

## Article

# Development of a Citrus Drink Using Mixture Design: Sensory Evaluation, Total Polyphenols and Vitamin C

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**Abstract:** The development of beverages with functional potential and maximum sensory acceptability are priorities for the food industry. This study investigated the effect of orange juice (OJ), lemon juice (LJ) and ginger juice (GJ) concentrations on overall acceptability (OA), total phenolic compounds (TPC) and vitamin C (VC). The experimental design used was simplex-centroid mixtures, and maximization was achieved using response surface methodology (RSM). The bioactive content of TPC and VC was measured using the Folin–Ciocalteu method and the dichlorophenol indophenol method, respectively; sensory evaluation was conducted with 100 panelists on a continuous scale acceptability primer (0–10 points). The RSM results indicated that the optimum formulation was obtained with: OJ = 79.8 mL, LJ = 5.7 mL and GJ = 4.37 mL, yielding maximum values of OA = 6.81 points, TPC = 14.64 mg GAE/100 g and VC = 32.3 mg/mL. The optimized beverage presented a free radical scavenging capacity of 65.72% (DPPH method). The regression models were validated and significantly ( $p < 0.05$ ) predicted sensory acceptability, total phenolic compounds and vitamin C content. The development of this beverage and its potential application in the food industry is attractive due to its high sensory acceptability and bioactive content.

**Keywords:** functional beverage; optimization; health benefits; bioactive; traditional foods



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## 1. Introduction

Currently, there is a growing trend in developing food beverages with nutritional and functional properties aimed at improving consumers' health [1]. Functional foods emerge as an attractive alternative for disease prevention and physiological benefits. These health-promoting effects are attributed to functional molecules or biologically active compounds naturally present in foods or industrially added to them [2].

The increasing demand for these foods can be explained by the rising costs of health-care, the steady increase in life expectancy and the desire to improve quality of life [3–6]. The development of functional beverages has been explored extensively. For example, Maharan and Fernandes [7] developed a novel functional fresh apple juice enriched with prebiotic fibers, ginger extract and cardamom essential oil, enhancing its nutritional value and sensory attributes while maintaining high organoleptic scores. Similarly, fruits and berries have been demonstrated as viable raw materials for producing functional beverages without artificial colorants or flavor enhancers [8].

In Peru, the wide diversity of fruits and vegetables rich in bioactive compounds serves as an excellent resource for developing functional beverages [9,10]. Bioactive compounds in sweet orange (*Citrus sinensis*), such as alkaloids, flavonoids and vitamins, offer numerous cardiovascular health benefits, including reducing hyperlipidemia, improving endothelial function and preventing myocardial infarction and cardiac hypertrophy [11]. Lime juice (*Citrus aurantifolia*) contains bioactive substances such as lycopene and vitamin C, which promote white blood cell proliferation and exert protective effects [12].

Ginger (*Zingiber officinale*) is consistently associated with alleviating nausea and vomiting during pregnancy, reducing inflammation, improving metabolic syndromes, enhancing digestive function and mitigating colorectal cancer markers [13]. Its molecular components, including phytoestrogens, may provide health benefits and chemoprevention against various conditions [14]. Additionally, ginger is an effective antimicrobial herb against Gram-positive bacteria and a potential source of natural antioxidants [15]. Natural antimicrobials are gaining interest among food technologists as alternatives to physical and chemical antimicrobial treatments. Numerous plant extracts or derivatives exhibit broad-spectrum antimicrobial properties, with thousands of phytochemicals showing inhibitory effects on various microorganisms in vitro [16,17].

The mixture design in food formulation is a statistical technique used to determine the optimum proportion of ingredients, with the objective of obtaining the desired properties in the final product, such as flavor, texture or nutritional value, without sacrificing organoleptic characteristics [18,19]. It is based on mathematical models that predict the behavior of the mixture according to the proportions of its components. In the food industry, it is applied to develop new products, improve existing ones or reduce costs without compromising quality [20].

This study aims to investigate the development of beverages with functional potential and maximum sensory acceptability. The effects of orange juice (OJ), lemon juice (LJ) and ginger juice (GJ) concentrations on bioactive characteristics, including total polyphenols and vitamin C, as well as sensory acceptability, were analyzed. The experimental design employed simplex-centroid mixtures, and optimization was conducted using response surface methodology (RSM).

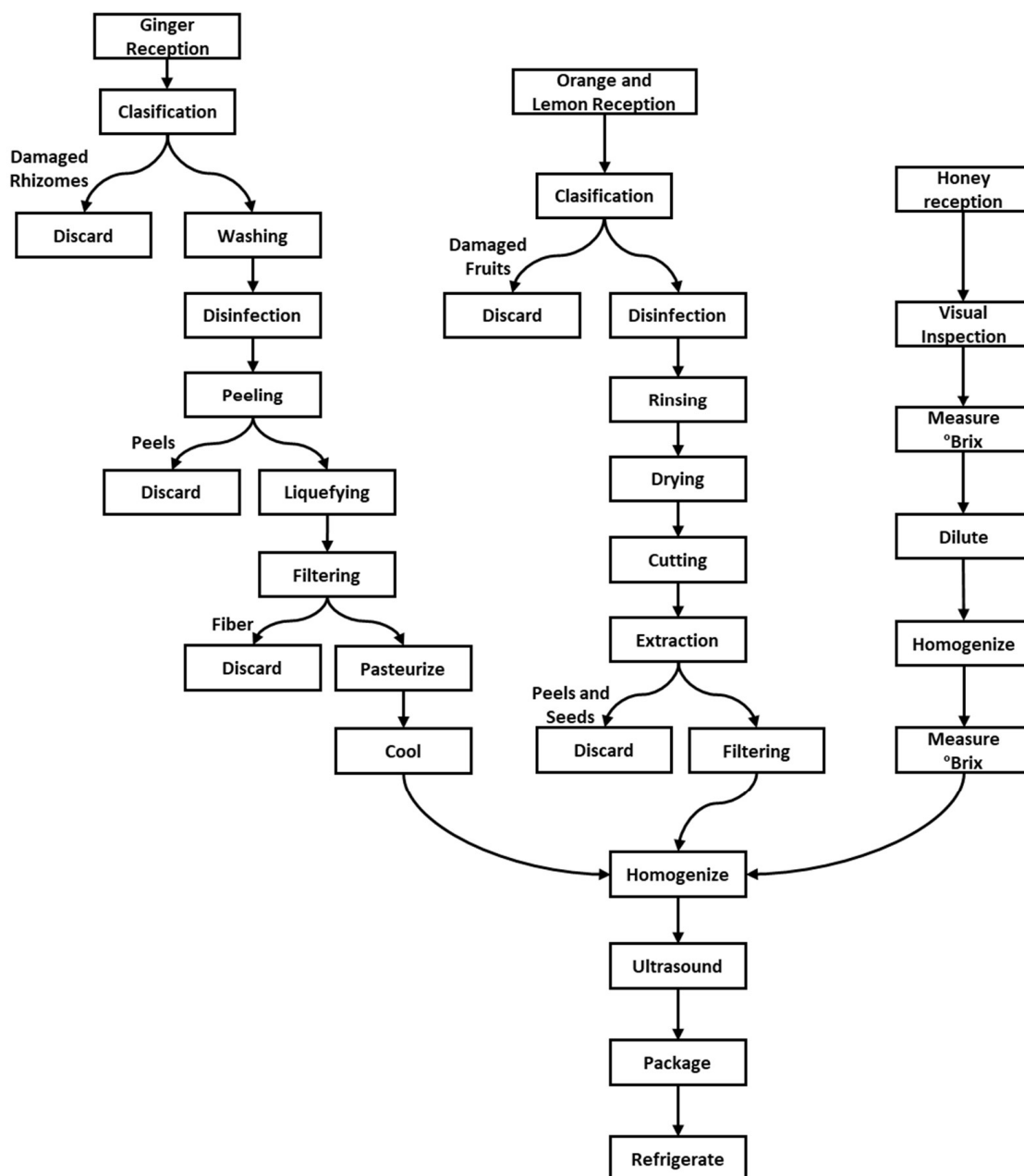
## 2. Materials and Methods

### 2.1. Materials

Orange (*Citrus sinensis*) Valencia variety from the San Martin region, Peru; lemon fruits (*Citrus aurantifolia*) from the Piura region, Peru and ginger (*Zingiber officinale*) from the Junin region were used. These samples were transported in polyethylene bags to the quality control laboratory at the Universidad Nacional del Santa, Chimbote, Peru. Moldy and wounded fruits were excluded to avoid contamination and changes in color, taste or flavor of the juice. Pasteurized honey was acquired from the locality of Pamparomás (Jimbe, Áncash, Peru). The water was obtained from the agro-industrial pilot plant of the Universidad Nacional del Santa, Chimbote, Peru.

## 2.2. Elaboration of the Beverage

The beverage was made following the process flow chart in Figure 1; the stages are described below. The fruits, such as oranges and lemons, were immersed in potable water for washing and then rinsed in water with a 10 ppm chlorinated solution to eliminate microorganisms and organic residues. A total of 35 kg of orange fruits and 52 kg of lemon fruits were used, from which the juice was extracted by a manual mechanical process using a stainless-steel instrument (Manual Juicer, Jinhua, China) and passed through a filter using a fine mesh (Able Brewing, Kone Filter, Portland, OR, USA) with a size of 100  $\mu\text{m}$ ; finally, the orange juice (OJ) and lemon juice (LJ) were refrigerated at a temperature of 5 °C and stored in glass containers until the formulation of the beverage.



**Figure 1.** Process flow chart to produce the citrus beverage.

For ginger, 7 kg was used, and the washing procedure was the same as for lemons and oranges; previously, the rhizomes were removed to ensure the complete removal of the soil. The ginger was weighed and cut into smaller sizes, then peeled off the husk and

mixed with mineral water (1:2) *w/w* using an electric blender (Oster, USA). The ginger showed a fibrous texture, so it was filtered using a fine mesh (Able Brewing, Kone Filter, Portland, OR, USA) with a size of 100  $\mu\text{m}$ . The obtained ginger juice (JG) was subjected to a temperature of 80 °C for 15 min with the aim of inactivating the enzymes and softening the strong and pungent flavor of the ginger. Finally, the JG was cooled and stored refrigerated at 5 °C in glass containers, ready for beverage formulation. The honey (polyfloral character) was acquired from the locality of Pamparomás (Jimbe, Áncash, Peru), which was carefully inspected visually to see if it contained undesirable particles. Prior to the formulation of the functional beverage, the honey was diluted in water (1/2) *w/v*, as indicated in Table 1.

**Table 1.** Simplex-centroid experimental design formulations.

Formulation (Runs)	Orange Juice (mL)	Lemon Juice (mL)	Ginger Juice (mL)	Honey (g)	Water (mL)
1	90.00	0.00	0.00	10.00	20.00
2	0.00	90.00	0.00	10.00	20.00
3	0.00	0.00	90.00	10.00	20.00
4	45.00	45.00	0.00	10.00	20.00
5	45.00	0.00	45.00	10.00	20.00
6	0.00	45.00	45.00	10.00	20.00
7	30.00	30.00	30.00	10.00	20.00
8	60.00	15.00	15.00	10.00	20.00
9	15.00	60.00	15.00	10.00	20.00
10	15.00	15.00	60.00	10.00	20.00
11 *	90.00	0.00	0.00	10.00	20.00
12 *	0.00	90.00	0.00	10.00	20.00
13 *	0.00	0.00	90.00	10.00	20.00

\* Replicated value.

### 2.3. Beverage Formulation

The matrix generated by the mixture design shown in Table 1 was used to formulate each beverage. Water–honey was added in an amount of 20 mL of water and 10 mL of honey to complete the 120 mL in each bottle; the water was heated to 60 °C to dilute and make the honey more fluid. Each formulation was added to a stainless-steel bowl. It was packaged in sterile bottles labeled according to the formulation code. Once the juice was mixed, it was passed through ultrasound (Branson Ultrasonics model 5800 series CPXH-E, Danbury, CT, USA) at 40 Hz for 30 min. Immediately after the ultrasound treatment, for the purpose of preserving the product, the beverage was analyzed for sensory and bioactive compounds.

### 2.4. Determination of Vitamin C

For the determination of vitamin C, the necessary reagents were prepared. Four grams of oxalic acid were weighed and diluted in a 1 L volumetric flask with distilled water to obtain a 0.4% (*w/v*) oxalic acid solution. Subsequently, a 0.1% (*w/v*) ascorbic acid solution was prepared by dissolving 0.1 g of the compound in a 100 mL volumetric flask using the prepared oxalic acid solution. Finally, the 2–6 dichlorophenol indophenol (DCPIP) reagent was prepared by dissolving 0.006 g in a 500 mL flask with distilled water. This reagent was protected from light by wrapping the flask in aluminum foil and refrigerating it for 24 h before use. To generate the standard curve, five standard ascorbic acid solutions with concentrations ranging from 1 to 5 mg/100 mL were prepared, adjusting the volume with 0.4% (*w/v*) oxalic acid. These solutions were mixed in a vortex and labeled as standards 1 to 5. Readings were performed using a multimode reader (Synergy H1, BioTek Instruments, Winooski, VT, USA) at 520 nm, and the curve was calculated using linear regression with

the equation:  $Y = 0.0219X + 0.0043$  ( $R^2 = 0.9989$ ). Juice samples were prepared by diluting them in 0.4% ( $w/v$ ) oxalic acid, subjecting them to ultrasound for 10 min and centrifuging them at 4000 rpm for 20 min. Results were expressed as milligrams of ascorbic acid per 100 g of juice (mg/100 g).

### 2.5. Determination of Total Phenolic Compounds

Total polyphenols were determined using the adapted method of Nagaraj et al. [21] on 96-well plates. Key reagents were prepared, such as a 450  $\mu\text{g/mL}$  gallic acid solution, 20% ( $w/v$ ) sodium carbonate and 2N Folin–Ciocalteu reagent. Additionally, for sample extraction, methanol/water solutions (50:50  $v/v$ , acidified to pH 2) and acetone/water solutions (70:30  $v/v$ ) were used. The standard curve was obtained by preparing different concentrations of gallic acid, reacting them with Folin–Ciocalteu and sodium carbonate and letting the solutions rest for two hours before reading at 765 nm on a Synergy™ H1 multimode microplate reader (BioTek Instruments, Winooski, VT, USA). The equation obtained was  $Y = 0.053X - 0.0365$  ( $R^2 = 0.9997$ ). Juice samples were processed using ultrasound and centrifugation (Orto Alresa, Digicen 21 R, Madrid, Spain) to extract supernatants, which were reacted with the reagents and measured following the described protocol.

### 2.6. Free Radical Scavenging Ability of the Optimal Formula

The ability of the samples to scavenge free radicals was evaluated using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. Specifically, 0.2 mL of an aliquot of each optimized formulation beverage sample (0.5 mL of each juice sample homogenized with 10 mL of methanol) was added to a 7.6 mL solution of 0.4 Mm methanol containing DPPH (1 mM). One milliliter (1 mL) of each resulting mixture was shaken vigorously and allowed to stand for 30 min at room temperature. Ascorbic acid (standard) and blank were also prepared at similar concentrations using methanol and DPPH. Subsequently, the resulting mixtures of samples and standard were incubated in a water bath at 37 °C for 30 min, and their absorbance was read against the blank at 517 nm on a Synergy™ H1 multimode microplate reader (BioTek Instruments, Winooski, VT, USA). Percent inhibition was expressed as:

$$\% \text{ Inhibition} = \left[ \frac{(C - S)}{C} \right] \times 100 \quad (1)$$

where S = absorbance of the sample and C = absorbance of the blank. The 50% inhibitory concentration ( $IC_{50}$ ) was calculated as the quantity of the extract needed to react with one and a half of DPPH radicals.

### 2.7. Sensory Acceptability

The sensory acceptability of the 13 citrus beverage formulations developed was evaluated by 100 panelists between 18 and 50 years of age (50% men and 50% women); the place of evaluation was in controlled rooms with constant temperature (20–22 °C), white light and air conditioning, belonging to the facilities of the pilot plant of the Universidad Nacional del Santa (UNS). An unstructured 10 cm hedonic scale was used, where 0 represented “I dislike it very much”, 5 “I neither like nor dislike it” and 10 “I like it very much”. Each panelist evaluated an average of 5 mL of juice, which was served in 25 mL plastic cups coded with 3 digits in a monadic manner and in a balanced order. Samples were presented in neutral containers, and between each sample, panelists cleansed their palate with water and soda crackers. The evaluation lasted between 30 and 45 min, ensuring objective conditions. The results helped identify the formulation with the highest overall acceptability among consumers. The study was conducted in accordance with the Declaration of Helsinki and approved by the Comité de Ética de Investigación—UNS.

## 2.8. Statistical Analysis

A “simplex-centroid” mixture design was performed to determine the optimal combination of the functional beverage formulated with orange juice ( $X_1$ : 0–90 mL), lemon juice ( $X_2$ : 0–90 mL) and ginger juice ( $X_3$ : 0–90 mL); the design mixtures are presented in Table 1 (honey (10 g) and water (20 mL) were constant). The responses evaluated ( $Y$ ) in the study included sensory acceptability, vitamin C content and total polyphenols. The regression model used to fit the responses was automatically determined by Design Expert software (Version 12, USA), selecting the most significant model based on  $p < 0.05$ . For each response, the software evaluated:

Linear models:

$$Y = \sum_{i=1}^q \beta_i \cdot x_i \quad (2)$$

Quadratic models:

$$Y = \sum_{i=1}^q \beta_i \cdot x_i + \sum_{i<j} \sum_{j=2}^q \beta_{ij} \cdot x_i \cdot x_j \quad (3)$$

Cubic models:

$$Y = \sum_{i=1}^q \beta_i \cdot x_i + \sum_{i<j} \sum_{j=2}^q \beta_{ij} \cdot x_i \cdot x_j + \sum_{i<j} \sum_{i<k} \sum_{k=3}^q \beta_{ijk} \cdot x_i \cdot x_j \cdot x_k \quad (4)$$

Non-significant regression coefficients were removed from the final model. To determine the adequacy and goodness of fit of the model, the coefficient of determination ( $R^2$ ) and the adjusted ( $R^2$  adj) were calculated. Finally, for the analysis of variance (ANOVA), the statistical significance of each overall model, as well as the coefficients of the model terms and the lack of fit (error), were assumed to be significantly different at the 95% confidence level ( $p < 0.05$ ).

## 3. Results

To analyze the interaction of the beverage ingredients on the response variables, a simplex mixture design with centroid was used with three ingredients, orange juice (OJ), lemon juice (LJ) and ginger juice (GJ). The combination is described in Table 1. The responses were overall acceptability (OA), total phenolic compounds (TPC) and vitamin C (VC), as shown in Table 2. With these results, analysis of variance (ANOVA) and regression coefficients were performed, as shown in Table 3.

**Table 2.** Response value of the design of experiments.

Formulation (Runs)	Overall Acceptability	Total Phenolic Compounds (mg GAE/100 g)	Vitamin C (mg/100 g)
1	7.85 ± 1.22	14.82 ± 0.71	35.46 ± 0.82
2	2.40 ± 1.68	5.14 ± 0.29	20.72 ± 0.61
3	1.34 ± 1.18	5.12 ± 0.12	1.79 ± 0.32
4	3.58 ± 2.39	9.60 ± 0.27	30.52 ± 0.31
5	2.96 ± 1.91	8.49 ± 0.34	14.47 ± 2.17
6	2.22 ± 1.44	5.29 ± 0.17	13.97 ± 2.16
7	4.05 ± 2.08	7.46 ± 0.26	15.13 ± 1.11
8	4.90 ± 1.94	11.15 ± 0.25	27.41 ± 0.86
9	2.26 ± 2.08	6.05 ± 0.17	22.33 ± 0.18
10	2.57 ± 2.08	6.24 ± 0.26	10.20 ± 1.3
11	7.65 ± 1.54	15.65 ± 0.10	30.97 ± 6.71
12	1.97 ± 1.56	6.02 ± 0.25	24.45 ± 0.18
13	1.46 ± 1.26	5.39 ± 0.13	1.95 ± 0.24

Average value ( $\bar{X}$ ) ± Standard deviation (SD).

**Table 3.** Coefficients and variance analysis for each response variable.

Model	OA			TPC			VC		
	Coefficient	p Value	Sig.	Coefficient	p Value	Sig.	Coefficient	p Value	Sig.
Linear									
B1	+0.086067	<0.0001	****	0.16982	<0.0001	****	0.36662	<0.0001	****
B2	+0.023378	0.0018	**	0.061203	<0.0001	****	0.25977	0.0057	**
B3	+0.015921	0.0001	**	0.058687	<0.0001	****	0.012986	<0.0001	****
Quadratic									
B12	$-7.46576 \times 10^{-4}$	0.0053	**	$-4.18066 \times 10^{-4}$	0.0672	NS	-	-	-
B13	$-7.77994 \times 10^{-4}$	0.0043	**	$-8.13820 \times 10^{-4}$	0.0040	**	-	-	-
B23	$+1.74061 \times 10^{-4}$	0.3582	NS	$-9.43901 \times 10^{-5}$	0.6400	NS	-	-	-
Cubic									
B123	$+4.43029 \times 10^{-5}$	0.0204	*	-	-	-	-	-	-
R <sup>2</sup>	0.9886			0.9932			0.9612		
R <sup>2</sup> adj	0.9773			0.9883			0.9534		

Sig. Significance: \*  $p < 0.05$ , \*\*  $p < 0.01$  and \*\*\*\*  $p < 0.0001$ ; NS: non-significant. OA: overall acceptability, TPC: total phenolic compounds, VC: vitamin C. B1, B2, B3, B12, B13, B23, B123: constants, R<sup>2</sup>: coefficient of determination, R<sup>2</sup> adj: coefficient of determination adjusted.

### 3.1. Analysis of Experimental Formulations

A total of thirteen experimental formulations were evaluated to optimize a beverage based on orange (B1), lemon (B2) and ginger (B3) juices. The binary interactions (B12, B13, B23) represent the interactions between orange–lemon, orange–ginger and lemon–ginger, respectively, while the ternary interaction (B123) represents the interaction between the three components simultaneously. Formulation 1 stood out significantly in terms of overall acceptability and vitamin C, reaching values of  $7.85 \pm 1.22$  and  $35.46 \text{ mg}/100 \text{ g}$ , respectively. Regarding the content of total phenolic compounds, formulation 11 showed the highest value, with  $15.65 \pm 0.10 \text{ mg GAE}/100 \text{ g}$ , followed by formulation 1, with  $14.82 \pm 0.71 \text{ mg GAE}/100 \text{ g}$ . In contrast, formulation 3 showed the lowest values for all three variables, with an acceptability of 1.34, a TPC of  $5.12 \pm 0.12 \text{ mg GAE}/100 \text{ g}$  and a vitamin C of  $1.79 \text{ mg}/100 \text{ g}$ .

### 3.2. ANOVA of Mathematical Models

The results of the analysis of coefficients and variance for each response variable are detailed in Table 3. For overall acceptability (OA), all linear terms showed significant positive effects, with coefficients of  $+0.086067$  ( $p < 0.0001$ ),  $+0.023378$  ( $p < 0.01$ ) and  $+0.015921$  ( $p < 0.001$ ) for B1, B2 and B3, respectively. The binary interactions B12 ( $-7.46576 \times 10^{-4}$ ) and B13 ( $-7.77994 \times 10^{-4}$ ) evidenced significant negative effects ( $p < 0.01$ ), while the ternary interaction B123 ( $+4.43029 \times 10^{-5}$ ) showed a significant positive effect ( $p < 0.05$ ).

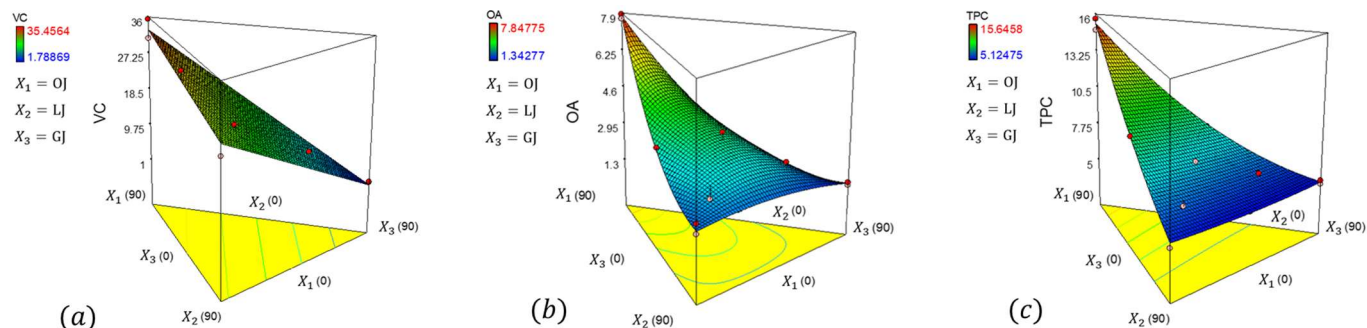
For total phenolic compounds (TPC), the linear terms were highly significant ( $p < 0.0001$ ), with coefficients of 0.16982, 0.061203 and 0.058687 for B1, B2 and B3, respectively. Only the binary interaction B13 showed a negative significant effect ( $-8.13820 \times 10^{-4}$ ,  $p < 0.01$ ), suggesting a possible interference between the corresponding components.

For vitamin C (VC), only the linear terms showed statistical significance, with coefficients of 0.36662 (B1,  $p < 0.0001$ ), 0.25977 (B2,  $p < 0.01$ ) and 0.012986 (B3,  $p < 0.0001$ ), with no evidence of significant interactions between the components.

### 3.3. Validation of Mathematical Models

The mathematical models developed for the three response variables demonstrated a high degree of fit, as evidenced by their coefficients of determination (R<sup>2</sup>) and adjusted coefficients of determination (R<sup>2</sup> adj). The model for TPC presented the best fit with  $R^2 = 0.9932$  and  $R^2 \text{ adj} = 0.9883$ , followed by the model for OA ( $R^2 = 0.9886$ ,  $R^2 \text{ adj} = 0.9773$ ) and VC ( $R^2 = 0.9612$ ,  $R^2 \text{ adj} = 0.9534$ ). These values indicate that the models are highly significant and have a robust predictive ability for the variables studied. The high significance of the linear terms in all response variables suggests that the individual properties of each

component exert a predominant influence on the final beverage characteristics. Significant binary and ternary interactions in OA indicate that combinations of components can modulate the acceptability of the product, a crucial factor for its potential marketability. Overall, the models were good at predicting the response outcomes. Figure 2 shows the geometric representation of the independent variables as a function of the responses.



**Figure 2.** Tridimensional surface plot expressing the mixture: (a) overall acceptability; (b) vitamin C; (c) phenols compounds. The red dots represent the response variables of the interactions between the components (X1, X2 and X3), while the pink dots indicate the response variables with a single component. On the other hand, the green lines represent the contour lines of the response surface projected on the yellow area.

### 3.4. Model Validation and Antioxidant Capacity

The optimization criteria are that the beverage is a mixture of the three components present and that the three response variables are maximized, with sensory acceptability being given the highest importance; the criteria adopted are those shown in Table 4.

**Table 4.** Criteria for optimizing responses.

Name	Goal	Importance
OJ	is in range	3
LJ	Maximize	1
GJ	Maximize	1
OA	Maximize	5
VC	Maximize	4
TPC	Maximize	4

OJ: orange juice, LJ: lemon juice, GJ: ginger juice. OA: overall acceptability, TPC: total phenolic compounds, VC: vitamin C.

By setting the initial criteria for all relevant parameters of the independent variables and the responses during the optimization process, the following optimal formulation is indicated OJ = 79.8 mL, LJ = 5.7 mL and GC = 4.37 mL. The formulation was analyzed with the same analysis methodology as the other samples and obtained acceptability values of 6.81 points, phenolic compound content of 14.64 mg GAE/100 g and vitamin C = 32.3 mg/mL, as shown in Table 5.

**Table 5.** Predicted and experimental values of the optimal beverage.

	Experimental Responses	Predicted Responses
OA	6.81 ± 0.42	6.55822
VC	32.3 ± 0.65	30.8351
TPC	14.64 ± 0.12	13.6952

OA: overall acceptability, TPC: total phenolic compounds, VC: vitamin C.

After determining the optimum formulation of the beverage (Table 5), the percentage of free radical inhibition (DPPH method) was obtained, with a value of  $65.720 \pm 0.502\%$ .

#### 4. Discussion

In the case of oranges, the juice was extracted for analysis of vitamin C and polyphenols, revealing 40.79 mg of vitamin C per 100 mL of juice. This value is close to that reported by Ore Quiroz [22], which indicated 50.2 mg. The values of vitamin C can vary between 40 and 70 mg depending on the state of ripeness, with the ripest oranges containing the highest vitamin content, as indicated by the same author. The variety used in this study was at an early stage of ripening. Meanwhile, the reported polyphenol content was 14.83 mg gallic acid equivalent/100 g (mg GAE/100 g), differing from the values reported by Ashari et al. [23], who observed values between 2.84 and 11.42 mg/100 mL, depending on factors such as processing methods and storage conditions.

In the case of lemons, juice was extracted for analysis of vitamin C and polyphenols, revealing 24.45 mg of vitamin C per 100 mL of juice. This value is close to that reported by Dominguez & Ordoñez [24], which indicated 21.1 mg vitamin C/100 mL juice. Meanwhile, the polyphenol content indicated was 6.02 mg GAE/100 g, differing from the value indicated by Torres [25], which indicated 8.10 mg GAE/g at room temperature. This discrepancy may be due to differences in the measurement method: In Torres [25], ultrasound-assisted extraction was used, whereas in this study, double extraction with methanol, acetone and ultrasound was used.

The ginger was peeled and mixed with water at a 50% (*w/v*) or 1:2 dilution. Results for vitamin C and polyphenols were adjusted to reflect this dilution, showing 1.78 mg of vitamin C per 100 g sample. This value differs from the 0.93 mg/100 g reported by García et al. [26].

Meanwhile, the polyphenol content reported was 10.78 mg GAE/100 g, which differs from the 73.6 mg GAE/100 g reported by Ozola [27], who stated that the values depend significantly on the extraction method. Kumari & Gupta [28] reported a higher total phenolic content in ginger root powder from India, with 776.2 mg GAE/100 g. Subsequently, Ghasemzadeh et al. [29] from University Putra Malaysia reported values with total phenols in ginger amounting to 39.1 mg GAE/100 g.

In the Tiencheu et al. [30] study, the most accepted formulation scored 6.5 on a 9-point scale, while in our study, the maximum acceptability was 7.85 on a 10-point scale. Both investigations highlight the importance of the balance between acidity (lemon) and spice (ginger) for sensory acceptability, as formulations with moderate proportions of these ingredients were the most preferred.

However, the variation in acceptability ranges between the two studies can be attributed to the experimental method used. While Tiencheu et al. [30] applied a factorial design, our study used a simplex-centroid mixture design, which allowed a wider variety of ingredient combinations and proportions to be explored. This explains the wider range of acceptability observed in our case, reflecting the differences in the interactions between the components of the formulations evaluated. Mixture designs are useful tools in food science and technology to study the effects of ingredients and components, but their use is limited to a few products and requires a wider scope of application [31]. Both approaches confirm the importance of optimizing the proportions of key ingredients, but mixture design provides a more detailed perspective on the interactions between them and their impact on sensory perception.

In terms of sensory acceptability, the results of the present study identified that the formulation with the highest concentrations of orange juice had the highest overall acceptability values ( $7.85 \pm 1.22$ ), while the formulations with the highest concentrations of ginger

had the lowest scores. This is in line with similar studies conducted in Cameroon, where ginger, honey and citrus-juice-based drinks were evaluated. In this case, combinations with balanced proportions of the three components also proved to be the most sensorially appreciated [31]. The application of the mixture design and response surface methodology allowed the identification of significant interactions between the components.

The total phenol and vitamin C content is another key aspect in the development of functional beverages. In this study, the formulations showed a range of phenols between  $5.12 \pm 0.12$  and  $15.65 \pm 0.10$  mg GAE/100 g, values that are comparable with those obtained in similar beverages described in the literature. Emmanuel et al. [32] reported that combinations of ginger and honey significantly increase the content of total phenols, reaching up to 18.48 mg GAE/g in pure ginger extracts.

TPC is a crucial indicator of antioxidant activity in functional foods and beverages. Phenols act as free radical scavengers due to their hydroxyl groups, which confer protective properties against oxidative stress [32]. In this context, formulations based on combinations of lemon juice, orange juice and ginger showed a remarkable increase in TPC when properly combined, highlighting the impact of synergistic interactions between these ingredients. Previous studies have also confirmed that the incorporation of honey in beverages enhances phenolic content, due to the richness of bioactive compounds present in this natural ingredient [33]. For example, Bekkouch et al. [33] reported that lemon juice contains mainly eriodictyol, rutin, hesperidin and isorhamnetin, while ginger contributes various gingerols. These compounds, when combined, generate synergistic effects such as those observed in this study.

The functional potential of these beverages also extends to the field of health. Studies such as that of Bekkouch et al. [33] highlight the hepatoprotective properties of ginger and lemon, which may prevent toxic-induced liver damage through their antioxidant capabilities. These findings underline the relevance of promoting natural beverages as healthy alternatives to commercial options with less nutritional benefits.

In the Peruvian context, the abundance of fruits rich in bioactive compounds, such as oranges and lemons, represents a competitive advantage for the local industry. Furthermore, the incorporation of traditional ingredients such as ginger highlights the opportunity to merge innovation and tradition, thus satisfying consumer trends towards functional foods [30,32,33].

Despite the promising results, it is important to consider certain challenges. Variability in the quality of ingredients, as well as limitations in production scales, may affect the replicability of formulations. Further studies assessing the stability of bioactive compounds over time and under different storage conditions are therefore recommended.

The values predicted by the mathematical models of the optimized beverage were validated and were close to the theoretical values. For example, the percentages of accuracy were high: OA (96.18%), VC (95.46%) and TPC (93.55%). It is important that these percentages, obtained from experimental values from the independent variables, are very close to the theoretical values in order to control the process of elaboration of a product. Several investigations have validated their predicted results from experimental tests with their optimum parameters. Monzón et al. [34] validated the content of phenolic compounds in avocado seeds and peels, extracted by ultrasound and using neural network models; the results showed an accuracy of 99% between experimental and predicted values. Hurtado-Soria et al. [35] performed an external validation (%Val) for models obtained with the optimal values of total phenolic compounds (TPC) and antioxidant capacity (FRAP method and DPPH method) during the cocoa roasting process. The experimental and pre-determined values were approximately equivalent (98.44–99.66%) and, therefore, the model was validated and could be replicated. In the case of beverages, Yildiz & Maskan [36]

developed a green tea beverage enriched with honey and bee pollen, managing to validate the model with optimal response values; these experimental and predicted values were 614.87 and 578.67 for total phenolic compounds (TPC), 95.53 and 94.463 for antioxidant capacity (AC) and 3.89 and 3.79 for sensory evaluation, respectively. It was observed that the experimental results were quite close to those predicted. Therefore, the model can be considered satisfactory.

On the other hand, the optimized beverage obtained a significant presence of antioxidant capacity, with a value of % inhibition (DPPH) of  $65.720 \pm 0.502\%$ ; this value is lower than that reported by Raji et al. [37], whose optimized fresh beverage was based on bitter orange (25%) and pineapple (75%) with a value of  $78.82 \pm 1.84\%$ , with pasteurization treatment ( $97^\circ\text{C}$  and 5 min); likewise, this value is higher than that reported by Khalil et al. [38], whose optimized fresh beverage was based on carrot (70%) and orange (30%) with a value of  $34.71 \pm 0.78\%$ , with ultrasonic preservation treatment (28 Hz,  $20^\circ\text{C}$  and 25 min). Therefore, beverages formulated with citrus fruits represent an opportunity to develop foods with potential beneficial effects on human health.

## 5. Conclusions

The study conducted demonstrates the potential for the development of a functional beverage that not only offers high sensory acceptability but also retains a significant amount of bioactive compounds, such as total polyphenols and vitamin C. Using simplex-centroid mixture experimental design and response surface methodology (RSM), an optimal formulation composed of 79.8 mL of orange juice, 5.7 mL of lemon juice and 4.37 mL of ginger concentrate was identified, which achieved a balance between sensory acceptability (6.81 points) and bioactive compound levels (14.64 mg GAE/100 g of total phenolic compounds and 32.3 mg/mL of vitamin C). In addition, this optimized beverage presented a free radical scavenging capacity of 65.720% (DPPH method). On the other hand, regression models (linear, quadratic and cubic) proved to be adequate to predict sensory acceptability and bioactive compound content. In conclusion, this work contributes to the development of functional beverages with an adequate balance between sensory enjoyment and health benefits, which could boost their market acceptance and commercial potential, especially in regions where ingredients such as orange, lemon and ginger juice are widely available.

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## Abbreviations

The following abbreviations are used in this manuscript:

OJ	Orange Juice
LJ	Lemon Juice
GJ	Ginger Juice

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