

Production of a mixed fruit juice powder using mixture analysis and a fuzzy model

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Abstract

The present work aimed to find a mixed fruit juice powder with high antioxidant activity and sensory score. A two-step fuzzy algorithm and fuzzy toolbox were applied to produce acceptable sensory data for the mixture analysis design. The mixture design was then analysed using two responses of sensory and antioxidant activity, and the optimised beverage consisted of red grape (20.4%), mulberry (75.6%), and strawberry (4.0%). As compared to single fruit juices, the selected mixture yielded high content of phenolic compounds with desirable antioxidant activity and sensory score. Spray- and freeze-drying were then compared for the fruit juice powder production from selected mixed juices formulated with 20% maltodextrin (DE = 20). A significant ($p < 0.05$) difference was observed between the powder yield (82.0% in comparison to 51.7%), free radical scavenging activity (85.6% in comparison to 75.4%), and total phenolic content (2958.8 in comparison to 2791.4 mg GAE/L) of the freeze- and spray-dryer, respectively. Furthermore, the spray-dried powder was much lighter than the freeze-dried powder, with a lower chroma index, redness, and higher hue angle. Overall, freeze-drying was less destructive than spray-drying in the powder production from the mixed juice.

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Introduction

Fruit juices are popular due to various advantages including the presence of valuable nutrients such as minerals, vitamins, antioxidants, polyphenols, bioactive compounds (Vilela and Cosme, 2016), and their refreshing taste and aroma (Dhar *et al.*, 2021). Antioxidants exist in many fruit juices, and reduce the level of free radicals, thus resulting in a reduction of the damage continually occurring in cells and tissues (Vilela and Cosme, 2016). Consumption of fruit juices with high phenolic compounds is associated with a decrease in heart and brain diseases, and cancer-related mortality (Vilela and Cosme, 2016; Zhang *et al.*, 2018). Furthermore, for many seasonal fruits, fruit beverages and powders have a higher shelf life than fresh fruits. Therefore, many fruit juices are now accessible in liquid and powder forms, thus facilitating their shipping and improving storage.

The production of mixed fruit beverages has been explored in previous study as mixing could resolve and mask some unpleasant characteristics of

individual fruit juices, and introduce new natural beverages. For example, Dhar *et al.* (2021) have introduced a mixed fruit beverage with high sensory acceptability to mask the unpleasant taste of *amla*. Mulberry (Mu), pomegranate (Po), red grape (Gr), and strawberry (St) are four popular seasonal fruits in Iran. These fruits have several benefits, such as significant amounts of biologically active compounds with antioxidant activity (Zhang *et al.*, 2018; Dzhhanfezova *et al.*, 2020; Ghalegi Ghalenoe *et al.*, 2021; Orellana-Palma *et al.*, 2021). However, not only is the access time for these fruits short throughout the year, but they are also not available in all parts of the country due to their low shelf life, and the fact that they wither quickly. One popular way to increase and facilitate the accessibility of fruits is the production of beverages and powders from these fruits.

Nevertheless, there are some drawbacks. Mu, and especially St juices, have a rich and favourable aroma and flavour while their viscosities are usually high, which sometimes affects the quality of juices (Fathi *et al.*, 2019; Schiassi *et al.*, 2020). In this

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regard, Po and Gr, as popular fruit beverages, have been applied to improve the rheological and sensory properties of mixed products (Roy *et al.*, 2019; Pan *et al.*, 2019). Therefore, well-designed mixed fruit juice or powder from these fruits may improve its health benefits (such as antioxidant activity) and sensory properties, as well as improve accessibility and shelf life (Hadijah *et al.*, 2015).

Sensory analysis is the main index for developing a new mixed beverage (Dhar *et al.*, 2021). Several methods are applied to execute sensory, most of which are based on classification and sorting of samples. In most of these techniques, the preference for food products is categorically graded based on acceptability (Vivek *et al.*, 2020). Overall, sensory data are vague, and include uncertainty and imprecision (Meena *et al.*, 2016; Vivek *et al.*, 2020; Dhar *et al.*, 2021). This issue limits sensory data analysis, and inhibits precise evaluation through a quantitative mode. The fuzzy logic technique is a powerful method for assessing vague, imprecise, and uncertain data. The technique has been used for ranking and producing convincing inferences about the acceptance and rejection of food products (Meena *et al.*, 2016; Vivek *et al.*, 2020). The basis of the method is to create triplets from sensory scores of each attribute, and then find membership functions in a normalised fuzzy model. An interesting characteristic of sensory analysis using a fuzzy logic model is inserting the weightages of sensory attributes in the final results. Since the strengths and weaknesses of different sensory attributes in the final decision are unknown, the fuzzy logic technique can help distinguish their intensity, which improves and create a better understanding of the sensory analysis.

The fuzzy logic model can also be applied to quantify sensory data, an essential feature of responses in many experimental design methods such as response surface methodology and mixture analysis (Meena *et al.*, 2016). Recently, Dhar *et al.* (2021) introduced a fuzzy logic algorithm combined with a D-optimal mixture design to optimise mixed fruit beverages based on sensory evaluation. Sugumar and Guha (2022) also applied the method to analyse a soup produced from European black nightshade (*Solanum nigrum* L.). The basis of the method is the production of polynomial models from sensory data, and the calculation of a single sensory score from different attributes (Oganesyants *et al.*, 2020; Dhar *et al.*, 2021). In addition to the fuzzy algorithm, the fuzzy toolbox has also been attended as a powerful

method for ranking and scoring food samples (Zare and Ghazali, 2015). The method is simple and practical, allowing for precedence and delay in input variables. By combining the fuzzy logic algorithm and fuzzy toolbox, a more precise analysis of sensory data will be possible, as precedence and delay in sensory attributes will be applied besides considering the weightages. Therefore, such an approach can guide the food industries in the production of food products based on the behavioural characteristics of consumers.

Spray- and freeze-drying are two popular methods of producing fruit juice powders (Saikia *et al.*, 2014; Ghalegi Ghalehoe *et al.*, 2021). However, each method includes some benefits and drawbacks. Spray-drying has often been mentioned as a low-cost and fast method; however, it has a destructive effect on thermo-sensitive components. In contrast, freeze-drying has a milder effect on heat-sensitive components, but the process is more prolonged (Chen *et al.*, 2021; Shuen *et al.*, 2021). Unlike many discrete investigations on the impact of spray- and freeze-drying on nutritional characteristics and yield of fruit juice powders (Shishir and Chen, 2017), simultaneous comparison, especially in mixed fruit juice, has been given less focus.

In the present work, a combined fuzzy logic algorithm and fuzzy toolbox were designed to produce sensory data in a quantitative mode, and a mixture analysis design was applied to find an optimum mixture of fruit juices, including Mu, Po, Gr, and St, with high antioxidant activity and sensory acceptance. For the first time, the present work introduced a novel method for considering precedence and delay besides weightage for sensory attributes. Furthermore, the physicochemical properties of the optimised mixed fruit juice were compared with single fruit juices. The capability of freeze- and spray-drying was also evaluated for the production of fruit juice powder using a comparison of yield, antioxidant activity, total phenol, and colour analysis.

Materials and methods

Preparation of fruit juices

Fresh ripe mulberry (*Morus nigra* L.), ripe strawberry (*Fragaria × ananassa* cv. Paros), large seedless ripe red grapes (*Vitis vinifera* L.), and fresh coarse ripe pomegranate (*Punica granatum* L.) were purchased from the Tajrish fruit market in Tehran

between August and September of 2020. Fruits were examined for assurance of freshness and care, washed, and their surfaces were dehydrated with damp cloths. Mu, St, and Gr juices were prepared with a Tefal home juice maker (model: ZE-350). Po juice was extracted using a hand-operated domestic press. Fruit juices were then centrifuged at 3,000 g (Beckman J2-21, Beckman Coulter Inc., CA, USA) at 10°C for 20 min, and the clarified fruit juices (supernatant) were then used to prepare different mixtures.

Design of mixture analysis

A simplex lattice design degree 2 augmented with a centre and axial points was applied to optimise the four independent variables of Mu, Po, Gr, and St juices. The level of each independent variable was defined between zero and 100%. Three duplications in axial points were selected based on the software proposition to evaluate the lack of fit. Based on the design, 18 trials were defined by the software, as

indicated in Table 1. The sensory and free radical scavenging activity were applied as a response.

Sensory evaluation

A five-point hedonic sensory evaluation was applied to collect data as a sensory response, and four sensory attributes including appearance (Ap), aroma (Ar), taste (T), and mouthfeel (M) were selected (Kim *et al.*, 2013; Zare and Ghazali, 2015). At first, 18 different mixtures of juices were prepared based on Table 1, and coded. Afterward, in one day, samples in identical containers were presented to 32 semi-trained panellists, including men and women, between 18 and 50 years. For this purpose, they were instructed to express their feelings about the samples by scoring the sensory attributes using a hedonic scale (poor, fair, good, very good, and excellent) (Dhar *et al.*, 2021). Prior to the evaluation, three salty and sweet solutions were introduced to evaluators, and the individuals were asked to rank them in a decreasing trend to confirm taste health.

Table 1. Experimental design for optimisation of a mixture of four fruit juices.

Variable	Independent				Dependent	
	Mulberry juice	Red grape juice	Pomegranate juice	Strawberry juice	Sensory acceptance (G score)	Radical scavenging activity (DPPH%)
Minimum	0	0	0	0		
Maximum	100	100	100	100		
Trial	Ratio of component (%)					
1	0	0	100	0	18.9	92.72
2	50	0	50	0	18.5	75.57
3	0	0	0	100	17.1	84.15
4	12.5	12.5	12.5	62.5	18.1	75.04
5	0	0	100	0	18.8	87.21
6	0	100	0	0	17.5	15.30
7	62.5	12.5	12.5	12.5	18.0	81.39
8	0	0	50	50	17.4	86.90
9	100	0	0	0	18.4	90.42
10	12.5	12.5	62.5	12.5	19.1	99.77
11	50	0	0	50	17.0	86.00
12	12.5	62.5	12.5	12.5	19.0	61.17
13	0	100	0	0	17.4	18.40
14	50	50	0	0	18.8	73.27
15	0	50	0	50	18.6	48.54
16	0	50	50	0	18.8	80.55
17	100	0	0	0	18.2	94.14
18	25	25	25	25	18.8	69.14

Fuzzy model of sensory analysis

Preparation of crisp score

The model applied for analysing sensory data was accomplished using a method explained by Dhar *et al.* (2021), combined with a fuzzy toolbox algorithm. At first, the linguistic score counts (LSC) of each trial of mixture analysis (Table 1) were calculated separately for each scale of sensory attributes. For example, in trial 1, how many volunteers in panellists voted poor for the appearance, or how many voted fair were counted. This approach was conducted for all attributes producing 20 columns with 18 rows for each attribute. These data were introduced to the designed mixture in a polynomial special cubic model, and then, 20 series of coefficients were obtained. The coefficients were then applied to calculate LSC using Eq. 1, while the values of Po, Gr, Mu, and St, and their interactions were calculated in the form of coded values between 0 to +1.

$$LSC = q_1Po + q_2Gr + q_3Mu + q_4St + q_5PoGr + q_6PoMu + q_7PoSt + q_8GrMu + q_9GrSt + q_{10}MuSt + q_{11}PoGrMu + q_{12}PoGrSt + q_{13}PoMuSt + q_{14}GrMuSt \quad (Eq. 1)$$

The values of LSC lower than zero were considered zero. Then LSC was applied to define related fuzzy triplets of Ap, Ar, T, and M in MSExcel software. Based on the fuzzy scale of 0 - 100, LSC was fuzzified using the calculation of fuzzy triplets. The final membership values (μ_x) were calculated for each attribute separately by calculating the relationship of fuzzy triplets and membership functions USING Eq. 2 (Dhar *et al.*, 2021).

$$\mu_x = \text{Max of (Min of } [x-(a-b)/b, (a+c)-x/c] \text{ and } 0) \quad (Eq. 2)$$

Based on the calculated (μ_x), a simplified series of μ_x° were calculated using Eq. 3 as follows:

$$\begin{aligned} \text{If } x = 0, \mu_x^\circ &= 0.75 \mu_x + 0.25 \mu_{x+12.5} \\ \text{If } x = 25-75, \mu_x^\circ &= 0.25 \mu_{x-12.5} + 0.50 \mu_x + 0.25 \mu_{x+12.5} \\ \text{If } x = 100, \mu_x^\circ &= 0.25 \mu_{x-12.5} + 0.75 \mu_x \end{aligned} \quad (Eq. 3)$$

Finally, the crisp score (CS) of each attribute was calculated separately through defuzzification using Eq. 4:

$$CS_{Ap,Ar,M,T} = \frac{(1 \times \mu_0^\circ) + (2 \times \mu_{25}^\circ) + (3 \times \mu_{50}^\circ) + (4 \times \mu_{75}^\circ) + (5 \times \mu_{100}^\circ)}{\mu_0 + \mu_{25} + \mu_{50} + \mu_{75} + \mu_{100}} \quad (Eq. 4)$$

Based on Eq. 4, four CS values were obtained for each trial, thus indicating overall acceptance of Ap, Ar, T, and M, and expressed as CS_{Ap} , CS_{Ar} , CS_T , and CS_M .

Further fuzzification using a fuzzy toolbox

Memberships and labelling

This step applied a fuzzy toolbox model (FTM) to produce a single response from individual CS values. Furthermore, the importance of the attributes was inserted into the final response.

During fuzzification, input variables of sensory evaluation, including Ap, Ar, T, and M, were defined and interpreted based on the fuzzy model. Memberships and labelling of each variable were defined in low, medium, and high acceptability levels. Triangular structures of input variables were defined for all attributes using Eq. 5 (Zadeh, 1983):

$$\mu_x = \begin{cases} \text{low: } x < 2.5, (0, 0, 2.5) \\ \text{medium: } 0 < x < 5, (0, 2.5, 5) \\ \text{high: } x > 2.5, (5, 0, 0) \end{cases} \quad (Eq. 5)$$

where, μ_x = membership values in the fuzzy toolbox input variables.

The inferencing process was defined in the next step based on "IF-THEN" rules (Zadeh, 1983). The definition of 81 rules was possible by considering four input variables in three levels, as explained in the fuzzification step (Dhar *et al.*, 2021). Therefore, 81 rules were defined as follows:

IF CS_{Ap} is (low, medium, high), and CS_{Ar} is (low, medium, high), and CS_T is (low, medium, high), and CS_M is (low, medium, high), THEN the fruit juice grade is 0 - 25.

Ranking of sensory attributes, rule definition, and output variable

In order to find the importance of each sensory attribute, a professional sensory team including six specialists in fruit juice (two people from the research and development sections of food factories, one specialist from the food and drug administration of Iran, and three members of the scientific board with

food and beverage specialty) were chosen and asked to rate the degree of importance of four attributes of Ap, Ar, T, and M through discussion. The final ranking was then applied to define the output variable in the fuzzy toolbox. The scores of 0, 2.5, and 5 were considered for each attribute's low, medium, and high acceptability. To define the related rules, two main principles were applied as follows:

IF at least one input variable (CS value) is in low level, THEN juice grade (G) = $[0.8 \times (CS_{Ap} + CS_{Ar} + CS_T + CS_M)]$

IF all input variables (CS values) are higher than low level, THEN juice grade (G) = $[1 \times (CS_{Ap} + CS_{Ar})] + [1.5 \times (CS_T + CS_M)]$

where, CS values of Ap, Ar, T, and M were calculated from Eq. 4, and coefficients of 0.8, 1, and 1.5 were extracted based on professional sensory team conclusions.

Then, the values for a rating of each attribute, proposed by the special team, were multiplied by the related scores of the attribute. Afterward, scores of all attributes, including Ap, Ar, T, and M, were summed, and the results were considered the score of each rule. This method was applied to complete all rules in the fuzzy processing section.

For example:

IF CS_{Ap} is low (0×0.8), CS_{Ar} is high (5×0.8), CS_T is high (5×0.8), and CS_M is low (0×0.8), THEN crisp overall score (COS) is 8 and G = 8.

Or:

IF CS_{Ap} is high (5×1), CS_{Ar} is medium (2.5×1), CS_T is high (5×1.5), CS_M is medium (2.5×1.5), THEN COS is 18.75 and G = 19.

Following the definition of rules, a total of 26 grades were defined as the score of acceptance of fruit juices, and considered output variables. The range of output variables was from 0 - 25.

Defuzzification

In the defuzzification step, analysis and processing of input data are accomplished by the established rules, and then the final results are obtained through the defuzzification of each trial based on defined output variables (Zadeh, 1983).

Defuzzification of output variables was accomplished based on the centre of gravity method.

Ultimately, practical CS values of CS_{Ap} , CS_{Ar} , CS_T , and CS_M for each trial of mixture design (Table 1) obtained from Eq. 4 were inserted in the designed program in MATLAB software, and the final overall score (G score) of each trial was obtained, automatically. The obtained scores, grades of mixed fruit juices (between 0 and 25), were applied as a numeric response for analysis of mixture design.

Physicochemical properties

Free radical scavenging activity

The scavenging activity for DPPH radicals was determined using a UV-vis spectrophotometer (Unicam 8620, Thermospectronic, UK) based on the method described earlier with some modifications (Rana *et al.*, 2019; Shariati *et al.*, 2019). Briefly, 200 μ L of a solution of diluted fruit juice in methanol was added to 800 μ L of DPPH solution in methanol (0.004 g/100 mL), and kept in the dark for 60 min (Sample). A solution of DPPH (800 μ L) and MeOH (200 μ L) was applied as a control. A solution of diluted fruit juice (200 μ L) and MeOH (800 μ L) was applied as blank. The percentage of free radical scavenging was obtained using Eq. 6:

$$\text{Scavenging activity (\%)} = (1 - [(\text{sample Abs} - \text{blank Abs}) / \text{control Abs} - \text{MeOH Abs}]) \times 100 \quad (\text{Eq. 6})$$

IC_{50} of fruit juices was calculated based on the juice concentrate, which inhibited 50% DPPH free radicals (Wern *et al.*, 2016; Rana *et al.*, 2019).

Ferric reducing antioxidant power

Briefly, a solution of FRAP reagent was prepared by mixing 2,4,6-tripyridyls-triazine (10 mmol/L), $FeCl_3$ (20 mmol/L), and acetate buffer (300 mmol/L) solutions in a ratio of 1:1:10 (v/v). Then, 40 μ L of fruit juices were added to 3 mL of FRAP reagent, and placed at 37°C for 30 min. Afterward, the absorbance of solutions was recorded at 593 nm (Unicam 8620, Thermospectronic, UK). A standard calibration curve was plotted using different concentrations of Fe^{2+} , and finally, FRAP values of fruit juices were calculated as μ mol Fe^{2+} /mL fruit juice (Saikia *et al.*, 2014).

Phenolic compounds

The total phenolic compounds of single fruit juices and the selected mixture were estimated as

described earlier (Ghaderi *et al.*, 2019; Rana *et al.*, 2019), and gallic acid in 0 - 250 mg/L was used to plot the standard calibration curve. The content of phenolic compounds was reported as gallic acid equivalent (mg GAE/L).

Total soluble solids, pH, and acidity

The pH of fruit juices was directly determined using a pH meter (EcoMet, Korea), and total soluble solids (TSS) were measured by a refractometer (ATAGO SPR-T2, Japan). The acidity of the fruit juices was also calculated based on citric acid using the titration method (Adetoro *et al.*, 2020; Ghalegi Ghalehoe *et al.*, 2021).

Powder production from mixed fruit juice

Comparison between spray- and freeze-dryers

An equivalent formulation of mixed fruit juices was applied as a feed for the spray- and freeze-dryer, to find a precise comparison of the effect of spray- and freeze-drying, on the physicochemical properties of fruit juice powders. Briefly, around 400 mL of clarified selected mixed fruit juices with total suspended solids (TSS) of $15.7 \pm 0.1\%$ was prepared. The juice was then formulated using 20% maltodextrin (DE = 20), and the final volume reached 500 mL in a volumetric flask using the clarified selected mixed fruit juices. The brix of the solution was $33.6 \pm 1.3\%$. The mixture was then divided into two parts. One part (250 mL) was poured into 15 cm diameter glass plates until around 1 cm deep, transferred to a freezer at -80°C for 24 h, and freeze-dried using a freeze-dryer (Leybold Heraeus, Cologne, Germany) for 48 h until the juice was completely dried. The shelf temperature varied between 26 to 38°C , and the pressure was less than 0.1 mbar.

The second part of the formulated mixture of fruit juice (250 mL) was introduced to a Dorsa (DSD-02, Dorsa Tech, Tehran, Iran) spray-dryer. The air temperature of the inlet was regulated at 120°C while the outlet varied between $82 \pm 1^\circ\text{C}$. The flow type of the inlet air was concurrent with, and the feeding rate of the juice was around 200 mL/h.

At the end of drying, the dried powder in the final chamber of the spray-dryer was collected in a sealed polyethylene bottle, placed in a desiccator in the presence of silica gel, and stored at 4°C before analysis. Applying both the sealed bottle and desiccator ensured the inhibition of humidity absorption of the powders before each analysis. The

dried powder obtained from the freeze-dryer was first collected and blended using a blender, and then stored similarly. Afterward, the powders were evaluated for free radical scavenging activity and total phenolic content.

The yield of freeze- and spray-dried powder

The dry weight of fresh fruit juice was obtained by placing 100 mL of the selected mixed fruit juice in an oven with a temperature of 90°C up to 24 h until reaching a constant weight. Then, the yield of both freeze- and spray-dried powders was calculated using Eq. 7 (Obón *et al.*, 2009; Saikia *et al.*, 2014; Muzaffar and Kumar, 2015):

$$\text{Yield (\%)} = \frac{\text{Collected powder (g)}}{\text{Dry weight of formulated juice feed}} \times 100 \quad (\text{Eq. 7})$$

The moisture content of the powder was also measured by placing 1 g of powder in a vacuum oven (Nuve EV 018) at 85°C for 8 h, and finding the difference in weight before and after drying (Shuen *et al.*, 2021).

Free radical scavenging activity and total phenolic compounds

Free radical scavenging activity and total phenolic compounds of both spray- and freeze-dryer powders were estimated as described earlier. In order to find comparable data with fresh fruit juice, powders were dissolved in distilled water based on dry weight, and free radical scavenging activity and total phenolic content were calculated in a solution similar to the fresh fruit beverage.

Colour analyses

Important attributes of colour, including L^* (lightness/darkness), a^* (redness/greenness), and b^* (yellowness/blueness) were determined, and then chroma (C) and hue angle (hu) were calculated. Attributes were specified in reflectance situations. Briefly, 1 g of fruit juice powder was pressed in a glass holder, and placed in front of the light source of Spectroradiometer CS-2000 (Konica Minolta INC., Tokyo, Japan) with an observing angle of 10° and illuminant D65, and the reflectance of the sample was analysed. Before analysis, black and white tiles were applied to calibrate the device (Ghalegi Ghalehoe *et al.*, 2021; Shuen *et al.*, 2021). The hue angle was calculated using Eq. 8:

$$\text{Hue angle} = \tan^{-1}(b^*/a^*) \quad (\text{Eq. 8})$$

The chroma was calculated using Eq. 9:

$$C = \sqrt{(a^{*2} + b^{*2})} \quad (\text{Eq. 9})$$

Statistical analysis

Significant differences between means of free radical scavenging activity and phenolic compounds were calculated by analysis of variances (ANOVA) using Tukey's multiple range test with a confidence interval of 95% ($p < 0.05$). The sample size for experiments analysed by ANOVA was triplicate. The software for defining the mixture design and calculating the ANOVA was Design Expert 11 (Statease Inc., Minneapolis, MN 55103, USA) and Minitab 16 (Minitab Inc., USA), respectively. The designing of the fuzzy model, including fuzzification, data processing, and defuzzification was accomplished by MATLAB and Statistics Toolbox Release R2019a (The MathWorks, Inc., Natick, Massachusetts, USA).

Results and discussion

Regression coefficients of defined polynomial models and crisp scores

Various methods, such as spider graphs and principal component analysis (PCA), are commonly applied to describe sensory data. Although they are efficient to analyse sensory data, the results cannot be applied as a numerical response in many experimental designs, such as mixture analysis. In the present work, the quantification of sensorial data was accomplished, and then used in mixture design as a response. Based on Eq. 1, 20 models were calculated using raw sensory data. Overall, significant models ($p < 0.05$) and non-significant lack of fits ($p \geq 0.05$) were obtained for all 20 calculated models, and data were in agreement with Dhar *et al.* (2021). The levels of R^2 were also between 89.40 and 99.74, meaning that data introduced for polynomial models were trustable. The coefficients ranged between -1532 and 1282, and the calculated LSC of coefficients were 360 values which were fuzzified to triplets. Finally, the CS values of each attribute were calculated using Eq. 4, as indicated in Table 2. The CS values were numerical sensory data of 18 mixture design trials, calculated separately from hedonic data for each attribute. CS values of Ap, Ar, T, and M varied between 2.566765 and 4.386903 (range = 1.82), 2.964910 and 3.990372

(range = 1.03), 2.790662 and 4.096875 (range = 1.30), and 2.992390 and 4.199035 (range = 1.20), respectively (Table 2). Therefore, the highest and lowest variations in numerical sensory data (CS values) of attributes were observed in the sensory of Ap and Ar, respectively, leading to the conclusion that the impact of mixing different fruit juices on Ap was more effective than other sensory attributes. Based on the range of the impacts, attributes could be arranged as $Ap > T > M > Ar$. However, a different impact of attributes was observed by Bhalerao *et al.* (2020) and Dhar *et al.* (2021). This could be due to different strategies for producing mixed fruit juice, as in their study, the main goal was masking the bitter taste of *amla*.

Production of separate CS values allowed us to evaluate correlations between sensory attributes for the first time, which was also interesting (Table 2). Unlike the low correlation among most attributes, a moderate correlation between Ap and Ar, and a strong correlation between T and M, were observed. It could be concluded that the panellists evaluated attributes in basically two categories, Ap and Ar, and T and M. It can also be inferred that there was dependence between sensory attributes T and M, which panellists gave almost similar scores. Bhalerao *et al.* (2020) also observed a strong relationship between appearance and taste in sensory acceptance of a mixed fruit juice using a 9-point hedonic test. Although a relationship between sensory data and sensory attributes has been reported by some previous studies, finding dependence between different sensory attributes may be important in the analysis of food products, and has not yet been studied.

Data collection from sensory evaluation using fuzzy toolbox

Fuzzy toolbox is a powerful method for defining the precedence and weightage of input variables. In the present work, the weightage, precedence, and delay of sensory attributes were involved in the final sensory results through the fuzzy toolbox for the first time. Furthermore, calculated CS values of the four sensory attributes (Ap, Ar, T, and M in Table 2) were combined to produce one response. By definition of the present rules, and considering current weightages (described in the material and methods section), the role of Ap and Ar was highlighted before T and M. This means that decisions about Ap and Ar by sensory panellists could moderately determine the category of the final score

Table 2. Calculated CS values obtained from polynomial models, and final overall scores of 18 trials obtained from the fuzzy toolbox (Part 1) along with Pearson correlations calculated for each pairwise of attributes (Part 2).

Part 1					
Mix	CS values				Final overall score (G score)*
	Appearance	Aroma	Taste	Mouthfeel	
1	3.752602	3.443604	4.090665	4.178265	18.9
2	4.018721	3.360749	3.861161	3.729156	18.5
3	3.672222	3.586452	2.954158	3.035722	17.1
4	3.27332	3.674352	3.555383	3.568875	18.1
5	3.763241	3.422989	4.031943	4.129352	18.8
6	2.566765	3.287746	4.027266	4.120746	17.5
7	3.936991	3.556023	3.265244	3.399483	18.0
8	3.728055	3.444436	3.089448	3.045110	17.4
9	4.160371	3.045810	3.988639	3.723941	18.4
10	4.386903	3.842405	4.096875	4.199035	19.1
11	4.244386	3.409653	2.790662	2.992390	17.0
12	4.346456	3.603466	3.887357	3.979028	19.0
13	2.667341	3.088243	4.009122	4.021843	17.4
14	4.341934	3.734290	3.746794	3.636559	18.8
15	4.334626	3.502621	3.491795	3.523132	18.6
16	4.277197	3.540551	3.550662	4.103636	18.8
17	4.071298	2.964910	3.849702	3.623152	18.2
18	4.122155	3.990372	3.886125	3.982948	18.8

Part 2	
Term	Pearson correlations
Ap*Ar	0.35045
Ap*T	-0.15436
Ap*M	-0.13627
Ar*T	-0.12822
Ar*M	0.05370
T*M	0.90366

Final overall score (G score) was calculated by inserting CS values into the fuzzy toolbox model.

of the product before sensing the taste or mouthfeel. In fact, if volunteers score only one attribute of Ap and Ar poorly, the final score never passes medium (around 12.0), even if all other attributes got excellent score. It should be mentioned that consumers usually evaluate Ap and Ar before trying a product, and do not consume it if these two attributes are not acceptable to them. The domination of Ap in sensory acceptance of mixed fruit juice has also been observed by Bhalerao *et al.* (2020), which is in agreement with the present work. Based on the surface plot view, the G score did not exceed medium

when at least one attribute was at a low level (Figure 1a and 1c). For instance, output changed between 0 to 8 when at least two attributes in *ref input* (Figure 1a) were regulated in low (0), and the others, including axes of taste and aroma, were up to high (5) (Figure 1a), while the grade of the product increased up to 25 when two attributes in *ref input* (Figure 1b) were regulated in high (5) and two others varied between 0 - 5.

Interestingly, the score of sensory never passed from 12.5 when one of the attributes was at a low level in *ref input* (Figure 1c), even if all others were

high (Figure 1c). In fact, when Ap was at a low level (0 in *ref input*), even if M was regulated at a high level (5 in *ref input*), and Ar and T changed between low to a high level, the highest possible grade was around 12 (Figure 1c). In contrast, when Ap and M were regulated at the medium (2.5 in *ref input*), and Ar and T changes from low to high, the highest possible grade was around 20 (Figure 1d). In both situations,

the sum of CS values for attributes were 15 (0, 5, 5, and 5 vs. 2.5, 2.5, 5, and 5) while the maximum scores assigned as final sensory scores differed (12 vs. 20 in Figures 1c and 1d). This could be due to setting priority and latency to attributes. A logical and positive trend with different ranges for each attribute was observed in the surface plots, thus proving the method's accuracy.

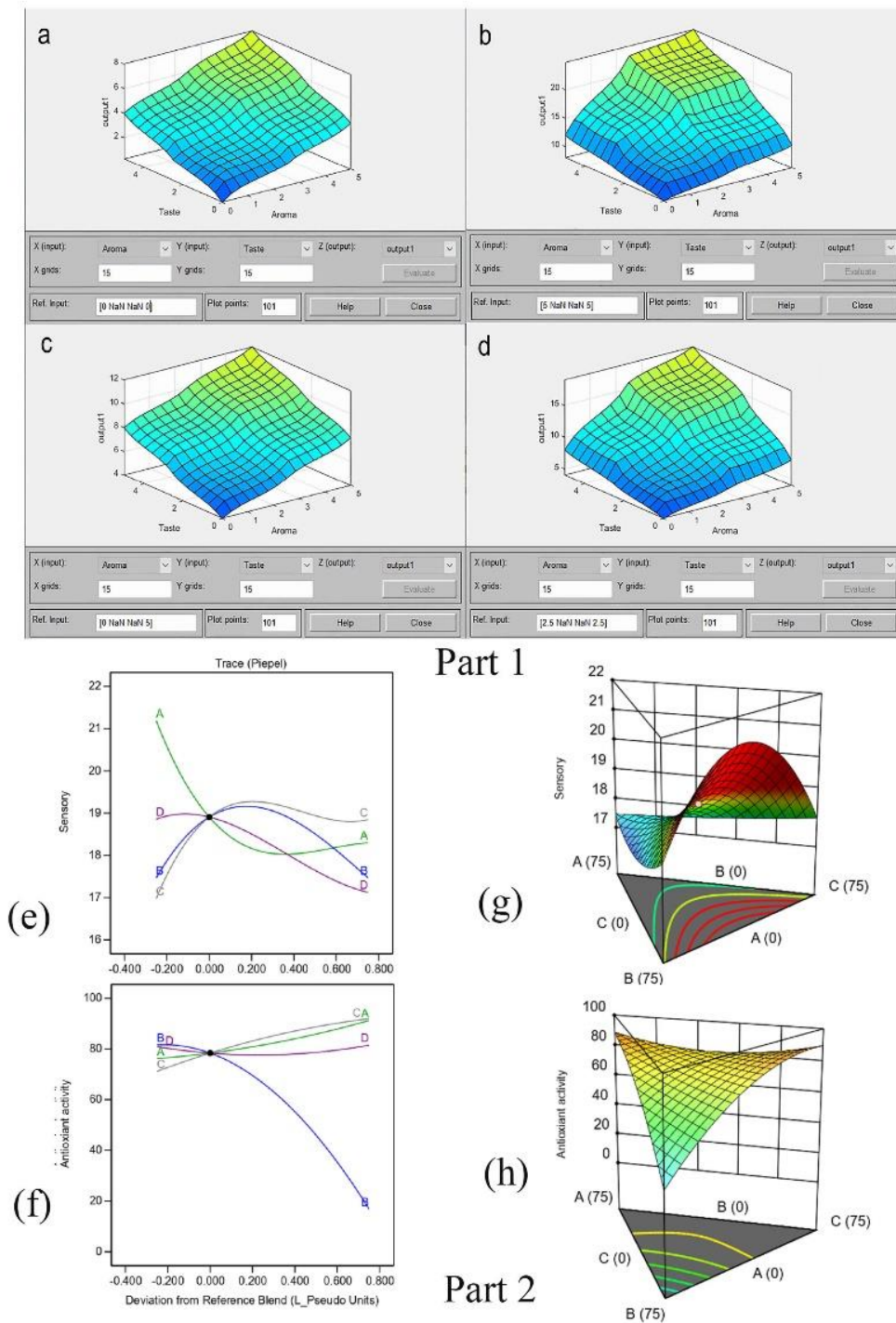


Figure 1. Surface plots of appearance, aroma, taste, and mouthfeel vs. output variable in fuzzy toolbox model (part 1); Piepel graphs (e and f) and 3D surface plots (g and h) of sensory and antioxidant activity (part 2).

The outcome of these results could be followed by inserting some dummy data. For instance, by placing CS values of 5, 5, 2.5, and 2.5 for Ap, Ar, T, and M, respectively, the final G score was 17, while by relocations of scores to 2.5, 2.5, 5, and 5, the final G score was 20. In fact, while the sum of CS values was the same in both situations, the final scores were different (17 vs. 20). This was possible because the weightage considered for T and M differed from Ap and Ar. For example, when a product is introduced to the consumer, the first reaction is to evaluate its appearance and aroma. The product will be tried if these two attributes are acceptable, at least in medium ranges. Therefore, the product must take the consumer's medium (2.5) scores of Ap and Ar. Afterward, scores of medium to high for Ap and Ar are not as important as the ones for T and M. In fact, the role of Ap and Ar is highlighted before T and M, but after moderate acceptance of these two attributes, T and M will take the prominent roles. Involving the weightage of attributes in the final decision of sensory evaluation is important for precise sensory evaluation. Fuzzy logic methods have already been applied to insert the weightage of sensory attributes (Oganesyants *et al.*, 2020; Dhar *et al.*, 2021). The fuzzy logic method (similarity analysis) has also been applied to rank the attributes (Kaushik *et al.*, 2015; Sugumar and Guha, 2022). For instance, Kaushik *et al.* (2015) ranked the sensory attributes, and applied the method for inserting the weightage to the final ranking of food products.

In the present work, we introduced a new fuzzy toolbox method for involving attribute weightage, which is also capable of inserting the priority and latency of attributes. Using this approach, a variety of rules can be defined based on experience, trial, *etc.* Therefore, it is possible to alter the rules to fit desired product based on any special idea, and this is the main advantage of the method. It is even possible to define all rules one by one based on the characteristics of final products, the application of different technologies, or even the influence of cultural customs.

Based on the output obtained from the surface view (Figure 1), the ranking of final scores was as follows: < 8, poor; 8 - 12.5, fair; 12.5 - 16, good; 16 - 20, very good; and > 20, excellent.

Therefore, CS values obtained in Table 2 were inserted into fuzzy inference, and the final G scores for the 18 trials were obtained.

Mixture analysis design

Data produced by the fuzzy model (G score in Table 2) and free radical scavenging activity were introduced to the mixture analysis design as responses (R_1 and R_2 in Table 1, respectively). The software did not propose data transformation, as both responses were normal. Therefore, the fuzzy model's method was suitable for producing normal data for mathematical design methods. Based on the results, two different models were proposed for sensory and antioxidant activity. For the sensory response, the selected model was modified-special cubic, a favoured model in mixture design (Cornell, 2002). The same inference was reported by Bhalerao *et al.* (2020) and Dhar *et al.* (2021). The significant p -value of the model (0.011), non-significant lack of fit (0.438), suitable R^2 (0.99), adjusted R^2 (0.97), and closeness of predicted R^2 (0.79) to adjusted R^2 were evidence of model adequacy. The Piepel graph demonstrates the main effect of the factors, and was applied to compare the effect of all factors, simultaneously. The present work showed that St and Mu were factors with cubic behaviour, while the others showed quadratic behaviours. A synergistic or antagonistic characteristic may exist between independent variables in cubic and special cubic models. Therefore, it could be concluded that there was dependence between components to produce such a model, and fruit juice components could not be varied independently to find the optimum sensory. The final equation for sensory response in the form of the real component was as follows:

$$\begin{aligned} \text{Sensory} = & 18.29621(\text{Po}) + 17.46324(\text{Gr}) + \\ & 18.84621(\text{Mu}) + 17.12315(\text{St}) + 3.69194(\text{Po} * \text{Gr}) - \\ & 0.410216(\text{Po} * \text{Mu}) - 2.84120(\text{Po} * \text{St}) + 2.59194(\text{Gr} \\ & * \text{Mu}) + 5.36096(\text{Gr} * \text{St}) - 2.34120(\text{Mu} * \text{St}) - \\ & 36.15812(\text{Po} * \text{Gr} * \text{St}) + 74.24188(\text{Gr} * \text{Mu} * \text{St}) \end{aligned} \quad (\text{Eq. 10})$$

The software also proposed a quadratic model for the antioxidant activity response. Adequate model significance ($p < 0.0001$), lack of fit non-significance ($p = 0.126$), and very high regression criteria ($R^2 = 0.96$, R^2 (adj) = 0.94; and R^2 (pred) = 0.90) were observed, which indicated that the model has been correctly selected. Although the model was adequate and correct, the behaviour of Po and Mu was close to linear. Furthermore, it could be derived that increasing the percentage of Gr decreased the

mixture's free radical scavenging activity level. The final equation for antioxidant activity response in the form of the real component was as follows:

$$\begin{aligned} \text{Antioxidant activity} = & 91.22539(\text{Po}) + 16.85947(\text{Gr}) \\ & + 91.92416(\text{Mu}) + 81.48188(\text{St}) + 72.24436(\text{Po} * \text{Gr}) \\ & - 59.39021(\text{Po} * \text{Mu}) + 115.30609(\text{Gr} * \text{Mu}) \end{aligned} \quad (\text{Eq. 11})$$

Based on the selected models, the optimisation section was run to find the optimum mixture with the highest sensory acceptance and antioxidant activity. Numerical optimisation and point prediction in post-analysis proposed a mixture including Po, 0%; Gr, 20.4%; Mu, 75.6%; and St, 4.0% as the first solution. The software predicted 19.1 - 19.5 score as 95% confidence interval of low and high means for the sensory response. The predicted low and high means with 95% confidence interval for antioxidant activity were 86.2 and 101.6, as well. The predicted means obtained in the confirmation section were 19.3 and 93.9 for sensory and antioxidant activity, respectively.

In the present work, a novel mixed beverage with high quality and free radical scavenging activity was introduced by mixture design, while the sensory characteristics of the beverage was also improved. Similar investigations have also inferred that mixing fruit juices can promote the sensorial acceptance and physicochemical properties of mixed fruit beverages (Curi *et al.*, 2017; Schiassi *et al.*, 2020). This phenomenon has been attributed to the composition of the characteristics of fruits. However, the results were exciting and unexpected as the software did not include Po juice, a popular and well-known beverage, in the predicted mixture. This finding encouraged us to investigate the reasons for this prediction. Po contains high phenolic compounds and antioxidant activity (Ghalegi Ghalehnoe *et al.*, 2021), with various phenolic and anti-tumour compounds such as pelargonidin, cyanidin, delphinidin, and punicalcortin (Sreekumar *et al.*, 2014; Noomsiri and Lorjaroenphon, 2018). Furthermore, based on the literature, Po cultivars contain a remarkable content of reducing and non-reducing sugars, while their taste is commonly sweet and sour (Noomsiri and Lorjaroenphon, 2018).

A glance at Piepel graphs indicated that deviation of A (Po) from the centre point to the left and right had a positive and negative impact on mixed

beverage sensory, respectively (Figure 1e). Therefore, it can be concluded that Po had good acceptance in its single form, while the overall acceptance decreased when added to other juices. In contrast, a deviation of B (Gr) from the centre point to the left and right was ultimately accompanied by a negative impact. Therefore, it can be concluded that Gr had moderately low acceptance in its single form, but could improve overall acceptance in a juice mixture (Figure 1e). Negative interaction between Po and Mu (-0.41) in comparison to positive interaction between Gr and Mu (2.59) may also help explain this phenomenon, and indicated that Gr and Mu were more compatible with improving the juice mixture's aroma, colour, taste, and mouthfeel. This could be related to the nature of beverages, such as sweetness capability, viscosity, and aroma. For instance, the sensory panel described that the sweetness of applied Mu was low, which was expected, and the Gr juice used in the present work was very sweet.

On the other hand, Gr in low levels did not considerably affect the antioxidant activity, while by increasing higher than 25%, the total antioxidant activity of mixed beverage decreased (Figure 1f). Unlike Po and Gr, almost ascending trend in both sensory and antioxidant activity was observed for Mu. This could explain why Mu received the highest percentage in the selected mixture juice.

The relationship between dependent and independent variables can also be explained in the 3D response surface plots, where the participation of B (Gr) and C (Mu) increased sensory acceptance, and the negative role of Gr in antioxidant activity was evident (Figures 1g and 1h). Finally, the selected mixture was evaluated by the same panellists, a polynomial model was developed for data, and CS and G scores were calculated. The G score obtained for the selected mixture using the present method was 19.2 ± 0.1 , staying in the very good category, and between the 95% confidence interval predicted by the software. Therefore, the result was valid and trusted. The free radical scavenging activity of the selected mixture was 91.82 ± 2.05 , within the 95% confidence interval predicted by the software.

Comparison of physicochemical properties of selected fruit juice mixture and single fruit juices

The physicochemical properties of single fruit juices and the selected mixture are shown in Table 3. It could be concluded that the taste of these juices was

Table 3. Physicochemical properties of selected mixed fruit juice and single fruit juices.

Juice	pH	Titrateable acidity (citric acid %)	Total soluble solids (%)	Total phenol (mg GAE/L)	Radical scavenging activity (DPPH %)	DPPH IC ₅₀ (µL/mL)	FRAP (µmol/mL)
Pomegranate	3.59 ± 0.03	1.66 ± 0.05	13.43 ± 0.06	2407.33 ± 115.92 ^b	89.96 ± 3.78 ^a	16.06	290.7 ± 3.7 ^b
Red grape	3.71 ± 0.02	1.03 ± 0.03	17.39 ± 0.14	1235.42 ± 126.71 ^c	16.85 ± 1.47 ^c	154.86	69.5 ± 4.8 ^e
Mulberry	5.36 ± 0.01	1.09 ± 0.03	18.21 ± 0.48	3217.33 ± 113.70 ^a	92.45 ± 3.12 ^a	10.54	312.6 ± 6.3 ^a
Strawberry	5.14 ± 0.03	1.45 ± 0.04	7.79 ± 0.12	1415.15 ± 96.86 ^c	84.15 ± 1.39 ^b	18.64	272.2 ± 5.7 ^c
Selected mixture	4.54 ± 0.03	1.18 ± 0.04	15.73 ± 0.14	2887.42 ± 155.69 ^a	91.82 ± 2.05 ^a	17.92	213.6 ± 2.9 ^d

Values are mean ± standard deviation of three replicates ($n = 3$). Means within columns with different lowercase superscripts are significantly different ($p < 0.05$).

sour-sweet as the fruit beverages' pH was moderately low, with each single fruit juice containing moderately high acidity while also including some sugars. Lachowicz and Oszmiański (2018) also concluded that the acidity and sweetness of different fruit juices could complement each other, which agrees with the present work.

With regard to the single fruit juices, the highest and lowest total phenolic content and free radical scavenging activity belonged to Mu and Gr, respectively. Results also indicated that the free radical scavenging activity of the selected juice mixture was significantly higher than any two single fruit juices, including Gr and St (Table 3).

Different fruit juices have distinct free radical scavenging capacities, possibly due to the polyphenolic compounds' additional content and diversity. Hence, a combination of fruit drinks may have more biological activity than a single juice. This phenomenon can be explained by several types of interactions and synergistic or antagonistic properties of phenolic compounds (Sreekumar *et al.*, 2014). As indicated in the analysis of the mixture design, the model was quadratic with a positive interaction between Mu with Gr ($Gr*Mu = +115.31$). Therefore, the considerable free radical scavenging activity of the juice mixture would be logical. Some study have also reported this phenomenon (Lachowicz and

Oszmiański, 2018). A positive Pearson correlation (0.70) was also observed between total phenolic compounds and free radical scavenging activity (DPPH%), thus resulting in the conclusion that the majority of free radical scavenging activity of juices is due to phenolic compounds. This agreed with previous studies (Curi *et al.*, 2017; Lachowicz and Oszmiański, 2018; Okatan, 2020). A negative Pearson correlation (-0.99) between IC_{50} and free radical scavenging activity was obtained, which was the reason for adequate accuracy of fruit juice DPPH inhibition results.

The capability of reducing ferric iron accomplished by FRAP confirmed the results obtained by DPPH. Overall, Mu and Gr were fruit juices with the highest and lowest antioxidant activity. A positive Pearson correlation (0.92) was also observed between the FRAP and DPPH results.

Comparison of freeze- and spray-dried powder Yield, free radical scavenging activity, and total phenolic compounds

A significant ($p < 0.05$) difference was observed between the powder yield of the spray-dryer ($51.7 \pm 5.9\%$) and the freeze-dryer ($82.0 \pm 2.6\%$) (Figure 2a). In fact, the yield of the freeze-dryer was 30.3% higher than the spray-dryer for the production of mixed fruit beverage powder. This could be due to

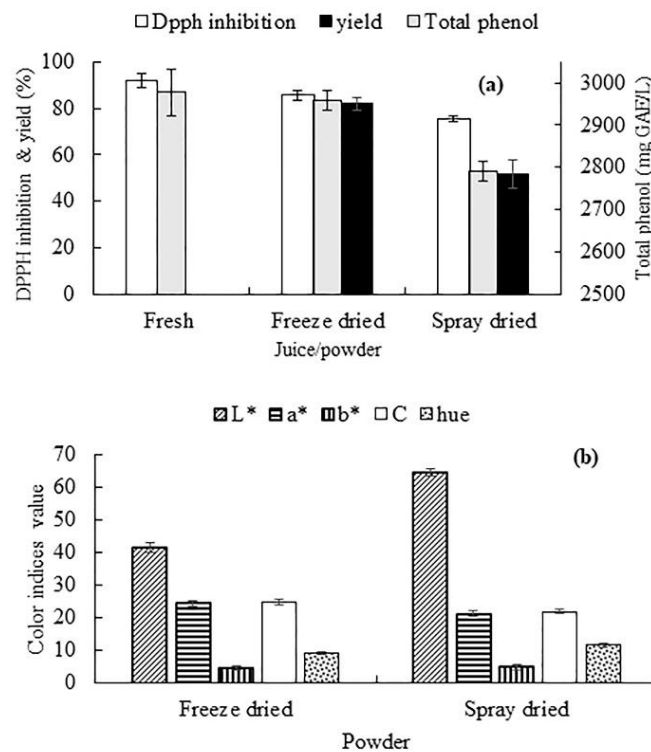


Figure 2. Yield, antioxidant activity, total phenolic content (a), and colour analysis (b) of freeze- and spray-dried powders of selected mixed juice.

very low product escapement in the freeze-dryer, unlike the spray-dryer which led to an expected high yield. Two main reasons have been mentioned for low yield in spray-drying: powder particle escapement through the outlet air and adherence of particles to the inside wall of the spray-dryer chamber (Maury *et al.*, 2005). However, the moisture content of freeze-dried powder was $5.9 \pm 0.65\%$, and when considering the moisture content in the yield formula, it decreased to 76.1%. Considering the spray-dryer powder moisture content ($4.9 \pm 0.38\%$), the calculated yield was reduced to 46.8%, as well. Due to the lack of similar reports in the simultaneous drying of related mixed beverages by spray- and freeze-dryer, a precise comparison of results may not be possible. Shuen *et al.* (2021) compared the drying methods for the production of kuini powder, and showed that the yield in the freeze-drying method was as high as 2.5 times when compared with the spray-drying method, which was in agreement with the present study. Adetoro *et al.* (2020) evaluated freeze-dryer recovery on the production of pomegranate powder, and reported 46.6% as the highest yield. However, their applied method for calculating the yield was based on the fresh weight of feed, which was different from the technique carried out in the present work. Regarding the spray-dryer, the yield of the powder depends on many factors, including inlet and outlet temperature, air and feed flow rate, type, and content of carrier. Overall, the value obtained for yield in the present work was in a moderate range of yield for fruit juice powder production by spray-dryer (Saikia *et al.*, 2014; Shuen *et al.*, 2021). It is noteworthy to mention that further optimisation could improve the yield in the spray-dryer. Besides yield, free radical scavenging activity and total phenolic content of the powder obtained by the freeze-dryer were significantly ($p < 0.05$) higher than that obtained by the spray-dryer. Free radical scavenging activity of the powder obtained by freeze-dryer was 6.5% less than fresh mixed fruit beverage, while it decreased by 16.7% when a spray-dryer was applied (92.1, 85.6, and 75.4% for fresh, freeze-dried, and spray-dried, respectively). No significant ($p \geq 0.05$) decrease in the total phenolic content of freeze-dried powder as compared to fresh mixed beverage could also indicate that freeze-drying is less destructive than spray-drying, as the entire phenolic content of the spray-dryer was significantly ($p < 0.05$) less than the freeze-dryer (2958.8 in comparison to 2791.4 mg GAE/L,

respectively) (Figure 2a). To find comparable data with fresh fruit juice, powders were dissolved in distilled water based on dry weight, and free radical scavenging activity, and total phenolic content was calculated in the solution similar to the fresh fruit beverage.

Spray-drying involves heating during the drying process. Therefore, heat-sensitive components are at risk of decomposition. Additionally, previous study reported that the yield of powder production in spray-dryers is usually lower than in freeze-drying methods (Shuen *et al.*, 2021). However, the efficiency of a freeze-dryer is much lower than a spray dryer, as the drying process is time-consuming. Therefore, unlike the clear superiority of the freeze-drying method in the yield and quality of the produced powder, the spray-drying method may be more attractive because of its efficiency and lower cost. Other criteria for the quality of the powder, such as water activity, hygroscopicity, water-solubility, *etc.*, could also be compared to find a desirable method but have not been discussed here to avoid prolonging the study.

Colour analysis

The effect of the drying method on some surveyed indices was significant ($p < 0.05$). The powder obtained by the spray-dryer was much lighter in colour than the freeze-dryer ($L^* = 64.45$ and 41.33 , respectively) (Figure 2b). This could also be visually observed. Furthermore, the redness of freeze-dried powder was significantly higher than the one obtained by the spray-dryer. However, the method of drying did not result in a significant effect on yellowness. Higher chroma index and lower hue angle were also observed in the powder obtained by the freeze-dryer as compared to the spray-dryer (24.80 and 9.16 compared to 21.85 and 11.65, respectively).

A decrease in redness when increasing the inlet temperature of the spray-dryer was mentioned by Ghalegi Ghalenoi *et al.* (2021) during Po powder production. The same phenomenon has also been observed for spray-dried powders of carrot and *amla*, which could be attributed to the destruction of thermo-sensitive components such as anthocyanin pigments (Chen *et al.*, 1995; Mishra *et al.*, 2014). In a well-designed experiment, the spray-dryer caused higher lightness as compared to the freeze-dryer during pea powder production, while the redness of the product decreased (Chen *et al.*, 2021). The freeze-

dryer retained the yellowness of pea powder much better than the spray-dryer, which was attributed to a low temperature applied during the process. In conclusion, the increasing lightness value and hue angle, and decreasing a* observed in the present work for the spray-dried powder of the mixed beverage were strong proofs of the much better capability of freeze-drying for the production of darker juice powder, similar in the colour to fresh mixed fruit juice.

Conclusion

Mixture analysis and a fuzzy logic algorithm were successfully applied to optimise the mixture of fruit beverages. The optimised mixed fruit juice consisted of grape (20.4%), mulberry (75.6%), and strawberry (4.0%). The optimised mixed beverage included more varied phenolic content than single fruit juices. Both freeze- and spray-dryer were successfully applied to produce fruit juice powder. The freeze-dryer generated a powder with a higher yield, free radical scavenging activity, and phenolic content than the spray-dryer. In addition, the freeze-dryer produced a darker powder with more increased redness and chroma index than the spray-dryer. Overall, the freeze-dryer's impact on fruit juice powder production was less destructive than the spray-dryer.

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