally dropping to 17.95% at a ratio of 5:5.

The higher the EE value, the better the matrix in coating/wrapping the active ingredient [45] and the higher the %EE value, the more efficient the microencapsulation process takes place [46]. The results explain that the %EE value is greatly influenced by the type and concentration of the coating material, the addition of the matrix to the optimum limit will increase the binding strength of the active material, but too high a viscosity of the mixture can inhibit the atomization process and reduce the efficiency of the coating.

3.8. Microbiological Tests

The results of the Total Plate Count (TPC) test on the sample of yellow pumpkin seed powder and only the formula F9 microcapsule (4.5:5) are presented in Table 7.

Table 7. TPC Test Results of Yellow Pumpkin Seed Microcapsules

Sample	Dilution	Total Plate Count (TPC)	Maximum Limit (TPC) [47]
Pumpkin Seed Powder	10-1	3.8 x 10 ² cfu/g	≤5 x 10 ⁷ cfu/g
	10-2	3.4 x 10 ³ cfu/g	≤5 x 10 ⁷ cfu/g
	10-3	3.1 x 10 ⁴ cfu/g	≤5 x 10 ⁷ cfu/g

Sample	Dilution	Total Plate Count (TPC)	Maximum Limit (TPC) [47]
Microcapsule 4.5:5 (Best Sample)	10-1	5×10^2 cfu/g	$\leq 5 \times 10^7$ cfu/g
	10-2	4.1×10^2 cfu/g	$\leq 5 \times 10^7$ cfu/g
	10-3	3.4×10^2 cfu/g	$\leq 5 \times 10^7$ cfu/g

The results of the Yeast Mold Count (YMC) test on the sample of yellow pumpkin seed powder and only the best formula microcapsule F9 (4.5:5) are presented in Table 8.

Table 8. YMC Test Results of Yellow Pumpkin Seed Microcapsules

Sample	Dilution	Yeast Mold Count (YMC)	Maximum Limit (YMC) [47]
Pumpkin Seed Powder	10-1	1.5×10^2 cfu/g	$\leq 5 \times 10^5$ cfu/g
	10-2	1.4×10^3 cfu/g	$\leq 5 \times 10^5$ cfu/g
	10-3	1×10^4 cfu/g	$\leq 5 \times 10^5$ cfu/g
Microcapsule 4.5:5 (Best Sample)	10-1	2.2×10^2 cfu/g	$\leq 5 \times 10^5$ cfu/g
	10-2	1.7×10^2 cfu/g	$\leq 5 \times 10^5$ cfu/g
	10-3	1.2×10^2 cfu/g	$\leq 5 \times 10^5$ cfu/g

The results of the microbial contamination test of *Salmonella* sp. on the sample of yellow pumpkin seed powder and only the best formula microcapsule F9 (4.5:5) are presented in Table 9.

Table 9. Microbial Contamination Test Results of *Salmonella* sp.

Sample	Observations	Results
Pumpkin Seed Powder	No blackheads	(-) Negatives
Microcapsule 4.5:5 (Best Sample)	No blackheads	(-) Negatives

Microbiological testing was conducted to evaluate the safety parameters of the pumpkin seed powder microcapsules. The results indicated that the tested parameters met the reference limits [47]. Specifically, the Total Plate Count (TPC) was recorded at 3.8×10^2 CFU/g and the Yeast and Mold Count (YMC) at 1.5×10^2 CFU/g calculated within the ranges of 30–300 and 10–150 colonies, respectively [28] values that are well below the established maximum limits of $TPC \leq 5 \times 10^7$ CFU/g and $YMC \leq 5 \times 10^5$ CFU/g. These results confirm the microbiological safety regarding the specific parameters tested and demonstrate the preparation's initial potential under laboratory conditions. However, these findings do not validate clinical safety, bioavailability, or efficacy in humans; therefore, further in vivo testing and clinical trials are required.

The results of the *Salmonella* sp. microbial contamination test in the sample were observed to have no black spots on the medium, indicating that the sample tested negative for *Salmonella* sp. According to [48] if *Salmonella* sp. develops in the SSA medium, it indicates the presence of a spherical colony, as well as having a black nucleus. The black color indicates that bacteria form H_2S due to the presence of sodium thiosulfate content in the medium reduced by H_2S which then reacts with iron salts [49].

3.9. Stability Test

The results of the microcapsule organoleptic stability test from cycle 0 to cycle 6 showed that the taste and odor parameters of the entire formula (0.5:5 to 5:5) were in a stable condition, i.e. they remained tasteless and had a distinctive aromatic odor. However, physical changes were observed in the shape and color parameters depending on the concentration of the formula and the number of cycles passed. In the shape parameters, the 0.5:5 and 1:5 formulas began to experience a decrease in physical stability in the 3rd and 4th cycles with a slight melting condition, until they finally melted completely in the 5th and 6th cycles. In contrast, the 1.5:5 to 5:5 formula managed to maintain its powder shape consistently from the beginning to the end of the test. Meanwhile in the color parameters, the 0.5:5 to 3.5:5 formula was found to be stable in maintaining a light yellow color throughout cycles 0 to 6. Color change occurred from the 3rd to the 6th cycle in formulas 4:5 and 4.5:5 which changed from light yellow to brownish yellow, and in the 5:5 formula which changed from an initial dark yellow to brownish yellow initial color. The stability test aimed to determine the effect of temperature on the microcapsule preparation [50].

Stability test results for the microcapsules across cycles 0 to 6 indicated that all formulations remained stable regarding taste (tasteless) and smell (characteristic scent). However, physical changes were observed in

some samples. Formulations with 0.5:5 and 1:5 ratios began to melt between the 3rd and 6th cycles, while those with ratios ranging from 4:5 to 5:5 discolored to a yellowish brown hue due to oxidation triggered by uncontrolled exposure to temperature and light [30]. Among the variations, the 1.5:5 formulation proved the most stable, successfully maintaining the powder's shape, color, taste, and aroma without degradation through the final cycle. Although F9 (4.5:5) exhibited discoloration, it was selected as the optimal formulation due to its balanced overall performance. F9 demonstrated highly stable powder morphology and superior active ingredient encapsulation capability compared to the 1.5:5 through 3.5:5 formulations. The discoloration in F9 was not considered a critical issue and could be mitigated by storage in airtight containers protected from direct sunlight. This study demonstrates that a higher gum arabic ratio optimizes the physical protection of pumpkin seeds without altering taste, while offering a practical and cost effective approach to local food fortification. Limitations of the study include the absence of large scale replication, active substance release profile data (in vitro release), and in vivo bioavailability assessment; therefore, gastrointestinal simulation tests, animal trials, and packaging optimization are recommended for future research.

ANOVA Results

ANOVA analysis showed that variations in the ratio of pumpkin seeds (*Cucurbita moschata*) to gum arabic had a significant effect on Fe content ($p = 0.002$), Zn content ($p = 0.001$), and encapsulation efficiency ($p = 0.007$), but had no effect on particle size ($p = 0.890$) or yield ($p = 0.929$). Interpretation of these data indicates that increasing the proportion of the active ingredient effectively optimizes the loading capacity of the gum arabic matrix to adsorb Fe and Zn minerals without compromising the structural integrity of the microcapsules, consistent with findings from previous studies reporting increased encapsulation efficiency as the core material loading increases up to the polymer saturation limit. Conversely, the absence of a significant effect on particle size (average 54.68–60.70 μm) is consistent with microencapsulation theory, which states that shear force and the constant mechanical energy of stirring during solvent evaporation are the primary factors controlling the droplet size of the primary emulsion. In general, these results suggest that gum arabic based microencapsulation systems offer high loading capacity flexibility for local food extracts without compromising the particles' basic physicochemical characteristics. The uniqueness of this study lies in the use of pumpkin seed waste as a source of dual mineral fortification (Fe and Zn), which was successfully and stably encapsulated using a simple solvent evaporation technique. The practical implications of these findings provide a strong scientific basis for the use of pumpkin seed microcapsules as a raw material for locally fortified dietary supplements to support efforts to address stunting. However, the limitations of this study include fluctuations in yield (38.8%–77.0%) due to technical constraints at the laboratory scale, such as imperfect emulsification efficiency and retention of the formulation on the filter medium; therefore, it is recommended to optimize the technical process and conduct an in vitro release study, assess (bioavailability), and clinical efficacy in future studies.

4. CONCLUSION

The microencapsulation process of yellow pumpkin (*Cucurbita moschata*) seed powder using gum arabic as a coating via the solvent evaporation technique produced microcapsules with characteristics that meet pharmaceutical standards. The ratio of active ingredient to polymer significantly affected the levels of Fe and Zn as well as encapsulation efficiency, but had no significant effect on yield or particle size. An increase in the proportion of active ingredient is directly proportional to an increase in mineral retention, with Formulation F9 (ratio 4.5:5) identified as the optimal formulation because it yields the highest levels of Fe (101 mg/L) and Zn (71 mg/L), the smallest particle size (54.68 μm) with a round, homogeneous, and well-separated morphology, and is microbiologically safe. These findings validate the concept that a gum arabic matrix up to a 4.5:5 ratio can optimize mineral loading without compromising the physicochemical integrity of the microcapsules, thereby implying significant potential as a raw material for local food fortification supplements to address stunting, although further testing regarding release profiles (release studies), bioavailability, and clinical efficacy is still required before commercial claims can be made.

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AUTHOR CONTRIBUTIONS

Conceptualization, A.M. and R.; Methodology, D.; Software, L.; Validation, R., A.M., and H.P.; Formal Analysis, H.P.; Investigation, A.M.; Resources, D.; Data Curation, L.; Writing-Original Draft Preparation, A.M., H.P., L., D., and R.; Writing-Review and Editing, A.M. and R.; Visualization, H.P.; Supervision, R.; Project Administration, L. and D.; Funding Acquisition, A.M., H.P., L., and D.

CONFLICTS OF INTEREST

The author(s) declare no conflict of interest.

INFORMED CONSENT STATEMENT

Prior to participation, each subject was provided with a detailed explanation of the study's objectives, procedures, potential risks, and benefits. All participants voluntarily agreed to participate and signed a written informed consent form. All images in this article have been authorized and are the property of the authors.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that no artificial intelligence (AI) tools were used in the generation, analysis, or writing of this manuscript. All aspects of the research, including data collection, interpretation, and manuscript preparation, were carried out entirely by the authors without the assistance of AI-based technologies.

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