Macedonian - Chinese Scientific and Technological Cooperation

New Project Proposal for 2018-2019

Title of project proposal: Characterization of multi-element profiles and multi-isotope ratios for determination of the geographical origin of various plant food and beverages	Project number

Macedonian organization: Faculty of Agriculture, Goce Delčev University – Štip, Republic of Macedonia

Chinese organization: College of Information Engineering, Shenyang University of Chemical Technology, Shenyang, Liaoning Province, China.

Project period: 2018-2019

Expected period of stay in China for the Macedonian Researchers:
  4 persons, **7** days
  From **11.06. 2018** to **17.06.2018**

Expected period of stay in Macedonia for the Chinese Researchers:
  4 persons, **7** days
  From **18.03. 2019** to **24.03.2019**

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Project description:

INTRODUCTION

Historically, food products have always been linked with a specific geographical origin. Food-consumption habits were created by the local natural resources and the social or cultural factors of the community. Such links have disappeared over the time for various reasons, mostly because of the globalization of the food industry, following the extensive growth in technological means over the past century. Thus, food consumption in a region no longer necessarily reflects food production of that area. However, in recent years, consumers have renewed their interest in food strongly identified with a place of origin. The reasons for this increasing interest of consumers vary from the global trend for organic and health products to their concern about animal welfare and environmental friendly methods of production. Resulting from this trend, local products around the world regained their fame and brought wealth to local producers, so product authenticity and authentication are emerging topics (Karoui et al 2004). Consumers in developed countries demand food products of high quality, one of the basic parameters being origin. A modern trend is the consumption of food produced locally, in order to reduce energy footprint and pollution through transportation. Determination of food authenticity is an important issue in quality control and food safety. In recent years, many serious diseases appeared related to foodstuffs, so providing the motivation for the scientific community to work more intensively in this area. Authenticity is a quality criterion for food and food ingredients, and is required more and more worldwide, as a result of legislative protection for regional foods. Foodstuffs authentication may cover many different aspects, including mislabeling, misleading claims about origin, and adulteration, which is defined as a process by which the quality or the nature of a given substance is reduced by adding a foreign or an inferior substance and removing a vital element or characterization of the product. European Union (EU) quality schemes identify products and foodstuff farmed and produced to exacting specifications. Product designations fall into two categories: those linked to a territory and those relating to a particular production method. Geographical indications and designations of origin are names identifying a product as originating in a given territory and testifying to a link between a given quality, reputation or characteristic of the product and its geographical origin. The EU regulation allows the application of the following geographical indications to a food product: (1) protected designation of origin (PDO); (2) protected geographical indication (PGI); and, (3) traditional specialities guaranteed (TSG) [1]. PDO covers agricultural products that are produced, processed and prepared in a given geographical area using recognized know-how. PGI covers agricultural products and foodstuffs closely linked to the geographical area. At least one of the stages of production, processing or preparation takes place in the area. TSG highlights traditional character in composition or means of production. Reviews of analytical methods for the determination of geographical origin of food and beverages have been published . Methods based on elemental composition have been reviewed as have methods based on isotope ratios. Strategies employed to detect adulteration have relied on instrumental techniques and have evolved through time along with technology and instrumentation. Highperformance liquid chromatography (HPLC) gas chromatography (GC) nuclear magnetic resonance (NMR), infrared spectroscopy (IR) fluorescence spectroscopy capillary electrophoresis (CE), and more advanced techniques{e.g., proton transfer reaction mass spectrometry (PTR-MS), electronic nose coupled with MS DNA technologies and sensory analysis, have been proposed for food authentication. However, organic components of a food-crop production depend on various conditions (e.g., fertilization, history of the field, climatic conditions in the year of cultivation, geographic location and soil composition), so it is not always possible to determine the origin of a product by analyzing the organic components. Despite uncertainty about the organic compounds in a sample, the content of selected elements (trace and rare earth elements, REEs) in food positively reflects the growing conditions in the particular environment. In addition, multi-isotope or single-isotope ratios (e.g., strontium and lead) can provide unique, representative fingerprints that make it possible to discriminate the origin of food samples. Over the past decade, with the development of new advanced analytical techniques [e.g., thermal ionization MS (TIMS), inductively coupled plasma MS (ICP-MS) and dynamic reaction cell–ICPMS (DRC-ICPMS)], we can successfully retrieve elemental and isotopic compositions

of any given food sample and determine the geographic origin successfully. The growing concern of the consumers stimulated scientific research and publications in recent years, including multi-element and isotope-ratio methods of analysis in food authentication after statistical evaluation of the results.

 The main issues for the proposed investigation are:
  (1) Describe a new technique for evaluating spatially-explicit trace elements profile in various environmental samples (with organic and inorganic complex matrixes)
  (2) Evaluate whether element composition of the environmental samples can be attributed large-scale geographic trace element variation associated with underlying geology or fine scale spatial differences related to foraging habitat
  (3) Determine whether element profiles along the individual species are auto-correlated (i.e. occurring in a predictable or random pattern)
  (4) Proved spatially resolved multi-element information that could be used for identification of geographical origin of various environmental samples and foodstuffs.

[1] European Commission (EC), Geographical indications and traditional specialities, EC, Brussels, Belgium, 2007 (http://ec.europa.eu/agriculture/quality/schemes/index_en.htm, accessed 20 March 2012).

RESEARCH PROJECT

Various plant species, foodstuffs and beverages samples will be selected as media for identification of trace element profiles, isotopic ratio records and metal binding mechanisms. Soil samples will be used for identification of the background distribution and basis for the trace elements distribution (including REEs). Automorphic and alluvial soil samples will be used for that issue. Local plant species commonly used in the human dietary will be collected in order of improving the trace elements profile and isotopic ratio in different plant parts. Specific endemic plant species will be collected in order to examine their ability of use as biogeochemical markers in environment. Commonly used foodstuffs and beverages will be chemically characterized for determination of the geographical origin. Comparative analysis will be conducted for the obtained data matrix (R. Macedonia and R. China) in order to improve the large scale application of the validated methods.

Main media samples – organic/inorganic matrixes
Plant species, foodstuffs and beverages
The proposed matrixes that should be included in this investigation are divided in several groups:
-Plant varieties (not included in human dietary; it will be used as matrix for the applied method validation);
-Fruits and vegetables;
-Beverages (including bottled water and fruit juices);
-Honey products;
-Wine;
-Edible oils.

Analytical methods
Trace and rare-earth element analysis
The techniques used for the elemental fingerprinting of plant species, food and beverages are mostly those with multielement detection capability. For the proposed investigation two multi-element techniques will be used: Inductively coupled plasma with atomic emission spectrometry (ICP-AES) and Quadrupole-inductively coupled plasma with mass spectrometry (Q-ICP-MS):
  -    ICP coupled to atomic emission spectroscopy (ICP-AES) provides high reproducibility reproducibility and a quantitative linear range greater than conventional methods with arc or

spark, allowing the simultaneous determination of metals and non-metals. ICP-AES uses a very high-temperature excitation source and molecular interferences are greatly reduced. However, analysis is more expensive and all samples have to be introduced in a dissolved form. Furthermore, in ICP-AES, complex samples usually provide patterns that are very difficult to interpret. ICP-AES is also a fast, multi-element technique with an extended dynamic linear range and moderate-to-low limits of detection (LODs). Up to 60 elements can be screened per sample run in less than 1 min. ICP-AES monitors wavelengths down to 165 nm that provide high sensitivity for phosphorous and sulfur determinations. For the present investigation the ICP-AES instrument model 8200 Shimadzu, at UNILAB, Faculty of Agriculture, Goce Delcev University-Stip, R. Macedonia

- ICP-MS is a powerful tool for elemental analysis, providing LODs for more than 70 elements at low concentrations,typically parts per billion or trillion (ppb or ppt). Conventional ICP-MS systems cannot provide the lowest LODs, because of the polyatomic interferences that can enhance the background signal or overlap the signals of the most abundant isotopes. The use of a DRC can remove unwanted interferences by creating specific chemical reactions with a supplementary gas, which improves the selectivity and the sensitivity dramatically. For the present investigation the Q-ICP-MS instrument model 7500cx, Agilent Technologies, at UNILAB, Faculty of Agriculture, Goce Delcev University-Stip, R. Macedonia

Multi-isotope ratio

Investigations of the ratios of stable isotopes and the contents of unstable isotopes (radioisotopes), especially heavy elements (e.g., uranium, strontium, thorium or lead) have been used primarily in geological sciences for age determination. For heavier elements (e.g., Pb and Sr), fractionation is insignificant compared to the original isotopic ratios because the nuclides have high mass compared to the mass differences of the isotopes. In this case, stable isotopic ratios depend essentially upon the origin of the ore body and can be used for identification of the source of a material or characterization of its transportation history (e.g., 208Pb/207Pb). Strontium is the commonly used heavy metal for isotope-ratio analysis. Clearly, the present 87Sr/86Sr ratio is greater for samples that have greater ages, t, and samples with larger Rb/Sr ratios. The 87Sr/86Sr ratio therefore varies between different rock types and formations. Since Rb is an alkali metal and Sr is an alkaline earth, these elements behave differently in geological processes, creating large variations in Rb/Sr, and, so, large variations in 87Sr/86Sr. The 87Sr/86Sr ratio has been shown to vary widely in surface rocks, so any Sr released into soils, rivers, and groundwaters has an isotopic signature that reflects its source. Sr isotopes have also been used to trace agricultural products, which have incorporated Sr, along with Ca, from soils incorporating the Sr-isotope ratios of the underlying rocks. For the present investigation the Q-ICP-MS, with Octopole Reaction System (ORS in He-mode), model 7500cx, Agilent Technologies, at UNILAB, Faculty of Agriculture, Goce Delcev University-Stip, R. Macedonia

Metal binding scan analysis

To better understand the factors leading to metal binding in complex organic matrix and trace metal fate, electron microscopic characterization of samples will be conducted. Scanning electron microscopy can revealed the presence of trace metal specific incorporation in organic matrix in close association with biological structures with morphologies consistent. For the present investigation, scanning electron microscope model Tescan Vega3-LMU, will be used in order of characterization of the morphology, structure, and metal binding mechanisms.

Chemometric tools

1. Analysis of Variance (ANOVA)

ANOVA is a collection of statistical models, and their associated procedures, in which the observed variance in a particular variable is partitioned into components attributable to different sources of variation. In its simplest form, ANOVA provides a statistical test of whether or not the means of several groups are all equal, and therefore generalizes t-test to more than two groups. Performing multiple two-sample t-tests would result in an increased chance of committing a type I error. For this reason, ANOVA is useful in comparing two, three, or more means and has been used to compare elemental profiles of foods of different origin.

2. Principal Component Analysis (PCA)

PCA is a technique that, by the reduction of the data dimensionality, allows their visualization, while retaining as much as possible of the information present in the original data. So, PCA transforms the original measured variables into new uncorrelated variables called principal components (PCs). Each PC is a linear combination of the original measured variables. This technique affords a group of orthogonal axes that represent the directions of greatest variance in the data. The first PC (PC1) accounts for the maximum of the total variance, the second (PC2) is uncorrelated with the first and accounts for the maximum of the residual variance, and so on, until the total variance is accounted for .

3. Cluster Analysis (CA)

CA or clustering is the task of assigning a set of objects into groups (called clusters) so that the objects in the same cluster are more similar (in some sense or another) to each other than to those in other clusters. In this way, we categorize the samples in order to succeed with discrimination.

4. Linear Discriminant Analysis (LDA)

LDA is probably the most frequently used supervised pattern-recognition method and the most studied one. LDA is based on the determination of linear discriminant functions, which maximize the ratio of between-class variance and minimize the ratio of within-class variance. In LDA, classes are supposed to follow a multivariate normal distribution and be linearly separated. LDA can be considered, as PCA, a feature-reduction method in the sense that both, LDA and PCA, determine a smaller dimension hyperplane on which the points will be projected from the higher dimension .

5. Canonical Discriminant Analysis (CDA)

CDA is a dimension-reduction technique related to PCA and canonical correlation. Given a classification variable and several interval variables, CDA derives canonical variables (linear combinations of the interval variables) that summarize between-class variation in almost the same way that PCs summarize total variation.

6. Classification and Regression Trees (CARTs)

CARTs is a powerful and flexible classification tool. It handles both ordered and categorical predictor variables. The final classification rule has a simple form, which is easy to interpret and to use for future classification. CART takes into account the fact that different relationships may hold between variables in different parts of the data. It does automatic stepwise variable selection and calculates the importance rank of the variables. CART calculates misclassification error estimated by both re-substitution and cross-validation .

7. Artificial Neural Networks (ANN)

ANN are defined as structures comprising densely interconnected adaptive simple processing elements,

called artificial neurons (or nodes), which can perform massively parallel computations for data processing and knowledge representation .

Results and perspectives

Determination of the geographical origin of food and beverages has been a growing issue over the past decade for all countries around the world, mostly because of the concern of consumers about the authenticity of the food that they eat. An increasing number of research articles in the past five years have investigated the elemental composition and the isotope ratios as indicators to determine the origin of food and beverages. The proposed investigation will be the first attempt of application of validated method including multi-element and multi-isotope chemical characterization and the statistical tools in order to use for determination of the geographical origin of food and beverages in the R. Macedonia. Comparative analysis (R. Macedonia vs. R. China) will help in improving the large-scale application of the implemented proposed method.

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