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Development of a Novel Formulation of a Functional Tomato Sauce

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Abstract A functional tomato sauce was developed through the enrichment of high-lycopene tomato pulp (TomP) with pea protein (PP), freeze-dried olive powder (OP), and tomato peel powder (TPP) to achieve good sensory acceptability and a high antioxidant level using a 4-component (X_1-TomP, X_2-PP, X_3-OP, X_4-TPP) D-optimal mixture design. Responses: Y_1-color, Y_2-aspect, Y_3-aroma, Y_4-flow/texture, Y_5-taste, Y_6-sourness, and Y_7-aftertaste, were evaluated by a non-trained sensory panel to obtain polynomial models for all responses. Numerical optimization resulted in the formulation: TomP (93.05%), PP (1.82%), OP (1.66%), and TPP (3.47%). Sensory analyses of the developed sauce revealed that the product was appreciated by untrained and trained panelists. Sensory profile analyses by the trained panel consensus concluded that the sauce has a good flavor profile with well-balanced sweetness and saltiness, slightly perceptible astringent notes, and no off-taste. Analyses of antioxidant activity (ABTS and FRAP), polyphenols, lycopene and beta-carotene were carried out. The sauce had concentrations of 35.37±1.85 mg/100 g (lycopene), 5.72±0.52 mg/100 g (beta-carotene), and 58.30±0.91 mg GAE/100 g (phenolic content) while the pulp had concentrations of 23.54±0.76 mg/100 g, 3.03±0.01 mg/100 g and 39.06±2.99 mg GAE/100 g, respectively. The developed tomato sauce compares favorably with the pulp in terms of lycopene, beta-carotene, and phenolic content. The remarkable increase in phenolic content, lycopene, and beta-carotene (known for their antioxidant properties) in the sauce enriched with powdered additives contributes significantly to its antioxidant potential. Therefore, the developed sauce is a good source of such health-promoting compounds and has the potential to be consumed as a functional food.

Keywords High-lycopene, Tomato, Plant-based, Functional Food, Product Formulation, Mixture Design, Bioactive Compounds

1. Introduction

An important aspect of food processing involves developing new product formulations using different ingredients. However, a novel product's acceptance depends on the product's composition developed via the selection of ingredients and, more importantly, the specific amounts of each ingredient used in the formulation. Also, food's sensory, nutritional, and physicochemical properties are highly dependent on its ingredients [1]. Food scientists are interested in developing, modifying, or optimizing food and beverage formulations for the following purposes, depending on the end goal(s): (i) technological applications (such as extending product shelf life); (ii) nutrient-related goals (such as meeting the required fiber content to comply with health claims); and (iii) sensory qualities (such as improving the texture and taste of food products) [2].

The food industry seeks to develop, improve, or modify products without increasing their cost and production time. Scientific procedures or experimental design can be employed as cost-effective tools in determining the appropriate amounts of ingredients in food product development [1]. Mixture design is a powerful instrument for evaluating the combination of multiple ingredients and recording the resulting products' characteristics. This enables the determination of the best proportions of ingredients in a blend to obtain an optimized formulation [3]. The factors of the design are the components of a mixture (i.e. ingredients), and the responses vary as the quantities or proportions of the components vary, thus each response is affected by the change in ingredient quantity [4]. The most commonly reported mixture designs are the simplex-centroid, simplex lattice, extreme vertex, and D-optimal designs [5]. A D-optimal design was used in the present research because it is an experimental design that allows for optimization with a relatively small number of trials, taking into account the interactions among all the ingredients making it a cost-effective option compared to other designs such as the one-factor at a time design (OFAT) [6]. A D-optimal design has been used to optimize blends composed of tomato puree, onion puree, and extra virgin olive oil to produce tomato-based sauces rich in Z-lycopene with high total-lycopene bioaccessibility [7].

In recent times, the rise in unhealthy eating habits and stressful lifestyles has greatly amplified the development of a wide variety of chronic illnesses like coronary heart disease, obesity, and cancer [8]. Consequently, the demand for functional foods has increased because consumers are becoming more aware of the food's impact on their health. Functional foods are defined as naturally occurring or industrially processed foods that, when regularly ingested at appropriate levels as part of a varied diet, may have health benefits that go beyond providing basic nutrition, such as preventing and treating diseases [9]. Tomatoes (*Solanum lycopersicum* L.) and tomato-based products are good examples of these so-called "functional foods" because they are a great source of bioactive compounds such as phytosterols, tocopherols, phenolics, ascorbic acid, folates, and, carotenoids [10]. To meet the growing demand from growers, processors, and consumers for high-quality, nutrient-dense food, a large number of new tomato cultivars with elevated lycopene levels called high-lycopene tomato cultivars (e.g. H1311, HLY18, HLT-F61, Kalvert, etc.), have been developed using plant breeding techniques [11]. High-lycopene tomatoes, with their genetically enhanced content of bioactive compounds, are a valuable resource for enhancing the nutritional, sensory, and health-related properties of tomato-based processed foods. Experimental evidence has demonstrated that lycopene, the predominant tomato carotenoid, is highly effective in scavenging free radicals. Lycopene has the highest capacity to eliminate singlet oxygen than all other carotenoids with an antioxidant potential reported to be twice that of beta-carotene [10].

Tomato peel, usually discarded during tomato processing, contains a higher content of the antioxidant lycopene than the pulp and seeds [12]. Because of tomato peel's high concentration of antioxidants such as flavonoids, phenolic compounds, and lycopene, it has an excellent nutritional value, and its consumption lowers the risk of chronic diseases. The utilization of proteins derived from sources is deemed necessary when animal proteins are unable to meet the demand of the world's population [13]. Pea protein is a sustainable alternative protein source that is affordable, hypoallergenic, and nutritious, and provides health benefits such as antihypertensive properties and the ability to regulate intestinal flora activity. Pea protein can be used in foods as an isolate (> 80% protein content) or as a concentrate (< 80% protein content) [14]. Green olives used to produce olive powder (OP) are rich in phenolic compounds and their regular intake helps the prevention of chronic illnesses (e.g. cancer and cardiovascular diseases) [15]. As such, the use of these ingredients (high-lycopene tomato pulp, high-lycopene tomato peel, pea protein, and olive powder) in the development of functional tomato sauce combines their health-promoting properties in a single product. Developing healthier and functional foods is a promising strategy per the United Nations 2030 Agenda for Healthy and Sustainable Human Nutrition [16]. Besides health and nutritional benefits, sensory properties are of significant importance when developing functional foods to ensure that the developed products are appealing to consumers. Thus, this study aimed to optimize the formulation of a tomato sauce enriched with pea protein, olive powder, and tomato peel powder in terms of sensory acceptability using a D-optimal mixture design.

The novelty of the study lies in exploring the possibility of obtaining a sensory-acceptable tomato sauce via the combination of pea protein (a sustainable source of alternative protein), freeze-dried olive powder, and tomato peel powder (made from a by-product of the tomato-processing industry).

2. Materials and Methods

2.1. Experimental Design

The Design Expert 13.0.5 (DX13) software (Stat-Ease, Inc., Minneapolis, MN, USA) was used for designing the experiment, analyzing the responses (scores of each sensory attribute), and obtaining the best formulation. A D-optimal design was used to select an ideal number of experiments and to generate the initial set of mixtures.

Experimental input parameters included the mixture components, namely TomP (X_1), PP (X_2), OP (X_3), and TPP (X_4). To ensure that the experimental results would be satisfactory, the center points of the design were carefully chosen, with the ingredients being kept at levels that were expected to produce satisfactory results. As such, the lower and upper limits of each mixture component were selected based on preliminary experiments. Table 1 presents the experimental design, including coded values, actual values, and quantity ranges of the mixture components. Color (Y_1), aspect (Y_2), aroma (Y_3), texture (Y_4), taste (Y_5), sourness (Y_6), and aftertaste (Y_7) were the output parameters (responses) influenced by variations in the proportions of input parameters (mixture components).

The final design consisting of 24 formulations (runs) based on a four-component system was obtained (Table 2) and the formulations were partitioned into four blocks of six runs that each contained a duplicate.

Table 1. Ranges of independent variables applied in D-optimal mixture design. TomP (X_1), PP (X_2), OP (X_3), and TPP (X_4)

Component	Name	Low actual (%)	Coded Low	High Actual (%)	Coded High
X_1	TomP	88.50	0	100.00	1.00
X_2	PP	0.00	0	3.00	0.26
X_3	OP	0.00	0	5.00	0.43
X_4	TPP	0.00	0	10.00	0.87

Table 2. Optimal mixture design generated by software Design-Expert

Run	X_1 (%)	X_2 (%)	X_3 (%)	X_4 (%)
1	88.50	1.50	0.00	10.00
2	94.32	2.17	1.08	2.42
3	88.50	3.00	2.50	6.00
4	96.00	1.50	2.50	0.00
5	91.82	2.17	3.58	2.42
6	95.00	0.00	0.00	5.00
7	91.75	0.00	5.00	3.25
8	88.50	1.50	0.00	10.00
9	88.50	1.50	5.00	5.00
10	88.50	0.00	1.50	10.00
11	92.00	3.00	5.00	0.00
12	91.65	1.35	2.15	4.85
13	100.00	0.00	0.00	0.00
14	88.50	3.00	2.50	6.00
15	90.07	0.67	1.82	7.43
16	95.00	0.00	5.00	0.00
17	91.65	1.35	2.15	4.85
18	91.65	1.35	2.15	4.85
19	97.00	3.00	0.00	0.00
20	100.00	0.00	0.00	0.00
21	92.00	3.00	5.00	0.00
22	91.65	1.35	2.15	4.85
23	88.50	1.50	5.00	5.00
24	92.75	3.00	0.00	4.25

2.2. Ingredients

The fully ripe red tomatoes of the high-lycopene variety 'H1657' cultivated in Ribatejo (Portugal) were included in the present study. The tomato fruits were peeled and processed into pulp using a Thermomix TM6-1 (Vorwerk, Elektrowerke GmbH & Co. KG, Wuppertal, Germany). TPP production involved peeling tomatoes, drying peels at 50°C for 1 day in the oven (U50, Memmert GmbH + Co. KG, Büchenbach, Germany), and conversion to powder using a centrifugal mill (ZM1, Retsch GmbH, Haan, Germany). The OP was produced using a process that enables keeping its oil and phenolic content in a stable form resulting in particle size smaller than conventional powders, and this involves blending pitted olives with water, followed by high-pressure homogenization (HPH) at 1000 bars in the homogenizer (Panda Plus, GEA Niro Soavi, Parma, Italy). The obtained slurry was freeze-dried and converted to powder in a centrifugal mill (ZM1, Retsch GmbH, Haan, Germany). The PP (100% vegan) was purchased from Vegrano.

2.3. Preparation of Functional Tomato Sauce

A block of six mixtures was prepared each week. A hot break pretreatment was performed by heating the tomato pulp at 85 °C in a water bath and immediately transferring it to an ice water bath at 4 °C to halt the hot break process. Components X_1, X_2, X_3, and X_4 were added according to Table 2 to prepare the formulations. The mixtures were homogenized using a hand blender and transferred to the HPH (Panda Plus, GEA Niro Soavi, Parma, Italy), for further homogenization at a pressure of 100 bar (first stage) and 500 bars (second stage).

2.4. Sensory Evaluation

Untrained panelists were invited to participate voluntarily in the sensory analyses that took place in the Sensory Analyses Laboratory of the Food Engineering Department (University of Algarve, Faro, Portugal) which includes individual tasting booths with serving windows and controlled lighting. Although the panelists have not been specifically trained in our lab to taste our formulations, the panelists are all regular tasters of our Sensory Evaluation Laboratory and usual consumers of many tomato products, from fruits to soups and sauces as well as olives given that Portugal is a Mediterranean country and tomato and olives are main products of the Mediterranean diet, and their tastes are well known by the consumers in Portugal. The samples were subjected to sensory analysis on a 6-point hedonic scale by an untrained panel of 19 individuals composed of males and females aged between 18 and 60. The sensory panel evaluated one block of six samples per week. Panelists were presented with samples labeled with 3-digit codes. They were asked to rate their preferences according to Table 3 for each one of the responses/sensory attributes: color (Y_1), aspect (Y_2), aroma (Y_3), texture (Y_4), taste (Y_5), sourness (Y_6) and aftertaste (Y_7). Tap water was made available to the tasters to clear the taste between samples.

Table 3. Hedonic scale used for the evaluation of the sensory descriptors

Code	Description
1	Dislike extremely
2	Dislike moderately
3	Dislike slightly
4	Like slightly
5	Like moderately
6	Like extremely

Table 4 below describes the sensorial attributes used to evaluate the tomato sauce formulations in the sensory analysis.

Table 4. Sensory attributes and their corresponding definitions

Sensory attribute	Description
Color (Y_1)	Evaluation of the intensity of the red coloration
Aspect (Y_2)	The visual appearance of the tomato sauce
Aroma (Y_3)	Any property perceived by the olfactory sense (odor)
Texture/Flow (Y_4)	The degree of resistance to movement/flow, evaluated by the speed at which the sauce sample flows when poured from a spoon.
Taste (Y_5)	The sensation of flavor experienced when tomato sauce comes in contact with the tongue and mouth
Sourness (Y_6)	The sour taste that is detected on the tongue due to acid
Aftertaste (Y_7)	Taste lingering in the individual's mouth after they had tasted the sauce

2.5. Analyses of Responses (Fitting for the Optimal Model)

The obtained sensory scores were subjected to statistical analysis using Design-Expert 13.0.5 (Stat-Ease Inc., Minneapolis, United States). The polynomial models that best described the experimental results for each response were selected based on the goodness of fit estimated by analyses of variance (ANOVA). The adequacy of the suggested response models was evaluated according to model P-value (Prob > F), adjusted R^2 values, and lack of fit F-test. The P-values of models lower than 0.05 (95% confidence interval) indicate that the models are statistically significant. The closer the adjusted R^2 value to the predicted R^2 (an indicator of the model's ability to predict), the better the fit. Lack of fit measures how well the model fits the data. If the observed lack of fit F-value is significantly larger, it suggests a lack of fit. The response analysis was followed by optimization which involves finding out where the best ingredient proportions lie.

2.6. Formulation Optimization

Numerical optimization was performed to find the best formulation for the functional tomato sauce using Design Expert (DX13). In search of the optimum formulation, the desirability function D (Eq. 1) was applied by combining pre-determined desirability values (d_i) of each response (Y_i) [17].

$$D = \left(d_1(Y_1)d_2(Y_2) \ldots d_k\ (Y_k)\right)^{\frac{1}{k}} \quad (1)$$

where D is the overall desirability, k denotes the number of responses and d denotes the desirability values.

When selecting the responses' desirability ranges, the goal was to achieve concentrations above 1% for the powdered ingredients (OP, PP, and TPP) to boost the functionality of the sauce in such a way that the sensory acceptability would not be compromised. For this reason, estimates corresponding to hedonic scores within the range of 3 to 6 were imposed for aspect, aroma, flow, taste, and aftertaste with the goal of maximizing the hedonic scores (Table 5). For sourness, a hedonic score range of 2.70 to 4.0 was imposed and no goal was specified following the assumption that sourness can be evaluated simultaneously with the taste parameter. Also, a target score of 2.38 was imposed for color. Then, the search algorithm of the optimization tool was used to obtain the optimum formulation.

2.7. Tolerance Test

The tolerance test involved frequently evaluating the sensory properties of the optimum sauce. Three sensory analysis sessions of the optimum sauce were conducted at a frequency of one tasting per week, for three weeks by an untrained panel of 13 individuals who evaluated the desirability for color (Y_1), aspect (Y_2), aroma (Y_3), texture (Y_4), taste (Y_5), sourness (Y_6) and aftertaste (Y_7) as previously described in 2.4.

2.8. Trained Panel Test

Six trained tasters from SELUZ Fragrance & Flavor Company (Istanbul, Türkiye) evaluated the optimum sauce in three steps according to the consensus method described in the flavor profile methodology (ISO 6564:1985). The sauce was stored at 4 °C until the tasting session. In the first session, flavor terms (attributes) in the products were determined by the trained panel consensus and a list of definitions corresponding to each term was used (Table 6).

Each panelist then evaluated the product to rate the perception intensity of each attribute. A 0-5 scale was used in the evaluations (0: not perceived at all; 1: barely detected; 2: poorly perceived; 3: moderately perceived; 4: perceived; and 5: highly perceived). In the last session, all answers obtained were re-analyzed by the panel as a group to reach a unanimous description of the sauce's flavor profile. The flavor profile results were used to generate a spider diagram.

Table 5. Desirability ranges for formulation optimization

Response	Goal	Lower Limit	Upper Limit
Color (Y_1)	is target = 2.38	2.38	4.53
Aspect (Y_2)	maximize	3	6
Aroma (Y_3)	maximize	3	6
Texture/Flow (Y_4)	maximize	3	6
Taste (Y_5)	maximize	3	6
Sourness (Y_6)	none	2.70	4
Aftertaste (Y_7)	maximize	3	6

Table 6. List of flavor terms (attributes) agreed upon by the trained panel and their corresponding definitions

Term	Definition
Sweetness	Taste associated with sugars
Saltiness	Taste associated with sodium ions
Sourness	Taste associated with acids
Fresh tomato taste	Degree of freshness of the product perceived by visual estimation, the tomato taste with green/raw notes
Tomato skin or peel taste	Flavor of the outermost membrane of a ripe red tomato
Tomato paste taste	Perception of the cooked product in the nasal region, with intense fruit flesh notes and characteristic flavor
Olive taste	Characteristic flavor (taste and smell) to olives
Astringency	The taste perceived on the tongue and palate specific to products with high sourness
Off-taste	Flavor that is not expected to be in the characteristic flavor profile of the product
Overall acceptance	The overall balance of all taste characteristics of the product

2.9. Physicochemical Analyses

2.9.1. Lycopene and Beta-carotene Analyses

Analyses were done by HPLC based on a modified method previously reported [18]. Approximately 1 g sample (*e.g.* optimum tomato sauce formulation, tomato fruit, and tomato pulp) was weighed into a 15 ml falcon tube and extracted with 5 ml methanol, briefly mixed using a vortex mixer, sonicated for 30 seconds in an ultrasonic bath (Branson 3510, Branson Ultrasonics Corporation, Danbury, Connecticut, United States) and centrifuged for 5 minutes at 4000 RPM (Cencom 2, J.P. Selecta, Barcelona, Spain). The supernatant (methanolic extract) was decanted and reserved. The pellet was re-extracted with 5 ml hexane–acetone (1:1, v/v), briefly mixed, sonicated for 30 seconds, and centrifuged for 5 minutes at 4000 RPM. The extraction was repeated until the pellet was colorless. The supernatant was added to the methanolic extract (reserved supernatant). The combined supernatants were mixed with 10 ml of ultrapure water to induce phase separation. Then, 1 ml from the upper nonpolar (organic) hexane phase containing carotenoids was transferred into a 2 ml transparent Eppendorf tube wrapped in aluminum foil. The upper nonpolar (organic) hexane phase was dried under nitrogen gas. Dried samples were analyzed immediately or stored at -20°C until analysis.

For carotenoid quantification, samples were re-suspended in 1 ml ethanol-dichloromethane (1:1, v/v). The solutions were filtered through a membrane (PTFE, 0.45 µm) and 20 µl was injected into a HPLC system equipped with a diode array detector (DAD) (Jasco MD 2015 Plus, Tokyo, Japan). Isocratic separation was done on a C30 column (3µm x 150 mm x 4.6 mm) attached to a guard column (Surf C30, InChem, Voisins le Bretonneux, France) using the mobile phase; methyl tert-butyl ether, acetonitrile, and methanol (50:15:35, v/v/v). The HPLC analysis was performed for 15 minutes at a flow rate of 0.5 ml/min and the detection wavelength was set to 200-600 nm. The analyzed carotenoids were identified by comparing their retention times with those of the lycopene and beta-carotene standards. All determinations were replicated three times.

2.9.2. Total Phenolic Content

Ethanolic extracts obtained from the samples were used for measurements of total phenolic content and antioxidant activity. Briefly, 2 g sample and 15 ml ethanol 70% were mixed in an ultraturrax at 10000 rpm. The mixture was centrifuged at 5000 rpm for 5 minutes and used for measurements immediately or stored at -20 °C until analysis.

Phenolic content was quantified spectrophotometrically based on the method in [19]. For each sample, a mixture of 200 µl of ethanolic extract, 1000 µl Folin-Ciocalteu solution (90%, v/v), and 800 µl aqueous sodium carbonate (7.5%, w/v) was prepared in this order. The mixture was allowed to rest for 30 minutes in the dark. The absorbance of samples was determined at 765 nm using a ultraviolet–visible (UV-Vis) spectrophotometer (Shimadzu model UV-160A, Shimadzu Corporation, Kyoto, Japan). Phenolic content results were estimated in milligrams of gallic acid equivalents (GAE) per 100 grams (mg GAE / 100 g) using a gallic acid (0 - 100 µg/ml) calibration curve.

2.9.3. Antioxidant Activity

The radical scavenging activity against 2,2-azinobis(3-ethylbenzothiazoline)-6-sulfonic acid) (ABTS•+) cation was determined based on a method described by [20] with some modifications. Potassium persulphate (2.47 mM) and ABTS•+ (7 mM) were mixed and kept at room temperature for 16 hours in a dark environment to produce ABTS•+ cation. The ABTS•+ cation was diluted in ethanol 96% to obtain an absorbance of 0.70-0.80 at 735 nm. An aliquot of 20 µl of sample extract was added to 1980 µl of the cation solution. After six minutes of room temperature incubation, the decolorization resulting from cation reduction by the sample's antioxidants was measured at 735 nm. ABTS•+

scavenging activity was estimated using a Trolox (6 hydroxy-2,5,7,8-trimethyl-chroman-2-carboxylic acid) calibration curve (0-2000 µM) and the results were expressed as µmol/100 g Trolox equivalents (µmol/100 g TE).

Analysis of the Ferric Reducing Antioxidant Power (FRAP) was performed according to the modified procedure in [21]. The FRAP reagent was prepared by combining acetate buffer at pH 3.6 (300 mM), 2,4,6-Tri-(2-pyridyl)-s-triazin (TPTZ) (10 mM in HCl (40 mM)), and $FeCl_3$ (20 mM in distilled water) in a solution ratio of 10:1:1 (v/v/v), respectively. The FRAP reagent (1200 µl) was mixed with 40 µl of tomato product extract. Sample blanks were prepared simultaneously by mixing 40 µl of each sample with 1200 µl of a reagent blank that contained ultrapure water, 20 mM $FeCl_3.6H_2O$, and 300 mM acetate buffer in a ratio of 10:1:1 (v/v/v). The samples' absorbances were measured at 593 nm after incubation at 37 °C for 15 minutes. Standard solutions of $FeSO_4.7H_2O$ (0-2000 µM) were used for calibration, and the results were presented in µmol/100 g $FeSO_4.7H_2O$ (Fe[II]) equivalent.

2.10. Statistical Analyses

The phenolic, lycopene, beta-carotene, and antioxidant activity results were expressed as mean ± standard deviation. Variations in the contents of phytochemicals (phenol, lycopene, and beta-carotene) and antioxidant activity of the tomato and derived products were evaluated by ANOVA with Tukey HSD post hoc test (when a significant difference was observed) at a significance level of 0.05 ($P<0.05$). Analyses were performed using IBM SPSS Statistics 29.0.2.0 (IBM® Co., USA).

3. Results & Discussion

3.1. Response Analyses and Model Development

The hedonic rating (mean ± standard deviation) of each response is presented in Table 7. The obtained sensory scores were used to generate polynomial models for each response variable that enabled the production of the surfaces and contour plots for each response.

Table 7. Responses (sensory analysis scores) for the mixtures of tomato sauce recipes

Run	TomP	PP	OP	TPP	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6	Y_7
	X_1 (%)	X_2 (%)	X_3 (%)	X_4 (%)	Color	Aspect	Aroma	Texture	Taste	Sourness	Aftertaste
1	88.50	1.50	0.00	10.00	4.5±1.3	4.4±1.2	3.8±1.1	3.5±1.3	2.8±1.4	2.7±1.3	2.6±1.3
2	94.32	2.17	1.08	2.42	4.0±1.3	3.6±1.3	3.7±1.3	3.6±1.3	3.6±1.4	3.9±1.1	3.6±1.3
3	88.50	3.00	2.50	6.00	3.6±1.4	3.3±1.4	3.1±1.3	3.6±1.4	2.8±1.6	3.0±1.4	3.0±1.3
4	96.00	1.50	2.50	0.00	2.4±1.4	2.3±1.2	3.±1.3	2.6±1.3	2.9±1.1	3.2±1.4	3.0±1.2
5	91.82	2.17	3.58	2.42	3.2±1.2	3.1±0.9	3.6±1.1	3.6±0.8	3.3±1.0	3.6±0.9	3.4±1.2
6	95.00	0.00	0.00	5.00	4.5±1.2	4.4±1.2	4.3±1.2	4.3±1.0	3.9±1.2	4.0±1.1	4.1±1.0
7	91.75	0.00	5.00	3.25	3.3±1.2	3.2±1.1	3.3±1.1	3.5±1.4	2.6±1.1	2.9±1.3	2.7±1.4
8	88.50	1.50	0.00	10.00	4.2±1.5	3.9±1.5	3.8±1.3	3.7±1.5	2.9±1.4	2.7±1.2	2.5±1.3
9	88.50	1.50	5.00	5.00	3.8±1.3	4.0±1.1	3.5±1.4	4.0±1.4	3.0±1.6	3.0±1.4	3.3±1.6
10	88.50	0.00	1.50	10.00	3.7±1.5	3.1±1.7	3.4±1.3	3.1±1.5	3.1±1.2	2.9±1.2	2.9±1.4
11	92.00	3.00	5.00	0.00	3.1±1.2	3.1±1.0	2.9±1.0	3.0±1.1	3.0±1.2	3.3±1.0	3.0±1.1
12	91.65	1.35	2.15	4.85	4.5±0.9	4.4±0.8	3.8±1.5	4.0±1.1	3.3±1.5	3.2±1.5	3.4±1.6
13	100.00	0.00	0.00	0.00	3.7±1.6	2.8±1.4	3.2±1.2	2.5±1.3	2.8±1.1	3.4±1.5	3.3±1.4
14	88.50	3.00	2.50	6.00	3.8±1.4	3.4±1.4	3.4±1.4	3.5±1.3	3.2±1.3	3.1±1.1	3.0±1.2
15	90.07	0.67	1.82	7.43	3.5±1.4	3.4±1.2	3.5±1.1	3.7±1.4	2.9±1.2	2.9±1.2	2.8±1.3
16	95.00	0.00	5.00	0.00	3.1±1.2	3.1±1.3	3.1±1.2	2.8±1.1	3.0±1.4	3.3±1.5	3.1±1.5
17	91.65	1.35	2.15	4.85	4.±1.3	4.0±1.4	3.9±1.1	3.9±1.2	3.3±1.3	3.6±1.1	3.1±1.4
18	91.65	1.35	2.15	4.85	4.4±1.0	4.2±0.8	4.1±0.8	4.2±0.7	3.9±1.0	3.8±1.1	3.8±1.1
19	97.00	3.00	0.00	0.00	2.5±1.4	2.4±1.2	2.8±1.4	2.4±1.3	2.6±1.5	3.0±1.4	2.9±1.4
20	100.00	0.00	0.00	0.00	3.8±2.0	2.9±1.3	3.3±1.2	2.6±1.1	3.1±1.2	3.2±1.3	3.3±1.4
21	92.00	3.00	5.00	0.00	2.5±1.3	2.8±1.1	3.2±1.1	2.8±1.2	2.7±1.3	3.2±1.2	2.9±1.3
22	91.65	1.35	2.15	4.85	4.1±1.2	4.0±1.2	3.7±1.4	3.7±1.2	3.5±1.3	3.5±1.2	3.6±1.4
23	88.50	1.50	5.00	5.00	2.4±1.1	2.4±1.2	3.3±0.9	2.9±1.2	2.8±1.5	2.9±1.4	2.8±1.4
24	92.75	3.00	0.00	4.25	4.5±1.2	4.3±1.3	3.8±1.3	4.1±1.1	3.3±1.2	3.2±1.2	3.1±1.4

The mathematical models that were fitted to each of the responses are shown in Table 8. Response variables were explained through special cubic and reduced quadratic models. The statistical analysis results showed that the suggested models were statistically significant (P<0.05), showing that the models adequately describe the responses. The fitted equations of Y_4, Y_5, and Y_7 showed greater prediction accuracy because their adjusted R^2 values were closer to the predicted R^2. The lack of fit test was performed to evaluate the fitness of the response models. Table 8 shows that the reduced quadratic models of Y_2, Y_3, Y_4, Y_5, Y_6 and Y_7 passed the lack of fit test because the F-value shows that the lack of fit is not significant, indicating that the suggested models fit the actual data within the upper and lower limits that were studied while the special cubic model of Y_1 failed the lack of fit test. The significant lack of fit for color (Y_1) could be because of a little color variation between different formulations not perceived by the panelists. [22]. It is usual to have some level of lack of fit in mixture design models that could not be completely accommodated. At this point, it is necessary to verify whether the amount of lack of fit is important or of concern [23]. The lack of fit did not appear large enough to raise significant concerns. Additionally, findings could be significant from a statistical point of view but may still be of no or little importance among researchers and may not necessarily be relevant to the real world [24]. Since the Y_1 model could not be further improved and statistically significant results do not necessarily mean that the results will have a substantial impact in practice, the model was used for the optimization to determine the best ingredients' proportions.

Table 8. Statistical parameters of polynomial models built for determining the optimal levels of ingredients. P<0.05 indicates that the model terms are significant. The significant interactions between model terms are highlighted in bold in the model equation column

Response	Model Equation	Type of Model	Model	Lack of Fit	R^2 adj	R^2 pred
Color (Y_1)	$+4.32*X_1-10.41*X_2-9.25*X_3+2.90*X_4+11.45*X_1*X_2+17.49*X_1*X_3+3.98*X_1*X_4+106.02*X_2*X_3+24.58*X_2*X_4+21.82*X_3*X_4-214.79*X_1*X_2*X_3+29.84*X_1*X_2*X_4+18.47*X_1*X_3*X_4-180.02*X_2*X_3*X_4$	Special cubic	F =5.06 P-value=0.0195 Significant	F=8.43 P-value=0.033 Significant	0.73	/
Aspect (Y_2)	$+2.88*X_1+1.03*X_2+4.55*X_3+3.44*X_4-2.92*X_1*X_3+7.21*X_1*X_4+6.94*X_2*X_4-4.42*X_3*X_4$	Reduced Quadratic Model	F=7.15 P-value= 0.0012 Significant	F=5.21 P-value=0.063 Not significant	0.68	0.016
Aroma (Y_3)	$+3.22*X_1-5.31*X_2+3.06*X_3+3.40*X_4+9.26*X_1*X_2+3.92*X_1*X_4+12.22*X_2*X_3+13.30*X_2*X_4-1.41*X_3*X_4$	Reduced Quadratic Model	F= 12.23 P-value=0.0001 Significant	F=1.66 P-value=0.329 Not significant	0.82	0.50
Texture/Flow (Y_4)	$+2.46*X_1+2.29*X_2+2.01*X_3+3.22*X_4+2.40*X_1*X_3+6.81*X_1*X_4+3.29*X_2*X_3+4.24*X_2*X_4$	Reduced Quadratic Model	F= 36.11 P-value=<0.0001 Significant	F=1.98 P-value=0.265 Not significant	0.93	0.82
Taste (Y_5)	$+3.07*X_1-9.46*X_2+2.21*X_3+2.22*X_4+14.51*X_1*X_2+4.81*X_1*X_4+19.05*X_2*X_3+19.17*X_2*X_4$	Reduced Quadratic Model	F=8.66 P-value=0.0007 Significant	F=0.24 P-value=0.960 Not significant	0.73	0.51
Sourness (Y_6)	$+3.44*X_1-4.41*X_2+2.44*X_3+2.79*X_4+8.57*X_1*X_2+3.42*X_1*X_4+13.52*X_2*X_3+8.87*X_2*X_4$	Reduced Quadratic Model	F=9.18 P-value=0.0004 Significant	F=0.61 P-value=0.756 Not significant	0.74	0.47
Aftertaste (Y_7)	$+3.49*X_1+1.54*X_2+1.65*X_3+2.62*X_4+4.39*X_1*X_4+5.69*X_2*X_3+3.12*X_3*X_4$	Reduced Quadratic Model	F=7.56 P-value=0.0009 Significant	F= 0.60 P-value=0.769 Not significant	0.66	0.40

The mathematical models (Table 8) were used to build contour plots (Figure 1) to visualize the effect of the components (X_1, X_2, X_3, X_4) on the responses by altering the proportion of the mixture components within the studied ranges (Table 1). Significant interactions between the independent factors (mixture components) are indicated by plots with elliptical (oval-like) contours, and the center of the smallest ellipse denotes a maximum or minimum response point. Plots with circular contours also demonstrate the minimal interactions between the independent factors. All points in the contour plot that have a similar response are connected to produce contour lines of constant responses [25].

Figure 1 shows the contour plots of the responses (Y_1 to Y_7) based on their corresponding models in Table 8. In the figure below, the predicted hedonic scores are shown as a function of the combination of the mixture components when X_1 is set at 95% (a), 93.5% (b), and 91.5% (c) to illustrate the variation in response scores at different values of X_1, X_2, X_3, and X_4. For example, Y_2a in the figure represents the contour plot of Y_2 at X_1 concentration of 95% and Y_2b is the contour plot of Y_2 at X_1 of 93.5.

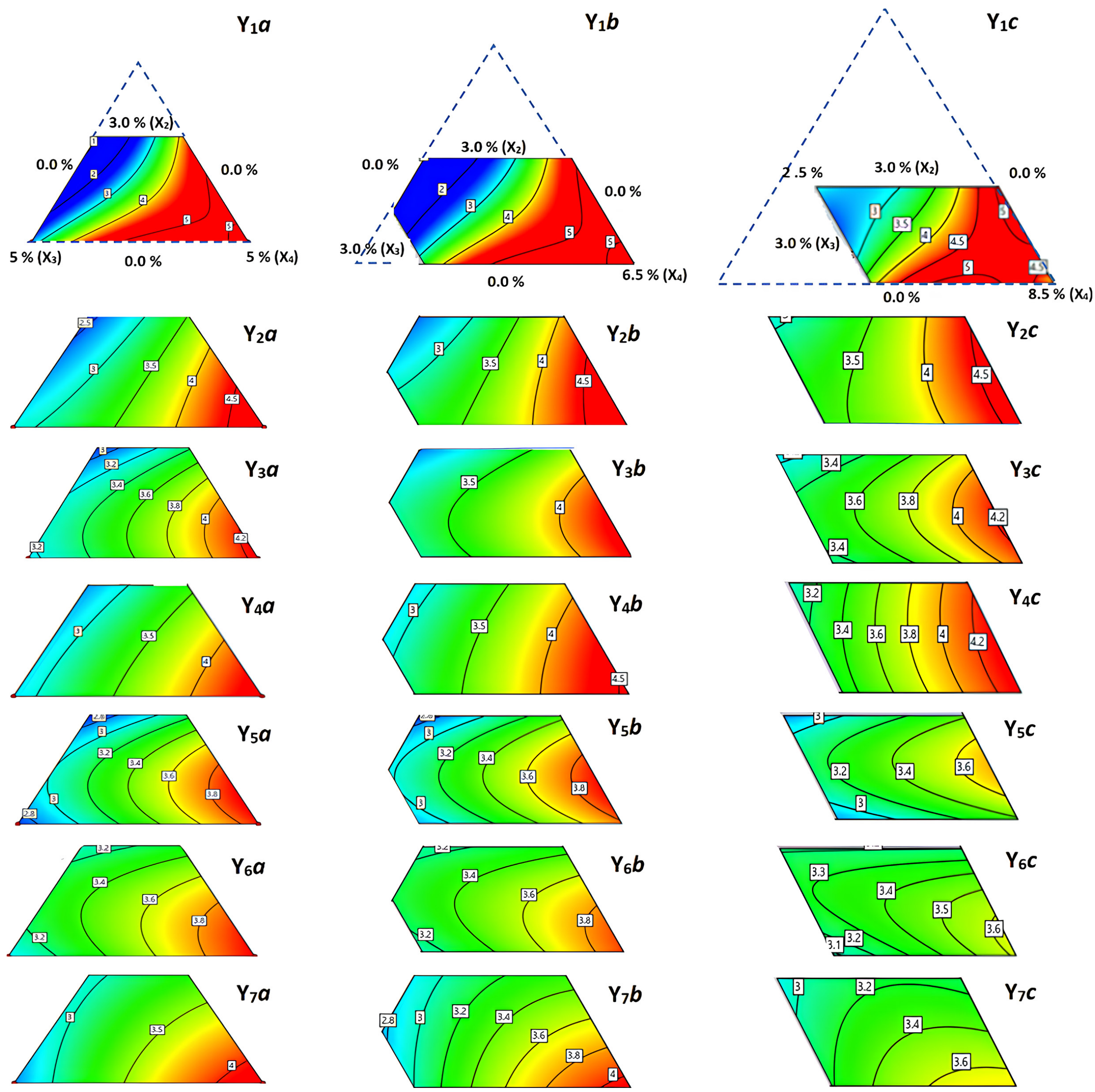


Figure 1. Contour diagram of responses (color (Y_1), aspect (Y_2), aroma (Y_3), texture (Y_4), taste (Y_5), sourness (Y_6), and aftertaste (Y_7)) when TomP (X_1) is set at different proportions. a=X_1 at 95% (X_4 from 0 to 5%), b=X_1 at 93.5% (X_4 from 0 to 6.5%), c=X_1 at 91.5% (X_4 from 0 to 8.5%). The red coloration corresponds to higher response values (higher acceptability) while the blue coloration corresponds to lower response values

Figure 1 shows that higher response scores are predicted with increasing concentration of X_1 from 91.5% to 95%, suggesting that increasing the quantity of tomato pulp within the range of 93-95% in particular, results in products with higher preference scores. This shows the importance of preserving the natural tomato taste of the sauce despite enrichment with PP, OP, and TPP. In addition, the desirability of the formulations decreases as the OP concentration increases when X_1 is at 95% and 93.5%. It can be seen from the contour plots that to obtain higher predicted scores for the responses, the OP (X_2) and PP (X_3) concentrations should be closer to lower limit values within the studied range. A possible explanation for this is that high concentrations of OP might increase the bitterness of the product. Phenolic compounds in the fruit are the primary cause of the characteristic bitter taste of olives. Oleuropein, the most abundant polyphenol in fresh olives, is primarily responsible for the bitterness of olives [26]. Lower preference for high PP concentrations could be associated with the characteristic beany flavor of pea typically caused by aldehydes, mainly hexanal, which is the most commonly reported compound responsible for this undesirable aroma of pea proteins [14]. This observation implies that it will be preferable to use lower OP and PP concentrations (e.g. 1-2%) to minimize the perception of the bitterness and beany flavor.

The peak region (corresponding to higher response scores) becomes wider as the TPP concentration varies from 8.5% to 5%, indicating that the formulations with TPP quantities closer to the center value (5%) within the studied range in Table 1 have higher predicted acceptability scores and will be more acceptable compared to those having TPP in quantities closer to the upper limit (e.g. 8.5%). These findings are in agreement with a study [27] that reported higher acceptability of a commercial sauce characterized by a lower consistency compared to sauces enriched with 10% and 15% whole tomato powder characterized by a strong consistency. This may be due to the desirable thickness and red coloration brought on by the incorporation of moderate TPP quantities. During the preparation of the sauces, it was noticed that TPP increases the thickness of the sauce compared to the other ingredients. The increase in viscosity can be attributed to the pectin content in tomato peels since tomato products with a higher pectin content have a higher viscosity [28]. In tomato sauce, hydrocolloids in pectin can thicken the sauce, increasing its viscosity. Moreover, the methoxylation degree, methoxylation pattern, degree of branching, size, composition, and conformation of pectin may affect the viscosity of tomato products by altering the interactions between pectin chains [29]. Additionally, a recent study found that TPP's soluble fibers reduce the mobility of water, thereby increasing the consistency of tomato juice [28].

3.2. Formulation Optimization and Tolerance Test

The second stage of this research involved obtaining the optimum tomato sauce formulation based on sensory properties by determining the optimum levels of ingredients using the desirability technique. The "desirability function" method introduced by Derringer and Suich in 1980 is commonly used in industries to optimize multiple factors simultaneously [17]. The idea is that if the quality of a product doesn't reach a minimum threshold, it is considered unacceptable. To achieve the overall Desirability (D), it is required to build a multi-response optimization by transforming individual responses into their associated desirabilities (d_i) [30]. As a compromise among all the goals imposed in Table 5, six formulations were generated by DX13 and are shown in Table 9. Formulation 1 (93.06% TomP, 1.82% PP, 1.66% OP and 3.47% TPP) was the optimum formulation of the functional tomato sauce because it yielded the highest acceptability scores for the sensory attributes resulting in a higher desirability score. Figure 2 shows the ramps of each sensory attribute for the optimum sauce and the respective d_i obtained for the optimum formulation.

Table 9. Suggested ingredient proportions of optimization study to develop the functional tomato sauce and predicted acceptability scores of sensory parameters of the suggested formulations (Formulation 1 in bold was selected)

Formulation number	TomP	PP	OP	TPP	Color (Y_1)	Aspect (Y_2)	Aroma (Y_3)	Flow (Y_4)	Taste (Y_5)	Sourness (Y_6)	After taste (Y_7)
1	**93.06**	**1.82**	**1.66**	**3.47**	**4.04**	**3.83**	**3.80**	**3.82**	**3.54**	**3.54**	**3.45**
2	93.89	2.05	1.00	3.06	4.17	3.85	3.78	3.80	3.55	3.55	3.46
3	93.67	1.00	2.38	2.95	4.19	3.69	3.75	3.71	3.48	3.55	3.49
4	92.27	3.00	1.00	3.73	3.91	3.87	3.61	3.84	3.26	3.25	3.23
5	96.09	1.00	1.00	1.91	4.35	3.62	3.72	3.50	3.55	3.66	3.60
6	90.05	1.00	1.00	7.95	4.41	4.04	3.87	3.87	3.23	3.27	3.20

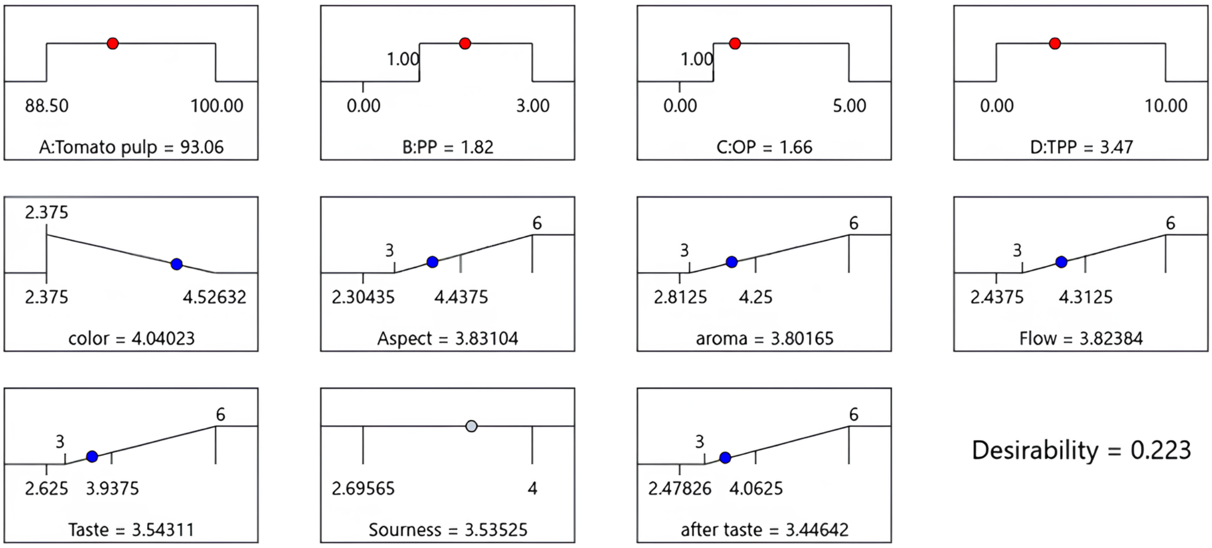


Figure 2. Ramps of the sensory responses with predicted scores of the optimum functional tomato sauce formulation (93.06% TomP, 1.82% PP, 1.66% OP, and 3.47% TPP)

After optimization, a tolerance test was performed with the optimized formulation by conducting sensory analyses to evaluate product acceptability in a real-world situation and compare experimental data to the predicted acceptability scores generated by the polynomial models. Tolerance tests are conducted to determine if a consumer's preferences would alter after being repeatedly served a particular item [31]. The results of the tolerance test carried out once per week for three weeks are summarized in Table 10. The level of acceptance for aroma and sourness showed little variability with the consumption frequency. Taste preference, on the other hand, showed a greater variability between tasting sessions. In week 1, Y_4 received the highest preference with an average score of 4.6 followed by color, aroma, aspect, sourness, aftertaste, and taste respectively, showing that panelists most likely preferred texture, color, and aroma in week 1. In week 2, color was the most preferred parameter with an average score of 5.2, followed by aspect, flow, aroma, taste, sourness, and aftertaste respectively. In week 3, color received the highest preference. Aspect and aroma received the same mean score of 4.2 in week 1 and week 3.

The differences observed during tasting might be because the developed sauce is a product with a new taste stimulus and the panelists still have low familiarity with this product. Consumers' familiarity with food products has been found to influence their discriminative power regarding their preferences for food and beverages [32]. The findings of a previous study revealed that consumers from Italy and the Czech Republic could more accurately distinguish the quality of foods they were familiar with compared to those they weren't [33]. Besides evaluating variations in product acceptance due to frequent tasting during the tolerance test, the actual sensory scores provided by the panelists for three weeks were compared to the predicted scores provided by the optimization tool of DX13 to ensure that the prediction models were adequate in predicting responses of the optimum sauce formulation. Figure 3 summarizes the mean sensory scores obtained through a 6-point hedonic evaluation method of the best sauce formulation as well as predicted sensory scores generated by DX13.

The week 3 mean sensory scores of the optimum formulation were more similar to the predicted response scores, suggesting that the fitted models were suitable for predicting the sensory scores of the best formulation [34]. Furthermore, the hedonic scores obtained showed satisfactory results because various parameters (e.g. color, aspect, aroma, flow) obtained scores of 4 or higher, showing that they were liked by the panel. Numerous herbs and spices can enhance the taste, aroma, and flavor of food products, including oregano, basil, pepper, cinnamon, and thyme [35]. Nevertheless, it is worth noting that consumers' preferences for a product can be influenced by factors such as ethnicity, education, socioeconomic status, dietary habits, age, and gender. This implies that product characteristics such as viscosity, color, or spiciness can be adjusted to cater to specific market preferences [36]. Taking these factors into consideration is crucial when developing food and beverages to ensure consumer satisfaction and market success. Given that the developed sauce product is destined to be used by consumers as an ingredient in the preparation of various recipes, the overall taste experience could be modified as required when used by different consumers.

Table 10. Results of the tolerance test of the optimum functional sauce formulation. Scores were given on a scale of 1-6

Response	Week 1	Week 2	Week 3
Color (Y_1)	4.4±1.3	5.2±0.8	4.3±1.1
Aspect (Y_2)	4.2±1.3	5.1±1.0	4.2±1.2
Aroma (Y_3)	4.2±0.9	4.6±0.8	4.2±1.1
Texture/Flow (Y_4)	4.6±1.1	4.8±1.0	3.9±1.0
Taste (Y_5)	3.7±1.1	4.0±1.0	3.2±1.3
Sourness (Y_6)	3.9±1.1	3.9±1.1	3.6±1.3
Aftertaste (Y_7)	3.9±1.1	3.8±1.2	3.2±1.5

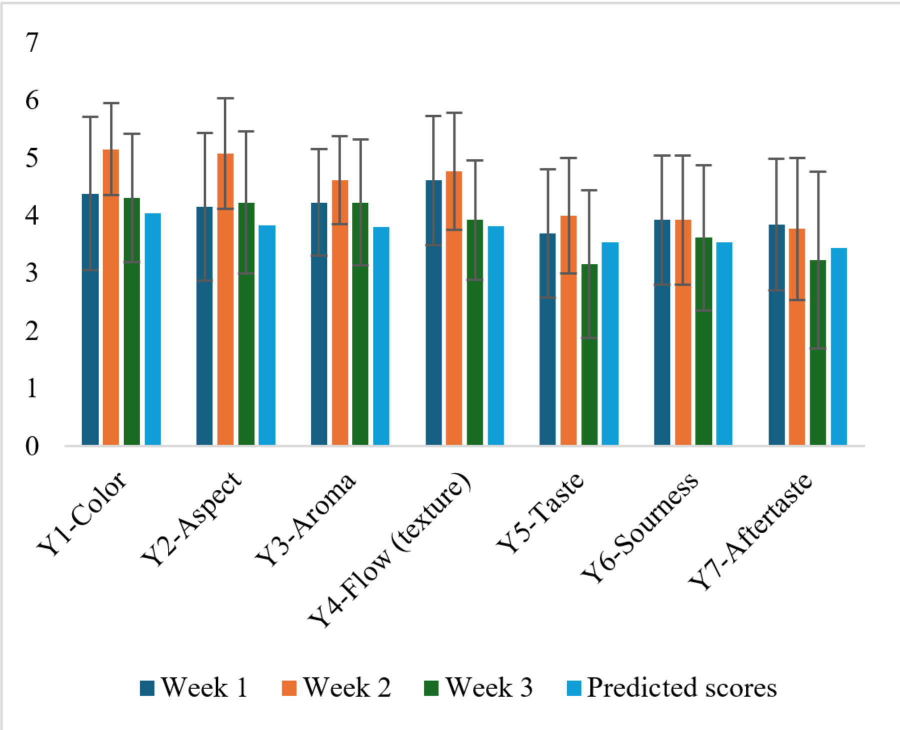


Figure 3. Sensory scores of optimum functional tomato sauce formulation compared to the predicted scores. 1= dislike extremely, 2= dislike moderately, 3= dislike slightly, 4= like slightly, 5= like moderately, 6= like extremely. Bars represent means and error bars represent standard deviations (n=13)

3.3. Trained Panel Test

An overview of the trained panel test results is presented in Figure 4 on a spider diagram. When interpreting the spider diagram, the overall acceptance value was the first parameter verified. The sweetness (3/5), fresh tomato taste (3/5), and tomato peel taste (2.5/5) were the most perceived attributes. Sourness (0.2/5), tomato paste taste (0.3/5), and off-taste (0/5) had the lowest perception intensities. In this product, tomato was perceived as clear and strong, and there was no off-taste perception, and sweetness and saltiness were balanced. However, there were astringent notes perception. Polyphenol-rich foods and beverages such as red wine, tea, and nut skins are frequently responsible for astringency. Tomato peel and olives are rich in phenolic compounds [37], [38]. Therefore, OP and TPP are potential contributors to the astringency perceived in the tasted sauce.

3.4. Lycopene and Beta-carotene Analyses

A significant increase in lycopene and beta-carotene was found in the optimum formulation of functional tomato sauce (Figure 5). The tomato sauce presented higher lycopene and beta-carotene contents than the raw tomato pulp with values of 35.37±1.85 mg/100 g and 5.72±0.52 mg/100 g respectively as compared to values of 23.54±0.76 mg/100 g (lycopene) and 3.03±0.01 mg/100 g (beta-carotene) obtained from the raw tomato pulp. A recent study recorded lower lycopene and beta-carotene concentrations of 5.21 mg/100 g and 1.09 mg/100 g in tomato pulp, respectively [39]. The higher carotenoid concentration in the sauce indicates that the added ingredients contributed to the significant increases in lycopene and beta-carotene, resulting in improved nutritional quality of the product, thus, fitting in a healthier diet after undergoing processing from tomato pulp to sauce. There is an inverse relationship between the development of prevalent human diseases and the regular consumption of fresh tomatoes or tomato-based products. The fruit's carotenoid content, specifically in lycopene and beta-carotene, has been primarily linked to this protective effect on cholesterol. Tomato juice containing 10.81±0.19 mg/100 g of lycopene, provided 3.5 mg of lycopene/day in male Sprague-Dawley rats after five weeks, leading to a significant reduction in total, LDL and HDL cholesterol [40].

It has been stated that tomato peels possess higher lycopene levels compared to the pulp and seeds. Tomato pulp may contain 110 mg/kg (11 mg/100 g) lycopene but the tomato peel contains 540 mg/kg (54 mg/100 g) lycopene [41]. Removing the peels of Portuguese tomato cultivars (*cereja*, *chucha*, *rama* and *redondo*) led to a significant decrease (65–80%) in lycopene [42].

When tomatoes are processed into sauces, significant amounts of carotenoids (and polyphenols) are lost because they are bound to the dietary fibers and proteins in the peels and seeds. Given that tomato peels and seeds are so rich in nutrients such as lycopene and beta-carotene, they shouldn’t be considered a waste causing an environmental concern, instead, they should be viewed as a valuable food ingredient. For example, tomato pomace (peel, seed, and pulp particles) was used in the formulation of ketchup with increased fiber content [36]. In another product developed, tomato peel flour was added to whole-meal durum wheat spaghetti at a maximum of 15%. These spaghetti samples had higher carotenoid content than the control group [43].

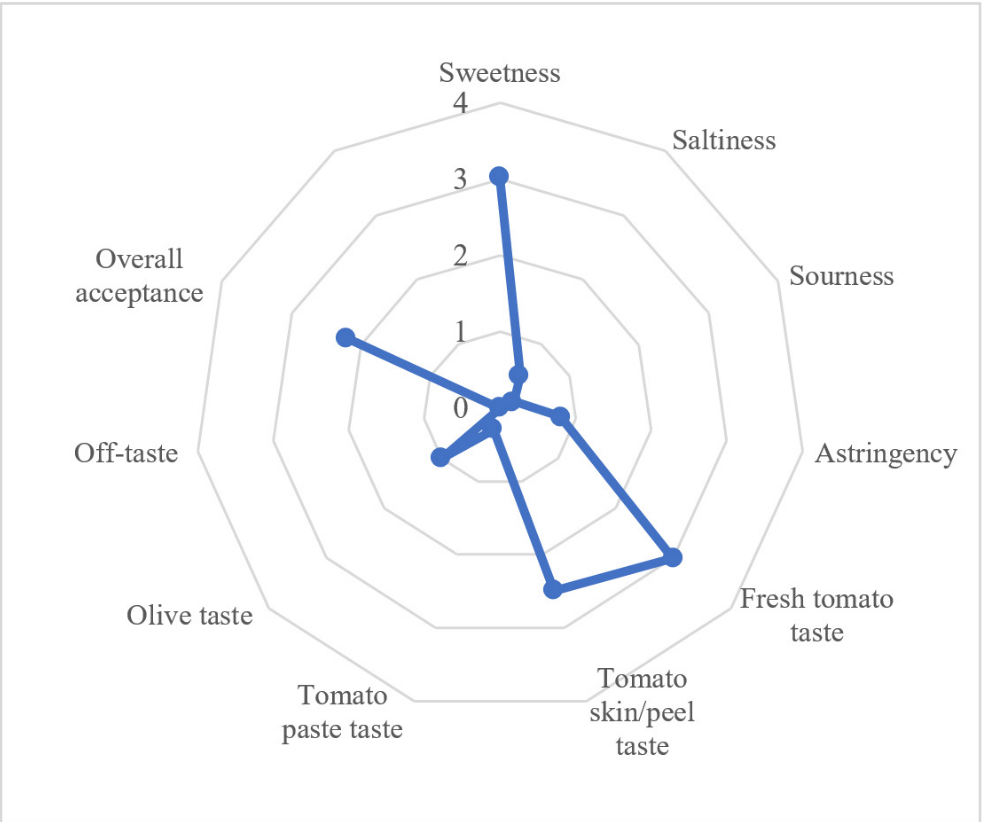
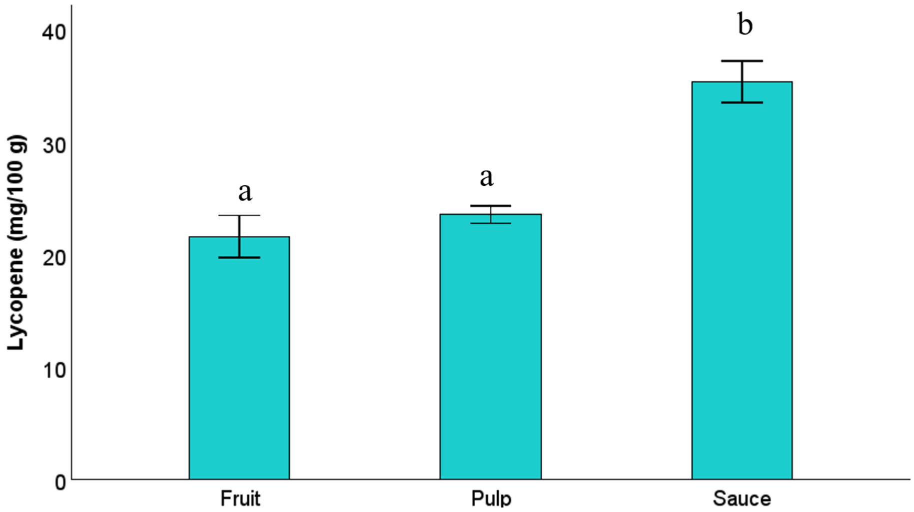


Figure 4. Flavor profile of the functional tomato sauce. The sauce was assessed by a trained panel based on intensity perception as follows, 0: not perceived at all; 1: barely detected; 2: poorly perceived; 3: moderately perceived; 4: perceived and 5: highly perceived



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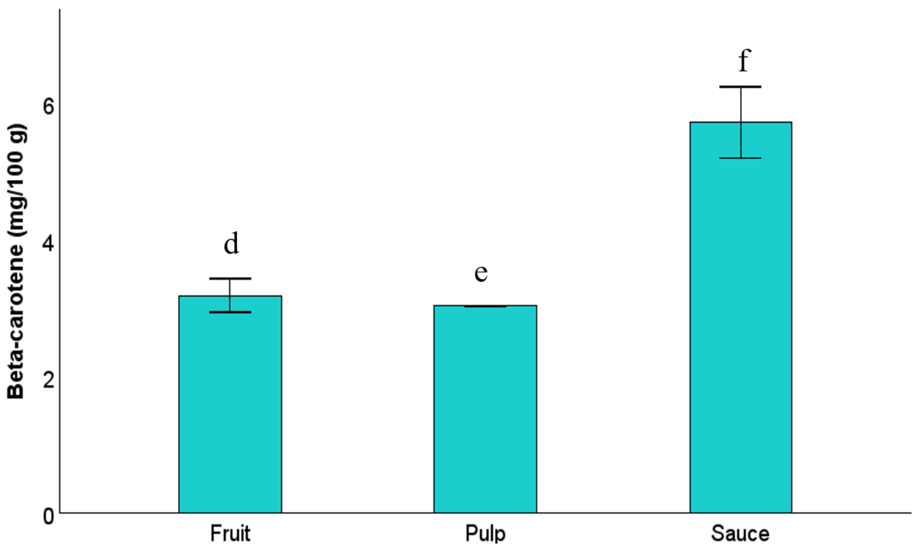


(ii)

Figure 5. Lycopene (i) and beta-carotene (ii) content of tomato fruit, tomato pulp and functional tomato sauce. Bars represent means and error bars represent standard deviations (n=3). Bars marked with the same letter are not significantly different (Tukey's Test, $P<0.05$)

Tomatoes and olives are staples of the Mediterranean diet, which is linked to a healthy lifestyle. It has been observed that co-milling olives with freeze-dried or defrosted tomato by-products result in a product enriched in antioxidants such as carotenoids [44]. Also, novel tomato sauces prepared with either 100% or 50% extracts of tomato pomace (consisting of seeds and peels) in a previous study showed greater levels of all carotenoids (primarily lycopene) and antioxidant capacity than conventional tomato sauces even though the bioaccessible total carotenoids were lower [45], implying that mixing tomato and olive powder in the sauce formulation could lead to higher carotenoid concentrations. The table below (Table 11) shows the published mean lycopene and beta-carotene contents of different tomatoes and derivatives.

The products analyzed in the present study had higher lycopene and beta-carotene contents than most of the products in Table 11. High-lycopene tomato varieties (Lyco 1, Lyco 2, HLY 02, HLY 13, HLY 18, and Kalvert) were reported to have higher lycopene and beta-carotene contents compared to an ordinary tomato variety (cv Donald). The HLY 18 had the highest levels of lycopene and beta-carotene, measuring 232.9 mg/kg (23.29 mg/100 g) and 19.4 mg/kg fw (1.94 mg/100 g), respectively [51]. According to [11], traditional tomato cultivars reach a maximum lycopene content of 150 mg/kg fresh weight (15 mg/100 g). The hp mutations in high-lycopene tomatoes have a beneficial impact on the amount of carotenoid, particularly lycopene. Consequently, high-lycopene tomatoes can accumulate more carotenoids including lycopene and beta-carotene.

Table 11. Lycopene and beta-carotene concentrations of different tomatoes and derivatives

Product	Lycopene (mg/100 g)	Beta-carotene (mg/100 g)	Ref
Tomato juice	10.81±0.19	N/A	[40]
Heirloom tomato-black krim	1.91±0.32	N/A	[46]
Heirloom tomato green zebra	0.35±0.02	N/A	[46]
Tomato line poly20	16.0±0.18	1.00±0.01	[47]
Tomato cv Motelle	16.90±0.68	0.47±0.01	[47]
Tomato line poly56	14.20±0.72	0.51±0.01	[47]
Tomato HLT-F61 pulp	28.0±1.0	N/A	[48]
Cherry Tomato	15.8±0.1	1.6±0.1	[42]
Plum Tomato	14.2±0.1	0.7±0.1	[42]
Tomato carrot juice blend	22.05±0.24	4.67±0.014	[49]
Tomato juice	4.18±0.09	0.18±0.01	[50]
Tomato juice fortified with vitamin C	4.07±0.19	0.19±0.01	[50]

3.5. Phenolic Content and Antioxidant Activity

Consumption of phenolic compounds, which are beneficial phytochemicals, reduces the incidence of non-communicable diseases because they exhibit powerful antioxidant properties. Consequently, boosting food products' phenolic content can significantly improve their nutritional value. Figure 6 shows that there was an increase in total phenolic content in the final product when comparing the fruit (51.39±2.91 mg GAE/100 g) and pulp (39.06±2.99 mg GAE/100 g) against the formulated sauce (58.30±0.91 mg GAE/100 g). The higher concentrations of phenolic acids in the peels and seeds could explain this result [45] because the peels and seeds of the tomato were removed during pulping and a 3.47% tomato peel powder was added in the sauce.

A study conducted with four Portuguese tomato cultivars also revealed that seed elimination reduced mainly the total phenolic contents [42]. Also, the incorporation of 10% tomato peel powder in soy protein-based high-moisture meat analogs led to a significant increase in phenolic content [52]. Another research revealed that adding tomato powder to corn snacks increased the concentration of bioactive compounds, particularly the total phenolic content [53]. Researchers observed a 2 to 3.6 times higher total phenolic content in tomato peels compared to the pulp [48]. This is because phenolic compounds function as defense chemicals against pathogens and predators as well as shielding against UV radiation. They tend to accumulate in higher concentrations in the peel compared to other tomato parts [48].

The OP produced from whole olive fruits, is one ingredient that contributes significantly to the increased phenolic content of the sauce when compared to the tomato pulp. The flesh of healthy olive fruits contains approximately 2-3% of antioxidant phenolic substances in the form of glucosides and esters. These phenolic compounds include glycides (e.g., oleuropein), alcohols and phenols (tyrosol, hydroxytyrosol), and flavonoids [26]. Moreover, pulses such as peas and their protein isolates contain different amounts of health-promoting bioactive compounds. This was observed by researchers who conducted a comparative analysis of bioactive compounds in pea and bean flours, and they observed that pea flour had the highest concentrations of anthocyanins, flavonols, and total phenolic compounds among the various groups of analyzed phenols [54].

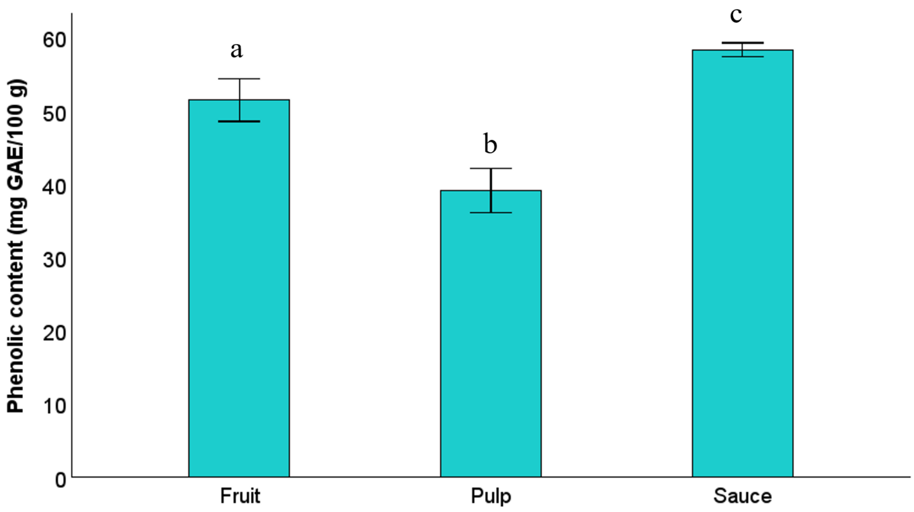
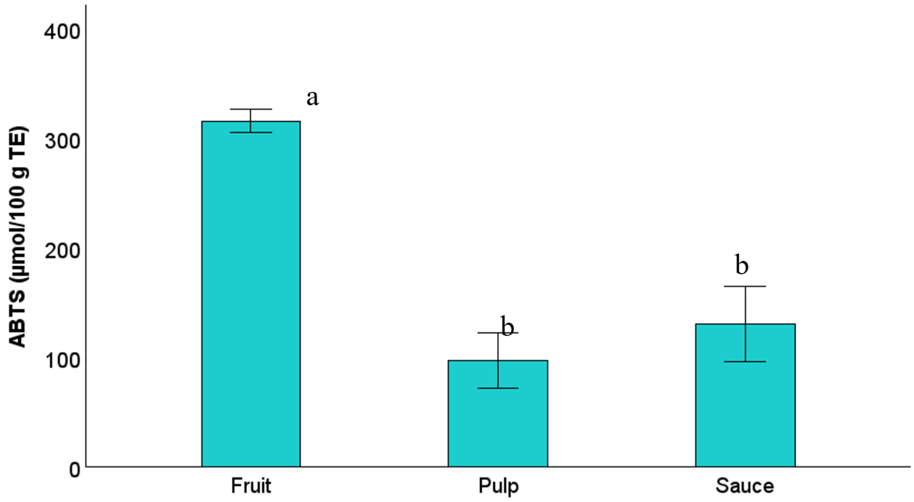
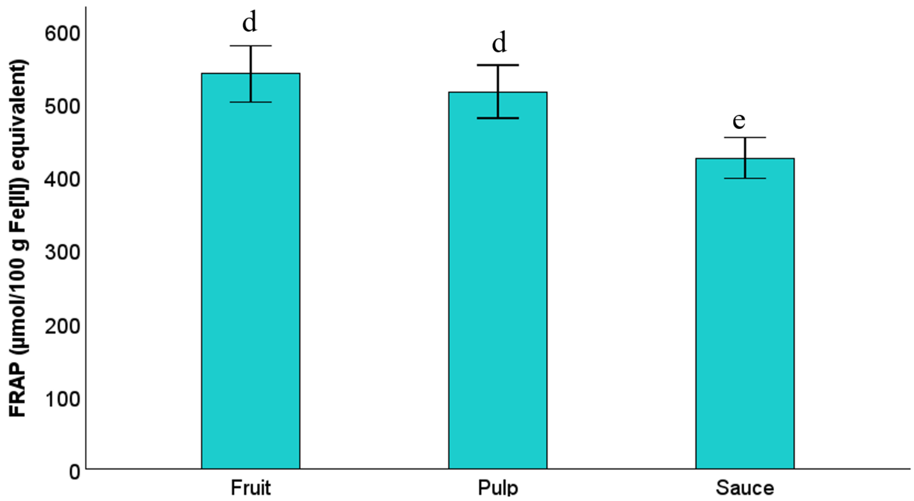


Figure 6. Total phenolic content of the tomato fruit, tomato pulp, and functional tomato sauce. Bars represent means and error bars represent standard deviations (n=3). Bars marked with different letters are significantly different (Tukey HSD Test, P<0.05)



(i)



(ii)

Figure 7. Antioxidant activity of tomato fruit, tomato pulp and functional tomato sauce analyzed by ABTS (i) and FRAP (ii) assays. Bars represent means and error bars represent standard deviations (n=3). Bars marked with the same letters are not significantly different (Tukey HSD Test, P<0.05)

Antioxidant capacity was determined by ABTS and FRAP methods. The tomato fruit was estimated to have the highest antioxidant activity in the ABTS (315.72±10.85 µmol/100 g TE) and FRAP (540.95±38.40 µmol/100 g Fe[II] equivalent) assays (Figure 7). The concentration of bioactive compounds in the final product depends on the tomato variety, fruit maturity, added ingredients, and processing methods, among others. The total phenolic content of tomatoes was reported to range from 4.43 to 25.84 mg GAE/100 g while the antioxidant capacity of tomatoes varies between 45 and 230 x 10^3 µmol TE/100 g [55]. The values recorded in this study fall within these ranges. The recorded values of antioxidant activity analyzed by the FRAP assay were not so different for the three samples. In the ABTS assay, there was an increase in the antioxidant activity of the formulated sauce (130.03±34.50 µmol/100 g TE) compared to the pulp (96.83±25.26 µmol/100 g TE). The antioxidant capacity results of the FRAP and ABTS assays do not follow the same tendency which may be explained by the different reactive species (i.e. polyphenols, carotenoids, tocopherols, phytosterols, metals, etc.) and mechanisms involved in oxidative stress [56]. Likewise, the authors have previously shown that results from different assays using the same material can vary significantly. They concluded that these differences could be caused by different types of antioxidants present in the samples, which react differently with the radicals used in the assays [57]. Several researchers have examined the phenolic content and antioxidant capacity of different tomato-based products and different parts of tomato varieties. Table 12 shows the mean values of phenolic contents and antioxidant capacity of different tomatoes and tomato-based products that were previously analyzed.

Carotenoids, polyphenols, and certain vitamins are the bioactive compounds found in tomato products that are linked to their antioxidant activity. The physicochemical characteristics and antioxidant activity (measured by ABTS and DPPH) of Korean-marketed ketchup were investigated, and the authors found that phenolic compounds highly contributed to antioxidant activity [61]. The antioxidant properties of lycopene can be increased by the presence of other biologically active substances, such as carotenoids, phenols and vitamins, through their combined antioxidant effects due to synergism [62]. A previous study indicated that the combination of phenolic acids (caffeic and p-coumaric acids) and carotenoids (beta-carotene, lycopene) showed a synergistic antioxidant effect, meaning that they had a greater antioxidant effect than when used separately. The researchers further stated that phenolic acids enhance the cellular uptake of carotenoids and the expression of their cell membrane transporters [63]. As such, the OP in the optimum tomato sauce contributes to the higher antioxidant capacity in comparison to the pulp because olives are rich in phenolic compounds, and a synergistic antioxidant effect linked to the presence of carotenoids (beta-carotene, lycopene) and phenolic compounds may have resulted from the combination of the ingredients in the tomato sauce.

Researchers assessed the antioxidant activity of tomato peels and found that they have a high concentration of antioxidants such as flavonoids, phenolic acids, lycopene, and ascorbic acid. This means that tomato peel can thus be utilized as a functional food ingredient to enhance the antioxidant content in food products and contribute significantly to increasing the amount of antioxidants consumed by humans [64]. Adding pea protein also contributes to the antioxidant activity of the optimum functional tomato sauce. Besides being a beneficial ingredient that can boost the protein levels in one's diet, they also exhibit biological properties like antioxidant properties [13]. Therefore, these results demonstrate that using the ingredients (OP, PP, and TPP) can be a viable option to enhance the antioxidant activity and ultimately, the functional properties of tomato-based sauces.

Table 12. Concentration of total phenolics and antioxidants (measured by ABTS and FRAP) present in tomatoes and tomato-based products

Product	Phenolic content (mg GAE/100 g)	ABTS (µmol/100 g TE)	FRAP (µmol/100 g Fe[II] equivalent)	Ref
Heirloom tomato (black krim)	15.29±2.93	1200±83	N/A	[46]
Heirloom tomato (Green Zebra)	16.97±4.03	1176±193	N/A	[46]
75% tomato + 25% strawberry ketchup sauce	61.90±4.51	774.49 ± 85.37	N/A	[58]
100% tomato ketchup	32.07 ± 9.60	388.32±67.55	N/A	[58]
Tomato juice	25.52±0.44	190.00±0.00	310.00±10.00	[50]
Tomato juice fortified with vitamin C	79.07±1.95	740.00±20.00	182±40.00	[50]
Tomato sauce prepared with green pepper (10 %), extra virgin olive oil (2 %), and salt (0.3 %)	72.63±3.03	N/A	N/A	[59]
Commercial tomato paste	210±17	616±227	N/A	[55]
Homemade tomato paste	112±53	267±221	N/A	[55]
Tomato cv EC-521083	456.00±29.00	49.00±46.00	456.00±29.00	[60]
Tomato cv EC-521086	460.00±28.00	53.00±64.00	467.00±23.00	[60]

4. Conclusions

The growing consumer demand for healthier food items presents an opportunity for developing new functional foods that are high in antioxidants and are a source of sustainable plant-based proteins. Reformulation of tomato sauce into a functional tomato sauce through a suitable combination of functional ingredients is a solution proposed within the scope of the FunTomP (Functionalized Tomato Products) Project to meet this growing consumer trend.

This study proposes a functionalized tomato sauce containing high-lycopene tomato pulp, tomato peel powder, olive powder and pea protein formulated based on a D-optimal mixture design developed using DX13, demonstrating that mixture design is a reliable and efficient approach for determining optimal ingredient quantities in food and beverages. The mean experimental and predicted sensory scores of the selected optimized functional tomato sauce were in good agreement with each other. This shows an accurate prediction of the product acceptance by the optimization tool of DX13.

Moreover, the sensory attributes of the tomato sauce formulation received good acceptability scores from members of the untrained panel. This observation was strengthened by the trained panel test which revealed a good flavor profile with a strongly perceived ‘tomato taste’, negligible sourness and no off-taste that was appreciated by the trained panelists. The significant enhancement in the levels of lycopene, beta-carotene, and phenolic compounds present in this sauce which has been fortified with powdered additives, plays a crucial role in boosting the antioxidant properties of the final product. However, the absence of in-vivo studies of the antioxidant activity is a limitation of this study. Also, all sensory analyses were conducted in Turkïye and Portugal, as such the findings of this study might differ in other populations beyond the group included in the study. In conclusion, the inclusion of the powdered additives produced a good flavor profile and elevated the antioxidant profile of the developed sauce, underscoring its potential as a functional food that can contribute positively to overall health. As a result, the combination of tomato peel powder, olive powder, and pea protein makes it possible to produce a functional tomato sauce.

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Data Availability Statement

The data supporting the findings of the study are presented within this article.

Conflict of Interests

The authors have stated that they do not have any conflicts of interest.

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