



(RESEARCH ARTICLE)



Optimum beverage formulation in terms of pH, viscosity, and DPPH free radical scavenging activity of bird's nest beverage

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Abstract

Bird's nest is widely used in Asia and good for consumers' health. The paper focused on optimizing for beverage formulation that contain Salanganes' Nest harvesting at Yen Island, Nha Trang, Khanh Hoa province, Vietnam. The input factors such as Bird's Nests essence, *Ginkgo biloba*, Chamomile, and Glucosamine were studied. Target functions were pH, viscosity, and DPPH free radical scavenging activity. The results showed that the optimized beverage formulation value is at the condition as following: Bird's nest essence of 24.79%, *Ginkgo biloba* of 0.18%, Chamomile of 0.23%, Glucosamine of 0.8%, pH of 5.5, a viscosity of 32.9 cP, and DPPH free radical scavenging capacity of 75.4%. Salanganes' Nest beverage possesses antioxidant activity and good status that is useful for healthy of human.

Keywords: Bird's Nests essence; Chamomile; DPPH; *Ginkgo biloba*; Glucosamine viscosity

1. Introduction

Aerodramus (echo-locating swift) and *Collocalia* (echo-locating bird's nest) are two species of insectivorous birds that secrete swiftlets known as valuable Edible Bird's Nest (Ma and Liu, 2012), mainly found in Southeast Asia (SEA) and southern China (Aswir and Wan, 2010). Edible Bird's Nest distributes in China, Indonesia, Malaysia, and Vietnam (Hobbs, 2004).

According to traditional medicine, a bird's nest has a sweet taste, is average, and has the effect of nourishing blood. Specialized for cases such as asthenia, bronchitis, asthma, gastritis, tuberculosis, and respiratory diseases, helping to keep the skin young and beautiful, preventing aging, improving the digestive system, restoring the mother's health after birth, and strengthening the immune system (Gruber et al. 1984; Zhao et al. 2016; Albishtue et al. 2018; Bizrah et al. 2019; Szatmari et al. 2019; Wang et al. 2019; Jamalluddin et al. 2019). A bird's nest contains all the essential amino acids, and protein is the most abundant component of the bird's nest (Ting et al. 2021; Azmi et al. 2021). Bird's nests are known with antioxidant, antiviral (Haghani et al. 2016). They also have six hormones, including testosterone and estradiol. Bird's nest also contains carbohydrates and small amounts of lipids (naturally occurring molecules including fat). Previous research has shown that bird's nest contains substances that can stimulate cell division and growth and enhance tissue growth and regeneration.

Today, products made from bird's nest are very diverse, from drinks to food in different forms, however, applying an optimization model to optimize the recipe of bird's nest drink with ingredients (Ting et al. 2021; Kasidate et al. 2022; Peggy et al. 2023). Various active ingredients are unpublished.

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Therefore, this study focuses on finding the optimal formula for a beverage rich in bioactive ingredients containing bird's nests harvested in Khanh Hoa, Vietnam.

2. Material and methods

2.1. Materials

Base materials: Walocel, Xanthan gum, refined sugar, rock sugar, Stevia, flavoring.

Additional and active ingredients: Bird's nest essence, *Ginkgo biloba*, Chamomile, Glucosamine.

2.2. Experiment design

Experiments were designed with four input factors composed of Bird's nest essence (20%-30%), *Ginkgo biloba* (0.1%-0.3%), Chamomile (0.1%-0.3%), and Glucosamine (0.3%-0.9%) (Table 2). The actual and code variables are converted and shown in Table 1. The target functions were pH, viscosity, and DPPH free radical scavenging activity of beverages.

Table 1 Actual and code variables

No.	Input factor	Code variant	Unit	Level		
				Lower -1	Basic 0	Upper +1
1	Bird's nest essence	Z ₁	%	20	25	30
2	<i>Ginkgo biloba</i>	Z ₂	%	0.1	0.2	0.3
3	Chamomile	Z ₃	%	0.1	0.2	0.3
4	Glucosamine	Z ₄	%	0.3	0.6	0.9

2.3. Analysis methods

pH value was measured using pH meter (HANNA HI2002-02 Dedicated pH/ORP Meter (-2.000~16.000 pH±1000.0 mV))

The viscosity of beverage was measured using capillary flow viscometers

DPPH free radical scavenging activity was evaluated according to Le et al. (2017)

2.4. Quantification of microorganisms

Determination of total aerobic microorganisms, Coliforms, *Escherichia coli*, *Salmonella*, *Clostridium perfringens*, and *Staphylococcus aureus* was according to ISO 4833-1:2013, ISO 4832:2006, ISO 7251:2005, ISO 6579:2002, ISO 7937:2004, and ISO 6888-3:2003, respectively.

2.5. Quantification of heavy metal content

The content of cadmium, lead, and mercury was according to AOAC 999.11, AOAC 999.11, and AOAC 971.21.

2.6. Data analysis

Experiments were triplicated and presented under average ± SD. Unnormal values were removed using Duncan method.

3. Results and discussion

3.1. Effect of the ingredients-to-active ingredients ratio on pH value of beverage

The pH value in food and beverage production is an essential indicator of quality, safety, and uniformity. Changes in pH of products lead to differences in taste, freshness, and shelf life. Thus, pH value is one of the most frequently controlled parameters before going to market.

Table 2 The results of the optimization for beverage formulation from bird's nest

No.	Bird's nest essence Z ₁ (%)	<i>Ginkgo biloba</i> Z ₂ (%)	Chamomile Z ₃ (%)	Glucosamine Z ₄ (%)	pH	Viscosity (cP)	DPPH (%)
1	-1	-1	-1	-1	5.97±0.05	32.39±0.45	66.96±0.98
2	1	-1	-1	-1	5.41±0.05	32.66±0.22	69.13±2.82
3	-1	1	-1	-1	5.58±0.07	31.31±0.34	70.61±0.85
4	1	1	-1	-1	5.27±0.02	30.81±0.95	71.8±0.98
5	-1	-1	1	-1	5.52±0.07	35.5±0.36	72.34±0.78
6	1	-1	1	-1	5.25±0.02	32.54±0.27	69.67±1.92
7	-1	1	1	-1	5.54±0.2	33.42±0.14	74.16±0.52
8	1	1	1	-1	5.54±0.03	30.67±0.31	70.96±0.7
9	-1	-1	-1	1	5.58±0.03	33.43±0.34	63.07±0.56
10	1	-1	-1	1	5.36±0.11	36.4±0.3	70.27±2.57
11	-1	1	-1	1	5.49±0.08	33.36±0.54	67.8±0.62
12	1	1	-1	1	5.51±0.06	36.43±0.33	73.96±0.82
13	-1	-1	1	1	5.46±0.07	33.99±0.64	68.54±0.37
14	1	-1	1	1	5.5±0.06	35.73±0.86	70.07±2.35
15	-1	1	1	1	5.73±0.03	34.83±0.41	70.22±0.98
16	1	1	1	1	6.27±0.12	35.9±0.85	72.14±0.48
17	-1	0	0	0	5.5±0.08	34.31±0.38	71.3±0.44
18	1	0	0	0	5.44±0.03	34.26±0.46	75.84±0.81
19	0	-1	0	0	5.5±0.01	31.48±0.38	75.74±0.39
20	0	1	0	0	5.55±0.03	30.71±0.24	76.08±0.56
21	0	0	-1	0	5.44±0.02	31.34±0.59	73.96±0.77
22	0	0	1	0	5.51±0.03	32.19±0.95	76.23±0.43
23	0	0	0	-1	5.46±0.03	31.67±0.28	76.53±0.56
24	0	0	0	1	5.54±0.02	33.07±0.37	74.75±0.52
25	0	0	0	0	5.5±0.06	31.59±0.34	76.08±0.74

Table 3 Optimization model parameters

No.	Factors	pH of beverage	Viscosity of beverage	DPPH free radical scavenging activity of beverage
1	Lack of fit (<i>p</i> -value)	0.343	0.07	0.11
2	R ²	0.912	0.931	0.96
3	Adjusted R ²	0.891	0.915	0.951
4	Q ²	0.858	0.892	0.937

The adjusted R^2 coefficient of the model is 0.891, which means that the impact of 04 factors (Salanganes nests essence, *Ginkgo biloba*, Chamomile, and Glucosamine) on the pH value was 89.1% and 10.9% due to the impact of factors out of the model. The lack of fit coefficient means that the errors in different software are not statistically significant. This coefficient must be greater than 0.05 ($p > 0.05$) to ignore the error in the software system because of the non-significant error. The lack of fit coefficient of the design model is 0.343, which is greater than 0.05, so we can assume that the errors in the software system are negligible and these ignored errors. The results of the regression analysis show that the coefficient R^2 (actual variation) is 0.912 and Q^2 (virtual variability) is 0.858, and the confidence level is 95% ($p < 0.05$) (Table 3 and Fig. 1), proving that the obtained model has meaningful and compatible with high reality.

pH	Coeff. SC	Std. Err.	P	Conf. int(±)
Constant	5.47531	0.0172899	0	0.0345846
Z1	-0.0446296	0.00943815	1.41277e-005	0.0188789
Z2	0.052037	0.00943815	7.84374e-007	0.0188789
Z3	0.0400004	0.00943815	7.88735e-005	0.0188789
Z4	0.0501853	0.00943815	1.63726e-006	0.0188789
Z1*Z1	-8.47142e-005	0.0250916	0.997397	0.0501902
Z2*Z2	0.0565819	0.0250916	0.0277939	0.0501902
Z3*Z3	0.00158199	0.0250916	0.949948	0.0501902
Z4*Z4	0.0265818	0.0250916	0.293671	0.0501902
Z1*Z2	0.0791664	0.0100107	7.02872e-011	0.0200241
Z1*Z3	0.0866665	0.0100107	3.73752e-012	0.0200241
Z1*Z4	0.0949998	0.0100107	1.49349e-013	0.0200241
Z2*Z3	0.11375	0.0100107	1.35996e-016	0.0200241
Z2*Z4	0.0837499	0.0100107	1.16632e-011	0.0200241
Z3*Z4	0.0870833	0.0100107	3.17785e-012	0.0200241

Figure 1 The results show the influence of + factors

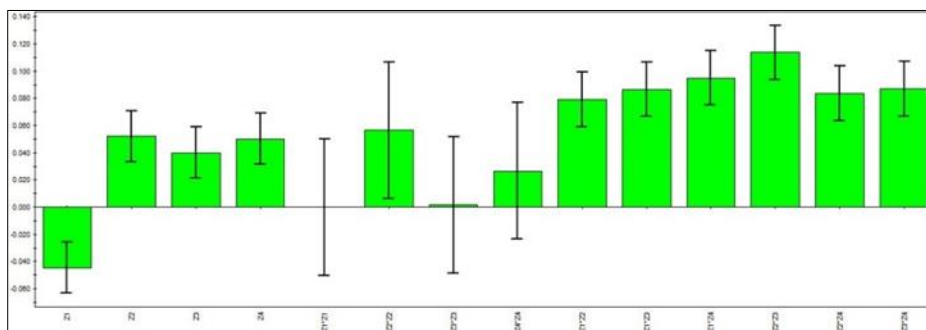


Figure 2 The coefficient of variation of the influencing factors

Using MODDE 5.0 software, the coefficients of the objective function have been calculated after removing the non-significant coefficients that the computer has calculated. The objective function follows the quadratic model of the ratio of the optimal supplemental ingredients and active ingredients of the pH value in the form as follow:

$$Y = 5.4753 - 0.0446Z_1 + 0.0520Z_2 + 0.04Z_3 + 0.0501Z_4 + 0.0565Z_2^2 + 0.0015Z_3^2 + 0.0265Z_4^2 + 0.0791Z_1Z_2 + 0.0866Z_1Z_3 + 0.0949Z_1Z_4 + 0.1137Z_2Z_3 + 0.0837Z_2Z_4 + 0.087Z_3Z_4$$

Bird's nest essence (Z_1) has a negative value that affects the pH value, and the interaction between the quadratic coefficients affects the pH value (Fig. 2).

3.2. Effect of the ingredients-to-active ingredients ratio on the beverage viscosity

The adjusted R² coefficient of the model is 0.915, which means that the effect of 04 factors (Salanganes nests essence, *Ginkgo biloba*, Chamomile, and Glucosamine) on viscosity is 91.5% and 8.5% due to the impact of these factors out of the model. The lack of fit coefficient means that the errors in different software are not statistically significant. This coefficient must be greater than 0.05 ($p > 0.05$) to ignore the error in the software system because of the non-significance error. The lack of fit coefficient of the design model is 0.07, which is greater than 0.05, so we can assume that the error in the software system is negligible. The results of the regression analysis show that the coefficient R² (actual variation) is 0.931 and Q² (virtual variability) is 0.892, and the confidence level is 95% ($p < 0.05$) (Fig. 3), proving that the obtained model has meaningful and compatible with high reality.

Do nhot	Coeff. SC	Std. Err.	P	Conf. int(±)
Constant	31.8709	0.135029	0	0.270095
Z1	0.157778	0.073709	0.0363836	0.147438
Z2	-0.370926	0.073709	4.69821e-006	0.147438
Z3	0.367595	0.073709	5.54322e-006	0.147438
Z4	1.23111	0.073709	3.10206e-024	0.147438
Z1*Z1	2.36895	0.195958	1.00518e-017	0.391969
Z2*Z2	-0.822722	0.195958	9.03269e-005	0.39197
Z3*Z3	-0.152721	0.195958	0.438834	0.39197
Z4*Z4	0.45228	0.195958	0.0244605	0.39197
Z1*Z2	-0.0712517	0.0781802	0.365745	0.156382
Z1*Z3	-0.544167	0.0781802	2.9221e-009	0.156382
Z1*Z4	0.924582	0.0781802	2.5603e-017	0.156382
Z2*Z3	0.00208254	0.0781802	0.978859	0.156382
Z2*Z4	0.491667	0.0781802	4.02464e-008	0.156382
Z3*Z4	-0.257917	0.0781802	0.00163518	0.156382

Figure 3 The results show the influence of optimization factors

The influence of optimization factors demonstrated that the factors Salanganes'Nest Essence (Z₁), *Ginkgo biloba* (Z₂), Chamomile (Z₃), and Glucosamine (Z₄) and the interaction between them all affect viscosity that was impacted by bird's nest essence more than Glucosamine as analyzing of the quadratic coefficient (Fig. 4).

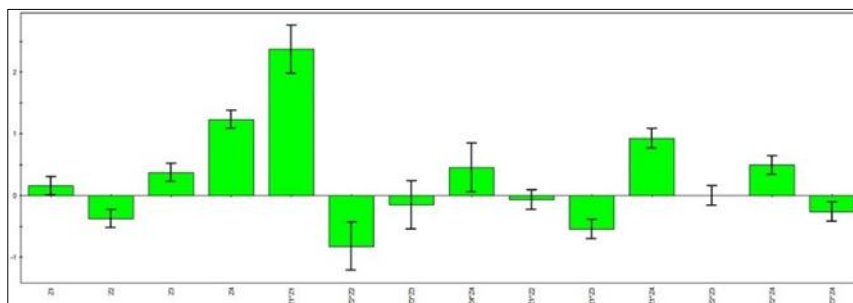


Figure 4 The coefficient of variation of the influencing factors

After removing the non-significant coefficients that the computer has calculated, the objective function according to the quadratic orthogonal model of the optimal ratio of ingredients and active ingredients of the viscosity has the form as follow:

$$Y = 31.8709 + 0.1577Z_1 - 0.3709Z_2 + 0.3675Z_3 + 1.2311Z_4 + 2.3689Z_1^2 - 0.8227Z_2^2 - 0.1527Z_3^2 + 0.4522Z_4^2 - 0.0712Z_1Z_2 - 0.5441Z_1Z_3 + 0.9245Z_1Z_4 + 0.002Z_2Z_3 + 0.4916Z_2Z_4 - 0.2579Z_3Z_4$$

The pH and viscosity values create the product's characteristics through sensory values and are influenced by independent variables composed of Bird's nest essence, *Ginkgo biloba*, Chamomile, and Glucosamine. Viscosity creates a stable, uniform state of the product solution over time and is desired by the manufacturer. With the above requirements of the dependent variable pH, viscosity plays a significant role in affecting product quality, and acted-seventy experiments for finding a suitable pH and viscosity threshold for the product.

3.3. Effect of the ingredients-to-active ingredients ratio on the antioxidant activity

The product is studied to add bird's nest essence and good nutritional active ingredients for the elderly with the ability to be antioxidant, strengthen the immune system, improve bones and joints, eat well, and sleep well. Therefore, the research team evaluated the DPPH free radical scavenging ability to determine the antioxidant capacity in the studied formulations. The coefficient of adjusted R² in the model is 0.951, which means that the influence of 04 factors (Bird's nest essence, *Ginkgo biloba*, Chamomile, and Glucosamine) on DPPH free radical scavenging ability is 95.1% and 4.9% was the impact of factors out of the model. The lack of fit coefficient means that the errors in different software are not statistically significant. This coefficient must be greater than 0.05 ($p > 0.05$) to ignore the error in the software system. The lack of fit coefficient of the design model is 0.11 (> 0.05), so we can assume that the error in the software system is insignificant and the ignored errors. The results of the regression analysis showed that the coefficient R² (actual variation) is 0.96 and Q² (real variability) is 0.937, and the confidence level is 95% ($p < 0.05$) (Fig. 5), proving that the obtained model has meaningful and compatible with high reality.

DPPH	Coeff. SC	Std. Err.	P	Conf. int(±)
Constant	76.4032	0.182552	0	0.365154
Z1	1.65735	0.0996507	3.82717e-024	0.199329
Z2	0.608167	0.0996507	8.26264e-008	0.199329
Z3	0.906709	0.0996507	6.73173e-013	0.199329
Z4	-0.687554	0.0996507	3.70909e-009	0.199329
Z1*Z1	-2.88615	0.264925	7.55687e-016	0.529922
Z2*Z2	-0.544162	0.264925	0.0443377	0.529922
Z3*Z3	-1.35766	0.264925	3.34503e-006	0.529922
Z4*Z4	-0.815325	0.264925	0.00314358	0.529922
Z1*Z2	-0.822837	0.105696	1.14099e-010	0.21142
Z1*Z3	-1.2235	0.105696	6.29491e-017	0.21142
Z1*Z4	1.14333	0.105696	1.00432e-015	0.21142
Z2*Z3	-0.465376	0.105696	4.46489e-005	0.21142
Z2*Z4	0.237291	0.105696	0.0284633	0.21142
Z3*Z4	-0.24971	0.105696	0.0214104	0.21142

Figure 5 The results show the influence of optimization factors

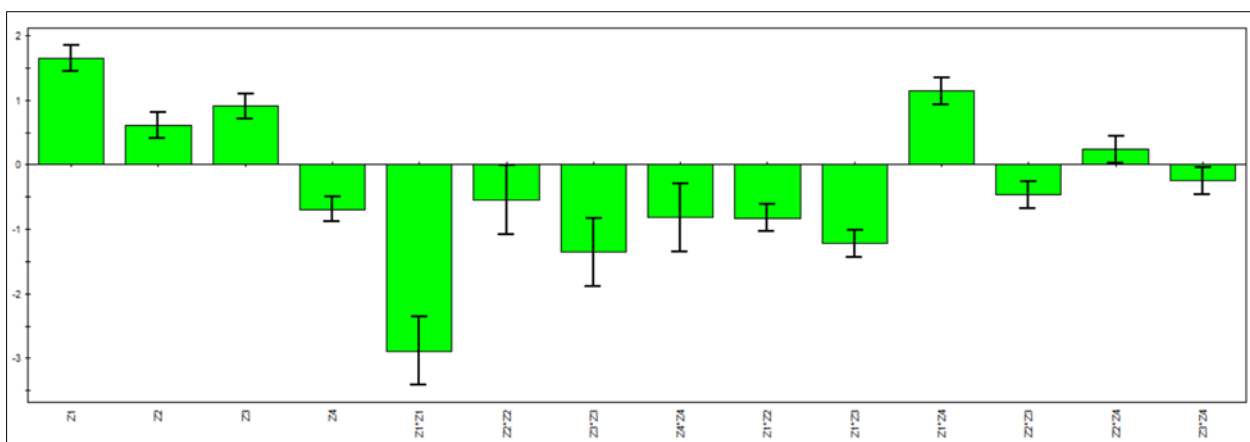


Figure 6 The coefficient of variation of the influencing factors

According to the results showing the influence of the optimization factors, the coefficient of the first-order variable according to descending in order of Bird's nest essence (Z₁), *Ginkgo biloba* (Z₂), and Chamomile (Z₃), Glucosamine (Z₄) affected DPPH free radical scavenging ability that increases following the concentration of Bird's nest essence. In

addition, the reciprocal interaction between variables Z_1 , Z_2 , Z_3 , and Z_4 also affects DPPH free radical scavenging ability (Fig. 6).

After removing the non-significant coefficients that the computer has calculated, the objective function follows the quadratic model of the optimal ratio of additional ingredients and active ingredients to the DPPH free radical scavenging ability has the following form:

$$Y = 76.4032 + 1.6573Z_1 + 0.6081Z_2 + 0.9067Z_3 - 0.6875Z_4 - 2.8861Z_1^2 - 0.5441Z_2^2 - 1.3576Z_3^2 - 0.8153Z_4^2 - 0.8228Z_1Z_2 - 1.2235Z_1Z_3 + 1.1433Z_1Z_4 - 0.4653Z_2Z_3 + 0.2372Z_2Z_4 - 0.2497Z_3Z_4$$

3.4. The optimization and test

The optimization value is pH of 5.5, a viscosity of 32.9 cP, DPPH free radical scavenging capacity of 75.4% corresponding to Bird's nest essence of 24.79%, *Ginkgo biloba* of 0.18%, Chamomile of 0.23%, and Glucosamine of 0.8% (Fig. 7). At the optimized condition, microorganisms and heavy metals of beverage were not detected leading to the safety of antioxidant nests beverage.

Bird's nest essence	Ginkgo biloba	Chamomile	Glucosamine	pH	Viscosity	DPPH
24.8192	0.1797	0.2321	0.7962	5.523	32.8749	75.4739
24.7918	0.1793	0.2322	0.7966	5.5225	32.8737	75.4549
24.8175	0.1792	0.2313	0.795	5.5214	32.8659	75.4761
24.7612	0.1794	0.2317	0.797	5.5222	32.8731	75.4386
25	0.2	0.22	0.84	5.5545	33.1714	76.1048
25	0.2	0.2	0.78	5.515	32.7724	75.6972
25	0.2	0.2	0.78	5.515	32.7724	75.6972
25	0.2	0.2	0.78	5.515	32.7724	75.6972

Figure 7 Optimal results of pH and viscosity values

4. Conclusion

Bird's Nest beverage formula is optimized, and the optimization condition of pH, viscosity, DPPH free radical scavenging capacity, Bird's Nest essence, *Ginkgo biloba*, Chamomile, and Glucosamine are as follows 5.5, 32.9 cP, 75.4%, 24.79%, 0.18%, 0.23%, and 0.8%, respectively.

The results showed that Salanganes' Nest beverage is suitable for antioxidants and improve human health. The beverage formula could be widely deployed in Vietnam and many countries that use and process the nests. This process and recipe is suitable for all sizes of production of anti-oxidation bird's nest beverage.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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