Food Forensics and Toxicology

Food Forensics and Toxicology

Titus A. M. Msagati

College of Science, Engineering and Technology
University of South Africa
Johannesburg, South Africa

WILEY Blackwell

This edition first published 2018

Registered Offices
John Wiley & Sons, Inc., 111 River Street, Hoboken, NJ 07030, USA
John Wiley & Sons Ltd, The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK

Editorial Office
The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK

For details of our global editorial offices, customer services, and more information about Wiley products visit us at www.wiley.com.

Wiley also publishes its books in a variety of electronic formats and by print-on-demand. Some content that appears in standard print versions of this book may not be available in other formats.

Library of Congress Cataloging-in-Publication Data

Names: Msagati, Titus A. M.
Title: Food forensics and toxicology / Titus A.M. Msagati.
Description: First edition. | Hoboken, NJ, USA : Wiley, 2017. | Includes bibliographical references and index. |
Identifiers: LCCN 2017031781 (print) | LCCN 2017036341 (ebook) | ISBN 9781119101390 (pdf) | ISBN 9781119101383 (epub) | ISBN 9781119101413 (hardback)
Subjects: LCSH: Food–Toxicology. | Food fraud. | Food industry and trade–Health aspects. | Food adulteration and inspection. | Fraud investigation–Case studies. | Food poisoning–Case studies. | BISAC: TECHNOLOGY & ENGINEERING / Food Science.
Classification: LCC RC965.F58 (ebook) | LCC RC965.F58 M73 2017 (print) | DDC 615.9/54–dc23
LC record available at https://lccn.loc.gov/2017031781

Cover Design: Wiley
Cover Image: © Andrzej Wilusz/Shutterstock

Set in 10/12pt Warnock by SPi Global, Pondicherry, India

10 9 8 7 6 5 4 3 2 1

Contents

Preface

Deliberate tampering of foods (food adulteration, changes to originality and composition, etc.) for whatever purpose, be it for ensuring unfair business advantages in winning markets for economic gain, or unlawful tendencies that result in trespassing on legal binding standards, and crimes harming other human beings, are on the increase. The tendencies to either add or subtract food components, replace food ingredients with inferior ones, or label inferior food products with those of higher grade components, have become a serious problem and are affecting people in various ways almost everywhere.

In some cases, food adulteration has involved the deliberate introduction of harmful substances into foods (chemical and biological poisonous agents), where foodstuffs such as fruits, seafood, milk, dairy products, and water have been poisoned and therefore have directly affected the health of consumers either individually or as a large part of the community, causing disease and illness, abnormalities such as neurological impairment, allergies, and even death. There is a heavy penalty to be paid by criminals who cause food poisoning by food adulterations, as there are now laws that govern the quality of foods, together with standards, guidelines, and regulations that have to be adhered to by food producers, food processing industries, vendors, and food distributors.

This book covers different types of cases that encompass food forensics and food toxicology. The book also surveys different methods and techniques that are useful in providing the evidence required to be presented in food forensics cases. The book will be of relevance to colleges and universities where forensics science modules are being taught, or to academics who are involved in research activities in food forensics or food toxicology. The book may also be useful in food forensics laboratories, to researchers in food forensics, and government departments that deal with health and the general public at large.

List of Acronyms and Abbreviations

2,4-D	4-Dichlorophenoxyacetic acid
2,4,5-T	2,4,5-Trichlorophenoxyacetic acid
3PBA	3-Phenoxybenzoic acid
4F3PBA	4-Fluoro-3-phenoxybenzoic acid
AAS	Atomic Absorption Spectroscopy
AC	Affinity Chromatography
AChE	Acetylcholinesterase
ACP	Ascorbyl-palmitate
ADA	Aliphatic dicarboxylic acid
ADI	Acceptable Daily Intake
AdSV	Adsorptive stripping voltammetry
AFM	Atomic Force Microscopy
ALT	Alanine aminotransferase
AM	Atrazine mercapturate
AMP_PCR	Anchored microsatellite primed-PCR
ANN	Artificial Neural Networks
ANOVA	Analysis of Variance
APCI	Atmospheric Pressure Chemical Ionization
API	Atmospheric Pressure Ionization
AST	Aspartate aminotransferase
ATR	Attenuated Total Reflection
BChE	Butyrylcholinesterase
BDDE	Boron-doped diamond electrode
BFE	Bismuth film electrodes
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
BOTX	Botulinum toxin
BSDA	Backward Stepwise Discriminant Analysis
BSE	Bovine Spongiform Encephalopathy
BSEs	Backscattered electrons
CA	Chromosomal aberrations
CART	Classification and Regression Trees
CCD	Charged Coupled Detector
CD	Circular Dichroism
CDT	Cytolethal Distending Toxin

CE	Capillary Electrophoresis
CEC	Capillary Electrochromatography
CGE	Capillary Gel Electrophoresis
CIEF	Capillary Isoelectric Focusing
CITP	Capillary Isotachophoresis
CK	Creatine kinase
CM	Carboxylmethyl
CMC	Carboxylmethyl cellulose
CMP	Caseinomacropeptide
CNE	Carbon nanotube electrodes
CNS	Coagulase-Negative Staphylococci
CPB	*Clostridium perfringens* Beta toxins
CPE	Carbon paste electrodes
CPE	*Clostridium perfringens* Enterotoxins
CPLA	Polylactide aliphatic copolymer
CP-MAS	Cross Polarization Magic Angle Spinning
CPS	Coagulase-Positive Staphylococci
CSIA	Compound-specific isotope analysis
CSV	Cathodic stripping voltammetric
CTAB	Cetyltrimethyl Ammonium Bromide
CYP	Cytochrome P
CZE	Capillary Zone Electrophoresis
DA	Discriminant Analysis
DAD	Diode Array Detector
DAP	Dialkyl phosphates
DBCA	3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid
DCCA	3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid
DDD	Dichlorodiphenyl dichloroethane
DDE	Dichlorodiphenyl ethane
DDT	Dichlorodiphenyl tetrachloroethane
DEAE	Diethyl aminoethyl cellulose
DEET	*N*,*N*-diethyl-m-toluamide
DEP	Diethyl phthalate
DEPT	Distortionless enhancement by polarization transfer
DG	Dodecyl gallate
DG	1,2-diacylglycerol
DLS	Dynamic Light Scattering
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
DRIFTS	Diffuse Reflectance Infrared Fourier Transform Spectroscopy
DSBs	Double Strand Breaks
DSC	Differential Scanning Calorimetry
ds-cDNA	Double Strand Complementary DNA
DSC	Differential Scanning Calorimetry
DTA	Differential Thermal Analysis
DTG	Differential Thermogravimetry
ECD	Electron Capture Detector

EELS	Electron Energy Loss Spectroscopy
EIA	Enzyme Immunoassay
EI-MS	Electron Ionization Mass Spectrometry
ELISA	Enzyme Linked Immunosorbent Assay
ELSD	Evaporative Light Scattering detector
EMR	Electromagnetic Radiation
ENPs	Engineered nano particles
EOF	Electroosmotic Flow
EPN	Ethyl Paraoxon
EPR	Electron Paramagnetic Resonance
ESCA	Electron Spectroscopy for Chemical Analysis
ESEM	Environmental Scanning Electron Microscopy
ESI–MS	Electrospray Mass Spectrometry
ETX	Epsilon toxin
F4	Flow Field-Flow Fractionation
FAAS	Flame-AAS
FCMs	Food contact materials
FFF	Field-Flow Fractionation
FINS	Forensically informative nucleotide sequencing
FIR	Far-Infrared
FITC	Fluorescein isothiocyanate
FMOC	9-Fluorenyhlmethyl chloroformate
FP	Fluorescence Polarization
FSCE	Free Solution Capillary Electrophoresis
FSDA	Forward Stepwise Discriminant Analysis
FTIR	Fourier Transform Infrared Spectroscopy
GBS	Guillain-Barré syndrome
GC	Gas Chromatography
GC FIS/NPD/FPD	Gas Chromatography Flame Ionization Detector/Nitrogen Phosphorus Detector/Fluorine Phosphorus Detector
GFAAS	Graphite Furnace-AAS
GGT	Gamma glutamyl transferase
GM	Genetically Modified Foods/Organisms
GPC	Gel Permeation Chromatography
GSTP1	Glutathione S-Transferase Polymorphisms 1
HA	Hemagglutinin
HCA	Hierarchical Cluster Analysis
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
HDC	Hydrodynamic chromatography
HFB	Heptafluorobutylated
HFBA	Heptafluorobutyric acid
HFBI	Heptafluorobutyrylimidazole
HMDE	Hanging mercury drop electrode
HMF	Hydroxymethylfurfural
HPAECPAD	High Performance Anionic Exchange Chromatography – Pulsed Amperometric Detector

HPAI	Highly Pathogenic Avian Influenza Virus
HPLC	High Performance Liquid Chromatography
HPMC	Hydroxypropylmethylcellulose
HPTLC	High Performance Thin Layer Chromatography
HUVECS	Human umbilical vein endothelial cells
IACE	Immunoaffinity capillary electrophoresis
IC	Ion Chromatography
ICP	Inductively Coupled Plasma
ICP-OES	Inductively Coupled Plasma Optical Emission Spectroscopy
IDT	Initial decomposition temperature
IEC	Ion Exchange Chromatography
IMPY	2-Isopropyl-4-methyl-6-hydroxypyrimidine
IRMS	Stable Isotope Ratio Mass Spectrometry
ISFET	Ion-selective field effect transistors
ISSR	Inter-sequence simple repeat
ITX	Iota toxin
kNN	k-Nearest Neighbours
LC-Q-TOF-MS	Liquid Chromatography-Quadruple-Time of Flight-Mass Spectrometry
LDA	Linear Discriminant Analysis
LDH	Lactate dehydrogenase
LDPE	Low-density polyethylene film
LIF	Laser-induced Fluorescence (detector)
LOS	Lipo-oligosaccharide
LPAI	Low Pathogenic Avian Influenza Virus
LPS	Lipopolysaccharide
MALDI-TOF-MS	Matrix-assisted Laser Desorption Time of Flight Mass Spectrometry
MALS	Multi Angle Light Scattering
MANOVA	Multivariate Analysis of Variance
MBTFA	*N*-methyl-bis(trifluoroacetamide)
MCF	Menthylchloroformate
MCHO	Mean Corpuscular Hemoglobin Concentration
MDA	Malathion dicarboxylic acid
MDM	Mechanically deboned meat
MEKC	Micellar Electrokinetic Chromatography
MES	2-(*N*-morpholino)ethanesulfonic acid
MFE	Mercury film electrode
MIR	Multiple Internal Reflection Spectroscopy
MLR	Multiple Linear Regression
MMA	Malathion monocarboxylic acid
MN	Micronuclei
MPK	Mitogen-activated Protein Kinase
MP-PCR	Microsatellite primed-PCR
MPV	Mean Platelet Volume
MRDT	Maximum rate of decomposition
MRI	Magnetic Resonance Imaging Microscopy
MRM	Mechanically recovered/reclaimed meat
MS	Mass Spectrometry

MSM	Mechanically separated meat
NA	Neuraminidase
NAD	Nicotinamide Adenine Dinucleotide
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NDGA	Nordihydroguaiaretic acid
NIR	Near Infrared
NMR	Nuclear Magnetic Resonance
NPs	Nano particles
NTE	Neuropathy Target Esterases
OG	Octyl gallate
OIDN	Organophosphate-Induced Delayed Neuropathy
OPA	o-phthalic anhydride
OPIDP	Organophosphate-Induced Delayed-Polyneuropathy
OPS	Oriented polystyrene
PAGE	Polyacrylamide Gel Electrophoresis
PARAFAC	Parallel Factor Analysis
PB	Basic Polymerase
PBDE	Polybrominated diphenyl ethers
PCA	Principal Component Analysis
PCB	Polynuclear Chlorinated Biphenyls
PCR	Polymerase Chain Reaction
PCL	Polycaprolactone
PCR	Partial Components Regression
PDO	Protected Designation of Origin
PET	Polyethylene terephthalate
PFE	Polymer film electrode
PFGE	Pulsed-Field Gel Electrophoresis
PFO	Perfringolysin O
PFPA	Pentafluoropropionic anhydride
PG	Propyl gallate
PGI	Protected Geographical Indication
PHA	Polyhydroxy-alkanoates
PHB	p-hydroxy benzoic acid
PHB	Polyhydroxybutyrate
PHBV	Copolymer polyhydroxybutyrate valerate
PHMG	Polyhexamethylene guanidine
PLA	Poly(lactic acid)
PLC	Phospholipase C
PLS	Partial Least Square
PLSR	Partial Least-Squares Regression
PNP	Para-nitrophenol
PON	Paraoxonase
POPs	Persistent Organic Pollutants
PP	Polypropylene
PS	Polyester
RAIRS	Reflection-Absorption Infra-Red Spectroscopy
RAMs	Randomly amplified microsatellites

RAPD	Random amplified polymorphic DNA
RBC	Red blood cells
RFLP	Restriction fragment length polymorphism
RI	Refractive Index
RIA	Radio Immunoassay
RNA	Ribose Nucleic Acid
RNPs	Ribonucleoproteins
RP-HPLC	Reversed-Phase High Performance Liquid Chromatography
RRS	Roundup Ready Soy
RT-PCR	Real Time Chain Polymerase Reaction
RT-PCR	Reverse Transcriptase Chain Polymerase Reaction
SANS	Small-Angle Neutron Scattering
SaPIs	*S. aureus* Pathogenicity Islands
SARS	Severe Acute Respiratory Syndrome
SAXS	Small-Angle X-ray Scattering
SBCEE	Surface-bound crown ethers electrodes
SCC	Staphylococcal Cassette Chromosome
SCE	Sister chromatid exchange
SCGE	Single Cell Gel Electrophoresis
SDA	Stepwise Discriminant Analysis
SDS-PAGE	Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis
SEC	Size Exclusion Chromatography
SEM	Scanning Electron Microscopy
SEs	Staphylococcal enterotoxins
SEs	Secondary electrons
SESANS	Spin Echo Small-Angle Neutron Scattering
SFE	Supercritical Fluid Extraction
SFG	Sum Frequency Generation Spectroscopy
SFP	Staphylococcal food poisons
SIMCA	Soft Independent Modelling of Class Analogies
SLN	Solid lipid nanoparticles
SNP	Single Nucleotide Polymorphism
SPAR	Single primer amplification reaction
SPE	Screen printed electrodes
SPE	Solid Phase Extraction
SPM	Scanning Probe Microscopy
SPT	Skin Prick Test
ss-DNA	Single Strand DNA
SSR	Simple sequence repeats
STM	Scanning Tunneling Microscopy
STRs	Short tandem repeats
TAPS	N-Tris[hydroxymethyl]methyl-3-aminopropanesulfonic acid)
t-BDMS	ter-butyldimethylsilyl
TBHQ	Tert-butylhydroquinone
TCBS	Thiosulphate Citrate Bile Salt Sucrose
TCPY	3,5,6-Trichloro-2-pyridinol
TDFs	Transcript-derived fragments

TDH	Thermostable Direct Hemolysin
TDH-TRH	Thermostable Direct Hemolysin-related hemolysin
TDPA	3,3′-thiodipropionic acid
TDS	Time Domain Spectroscopy
TEM	Transmission Electron Microscopy
TFA	Trifluoroacetates
TFAA	Trifluoroacetic anhydride
TGA	Thermal Gravimetry Analysis
TGA	Thermogravimetric
THBP	2,4,5-trihydroxybutyrophenone
THz	Tetrahertz
TIS	Transmission Infrared Spectroscopy
TLC	Thin Layer Chromatography
TMA	Thermomechanical Analysis
TMS	Trimethyl silyl
TNF	Tumour Necrosis Factor
TPC	*N*-trifluoroacetyl-L-prolyl chloride
TSG	Traditional Speciality Guaranteed
TXRF	Total X-ray Reflection Fluorescence
UVVis-	Ultraviolet-Visible (UV-Vis)
VAMP	Vesicle associated membrane protein
VNTRs	Variable number of tandem repeats
XMT	X-ray Microtomography
XPS	X-ray Photoelectron Spectroscopy

1

Food Forensics: Introduction

"Forensic" is derived from the Latin, with its meaning referring to law. Hence the definition of forensic science hinges on this meaning as a branch of science that applies scientific principles and methods to public domain cases related to criminology and civil law. It deals with the whole process of gathering and examining information that can be presented as evidence in courts of law, to enable the execution of law enforcement in relation to criminal or civil laws.

The application of science and scientific principles to point beyond reasonable doubt to criminals and crimes has been in existence for centuries. In the past, the strategy to catch and deal with criminals had many limitations, mainly due to the lack of standardized methods, which led to numerous flaws that allowed criminals to evade punishment due to lack of sufficient evidence. In those ancient times, criminal acts were investigated based on the testimonies of witnesses and from personal confessions. There was no application of any of the scientific methods and techniques which make use of the scientific principles and concepts that are available to us today. In these modern times, forensic science, as it is applied in a diverse number of fields, employs highly sensitive and selective methods and techniques in analyzing evidence obtained at a crime scene, even where the specimen presented as evidence is at the trace level. Among the areas of disciplines which make use of scientific principles to investigate foul play are in foods and foodstuffs.

Given its importance to life, food will remain the most fundamental of our needs, because it is the source of all the energy needed to carry out life's activities, including those which define the very characteristics of life. Food is essential to our physical well-being, and it is the main item that all the cares of the day are invested towards. Food is the source of energy needed for tissue repair, muscle movement and also plays an important role in the whole process of growth. One may dare to say that without food there is no life! On the other side of the same coin, food that is unfit for human consumption may be used to target and terminate life. For this reason, food is one of the items prone to be used or abused to threaten life.

There are many ways and instances where food composition has been compromised or tampered with, bringing negative effects to consumers. Normally the introduction/incorporation of either microbial (bacterial, fungal or viral) metabolites or chemical agents, compromises the safety of food that is meant for consumers and this constitutes a food fraud case. There are two main types of fraudulences as far as food is concerned. One such example involves a complete replacement of the entire authentic food

Food Forensics and Toxicology, First Edition. Titus A. M. Msagati.

product with a substitute product. This kind of fraud is termed as "crude fraud" and in many cases it involves expensive, highly moveable items such as alcoholic beverages. Another type of fraud that is practiced within the food business/industry is known as "sophisticated fraud", in which some food components are manipulated by either substituting quality ingredients with inferior ones or the entire food product is subjected to dilution (e.g. addition of water to milk, brine to frozen meat, glycerol to wine, etc.).

Again, of late, there have been many food scares, scandals and fraud cases reported widely that not only pose a large risk but have also caused loss of life globally. For example, the advent of genetic engineering technology in food as well as in food industries has introduced genetically modified foods/organisms (GMO), which could pose a potential risk to human existence, though not yet proved scientifically. Apart from the GMO issue, there have been plenty of other incidences, such as the outbreak of bird/avian and swine flu (H1N1, H5N1, H7N3, H7N7, H7N9, H9N2, etc.), and the outbreak of foot-and-mouth disease and bovine spongiform encephalopathy (BSE), also known as Mad Cow Disease. Other microbes, such as the strain of *Escherichia coli* (*E. coli* O157), are harmless resident flora in the gut of cattle but can cause disease in humans through the consumption of meat and meat products containing this bacteria strain. *Clostridium botulinum*, a spore-forming bacterium, produces botulism toxins that are fatal to consumers of food items containing this toxin.

There have also been numerous reports linking the incorporation of illicit food additives (which in some cases are not listed on the labels properly) in food products, and other malpractices by food producers and food industries. These reports have alerted the public and raised the awareness of the composition of food products, thus the high demand for tighter scrutiny, guidelines and regulations on additives in foods.

Incidences related to foul play in foods in terms of processing, labeling, distribution, food poisoning and intoxication, etc., have signaled law enforcers, nutritionists and health bodies to introduce more laws, set regulations and draw up guidelines to safeguard the health and well-being of consumers. Due to this, foodstuffs and food products are now analyzed and investigated to see if they comply with the requirements set for quality, nutritive contents, adulteration, compliance against legally set guidelines and regulations, and also whether the labeling requirements are accurate. These considerations are also meant for research and development purposes to improve the quality of food products.

All governments, as well as national and international agencies, have set regulations, guidelines and recommendations in place to ensure the quality and safety of foods and food products. Such regulations, guidelines and recommendations have to be observed and adhered to by all suppliers and food industries responsible for providing consumers with foods. The enforcement of rules and regulations ensure the wholesomeness and safety of foodstuffs, and satisfy the public need which insists that food suppliers and food industries inform consumers about the state and nutritional composition of their food products, to enable individuals make their choices with regard to their preferences or to provide an environment with fairness, in cases where there is more than one competing company or entity, to avoid any possibility of economic fraud.

Food regulatory bodies, and national and international agencies, have specified some standards to be observed by food suppliers and food industries with regard to quality and compositions of food products. Some of these standards are legally extremely strict and it is mandatory that they are adhered to by all responsible parties, while some others tend to be more flexible.

The strict standards which are mandatory include the quality standards in which quality specifications regarding stability, types and classes of color, mass/or volumes, tenderness, etc are being considered. Standards of identity are also mandatory by regulations and specifications regarding type, composition, ratio and amounts of various ingredients required for a specified food product, which must be made known so that where a threshold limit is set, the guidelines should be clear that it has not been violated. For example, in some foodstuffs, levels of fats are controlled by law and should not exceed certain specified levels. The standards of fill-of-containers are also mandatory, in which case a measure of fill/mass has to be known to avoid fraud or treachery of any sort. In this category of standards, the means or how to ascertain the fill/mass has to be made known as well. Flexible standards may as well include grading of food products.

Authorities responsible for food regulations also enforce a requirement that the origin and authenticity of food products be correctly included in the labeling, together with the labeling specifying food ingredients and their composition. This information is vital for the prevention of economic fraud and for ensuring that the correct type of food is supplied to the target people. For example, people of a particular religious group, or those who need food with certain ingredients, for instance, fat, sugar, salts, etc., at certain amounts. For this reason, food and food products need to be analyzed and ascertained for their safety and quality.

In some cases, microbial attack on food not properly stored leads to the presence of toxic metabolites. Therefore, storage conditions and techniques are some of the issues that are scrutinized, especially for the types of foods and food products that need to be transported to distant places or those which are not to be consumed immediately.

The presence of undesirable chemical and biological molecules prompts the enforcement of monitoring schemes to ensure safety and quality of foods and food products. The monitoring scheme has to have scientific methods and techniques in place that are sensitive and selective enough to exclude all other undesirable or non-targeted matrix molecules as well as to enrich the analytes of interest. The enrichment step is necessary because. in many instances, the target analytes are in trace amounts or dissolved/incorporated into a larger mass/volume of food products which causes the analyte to be highly diluted.

Normally, the analytical strategy involves the development of highly selective and sensitive methods that are capable of thorough clean-up and pre-concentrate the analytes before their introduction into the analytical system, such as HPLC, GC, AAS, ICP, etc. The data obtained from these analyses are meant to provide information regarding the safety of the foodstuffs or if a particular food item contains harmful microbes such as Salmonella or their metabolic by-products, or the presence of chemical molecules such as pesticide residues, or even the presence of some other foreign contaminants. Foodstuffs for human consumption imply that they are free from all such things, and food manufacturers as well as suppliers have a duty to ensure food safety by carrying out routine analysis of food products. Moreover, food industries are at all times faced with stiff competition from each other and are thus obliged to perform these routine checks to remain competitive and win more markets.

Generally, strict regulations and guidelines necessitate food industries to use methods and techniques that are reliable and which are capable of providing low detection and quantification limits to be able to comply with guidelines and regulations. Testing and analysis should normally be done strategically, before, during and after the

manufacturing and in some cases, long after the manufacturing to ensure stability under different sets of storage conditions, such that the final food product possesses the same properties and qualities including shelf life, appearance (color), flavor and texture in addition to chemical, physical and biological properties.

However, naturally foodstuffs do undergo deterioration and spoilage over time and under some conditions where the chemistry of raw material ingredients unpredictably changes, thus affecting the whole food product. Under such circumstances, what is required is to understand the chemistry of the ingredients and the mechanism by which each plays its role in relation to the final food product. This knowledge helps to predict the behavior of various ingredients under different sets of conditions and thus control the production processes accordingly, in order to avoid unnecessary food deterioration. For example, food color of some products, such as chips made from potatoes, is highly dependent on the concentrations of reducing sugars present in the potatoes during the manufacturing processes. If the reducing sugars are present at high concentrations in the potatoes, it will cause more browning of the potatoes as time goes on (Msagati, 2012). This implies that those responsible in the preparation have to ensure that the potatoes used have the required concentration of reducing sugars to avoid color changes with time or during the frying process.

Currently, the global trend with respect to attitudes towards foods has been highly shaped by the opinion of consumers, due to changes in consumer preferences towards the types of foods that are seemingly healthier. In some cases the origin of food, cost or those seen as exotic, have been an important factor for consumer preference. This trend has shaped the whole food industry, which has to conduct research and development for either the improvement of the existing products or formulate new food products that satisfy the changing demand of consumers. In this scenario, food industries engage in research activities that investigate the mechanisms of how various food ingredients and components work and also the roles they play individually or when introduced to others. The investigation process also encompasses the whole industrial processes that take place or occur during food manufacturing and how they affect the quality and composition of food, even after a long period of time when subjected to various physical/environmental conditions such as temperature, humidity, storage, etc. In other words, food development of new products always requires the knowledge of the food ingredients as well as the processing operations and how they interact to give food the desired quality.

On the other hand, the analytical procedures required by food analysts need to establish factors that will reveal the information about composition, physico-chemical properties of food, etc. This is due to the fact that food components (composition) tell how safe or nutritious the food is. The composition of many foods is complex, being made up of many different ingredients (vitamins, proteins, carbohydrates, fats, fibers, minerals, etc.) and in different ratios, both in terms of the types of atoms (e.g. C, H, N, O, S, Na, Se, etc.) and molecules (e.g. H_2O, sugars, essential fatty acids such as omega-3, essential amino acids, etc.). For food manufacturers and distributors to comply with regulations, they are thus obliged to declare compositions, ratios, levels and types of all the ingredients in their products.

Similarly, the attributes of foods in terms of their physical characteristics as measured by their rheological properties (stability, taste, color/optical, flavor, etc.), are very important to consumers, as they affect their appeal and choices. This shapes the food

industry since it requires that foods and food products be designed to contain the qualities that will appeal to consumers and at the same time comply with regulations and guidelines. The design and operating conditions must also ensure food stability under all conditions without affecting attributes such as color, smell/odor, feel or texture. Moreover, the design and operation processes during food manufacturing have to be such that they maintain the structure of the food and food products.

Food analysis will therefore require different types of analysis, methods and techniques, due to a variety of information and measurements that may be needed to ascertain the quality and safety of a particular food product. There are many sources where the information for the appropriate technique for measuring or ascertaining a particular food property can be obtained. For instance, there are specific books, journal publications and suppliers of instruments, reagents, chemicals and materials used in the analysis of foods. There are also official methods and techniques published by authorized bodies that deal with food analysis and quality assurance, as well as public domains such as the World Wide Web (internet).

Reference

Msagati, T.A.M. (2012) *The Chemistry of Food Additives and Preservatives*. Wiley-Blackwell, Oxford.

2

Food Provenance and Food Fingerprinting: Authentication and Traceability of Foods and Food Products

Is it important to know the origin of the food we eat? This may be a question to which some insist on concrete answers, though for some it may not matter whether the food they eat comes from west, east, north, or south. Generally speaking, there has been an interest in where food comes from, and how it reaches the consumers (knowledge of mechanisms and routes of distribution and supply chains). Some food products are in demand throughout the year, thus necessitating a well-known supply chain and this requires the knowledge of the origin of such food items be provided. Moreover, with concerns arising from environmental pollution, which directly negatively affects the whole of food safety, the food preference for many consumers is tilting towards organic foods (those that are grown with no application of long-lasting agrochemicals and fertilizers). All these factors have prompted authorities to introduce rules and regulations to govern the traceability mechanisms of food products, which will give confidence to consumers about the authenticity, quality, integrity, safety, and provenance of the food products of interest. At this juncture, there is a need to define the term "origin" as far as food provenance is concerned. The origin of the food's location refers to the geographical location where the food in question is either produced/obtained/found. In cases where the same food product is found in more than one geographical location, then the location where the last substantial transformation is recorded will be considered to be its geographical location of origin for that particular food product.

Introduction

In the modern day, consumers of various foodstuffs and food products demand to be provided with clear, accurate, precise, and succinct information and data about the origin and specification of the food products they consume. Authorities have put in force strict regulations that such information be outlined in the labels and labeling, which should adhere to the rules and regulations set by describing properly the geographical origin and authenticity of all that is claimed in terms of quality and composition on the packaging labels. The rules, guidelines, and regulations that have been set speak volumes about the legality of the whole issue about food provenance and fingerprinting, with laws being enacted to prosecute the culprits, offenders, and transgressors.

Food Forensics and Toxicology, First Edition. Titus A. M. Msagati.

Food Fraudulence and Adulteration

Food fraudulence refers to deliberate and intentional actions and tendencies to illegally tamper by substituting, adding, or mislabeling of foodstuffs, food ingredients, or even food packaging for whatever gain (Elliot, 2013). Food fraudulence involves all practices that lead to an intentional or pre-meditated addition or substitution of an ingredient in order to either raise its value or lower production/processing costs (Shears, 2010; Spink, 2013). Practices such as dilution of food products to levels that reduce their nutritive value or to an extent that risks the health of consumers and/or masking the effects of dilution by adding other inferior substances or replacing quality ingredients with either weaker/inert ones, false branding/misbranding or mislabeling, etc., are all part of food fraudulence (Ellefson *et al*., 2013; Elliot, 2013). This term "food fraudulence" also encompasses other tendencies, such as giving false, inaccurate, or misleading information or statements regarding a particular food product (Ellefson *et al*., 2013). In the context of this topic, food fraudulence will not deal with other forms of food fraudulency tendencies such as those that deal with economically motivated kinds of fraudulency, misbranding, smuggling, tax evasion, and food counterfeiting practices. This topic will only deal with intentional food fraudulency tendencies with motivations that affect food quality and food safety, which endangers the health of consumers.

For any food product to be considered fraudulent or adulterated it must contain the following:

- any substance or ingredient, or an added ingredient, that may cause a health hazard to consumers;
- an ingredient or component (added externally or internally) that is regarded as spoiled/decomposed, regardless of whether this ingredient was formed during processing, handling, or storage, or it came from a disease that has affected the plant or animal from which the food originated, from the container/packaging, or stores where the foodstuff was kept/stored;
- does not contain any valuable component whether omitted, damaged, concealed, abstracted, or present in an inferior form;
- harmful ingredients such as additives (coloring agents, flavor enhancers, etc.);
- wrong measure of fill (volume, mass/weight, bulkiness) or strength (e.g. color intensity, flavor sharpness, etc.);
- does not contain proper information, such as food items sold under a different identity (origin, type of food, etc.) and foods imitating others or foods with wrong definitions.

However, caution should always be taken when defining the cause of the incidences in food fraudulence cases. In the scenario where it is proved beyond reasonable doubt that there has been an unintentional and deliberate action of food contamination or adulteration through either improper food handling, or during processing, or spoilage by microorganisms or addition of ingredients that will poison the food products, all these will constitute what is termed as issues related to food safety. The situation will only be regarded as food fraudulence when it has been proven that there has been an intentional cause for the presence of adulteration in the food for whatever reason, especially the economic gain.

The common aspect between the two terms is that both food fraudulence and food safety result in health risks to consumers. When there has been an incidence of either food fraudulence or food safety endangering consumers' health, efforts are normally invested to address (prevent or reverse) the effects of intentional or deliberate adulteration that was caused for whatever motive, and this is defined as food defense. On the same note, cases that involve unintentional food adulterations (food spoilage or deterioration), which result in economic losses for the individuals or industry, constitute what can be termed as food quality issues. Food quality can be caused by various physical and chemical properties of the food. Now the efforts to prevent or measures put forward as intervention or responses to address food quality phenomena are known as food protection.

Bearing in mind the consequences of all these phenomena, authorities normally devise strategies and plans to prevent food adulteration incidences well before they take place. Among the strategies that are put in place are the enforcement of effective programs of risk-based intervention strategies well in advance and also the institution of rapid reaction measures to address effects that can be caused by food fraudulence once it is discovered. For convenience, food fraudulence can generally be categorized into a number of groupings as follows:

- counterfeiting: in which case the perpetrators infringe the legal owners' Intellectual Property, and produce a similar food product and package using similar packaging and without adhering to the standards;
- simulation: is another food fraudulence category that is similar to counterfeiting, where the design and formulation of simulated food products gives them a similar look of the real food product, but in reality it is similar and may have slightly different formulas or ratios of ingredients, and in most cases the simulated food products fail the compliance test in terms of quality assurance;
- adulteration: also a food fraudulence category that involves the addition of a foreign ingredient to a finished food product, for example adding brine to frozen meat and products, or adding melamine in milk;
- tampering: another category of food fraudulence that involves misusing either legitimate food products or food packaging, e.g. by changing the information, such as expiry period.

Currently, there is an ongoing fight against food fraudulence that is ever growing and expanding, covering almost all parts of the world. There is a widespread tendency of adulterations where foods, foodstuffs, food additives, and food supplements have been found to be contaminated or without the expected quality or labeling. The results of food adulteration cost the food industry greatly and affect consumers both health-wise and economically. The sources of adulteration may be traced from the geographical identity of the place of origin of that particular food product, the brand name and method of food processing, or even the time it has been stored. Food fraudulence is currently attracting the interest of many stakeholders, both in private as well as public sectors, as an emerging risk that is threatening society. This is partly due to the complex nature, trends, and principles that govern distribution and global food supply chains. Unscrupulous food manufacturers and distributors entertain the practice of tampering with the ingredients of foodstuffs by cutting down levels or replacing altogether some

of the ingredients in order to maximize profit (Elliot, 2013). The availability of numerous reports and publications suggests that there are major and serious food adulteration and contamination practices occurring, frequently and regularly (Ellefson *et al*., 2013).

For example, according to an article that appeared in the *Washington Post* on Tuesday, 30 March 2010; A01, which was entitled "FDA pressured to combat rising 'food fraud'" by Lyndsey Layton, *Washington Post* Staff Writer, reports on fraudulence involving sheep's milk cheese that was later discovered to be cow's milk; another case was sturgeon caviar that was later proved to be Mississippi paddlefish (source: http://phe.rockefeller.edu/news/wp-content/uploads/2010/04/FDA-pressured-to-combat-rising-food-fraud.pdf; accessed 11 September 2014). This article also pointed to instances where some honey-makers tended to dilute honey products with either sugar beet or corn syrup. Other instances involved the sale of frozen catfish fillets claiming to be other brands such as red snapper, etc. but they were actually from Vietnam (source: http://phe.rockefeller.edu/news/wp-content/uploads/2010/04/FDA-pressured-to-combat-rising-food-fraud.pdf; accessed 11 September 2014).

In general, deception in the food industry has been around for a long time, although the extent of its impact on consumers' health or the economic sector is difficult to ascertain. There are contributing factors such as the current trend toward urban migration, as well as globalization that play a role in its widespread increase, both in terms of magnitude and extent (Ellefson *et al*., 2013). These factors (urbanization and globalization) encourage a special trend of food supply chain to sustain the huge population concentrated in urban centres. To control such a complex food supply chain in the current global economy with limited resources, creates room for criminals to practice unscrupulous business deals with food products. As time goes by, they come up with new ways of practicing fraudulence, which creates more challenges to deal with the problem.

Scientific Methods and Strategies for Verification of Food Fraudulence Cases

There are a variety of profiling methods that generate useful data (those chemical signals obtained from analytical instruments that can be de-convoluted to give the composition of food), as well as many other chemical and biochemical techniques that are available to food forensic scientists, to ascertain any of the already mentioned food fraudulence cases. The advantage of profiling methods is that they can be applicable to complex samples such as foods and are also capable of performing a wide range of scientific measurements simultaneously. Among the chemical and biochemical techniques that are used to verify food adulterations or fraudulence are chromatography (e.g. liquid chromatography (LC), gas chromatography (GC), etc.); mass spectrometry (MS); nuclear magnetic resonance (NMR); infrared spectroscopy (IR); enzyme-linked immunosorbent assays (ELISA); and polymerase chain reaction (PCR), etc.) (Pomeranz and Meloan, 1994). Data from these techniques can further be subjected to statistical analyses, using multivariate techniques such as partial least square (PLS) or principal component analysis (PCA), to provide more in-depth information and variability of parameters that can reveal abnormal trends of behavior or lack of consistency.

Scientific Verification of Geographical Indications and Designation of Origin of Foods

There are three main types of geographical description or schemes for foods that play a significant role to protect the identity and names of foods, as well as other agricultural products. There is a scheme known as "Protected Designation of Origin" (PDO), which applies to those foodstuffs and food products originating/produced and/or prepared/processed in a particular geographical location using acceptable/known and standardized methods and techniques (Gonzalez *et al*., 2009).

The second type of scheme or geographical description of foods is known as a "Protected Geographical Indication" (PGI), which signifies that there is a connection with the geographical location or area in at least one step of either production, preparation, or processing of a particular foodstuff (Gonzalez *et al*., 2009). This second description of the geographical description (PGI) seems to be of more importance than PDO, due to the fact that it associates the food with a particular geographical location.

The third scheme is known as "Traditional Speciality Guaranteed" (TSG), which reveals the traditional nature or character in terms of either ingredients (composition) or the procedures followed during production.

Processes, Steps and Procedures to Distinguish the Geographical Origin of Foods

The identification of the origin of foods or the effects of different food production processes is not straightforward. The process must involve the identification of specific markers that distinguish food products, in order to ensure that there is no possibility of having either a false negative or a false positive. There must also be in-depth scientific knowledge of the chemical composition of foods, effects, or contribution of environmental factors (biological and physico-chemical factors), and other important differences between the target food items of interest to other similar food products found in other geographical locations. This calls upon the presence of analytical chemists who will establish procedures of correct identification of the exact discrepancy that defines the fraudulence in question (fraudulent food product and the type of fraudulence).

Analytical chemists will need to play an important role in developing appropriate methods that will reveal the exact chemical composition, as well as isotopic abundances of the elemental composition of that particular food product or other specific chemicals/molecules, spices, or flavors that may form part of the composition of the food products in question. Analytical chemists will also need to develop methods and strategies to identify products that may be formed during food processing or production, thus fingerprinting products by using appropriate analytical and bio-analytical techniques, including chromatography, spectroscopy, or mass spectrometry. Measurements of this kind of work require large sample sizes, as well as the application of statistics for validation, otherwise the results may not be considered authentic enough.

Geographical Identification, Quality Verification and Authentication of the Origin for Fruit Nectars, Fruit Juices, Vegetable Juices, and Non-alcoholic Beverages

The term "fruit juice" refers to a liquid derived from a fruit, while "fruit nectar" refers to an unfermented food product obtained by adding water to a fermentable food product. Generally, fruit nectars may contain additives such as sweeteners or honey. On the other hand, the general term "non-alcoholic beverages" includes products such as soft drinks, energy drinks, bottled water, carbonated drinks, and sports and isotonic drinks. Fruit juices, vegetable juices, fruit nectars, and other non-alcoholic beverages are normally verified on the basis of the composition of the additives incorporated for the purposes of adding or enhancing color and those that are added as flavoring or flavor enhancers. Also, these food products are verified on the basis of water content that forms part of the naturally derived fruit juice. Generally, for nectars and juices prepared from fruit, there are regulatory standard requirements that are supposed to be adhered to before the certification of the product for commercialization (sale). These include the requirement that they should possess and retain the same characteristic qualities of aroma (flavor and flavor sharpness), as well as the appearance (color and color intensity) to that of the fruit from which the product (fruit or nectar) was processed.

Another decisive factor that is crucial in the authentication or identification of the origin of fruit juices, vegetable juices, and nectars, is the measure of the main quality parameters that are normally required to be displayed on the label (showing composition). Verification of the measure of various ingredients in the fruits juices, vegetable juices, and nectar as claimed in the labeling, is always mandatory in order to prove compliance with the regulations. For instance, with some adulterations involving fruit juice blending, where a high-quality juice can be blended with an inferior one (adulteration for economic gain), the information as well as the measure and quality of ingredients as it may appear on the labels may be different from the actual chemistry of the juice.

The problem of food adulteration related to juices and nectars has prompted the need for the development of analytical methods, techniques, and regimes to analyze and verify the identity and authenticity of fruit juices and fruit nectars.

The development of analytical procedures for this purpose has been faced with challenges due to the complex nature of these food products, as well as the variability of fruit juices and nectars in terms of their composition. Another challenging consideration comes from the fact that the analysis requires skilled personnel as well as advanced analytical instruments. For fruit/vegetable juices and fruit nectar, the detection of identity, adulteration, and authenticity involve the detection of mainly the types and ratios of organic acids, amino acids, sugars, or other sweeteners, antioxidants such as polyphenols and flavonoids, as well as inorganic elements, and these compounds can thus be used as indicators of adulteration.

For example, one of the nutritive compositions in fruit is sugars, which is one of the components that actually impart to the fruit and juices the characteristic taste, aroma, and flavor. Sugars also play other roles, including the role of an indicator of the conditions for storage. The abundances and ratios of these sugars is characteristic to fruits, such that if there is any significant difference, then the situation will represent a possible scenario of adulteration. This is because any attempt to add either a sweetener, syrup, sugar, water, or an inferior type of juice blend, will alter the ratios of various sugars and

their abundances, thus confirming a fraud scenario. Sucrose (glucose and fructose), as well as sorbitol (a sugar alcohol), are the main types of sugars that are normally analyzed to confirm a fraud situation for fruit juices, vegetable juices, and nectars. The abundance and ratios of these sugars are qualitatively and quantitatively different for different types of fruit or vegetable juices and are dependent on a number of factors such as species, variety, geographical location where the fruits are grown (environment), and the maturation level of the fruit at the time of harvest. For example, pineapples, grapes, oranges, and apples from the same geographical location and maturation will have different profiles and ratios of sucrose, glucose, and fructose. Normally, glucose and fructose are found in higher levels in grapes and at the same time, sucrose in grapes is generally lower. The situation is the opposite in the case of apples, pineapples, and oranges. Sorbitol is generally lower in many fruits and fruit juices and where needed can only be added, in which case it has to be within the regulations and guidelines or else this will constitute an act of adulteration.

Sugars are the building molecules of carbohydrates and thus when analyzing sugars, methods used for carbohydrate analyses are normally applicable for sugars as well. The most common method involves the use of a high performance anion exchange chromatographic system equipped with a pulsed amperometric detector (HPAEC-PAD). Where necessary, this can further be confirmed by the use of stable isotope methods to ascertain the results for authenticity and identification of adulteration. This type of chromatography is normally preferred for the separation of anionic species, which can either be anions in their natural form or those that can be ionized at high pH values, for example sugars and carbohydrate. Where species that ionize at high pH are analyzed using HPAEC, the mobile phase for such kinds of species will constitute mainly hydroxide-based eluent solutions, because they are capable of producing anions from these analytes (e.g. sugars), which can form charges in neutral pH environments. The stationary phases (column packing materials) for HPAEC are normally prepared from non-porous resins, in which microbeads with anion-exchange moieties are attached to cation exchange resin particles to impart stability at all pH values. Moreover, the preference of non-porous polymeric resins as stationary phases for HPAEC comes from the fact that they minimize band broadening in chromatograms, thus highly improving the separation of analytes.

After separation, they can be detected without the need for derivatization using a pulsed amperometric detector (PAD) in which various (specific) electrochemical potentials are applied to a working electrode for a period of time, causing oxidation-reduction processes of the sugar's hydroxyl groups at the surface of the working electrode. The redox processes at the electrode surface will cause losses in protons, which will result in currents being generated and the resulting current will be proportional to the magnitude of the hydroxyl groups of the sugar samples.

Stable Isotope Ratio Analysis (SIRA) of Sugars in Fruit Juices and Fruit Nectars: Isotope Signature

By definition, stable isotopes are the non-radioactive atoms, with nuclei having the same number of protons but a different number of neutrons. Chemically, isotopes behave like other major elements such as carbon, hydrogen, oxygen, nitrogen, and