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Original Research Article

Artificial Neural Network Analysis of Xinhui Pericarpium *Citri Reticulatae* Using Gas Chromatography - Mass Spectrometer - Automated Mass Spectral Deconvolution and Identification System

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Abstract

Purpose: *To develop an effective analytical method to distinguish old peels of Xinhui Pericarpium citri reticulatae (XPCR) stored for > 3 years from new peels stored for < 3 years.*
Methods: *Artificial neural networks (ANN) models, including general regression neural network (GRNN) and multi-layer feedforward neural network (MLFN), were used to analyze the Gas Chromatography - Mass Spectrometer - Automated Mass Spectral Deconvolution and Identification System (GC-MS-AMDIS) data of the essential oils of the XPCR. The Root Mean Square (RMS) errors of each ANN model was obtained through judging the characteristic of old peels and new peels.*
Results: *The Root Mean Square (RMS) error of GRNN was 0.22, less than the error MLFN at different levels, indicating that GRNN model is more reliable and accurate for judging the characteristics of old peels and new ones.*
Conclusion: *The general regression neural network model is established to reliably distinguish between old peels and new peels.*

Keywords: *Artificial neural networks, Xinhui, Pericarpium, Citri reticulatae, Gas Chromatography, Automated Mass Spectral Deconvolution and Identification System, Peels*

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INTRODUCTION

Pericarpium *citri reticulatae* (PCR) is the dried peel of *Citrus reticulata* Blanco, commonly known as tangerine or orange, and its cultivars. Xinhui Pericarpium *citri reticulatae* (XPCR), which derived from *Citrus reticulata* 'Chachi', was regarded as the best national product of geoherbalism since ancient times. According to the number of years of storage, PCR can be divided into two types: 'new peels' and 'old peels'. Peels that have been stored for more than 3 years are known as old peels, while those stored for less than three years are known as new peels. According to clinical experience, the longer XPCR was stored, the better its effect is. However, it is difficult to identify the number of years of storage from the appearance of the peels (Fig 1), in addition most of the old peels are adulterated new peels during processing.

In the past few decades, much work has been done using PCR to identify the storage year [1,2]. However, all these works show certain

drawbacks without applying further exploratory data analysis to identify accurately the number of years the peels have been stored. Essential oil is one of the major effective components of XPCR and it is complicated. The ingredients in the oil exist in a nonlinear relationship, which is very suitable for artificial neural networks.

Pattern recognition through neural networks is an automatic processing and interpretation approach which uses mathematical techniques on computer [3]. It is envisioned that artificial neural networks (ANN) can be an alternative approach to distinguish between old peels and new peels. In this article, two artificial neural networks (ANNs) models—the general regression neural network (GRNN) and multi-layer feedforward neural network (MLFN) were used to analyze and interpret the GC-MS-AMDIS data of the essential oils of XPCR with different storage time. Thirty five batches of authentic XPCRs—21 batches with 12 months of storage time and 14 batches stored for 11 years— were collected to study the complicated and effective constituents with the use of GC-MS. The collected GC-MS data were then analyzed by two ANNs models to confirm the authenticity of the storage time of each batch. The model with smaller RMS errors was confirmed as the reliable judge for distinguishing 'old peels' from 'new peels'.

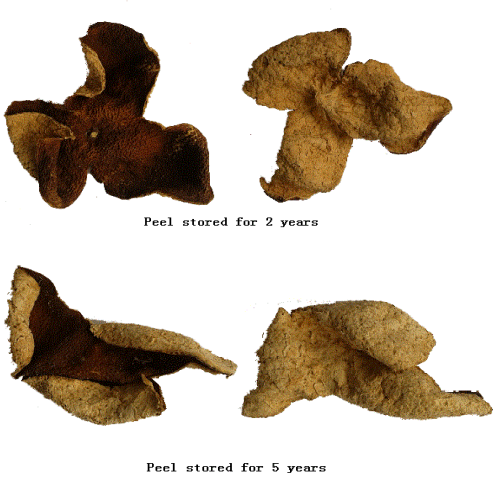

Fig 1: Peels stored for 2 years versus 5 years

EXPERIMENTAL

Materials and sample preparation

N-hexane (HPLC grade) was purchased from Fisher Scientific. Anhydrous sodium sulfate (analytical reagent) was acquired from Chengdu Kelon Co. Ltd. Thirty five batches of XPCR samples were collected from the Xinhui District, Guangdong Province. Through an identification process developed by Dr. Xianming Lu (Professor of Medicine Specimen Center of Chengdu University of Traditional Chinese Medicine), 21 batches of the peels were new and had been stored for at least 12 months; the remaining 14 batches were else old and had been stored for more than 4 years.

Extraction of essential oils

The samples were cut into small pieces (5 mm × 5 mm). In a typical experiment, 20 g samples were put in a round-bottom flask and steam distilled for 6 hours. The essential oils were collected and diluted by n-hexane, and then transferred to auto-sampler vials.

Analysis of the essential oils by GC-MS-AMDIS

The oils were analyzed by an Agilent 7890A-5975C instrument equipped with a quartz capillary column (0.25 mm × 30 m, 0.25 μm) and an Agilent 5975C MS detector. Temperature programmes are listed in Table 1. One μL of each sample was injected into GC-MS with a split ratio of 10:1. The temperature of the injection port and the detector were both 270 ^{o}C. High purity helium (> 99 %) was used as the carrier gas with a flow rate of 1 mL/min. All data were obtained by collecting the full-scan mass spectra within the scan range 30-550 amu. The database was NIST08s.L (National Institute of Standards and Technology). According to the mass spectrometer coupled with the automated mass spectral deconvolution and identification system (AMDIS) as well as previous studies, the presence or absence of individual components were all recorded for an ANN analysis.

Table 1: Temperature program

Ramp	Initial temperature (^{o}C)	Rate of temperature increase (^{o}C/min)	Final temperature (^{o}C)	Hold time (min)
First	60	-	-	-
Second	-	1	80	10
Third	-	10	150	0
Forth	-	2	180	5

Statistical analysis

To quantify the results of the model, the judgments generated by ANN model were presented as "1" or "0". "1" represents the characteristics of new peels while "0" represents the characteristics of old peels. For computer-based experiments, NeuralTools® Software (Trial Version, Palisade Corporation, NY, USA) was used to develop ANN models. The chemical components were set as independent variables while the quantified results (0 or 1) were taken as dependent variables. According to saliency analysis by Microsoft Excel, the level of statistical significance between new peels and old peels is less than 0.04, indicating that there is significant difference between the two kinds of samples in this study.

RESULTS

The GC-MS-AMDIS results of the 35 samples showed that 31 compounds were present, and the relative contents of the components were more than 90 % of the total content of 34 compounds. The main compounds were myrcene, 4-carene, d-limonene, g-terpinene, terpinolene, linalool, a-terpineol, methyl 2-(methylamino) benzoate, a-sinensal, n-hexadecanoic acid and d-limonene. Therefore, the contents of all the compounds were used as independent variables in the training of ANN models.

Model development

Two types of ANN models were used to establish the developed models for the peels: the general regression neural network (GRNN) model and the multi-layer feedforward neural network (MLFN) model. With the MLFN model, in order to find out the best number of nodes, different nodes were set from 2 to 20. The target of the first selection was to find out the best model of the judgment. Therefore, 28 sample groups were used as the trained set, while 7 sample groups were the tested set. The results are shown in Table 2.

According to the RMS errors of the different models in Table 2, it is obvious that the GRNN model is much better than the MLFN models in distinguishing old peels from new peels. In order to make a comparison between the different nodes of the MLFN models, a scatter diagram was obtained in Fig 2.

From Fig 2, it can be observed that the RMS error of the MLFN model is unstable and can be concluded by linear regression. The fluctuations of the RMS errors show that the MLFN models are less robust than the GRNN model during the classification process. In addition, the running time of different models shown in Table 2 indicate that the time for training MLFN models is much longer than those for GRNN model.

Table 2: The results of different ANN models for distinguishing new from old peels[a]

ANN model	RMS error	Training time
GRNN	0.22	0:00:00
MLFN 2 Nodes	0.65	0:02:40
MLFN 3 Nodes	0.53	0:02:06
MLFN 4 Nodes	0.53	0:02:17
MLFN 5 Nodes	0.53	0:02:12
MLFN 6 Nodes	0.65	0:02:55
MLFN 7 Nodes	0.72	0:02:32
MLFN 8 Nodes	0.65	0:03:03
MLFN 9 Nodes	0.65	0:04:18
MLFN 10 Nodes	0.53	0:03:55
MLFN 11 Nodes	0.65	0:05:01
MLFN 12 Nodes	1.18	0:05:58
MLFN 13 Nodes	1.01	0:04:31
MLFN 14 Nodes	0.92	0:06:18
MLFN 15 Nodes	0.55	0:07:31
MLFN 16 Nodes	0.63	0:05:06
MLFN 17 Nodes	0.36	0:47:15
MLFN 18 Nodes	1.31	0:09:55
MLFN 19 Nodes	1.49	0:15:30
MLFN 20 Nodes	1.23	0:16:44

[a] *The sample of the 28 trained samples and 7 tested samples were detected at the same time with the software auto-stopped*

Therefore, it can be concluded that the ANN model derived from the GRNN model is a more suitable and reliable than the ANN model derived from the MLFN model in distinguishing old peels and new peels.

GRNN model validation

In order to determine the robustness of the GRNN model, 50 % of the samples were set as the trained set and the rest were as the tested set. To ensure the persuasiveness of the test, experiments of the GRNN model were done 20 times, and each trained model was based on a different composition of samples. The average accuracy of both the old and new peels are relatively high (Table 3). In addition, according to the tested data of the GRNN model, the accuracy rates of the samples are mostly 100 %. This reveals that the judging processes of the characteristics of the old and new peels are robust and accurate. According to statistical analysis, all components had significant

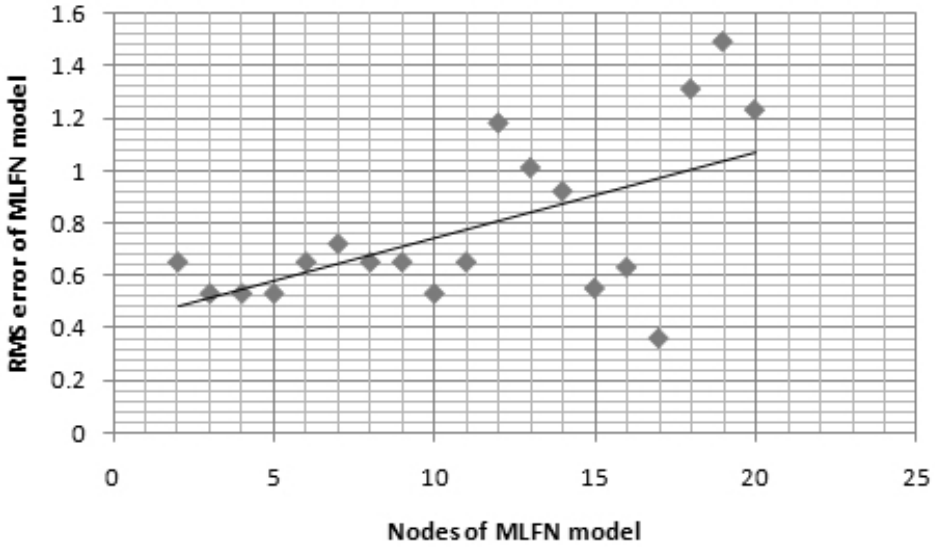

Fig 2: Comparison of the different MLFN models

contributions on these pattern recognition models. That is to say, every single independent variable for the sake of ensuring the robustness and precision of the model should not be ignored.

Table 3: Robustness of the MLFN-6 model

Peel	Sample size	Judging digit	Mean correct rate (%)
Old peel	14	0	97 %
New peel	21	1	95 %

DISCUSSION

Automated mass spectral deconvolution and identification system (AMDIS) was used to take chromatograms of complex samples to identify possible components [4] and these were widely applied in processing GC-MS data [5-9]. This identified automatically possible component peaks within the GC-MS data set in XPCR in this study. In previous studies, quality control and discrimination were done by developing analytical methods or coupled with some chemo-metric methods [10-12]. In the analysis by PCR (Polymerase Chain Reaction), studies on discrimination were about molecular authentication [13] and fingerprints [14]; most researches hammered at developing determining techniques [15-17]. The content of the components in the oil existed in a non-linear relationship and so ANNs can be used to analyze this relationship. The new analytical method may be used to distinguish between types of other Chinese herbs containing complex compounds.

CONCLUSION

A new and reliable method has been developed to distinguish between the characteristics of the old and new peels of XPCR accurately using ANN models. The results reveal that GRNN model, which is based on the comparison with MFLN, is robust and reliable. Neural networks can utilize actual data to differentiate old and new peels and can thus be applied to determine the specific storage times of old peels.

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