[1.]Kenechi NWOSU-OBIEOGU, [2.] Goziya DZARMA, [3.]Linus CHIEMENEM

ANFIS PREDICTION OF ANTIOXIDANTS YIELD FOR *LUFFA* OIL

[1-3.]Chemical Engineering Department, Faculty of Engineering, Michael Okpara University of Agriculture, Umudike, Abia, NIGERIA

Abstract: In this study, an Adaptive neuro-fuzzy inference system (ANFIS) was applied in predicting the optimum yield of terpineol and polyphenol for *luffa cylindrica* seed oil. The experiment was conducted at a temperature (60-80°C), time (4-6 hours) and solvent/seed ratio (8-12 ml/g). Fourier transform infrared spectroscopy (FTIR) confirmed the presence of terpineol and polyphenol at peaks of 1461.1cm^{-1} and 3008.0cm^{-1}, respectively. Parametric analysis showed that time and solvent/seed ratio had the most effect on terpineol yield, while time and temperature affected polyphenol yield significantly. The ANFIS prediction indices are thus; terpineol (R^2= 0.95614, MSE=1.11179) and polyphenol (R^2= 0.8258, MSE= 0.4253). This proves that the ANFIS technique is a reliable approach for predicting antioxidants from *luffa cylindrica* seed oil.
Keywords: *luffa cylindrica*, antioxidant, ANN, ANFIS, polyphenol, terpineol

INTRODUCTION

Antioxidants are naturally present in plants; they convert free radicals produced during the oxidation process to less reactive species at a low concentration. Thus, it plays a physiological role in the body defence system [1]. Antioxidants are primarily found in fruits and vegetables. Studies revealed that food rich in antioxidants positively impacts health; hence, their regular consumption reduces the risk of chronic diseases [2,3].

Luffa cylindrica is a non-edible plant commonly found in the tropics, a member of the family *Cucurbitaceae*; the fruits, leaves, flowers, and seeds draw considerable attention from researchers to harness its potentials [4]. However, phytochemicals in seed oils have been revealed to offer interesting nutritional and economic possibilities due to the high levels of polar antioxidants [5].

Reports have shown that *luffa* based derivatives contain antioxidants such as terpineol, polyphenol which are present in our food, employed in treating a various health condition, cosmetics and antiseptics production [6-12]. Extraction and analysis of polyphenols and terpineol from plants have been researched extensively [12,13]. However, the relationship between input extraction process parameters and measurable output (polyphenol/terpineol yield)) is imprecise, blur, non-linear and vague, as reported by earlier researchers [14-16].

Recent reports show the capability and efficiency of soft-computational models such as artificial neural network (ANN), Adaptive neuro-fuzzy inference system (ANFIS), Support Vector Machine (SVM), Non-Linear Multilinear Regression (NLMLR), Genetic Algorithm (GA) and Particle Swarm Optimization (PSO) for modelling intricate and complex processes [17,18]. Earlier studies on estimation and prediction of extraction process have been based on linear regression techniques [19,20]. Nonetheless, the relationship between extraction and dependent process variables cannot be elucidated and explained linearly. Therefore, neural network and fuzzy inference systems have gained increasing applications in modelling and controlling the relationship among process variables [17,18,21,22]

ANFIS seems to be an excellent model to map the interaction of process parameters on antioxidant yield. Yue et al [9] employed ANFIS to model biodiesel production from castor oil, and ANFIS proved superior compared to other predictive models in the literature. Roy et al [23] predicted maximum oil yield from almond seed considering pressure, temperature, heating time, and moisture content as input parameters and type-2 fuzzy logic predicted and improved the process parameters during extraction. Khoshnervisan et al. [24] predicted wheat grain yield based on energy inputs using the ANFIS model compared with ANN models; the result illustrated that the ANFIS model predicted the product more precisely. ANFIS has been widely applied as an efficient predictive model for a highly non-linear relationship due to its learning capability [25]. Thus, this study predicted polyphenol and terpineol yield from *luffa cylindrica* seed oil using the ANFIS model.

MATERIALS AND METHODS

— Sample preparation

Luffa cylindrica fruits, a natural precursor, were procured at Michael Okpara University of Agriculture Umudike, Abia State, Nigeria. Both matured and dried fruit of this tree was harvested in bulk quantity. First, the seed was winnowed, husks and dirt removed, after which it was sun-dried for easy removal of the shell and was also oven-dried at 60°C to constant weight before grinding to increase the surface area for oil extraction.

— Experimental procedure

The extraction of the *luffa* oil was carried out at the Department of Chemical Engineering laboratory, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria, using the solvent extraction method reported by Afolabi et al. [26]. It was done with the Soxhlet apparatus of 250 cm^3 capacity using *n*-hexane of analytical grade as the solvent. The parameters were 40 g of grounded *luffa* seed, extraction time of 4 to 6 h, extraction temperature between the ranges of 60°C to 80°C and the ratio of the solvent (*n*-hexane) to solute (biomass) from 8 to 12ml/g. The solvent used was recovered at every interval. The oil obtained was weighed; the experiment was repeated for other parameters, and the percentage yield was calculated using the equation below.

$$Y \; = \frac{M_o}{M_s} \times 100 \qquad (1)$$

where: Y = oil yield (%), M_o = mass of oil extracted (g), M_s = mass of luffa seed (g)

— Terpineol Concentration Determination

The terpineol concentration was determined using a method modified by Narayan et al. [27]. 0.1g of the *luffa* oil was introduced into a test tube, 1ml of methanol was added, placed in a water bath, it was stirred for 30minutes at a temperature of 100ºC, the mixture was removed, and 1ml of sulphuric acid was introduced, the colour turned to reddish-brown, it was allowed to stand for 30minutes then the absorbance was taken in a UV spectrophotometer, the standard curve was generated by treating the linalool as the sample with serial dilution

— Polyphenol yield determination

The total polyphenol content was determined using the Folin Ciocalteu method (Singleton and Rossi [28]. About 0.1g of the oil extract was weighed into a test tube, 1ml of methanol was introduced and was taken into a water bath and shaker, where it was allowed to shake for 30minutes at 40ºC. Next, the sample was removed, and 1ml of Folin-Ciocalteu and 2ml of 20% Na_2CO_3 were introduced; the mixture was allowed to stand for 10 minutes before it was stirred in a centrifuge 20minutes at 400rpm. The absorbance was taken using a UV spectrophotometer at 625nm. The standard curve was generated by preparing different concentration ranging from 10mg/l of Gallic acid.

— FT-IR analysis

The pure *luffa* seed oil was characterized using Fourier Transform Infrared (FTIR) Spectroscopy Technique to determine surface functional groups. The FTIR analyses were carried out on the samples using Shimadzu FT-IR-8400S Spectrophotometer with a resolution of 4 cm^{-1} in the range of 4000 - 500 cm.

— ANFIS Modelling Development

Artificial neural network (ANN) and Fuzzy Inference System (FIS) are integrated to form an Adaptive Neuro-Fuzzy Inference System (ANFIS) for solving and explaining imprecise and uncertainty problems.

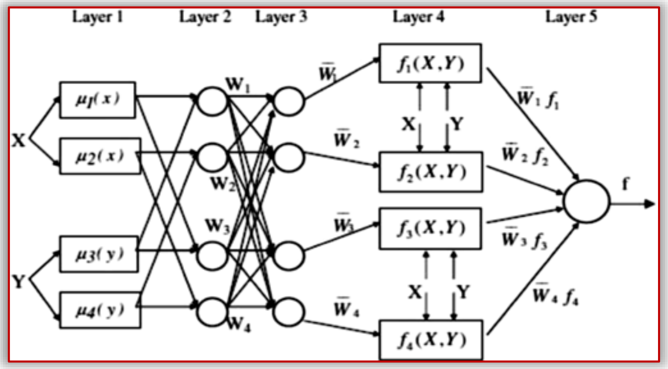

Figure 1: A basic structure of the ANFIS

The "exhsrchfunction" in MATLAB 8.4 (R2014b) software environment was implemented in an exhaustive search within the available inputs of the extraction process (temperature, time and solvent/seed ratio) to select the set of one and two variable input combinations that has the maximum influence on the terpineol/polyphenol yield. The 'exhsrch' function built an ANFIS model for each input variable and trained it for one thousand epoch reporting the performance achieved.

— Performance Evaluation of the Developed Models

Statistical parameters were applied to determine the performance of the proposed model for the prediction of the extraction process. MSE (Root Mean Square Error) and the R^2 value (Correlation Coefficient) were used as considered below:

$$R^2 = 1 - \frac{\sum_{p=1}^{p}(\quad DP - O_p)^2}{\sum_{p=1}^{p}(O_p)^2} \qquad (2)$$

$$MSE = \frac{1}{P}\sum_{p=1}^{p}(d_p - O_p)^2 \qquad (3)$$

The MSE value close to zero and the R^2 values close to one show the models' predictability and reliability. Li et al. [29] claimed that Soft-computing Model's evaluation should be based on ranges of RMSE as given in Table 1.

Table 1: Ranges of MSE for models performance

Ranges of MSE	Performance
‹ 0.009	Excellent prediction accuracy
0.009 ‹ MSE ‹ 0.09	Good prediction accuracy
0.09 ‹ MSE ‹ 0.5	Reasonable prediction
› 0.5	Inaccurate prediction

Source: Li et al. [29]

RESULT AND DISCUSSION

— FTIR Result

The FT-IR result of the oil yield from *luffa cylindrica* is shown in figure 2, the peak at 3008.0cm^{-1} can be ascribed to –OH stretching, which indicates the presence of polyphenol, two sharp-pointed peaks at 2922.2 cm^{-1} and 2855.1cm^{-1} indicated alkane group, another sharply pointed peak with value 1744 cm^{-1} indicates the presence of esters (6-membered lactone) with the structure C=O, hence the oil has high saponification value and could be recommended for soap production.

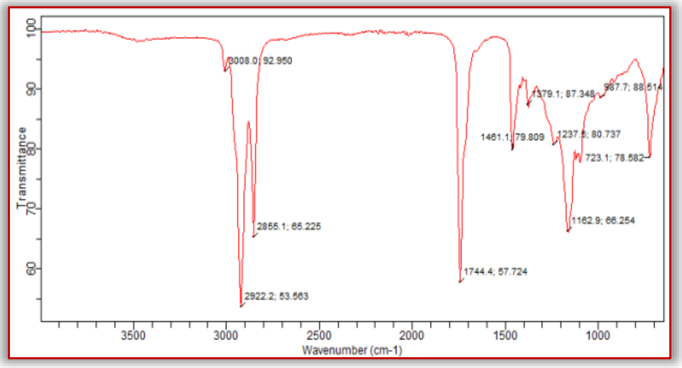

Figure 2: FT-IR result of the oil yield

The shorter, smaller pointed peak with a vibrational mode at 1461.1 cm^{-1} indicates the presence of terpineol (Agatonovic-kustrum et al. 2020), while a medium sharp peak was observed with a value of 1379 cm^{-1}, indicating an alkane of the gem dimethyl group. The peak at 1237.5cm^{-1} indicates an alkyl aryl ether with structure C – O – C, while 987.7cm^{-1} and 723.1cm^{-1} peak indicated alkene compounds. The presence of the unsaturated hydrocarbons makes the oil suitable for plastic and paint industries, as a drying agent in the production of cosmetics and maybe edible for animal feed [30].

— **ANFIS simulation results**

To obtain the best prediction of antioxidant yield, the developed ANFIS structure was simulated at various membership functions (MF) such as gauss mf, gauss2 mf, gbell mf, tri mf, trap mf, psig mf and dsig mf. The correlation coefficient (R^2) and the root mean square error (RMSE) were used as statistical criteria to evaluate the degree of reliability of the network. Table 2 and 3 summarizes the ANFIS results for terpineol and polyphenol yield of different input membership function types for linear and constant output mf, respectively. In Table 2, for linear output, the optimum value of R^2 (0.95614), MSE (1.4059) was obtained at tri mf, for the constant output mf, the trap of had the best prediction for R^2 (0.94799), with its corresponding MSE value (1.11179) obtained at tri mf, for polyphenol yield prediction in Table 3 trap and dsig mf has the best R^2 projection of (0.8150), (0.8258). In contrast, tri mf had the best MSE prediction of (0.4253), (13.9715) for linear and constant output, respectively; this affirms that optimum yield for terpineol was predicted by tri mf. In contrast, polyphenol yield was predicted by dsig and tri mf.

Table 2: Prediction efficiency of ANFIS model for terpineol yield

Input membership function	MSE (linear)	R^2 (linear)	MSE (constant)	R^2 (constant)
Gauss	19.5374	0.94628	1.15111	0.94646
Gauss2	69.9386	0.94623	1.11491	0.94641
Gbell	77.2027	0.94631	1.1178	0.94164
Tri	1.4059	0.95614	1.11179	0.94628
Trap	7.67826	0.91321	1.11537	0.94799
Pi	90.0508	0.94312	1.11356	0.94634
Design	77.2027	0.90315	1.11779	0.94164
Psig	40.106	0.93215	1.13363	0.94622

Table 3: Prediction efficiency of ANFIS model for polyphenol yield

Input membership function	MSE (linear)	R^2 (linear)	MSE (constant)	R^2 (constant)
Gauss	1.611	0.71012	15.1564	0.8093
Gauss2	6.3827	0.710135	36.2802	0.80104
Gbell	6.9954	0.71021	37.9403	0.82461
Tri	0.4253	0.71301	13.9715	0.82077
Trap	7.1824	0.8150	39.082	0.81567
Pi	8.9391	0.71019	42.988	0.79811
Dsig	6.9954	0.7314	37.9403	0.8258
Psig	3.0278	0.71013	21.8584	0.799955

— **Exhaustive search result**

Exhaustive search result for one input and two-input variable ANFIS model for antioxidant yields.

The ANFIS models using different input variable combinations were investigated with the exhaustive search method to determine the input variable that has the most significant effect on the antioxidant yield using RMSE as the performance indicator. Table 4 shows an exhaustive ANFIS model result with single/double input variables for terpineol yield. It was observed for single input, time had the least RMSE. In contrast, a combination of time and solvent/seed ratio had the least RMSE for double input; for polyphenol yield in table 5, temperature possessed the least RMSE. In contrast, a combination of temperature and time maintained the least RMSE; this indicated that time and solvent/seed ratio were the most relevant variable for terpineol yield. In contrast, time and temperature had a more significant effect on polyphenol yield.

Table 4: Exhaustive search result of one / two- input variable ANFIS model for terpineol yield

Input variable	RMSE training	RMSE Checking	Input variable	RMSE training	RMSE checking
Temperature	3.6582	3.5683	Temperature, time	1.86295	1.84745
Time	2.29572	2.29572	Temperature, solvent/seed	2.6176	2.6599
Solvent/ seed ratio	2.9658	2.893121	Time, solvent/seed ratio	1.31504	1.327939

Table 5: Exhaustive search result of one/two-input variable ANFIS model for polyphenol yield

Input variable	RMSE training	RMSE Checking	Input variable	RMSE training	RMSE checking
temperature	0.9077	0.89961	Temperature, time	0.556	0.5713
Time	0.930231	0.880086	Temperature, solvent/seed	0.7621	0.7412
Solvent/seed ratio	1.03552	0.98705	Time, solvent/seed ratio	0.8424	0.79106

CONCLUSIONS

This study has successfully developed an ANFIS model to optimize the process parameters for antioxidants (terpineol and polyphenol) yield from *luffa cylindrica* seed oil using statistical indicators (RMSE, R^2). The parametric analysis using exhaustive search showed that time, temperature and solvent/seed ratio significantly affected the antioxidants yield. The model developed can be used for process behaviour prediction, optimization and a learning tool for operators in the chemical industry.

References

[1] Yadav A, Kumari RI, Mishra JP, Srivtva S, Prabha SI, Antioxidants and its functions in human body- A review, 2016. Res. Environ. Life Sci. 9(11): 1328-1337
[2] Ohlsson T, Bengtsson N, Minimal processing technologies in the food industry, 2002
[3] Liyana-Pathirana CM, Shahidi F, Alasalvar C, Antioxidant activity of cherry laurel fruit (*Laurocerasus officinalis Roem.*) and its concentrated juice. Food Chemistry, 2006. 99: 121-128.
[4] Oboh IO, Aluyor EO, luffa *cylindrica*- an emerging cash crop, African Journal of Agricultural Research, 2009. 4(8):684-688.
[5] Oyetayo FL, Ojo BA, Food value and phytochemical composition of *luffa cylindrica* seed flour. American journal of biochemistry, 2012. 2(6):98-103.
[6] Shendge PN, Belemkar S,Therapeutic potential of *luffa rectangular*: A review on its traditional uses, phytochemistry, pharmacology and toxicological aspects. Frontiers in pharmacology, 2018. 9(1177).
[7] Park S, Lin YK, Friere OM, Cho E, Jin D, KookJ, Antimicrobial effect of linalool and α-terpineol against periodontopathic and cariogenic bacteria, Anaerobe, 2012. 18(3): 369-372.

[8] Akinsanmi AO, Oboh G, Akinyemi JA and Adefagha AS, Assessment of the nutritional, antinutritional and antioxidant capacity of unripe, ripe and overripe plantain (*Musa paradisiaca*) peels, 2015. International journal of advanced research. 3(2):63-72.
[9] Yu L, Jin I, Li X, Zhang Y, Optimization of bioactive ingredient extraction from Chinese herbal medicine Glycyrrhiza glabra: a comparative study of three optimization models, Evidence-based complementary and alternative medicine, 2018.1(2): 122-135.
[10] Okla K M, Alamri SA, Salem MZM, Ali HM, Behiry IS, Nasser AR, Alaraidh IA, Al-Ghtani SM, Yield, Phytochemical Constituents, and Antibacterial Activity of Essential Oils from the Leaves/Twigs, Branches, Branch Wood, and Branch Bark of Sour Orange (*Citrus aurantium L.*) Processes, 2019. 7 (363): 1-15.
[11] Zengan H, Daysal HA, Antibacterial and antioxidant activity of essential oil terpenes against pathogenic and spoilage-forming bacteria and cell structure-activity relationships evaluated by SEM microscopy, Molecules, 2015. 19: 17773-17798.
[12] Vladimir-Knezevic S, Blazevic B, Stefan MB, Babac M, Plant polyphenols as antioxidants influencing the human health, phytochemicals as Nutraceuticals-Global approaches to their role in nutrition and health, 2011. 9: 155-180.
[13] Campone L, Celano R, Rizzo S, Piccinelli A L, Rastrelli L and Russo M, Development of an Enriched Polyphenol (Natural Antioxidant) Extracts from Orange Juice (*Citrus sinensis)* by Adsorption on Macroporous Resins, 2020. Journal of Food Quality. 2(4):1-9.
[14] Sales A, Felipe OL, Bicas JL, Production, properties and application of terpineol. Food and bioprocess technology, 2020
[15] Molina G, Pessôa M G, Bicas JL, Fontanille P, Larroche C, Pastore GM, Optimization of limonene biotransformation for the production of bulk amounts of α-terpineol. Bioresource Technology, 2019
[16] Khaleel C, Tabanca N, Buchbauer G, α- Terpineol, a natural monoterpene: A review of its biological properties, 2017. Open Chem. 16: 349–361.
[17] Oke EO, Adeyi O, Okolo BI, Adeyi JA, Ayanyemi J, Osoh KA, Adegoke, T.S. Phenolic compound extraction from Nigerian *Azadirachta Indica* leaves Response surface and neuro-fuzzy modelling performance evaluation with Cuckoo Search multi-objective optimization, 2020. Results in Engineering, 8:100160.
[18] Soto J, Melin P, Castilo O, A new approach for time series prediction using ensembles of IT2FNN models with optimization of fuzzy integrators. Int. J. Fuzzy Syst. 2018. 20: 701-728.
[19] Jisieike C F, Betiku E, Rubber seed oil extraction, solvent polarity, extraction time, and solid-solvent ratio on its yield and quality, 2020. Biocatalysis and Agricultural Biotechnology. 24: 1-7
[20] Onoji S, Iyuke S, Igbafe IA, Daramola OM, *Hevea brasiliensis* (rubber seed) oil: modelling and optimization of extraction process parameters using response surface methodology and artificial neural network techniques, Biofuels, 2017.6:1–15
[21] Almeida JS, Predictive Non-linear Modelling of Complex Data by Artificial Neural Networks, 2002. Curr. Opin. Biotech. 2: 1372–1376.
[22] Ojediran O J, Okonkwo CE, Adeyi A J, Adeyi O, Olaniran FO, George NE, Olayanju AT, Drying characteristics of yam slices (*Dioscorea rotundata*) in a convective hot air dryer: application of ANFIS in the prediction of drying kinetics., 2020. Heliyon, 6, e03555.
[23] Roy K, Mukherjee A, Jana K D, Prediction of maximum oil yield from almond seed in the chemical industry: A novel type-2 fuzzy logic approach, S. Afr. J. Chem. Eng, 2019. 29: 1–9
[24] Khoshnevisan B, Rafiee S, Omid M and Mousazadeh H, Development of an intelligent system based on ANFIS for predicting wheat grain yield on the basis of energy inputs. Information processing in agriculture, 2014. 1: 14-22.
[25] Agatonovic-Kustrum S, Pustivojevic P, Valdimir G, Litrinova TU and Motron DW , Essential oil quality and purity evaluation via FT-IR spectroscopy and pattern recognition techniques, 2020. Applied science. 10: 1-12.
[26] Afolabi T J, Onifade K R, Akindipe VO and Odetoye TE, Optimization of benth seed oil (parinaripolyandra) using response surface methodology, 2014. British J. Appl. Sci. Tech: 5: 437–445.
[27] Narayan G, Sondipon C, Shamik G, Samir K.S, Suman B, Estimation of total terpenoids concentration in plants tissues using a monoterpene, linalool as standard reagent, Protocol Exchange, 2012. 5(10):1038-1041.
[28] Singleton VL, Rossi JA, Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, *Am J Enol Vitic*, 1965. 16(3): 144-158.
[29] Li M, Fan L, Liu H, Guo P and Wu W, A general model for estimating daily global solar radiation using air temperatures and site geographic parameters in Southwest China, Journal of Atmospheric and Solar-Terrestrial Physics, 2013. 92: 145-150.
[30] Oli CC, Onuegbu TU, Ezeudu EC, Proximate composition, characterization and spectroscopic analysis of luffa aegyptiaca seed. International journal of life sciences biotechnology and pharma research, 2014. 2250-3137.

ISSN: 2067-3809