Application of RSM and Multivariate Statistics in Predicting Antioxidant Property of Ethanolic Extracts of Tea-Ginger Blend

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Authors’ contributions

This work was carried out in collaboration between all authors. Author SAM carried out the research, statistical analysis and wrote the first draft of the manuscript. Author VNE reviewed the first draft of the manuscript. All authors read and approved the final manuscript.

ABSTRACT

The optimum conditions for ethanolic extraction of antioxidants from tea-ginger blend were determined using response surface modelling. The relationship between the colour, hue index and antioxidant properties of the extracts were also expressed as multivariate models using ordinary least square, principal component and partial least square regressions (OLSR, PCR, and PLSR). Results from the multi-response optimisation revealed the optimum conditions for the extraction as temperature of 50.16°C, concentration of 2.1 g (100 ml)⁻¹ and time of 5 minutes with a desirability of 0.68. The PLSR gave the most preferable model among the three multivariate regression techniques investigated. Hue index, A₅₁₀ and a* were able to predict total flavonoid content (R² = 0.933, Q² = 0.905) and diphenyl-picrylhydrazyl (DPPH) radical activity (R² = 0.945, Q² = 0.919). The a*, A₅₁₀, hue Index and hue were able to predict iron chelating activity (R² = 0.854, Q² = 0.794). The study revealed that colour and hue index property could give an indication of some antioxidant properties of ethanolic extracts of tea-ginger blend.

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1. INTRODUCTION

Ginger has gained popularity worldwide for its culinary and nutraceutical usage. Ginger root is one of the most heavily consumed dietary substances in the world [1,2]. The health benefits of ginger are derived mainly from its antioxidant property. Rats fed with ginger extract and methotrexate have been reported to have enhanced antioxidant levels compared with rats fed with methotrexate only which experienced a decline in antioxidant levels [3]. This indicates that ginger could play a role in reducing the effect of oxidative stress. Ginger contains many bioactive phenolic compounds, including non-volatile pungent compounds such as gingerols, paradols, shogaols and gingerones [4]. Tea (Camellia sinensis) is the most widely consumed beverage in the world after water [5]. Flavonoids are one of the major antioxidant components of tea. Tea flavonoid consumption has been linked to lower incidences of chronic diseases such as cardiovascular disease and cancer [6].

Antioxidants came to public attention in the 1990s, when scientists began to understand that free radical damage was involved in the early stages of artery clogging atherosclerosis and may contribute to cancer, vision loss, and a host of other chronic conditions [7]. Antioxidants have been known to prevent degenerative oxidative reactions. The antioxidant property confers on ginger and tea their ability to prevent oxidation of cells thus hindering malignant reactions. With the increase in oxidative stress in humans as a result of globalization and industrialization, the need to increase the consumption of antioxidants is quite germane. A combination of different antioxidants can help increase protection against free radical reaction. According to Halvorsen et al. [8], a combination of different redox-active compounds (i.e., antioxidants) may be needed for proper protection against oxidative stresses.

Various novel techniques have been employed to recover phenolics from plant matrices but from an industrial production point of view, solvent extraction is commonly chosen due to simplicity, efficiency of the procedure, and low investment costs required in terms of equipment [9]. Parameters having a great impact on the amount and composition of antioxidants in extracts, and thus on the measured antioxidant capacity, notably include the extraction solvent composition, temperature, extraction time (duration), solvent-to-solid ratio, and storage conditions [10].

Quality control is an essential part of the food manufacturing chain. An important quality check for ginger and tea is their antioxidant property. The measurement of quality parameters (i.e., antioxidants) is generally, carried out using traditional analytical techniques whose application in the food industry poses several problems: they require very long duration, are expensive and destructive [11]. Colour can be an important indication of the antioxidant properties of foods. An understanding of this relationship can help present a rapid analytical technique for the evaluation of the antioxidant content of tea-ginger extracts. This is possible because many food components – such as xanthophylls, lycopenes, tannins, anthocyanins and β-carotenes – are responsible for the colour of the food. The colour of foods will usually change when these food pigments undergo degradation. Degradation of these pigments can occur due to storage method used and processing method applied. It was reported that canned whole tomatoes packed in CaCl₂ juice were lighter than tomatoes packed in ordinary juice [12]. The advantage of relating colour property of food to their antioxidant property centres on the opportunity of doing rapid online in-process check in the factory to have an indication of the antioxidant property of the extract being produced. This means that the time for reagent preparation, sample preparation and incubation time are eliminated. The other advantage that would be presented by this new approach will be the reduced frequency in the use of analytical reagents. This means a reduced cost of evaluation. Another positive this approach offers is environmental friendliness – as the volume of reagent that will be used for antioxidant analysis will be reduced. It has been demonstrated that the antioxidant activity and total phenol content of carrots can be predicted from their colour [11]. Also colour measurements of intact tomatoes have been used as a non-destructive method to assess total antioxidant capacity of tomatoes [13].

In this study, we seek to: i) determine the optimum condition for ethanolic extraction of antioxidants from tea-ginger blend using response surface methodology (RSM), ii) investigate the relationship between colour, hue index and antioxidant properties of the ethanolic

**Keywords:** Antioxidants; tea-ginger blend; ethanolic extraction; optimisation; multivariate statistics.
tea-ginger extracts using multivariate statistics (ordinary least square regression – OLSR, principal component regression, PCR and partial least square regression – PL$	ext{SR}$).

To our knowledge, this is the first study looking at extraction of antioxidants from tea-ginger blend. Furthermore we are not aware of studies that have tried to predict antioxidant properties of tea, ginger and tea-ginger extracts from their colour property.

2. MATERIALS AND METHODS

2.1 Plant Material and Processing

Tea leaves were obtained from Obudu Mountain in Cross River state in Nigeria. The tea leaves were sun-dried, ground and passed through a 1.4 mm sieve. Ginger rhizomes were obtained from Kaduna state. Kaduna state is the leading ginger producing state in Nigeria. The ginger rhizomes were peeled, sun-dried and ground. The powder samples were passed through a 1.4 mm sieve. The obtained powders were packed in aluminium foil and stored under refrigerated condition until analysis.

2.2 Extraction

The extraction was done in a conical flask placed on temperature controlled magnetic stirrer (UC 152, Bibby Scientific, UK). The stirrer speed was set at scale 3. Ethanol was then introduced into the conical flask. The flask was covered with aluminium foil to minimize light penetration. To ensure the accuracy of the extraction temperature, a temperature controller (SCT 1, Bibby Scientific, UK) was placed inside the conical flask and connected to the temperature controlled magnetic stirrer. Once the required extraction temperature was reached, the required weight of blended powder sample of tea-ginger (2:1) was introduced into the conical flask. Tea-ginger (2:1) powder was selected after some preliminary investigation which revealed that the tea-ginger (2:1) powder had a higher total flavonoid content compared to the tea-ginger (1:1) and tea-ginger (1:2) extracts. The extraction was continued until the required extraction time was achieved. The extract was then filtered to remove the residues.

2.3 Response Surface Methodology

A face centered central composite design with three independent variables was used. The design consisted of 20 experiments: 8 factorial points, 6 axial points and 6 central points. The range of the independent variables investigated were: extraction temperature (TEM: 30-70 °C), powder to solvent ratio (CON: 0.12-2.10 g/100 ml), extraction time (TIM: 5-90 min). The response variables consisted of selected antioxidant properties of the extracts. The antioxidant properties were: total flavonoid content (TFC), total phenol content (TPC), 2,2’-azinobis (3-ethylbenzothiazoline sulfonate (ABTS) radical activity, diphenyl-picyrylhydrazyl (DPPH) radical activity, peroxide scavenging activity (PSA) and iron chelating activity (ICA). Data were fitted to different models. Models considered were linear, 2 factor Interaction and quadratic. Analysis of variance (ANOVA) was carried out to select the best model. The best model selected was further subjected to backward regression to remove redundant variables. Both single response and multi-response optimisation were done using the desirability concept. The optimisation was set to maximise all the antioxidant properties and the process conditions were set to be within the experimental range. The antioxidant properties were all given an equal weighting of 1 for the optimisation. The quality of the model was determined by evaluating the lack-of-fit, the coefficient of determination ($R^2$), Adjusted $R^2$, Predicted $R^2$, and adequate precision.

2.4 Prediction of Antioxidant Properties from Colour and Hue Index Properties of the Extract

Colour (CIE $L^*$, $a^*$, $b^*$), sample absorbance at 510 nm ($A_{510}$) and 610 nm ($A_{610}$) of the extracts were determined. $L^*$ is a measure of lightness with value ranging from 0 to 100. The $a^*$ and $b^*$ are chromaticity coordinates. From the $a^*$ and $b^*$ values, the hue and chroma of the extract were estimated. The hue index value was also estimated from $A_{510}$ and $A_{610}$. The hue index has been used in the caramel industry as an indicator of its colour [14]. The suitability of hue index in evaluating colour of tea has also been reported [15]. A multivariate regression was conducted on the obtained data. The dependent variables were the antioxidant properties. The independent variables were: $L^*$, $a^*$, $b^*$, hue, chroma, $A_{510}$, $A_{610}$, $A_{510}/A_{610}$ and hue index. The multivariate statistics used were: ordinary least square regression (OLSR), principal component regression (PCR) and partial least square regression (PLSR). The data were scaled and centered before running the regression analysis. In the PCR analysis, the
regression was run for components that explain between 90 to 99% of the variation in the independent variables. The dependent variables were also subjected to some transformation (log10, square root and inverse square root) to check if it improves the quality of the model.

2.5 Antioxidant Analysis

ABTS was assayed using the improved technique of Miliauskas et al. [16], as described by Spradling [17]. A phosphate buffer solution (PBS) was prepared by mixing 95 ml of sodium phosphate monobasic (2.98 g 100 ml⁻¹) and 405 ml of sodium phosphate dibasic (15.6 g 500 ml⁻¹), followed by 8.04 g of sodium chloride and filled to volume (1 l), lastly the pH was adjusted to 7.4 with 2M NaOH. The ABTS mother solution was prepared by mixing 44.8 mg of ABTS, 8.12 mg potassium persulfate, and 20 ml of distilled water. The solution was allowed to react in the dark for 12 h. The ABTS working solution was prepared by mixing 145 ml of PBS with 5 ml of the ABTS mother solution. Trolox was used as standard. To 2900 μl of the ABTS working solution, 100 μl of each extract or standard was added and allowed to react for 15 min before reading spectrophotometrically (Spectrumlab 23A, England) at 734 nm against a blank solution.

DPPH was measured as described by Sompong et al. [18]. The reaction mixture contained 1.5 ml DPPH working solution (4.73 mg of DPPH in 100 ml ethanol HPLC-grade) and 300 μl extract. The mixture was shaken and left to stand for 40 min in the dark at room temperature. The absorbance was read at 515 nm relative to the control (100%) using a spectrophotometer. The percentage of radical-scavenging ability was calculated by using the formula:

DPPH scavenging ability (%) =

\[ \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \]  

where \( A_{control} \) = Absorbance at 515 nm of control, \( A_{sample} \) = Absorbance at 515 nm of sample.

Iron chelating activity was measured by the method of Dinis et al. [19] as described by Ozena et al. [20]. The samples were added to a solution of 2 mM FeCl₂ (0.05 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml) and the mixture was shaken vigorously and incubated at room temperature for 10 min. The absorbance of the resulting solution was then measured at 562 nm. The iron chelating activity was calculated by the given formula:

Iron chelating activity (%) =

\[ \frac{(A_{control} - A_{sample})}{A_{control}} \times 100 \]  

where \( A_{control} \) = Absorbance at 562 nm of control, \( A_{sample} \) = Absorbance at 562 nm of sample.

Peroxide scavenging activity was measured by the method of Smirnoff and Cumbes [21] as described by Ozena et al. [20]. Peroxide radicals were generated by mixing of FeSO₄ and H₂O₂. The reaction mixture contained 1 ml FeSO₄ (1.5 mM), 0.7 ml H₂O₂ (6 mM), 0.3 ml sodium salicylate (20 mM) and appropriate volume of extracts. This was followed by incubation for 1 h at room temperature. The absorbance of the hydroxylated salicylate complex was measured at 562 nm. The percentage scavenging activity was calculated as:

The peroxide scavenging activity (%) =

\[ 1 - \frac{(A_1 - A_2)}{A_0} \times 100 \]  

Where \( A_0 \) is the absorbance of the control (without extract or standards), \( A_1 \) is the absorbance including the extract or standard and \( A_2 \) is the absorbance without sodium salicylate.

Total flavonoid content was measured as described by Prommuaka et al. [22]. A 0.5 ml of the extracted samples or catechin solutions was mixed with 1.5 ml of 95% ethanol (v/v), 0.1 ml of 10% aluminum chloride - AlCl₃·6H₂O (m/v), 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water, and the mixture was incubated at room temperature for 30 min. The absorbance of the mixture was then measured against a blank using a spectrophotometer at 415 nm. The blank contained all the reagents except the extract. Catechin was used as standard.

Total phenol was measured as described by Waterhouse [23], using the method of Slinkard and Singleton [24]. From the calibration solution, extract, or blank, 50 μl volume was taken and added to 1.58 mL water, and 100 μl of Folin-Ciocalteu reagent, and mixed well. After 8 min, 300 μl of the sodium carbonate solution was added. The solutions were left at room temperature for 1 h and absorbance of each solution was determined at 765 nm against the blank. The sodium carbonate solution was prepared by dissolving 200 g of anhydrous sodium carbonate in 800 ml of water and brought
to boil. After cooling, a few crystals of sodium carbonate powder were added. After 24 h, the solution was filtered and made up to 1 l. Gallic acid was used as standard.

2.6 Colour and Hue Index Analysis

Colour was measured with a spectrophotometer CM-700d (Konica Minolta Sensing). The spectrophotometer was calibrated against a white plate. The extracts were placed in a cuvette for the measurement. The CIE L*, a* and b* values were read from the spectrophotometer. Readings were taken in triplicate. Hue was calculated as θ using eq. 4.

\[ \Theta = \tan^{-1}(b*/a*) \] (4)

The following transformation were applied to the calculated θ [25]

If \( a^* > 0 \) and \( b^* > 0 \) then hue = θ \hspace{10cm} (5)
If \( a^* < 0 \) and \( b^* > 0 \) then hue = 180 + θ \hspace{10cm} (6)
If \( a^* < 0 \) and \( b^* < 0 \) then hue = 180 + θ \hspace{10cm} (7)
If \( a^* > 0 \) and \( b^* < 0 \) then hue = 360 + θ \hspace{10cm} (8)

Chroma was calculated with eq. 9.

Chroma = \( \sqrt{(a^* + b^*)^2} \) \hspace{10cm} (9)

The hue index was calculated from eq. 10.

Hue index = \( 10 \times \log(A510/A610) \) \hspace{10cm} (10)

The A610 and A510 values were determined by measuring the absorbance of the extracts against a distilled water blank in a spectrophotometer.

2.8 Software

The response surface analysis was carried out using Design Expert v 7.0.0 (Stat-Ease). The multivariate statistics was done with XLSTAT Pro, 2013 (Addinsoft)

3. RESULTS AND DISCUSSION

3.1 Single Response Optimisation

Table 1 shows the regression parameters for the extraction of antioxidants. The very low P-values (\( P < 0.05 \)) demonstrate that all the antioxidant extraction models were significant. An insignificant lack-of-fit (\( P > 0.05 \)) indicates that the entire extraction model fits the data well. The \( R^2 \) is a measure of the ratio of the mean sum of squares to total sum of squares (MSS/TSS). This gives a measure of the amount of variation in the data explained by the model. A value closer to one indicates that the model has a very good fit. However, the \( R^2 \) tends to increase with an increase in the number of variables in the model. With this situation a case may arise such that a model may have a very high \( R^2 \) but a low predictive quality. To compensate for the weakness in \( R^2 \), another parameter used to assess a model is the adjusted \( R^2 \). The adjusted \( R^2 \) does not necessarily increase with an increase in the number of variables in the models. The adjusted \( R^2 \) only increases if the new variable added to the model has a significant contribution to the model. The \( R^2 \) for the extraction models in this investigation range from 0.5410 to 0.9212 and the adjusted \( R^2 \) range from 0.4550 to 0.8848. This means that the extraction model with the least adjusted \( R^2 \) was able to explain 45.5% of the extraction process. The adjusted \( R^2 \) from this study indicates that the parameters included in the different extraction models could explain the various extraction process but this does not in any way tell us about the predictive ability or quality of the model. The predicted \( R^2 \) is a measure of the predictive quality of model. It measures the ability of a model to predict from new data. From Table 1, it is observed that the predictive quality of TPC and ABTS are very low with predicted \( R^2 \) of 0.1271 and 0.1779, respectively. The extraction models for TFC, PSA, ICA and DPPH have good predictive quality, with predicted \( R^2 \) of 0.8325, 0.5379, 0.6333 and 0.7396, respectively. The adequate precision is a measure of signal to noise ratio of the model. It is a measure that indicates if the model generated can be used to navigate the design space. The Design Expert software suggested that a value greater than 4.0 can be used to navigate the design space.

A square root transformation was used for the extraction model for TFC, as it gave a better model (\( R^2 = 0.8982 \), adjusted \( R^2 = 0.8791 \), predicted \( R^2 = 0.8325 \)) compared to the untransformed model (\( R^2 = 0.8326 \), adjusted \( R^2 = 0.8129 \), predicted \( R^2 = 0.7545 \)). Similar pattern was also observed in the TPC models and a square root transformation was found to give the best model. Temperature, concentration and quadratic effect of concentration had significant influence (\( P < 0.05 \)) on the extraction of flavonoids. Temperature, concentration and interaction of temperature and concentration had significant influence on extraction of phenolics.
The ABTS of the extract was influenced by temperature, concentration, time and quadratic effect of temperature. Temperature, concentration, time, temperature-concentration interaction, temperature-time interaction and quadratic effect of temperature had significant influence on peroxide scavenging activity of the extracts. The iron chelating activity of the extracts was significantly influenced by temperature, concentration, temperature-concentration interaction and quadratic effect of temperature. Temperature, concentration, time, temperature-concentration interaction, concentration-time interaction and quadratic effect of concentration had significant influence on the DPPH activity of the extracts. A look at the regression coefficients for all the extraction models indicated that concentration had the most significant impact on the antioxidant property of the extracts. The response surface graphs for the models are shown in Fig. 1. From Figs. 1a and c, the optimum temperature to maximise TFC and ABTS are around 60°C and 50°C, respectively. A numerical optimisation approach using the desirability factor approach was used to obtain the optimum conditions for the antioxidant extractions (Table 2). Using a central composite design model [26], the optimal conditions for extraction of total flavonoid from green tea using the desirability function was achieved at the extraction time of 32.5 min, ethanol concentration of 100% (v/v) and solid-to-liquid ratio of 1:32.5 (m/v). This extraction was performed at the boiling point of ethanol. In our study, the optimum condition for extraction of total flavonoids from tea-ginger was 58.14°C, 2.10 and a time of 9.99 min. The desirability for the single response optimisation extraction was defined to maximise each of the antioxidant properties in this study.

### Table 1. Response surface model for ethanolic tea-ginger (2:1) extraction

| Source     | TFC | TPC (mg) | ABTS | PSA | ICA | DPPH |
|------------|-----|----------|------|-----|-----|------|
| Transformation | Square root | Square root | 0.9615 | 30.3143 | 57.8835 | 111.6115 |
| INTERCEPT | -70.3393<sup>a</sup> | 36.8798 | 0.9615 | 30.3143 | 57.8835 | 111.6115 |
| TEM | 3.1403 | -0.08268 | 2.1437E-3 | 1.7701 | 0.3980 | -0.9668 |
| CON | 30.5182 | -7.7515 | -0.01279 | 12.4191 | -0.04034 | -0.9648 |
| TIM | -2.031E-4 | 0.1749 | -0.01724 | 3.7205E-3 | 9.192E-3 | 9.192E-3 |
| TEM*CON | 0.2211 | -0.2913 | 0.06233 | -0.1244 | -0.9668 |
| TEM*TIM | -4.907E-3 | 0.1749 | -0.01724 | 3.7205E-3 | 9.192E-3 |
| CON*TIM | -0.05581 | -0.9668 | 0.1749 | -0.01724 | 3.7205E-3 |
| TEM<sup>2</sup> | -0.02700 | -2.482E-5 | -0.01724 | -0.9668 | 0.1749 |
| CON<sup>2</sup> | -0.9668 | 0.1749 | -0.01724 | 3.7205E-3 | 9.192E-3 |
| TIM<sup>2</sup> | 20.759 | 7.762 | 9.872 | 11.160 | 11.750 | 18.615 |

<sup>a</sup> Regression coefficients are in actual factors.

### Table 2. Optimised conditions for ethanolic tea-ginger (2:1) extraction

| Source | TFC | TPC | ABTS | PSA | ICA | DPPH |
|--------|-----|-----|------|-----|-----|------|
| TEM (°C) | 58.14 | nd | nd | 30.00 | 67.14 | 30.00 |
| CON (g 100 ml<sup>-1</sup>) | 2.10 | nd | nd | 2.10 | 2.10 | 0.12 |
| TIM (min) | 9.99 | nd | nd | 90.00 | 5.20 | 5.35 |

<sup>nd</sup> = optimised conditions not determined due to the low predictive R<sup>2</sup>
Fig. 1. Response surface graphs showing effect of extraction variables on antioxidant properties (a), total flavonoid content (b), total phenol content (c), ABTS (d), peroxide scavenging activity (e), iron chelating activity (f), DPPH.
3.2 Multi-Response Optimisation

The different antioxidant properties have varied optimum regions (Table 2). This has an implication such that a particular antioxidant property might have reached its maximum and begins to degrade, whereby another antioxidant property may just start approaching its maximum. To resolve this kind of issue, the multi-response optimisation using the desirability approach was used. The desirability for the multi-response optimisation was defined to maximise all the antioxidant properties. The process variables were set to be within the range used in the investigation. The response surface graph for the multi-response optimisation is shown in Fig. 2. The required process condition for the multi-response optimisation is a temperature of 50.16°C, concentration of 2.1 g 100 ml⁻¹ and time of 5 min. This process condition resulted in a desirability of 0.68.

The confirmation run was done at 50.00°C, concentration of 2.1 g 100 ml⁻¹ and time of 5 min. The temperature was approximated to the nearest whole number by taking into account the operating convenience of the temperature controller. The values obtained from the confirmation runs were similar to the predicted values (Table 3).

3.3 Prediction of Antioxidant Properties from Colour and Hue Index Properties of the Extract

The OLSR, PCR and PLSR were run for all the antioxidant properties and a comparative analysis was done between the regression models. Multivariate model obtained by multiple linear regressions has been used for the prediction of antioxidant properties [11]. The PCR model having the highest adjusted R² was selected as the most preferred model among the PCR models. A sample of a PCR analysis is shown in Table 3. In most cases it was observed that the PCR with the highest adjusted R² had the lowest root mean square error (RMSE) and PRESS root mean square error (PRESS RMSE). The three components PCR for DPPH had higher adjusted R², lower RMSE and PRESS RMSE compared to the five components PCR (Table 4).

A comparative analysis of the different data mining technique is shown in Table 5. The extraction models for TFC, DPPH and ICA, gave good predictive quality with a R² that range from 0.591 to 0.960 and Q² that range from 0.461 to 0.919 (Table 5). The OLSR models had the highest R² values and the PLSR models had the highest Q² values for all the models. The PCR models recorded the lowest R² and Q² values among the regression techniques. These results indicated that the PLSR model has the highest predictive quality among the regression techniques due to its higher Q² values when compared to OLSR and PCR. The lower R² and Q² values of the PCR models can be attributed to the way the independent variable are selected. The PCR is an unsupervised regression technique [27], as it focuses on explaining the variability in the independent variables. The PLSR is a supervised regression technique [27], because the independent variables are selected in such a way that these selected variables are also able to explain the dependent variables [28]. The OLSR could be regarded as an all inclusive supervised technique because all the measured independent and dependent variables are involved in building the regression model. The OLSR extraction models of TPC and PSA had a good R² of 0.755 and 0.789, respectively, but a low Q² of 0.068 and -0.530, respectively. This infers that we have an OLSR model that is able to predict the current data well but has poor predictive quality with new data. This is a demerit of the OLSR technique. Hence, there is need to use other quality parameters (other than R²) to assess the quality of regression models. The PLSR models had the lowest RMSE for the TFC and DPPH prediction and the OLSR model had the lowest RMSE for the prediction of ICA (Table 5). In terms of model simplicity, the PCR and the PLSR gave the most parsimonious models. One of the considerations in model building is simplicity. In most cases, the rule of Occam's Razor, which states that the simpler explanation is the preferable one, is very useful, and is now applied to data analysis or data mining techniques in building models [27]. The PCR was able to predict TFC with a combination of: a*, hue index, hue, though with a very high RMSE of 1404.997 mg CE l⁻¹. The PLSR was able to predict TFC with a combination of: a*, A510, hue index with a low RMSE of 552.706 mg CE l⁻¹. Pace et al. [11], were able to predict the antioxidant activity and total phenol content of pigmented carrots using a regression equation built from L*, a* and b* properties of the carrots. Also Wold [13] reported that colour measurements of intact tomatoes can be used as a non-destructive method to assess total antioxidant capacity of tomatoes. They reported that a high negative correlation existed between
high values of L*, b* and ferric reducing ability of plasma (FRAP), and a high positive correlation between a*, hue, a*/b and FRAP values. A comparative analysis of the 3 regression techniques revealed the PLSR models as the most preferred model due to its higher R^2, higher Q^2, and low RMSE and less number of independent variables in the model. We have chosen the word preferred and not the best models because all the models have their merits and demerits. For example a look at the ICA extract models showed that the PLSR gave the highest R^2 and Q^2, the OLSR gave the lowest RMSE.

Table 3. Confirmation runs under multi-response optimisation conditions

| Response       | Prediction | 95% PI low | 95% PI high | Validation (n = 3)   |
|----------------|------------|------------|-------------|----------------------|
| DPPH (%)       | 70.51      | 62.90      | 78.13       | 78.08±1.74          |
| TPC (mg GAE / L)| 1579.44    | 883.49     | 2476.12     | 1266.61±44.19       |
| TFC (mg CE / L) | 6943.83    | 4008.13    | 10681.13    | 4725.00±530.33      |
| ABTS (mg TE / L)| 0.98       | 0.95       | 1.01        | 0.97±0.03           |
| PSA (%)        | 70.78      | 55.75      | 85.81       | 76.62±1.21          |
| ICA (%)        | 74.96      | 71.03      | 78.90       | 81.82±0.17          |

PI = Prediction interval

Table 4. Model quality parameters for principal component regression of DPPH

| Number of components | % Variation explained | P-value for model | R^2 | Adjusted R^2 | MSE | RMSE | PRESS | RMSE |
|----------------------|-----------------------|-------------------|-----|--------------|-----|------|-------|------|
| 3                    | 91.128                | 0.000583          | 0.653| 0.588        | 31.204| 5.586| 7.646 |
| 5                    | 99.463                | 0.00156           | 0.667| 0.579        | 31.916| 5.649| 9.454 |

TIM = 5.00 min

![Response surface graph for multi-response optimisation of antioxidant extraction](image)
Table 5. Comparative analysis of regression techniques for antioxidant prediction

| Components | R²   | Q²     | RMSE  |
|------------|------|--------|-------|
| **TFC**    |      |        |       |
| OLSR       | L*, a*, b*, hue, chroma, A510, A610, A510/610, hue index | 0.939 | 0.749 | 745.061 |
| PCR        | a*, hue index, hue | 0.655 | 0.561 | 1404.897 |
| PLSR       | a*, A510, hue index | 0.933 | 0.905 | 552.706 |
| **1/TPC**  |      |        |       |
| OLSR       | L*, a*, b*, hue, chroma, A510, A610, A510/610, hue index | 0.755 | 0.068 | 4.015E-07 |
| PCR        | a*, hue index, hue, L* | 0.397 | -0.030 | 5.141E-07 |
| PLSR       | -      | -      | -     |
| **DPPH**   |      |        |       |
| OLSR       | L*, a*, b*, hue, chroma, A510, A610, A510/610, hue index | 0.960 | 0.814 | 8.937  |
| PCR        | a*, hue index, hue, | 0.653 | 0.543 | 5.586  |
| PLSR       | hue index, A510, a* | 0.945 | 0.919 | 1.984  |
| 1 / ABT S^2|      |        |       |
| OLSR       | L*, a*, b*, hue, chroma, A510, A610, A510/610, hue index | 0.434 | -1.031 | 0.106  |
| PCR        | a*, hue index, hue  | 0.186 | -0.260 | 0.101  |
| PLSR       | -      | -      | -     |
| **ICA**    |      |        |       |
| OLSR       | L*, a*, b*, hue, chroma, A510, A610, A510/610, hue index | 0.789 | -0.530 | 0.0000790 |
| PCR        | a*, hue index, hue  | 0.109 | -0.157 | 0.000128 |
| PLSR       | -      | -      | -     |

* no suitable model was found because the antioxidant property had no positive Q² with any of the PLSR components

4. CONCLUSION

This work has identified optimum conditions for extraction of antioxidants from tea-ginger (2:1) blend using ethanol. Rapid procedures that could be useful for predicting the TFC, DPPH and ICA of ethanolic tea-ginger (2:1) extract have also been identified. A comparative analysis of the regression techniques indicated that the PLSR gave the most preferred models.

COMPE TING INTERESTS

Authors have declared that no competing interests exist.

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