Food authentication by microbiome analysis

Mummy: how can daddy possibly like that smelly cheese?

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Storyline

Food Protection is a very serious matter, since it may guarantee that our everyday meals do not have significant risk of contamination by hazardous physical, chemical or biological agents. These threats can enter the food supply unintentionally, but they can also get to our tables by the action of criminals who **adulterate** food. Instead of being a "treat" food may be a "trick" when adulterated.

Trick or Treat Sig, by Karolina Grabowska. From Pexels.

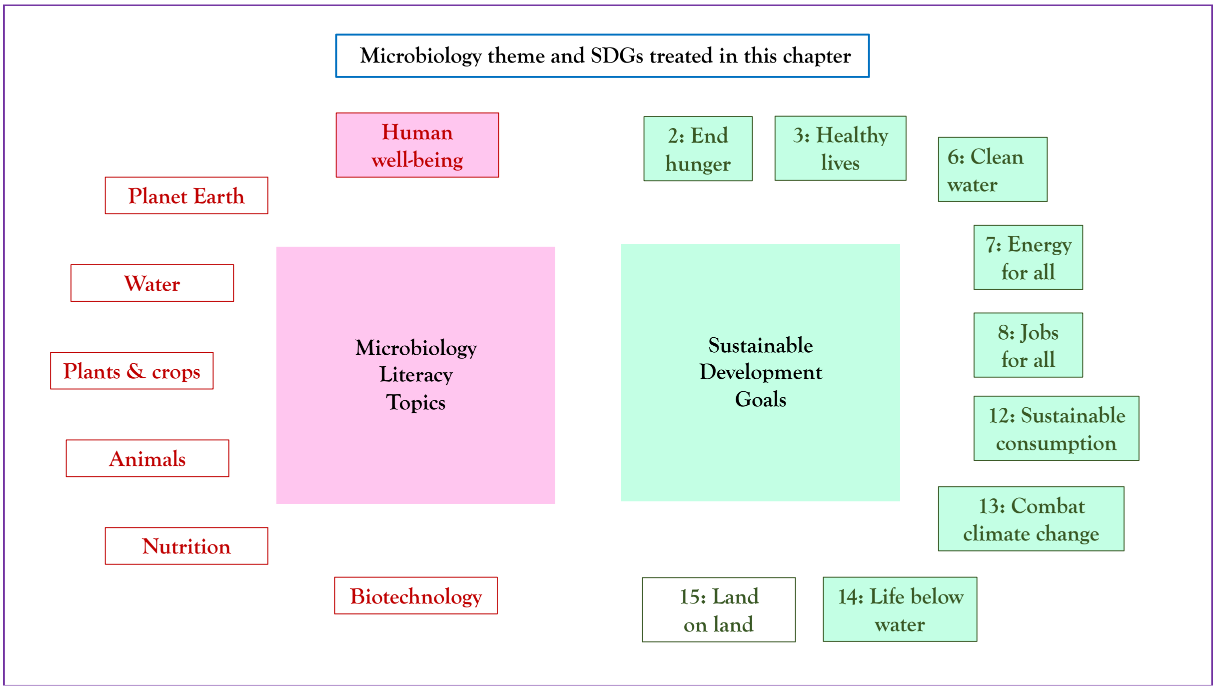
Crimes related to food adulteration cause losses worth billions of dollars every year and are of public health concern, since they often involve the addition to the food products of extraneous ingredients that can harm our health. The adulteration may have the objective of increasing profits (for example, by adding cheaper ingredients not declared on labels, which may cause allergies, intoxications or infections) or may also serve other bad intentions, such as **bioterrorism** (for example, by the deliberate addition of pathogens to food during production or transportation).

Fortunately, there are many prevention strategies in place during food processing, to avoid the contamination of our food supply. One such strategy is analysis of food microbiomes which reveals "microbial signatures" that can indicate food authenticity.

How can scientists find out if a finished food product is really what it is supposed to be? Am I really buying that fancy expensive cheese with *Designation of Origin*, or was it prepared carelessly elsewhere in the world?

The Microbiology and Societal Context

The microbiology: food microbiology; food microbiomes; DNA sequencing; metagenomes; *Sustainability issues:* health; food and energy, economy and employment; global warming.



Food authentication by microbiome analysis: the Microbiology

1. ***The importance of knowing what is in your food and its authenticity.*** The guarantee that a given food item is really what it is supposed to be involves the work of partners from the food industry, government bodies and academia. Also consumers and social organizations are stakeholders and play important roles to inhibit fraud in the food supply chain, by making smart choices from legal and approved traders.

Scientists make use of several methods to solve crimes implicated in food fraud, such as the determination of chemical composition, physical properties and biological characteristics, including microbiological analysis to determine what microbes are present and in what quantity. However, more recently, with widely available techniques for **massive DNA sequencing**, the search for microbial DNA directly in foods has also been used to trace the different origins of food products.

Intriguing, isn't it? Let's learn more about how microbes can help to discover in fact, what are the ingredients of our dinner plate…

2. ***Food microbial diversity: health, flavors, economics and laws.*** Think about how complex a food item may be… There are foods that you can more easily tell where they came from and what their ingredients are, such as pasteurized milk or eggs. However, things may get more complicated when you think of food such as frozen meals, hot dogs, fast food, packaged cookies and cakes, etc. There is also the challenge of the global food trade, with ingredients supplied by hundreds of countries around the world, with diverse sanitary and hygienic standards.

So, we get to "Food Science" - a complex field that involves biology, chemistry, physics, sensorial analysis, consumer behavior, agricultural practices, public policies, among others. Of pivotal importance in Food Science is Food Microbiology, which studies the **microbiota** of different foods.

"Microbiota" is a term that refers to all live microorganisms in a community, which is also influenced by external factors, such as temperature, pH, light, composition of the atmosphere.

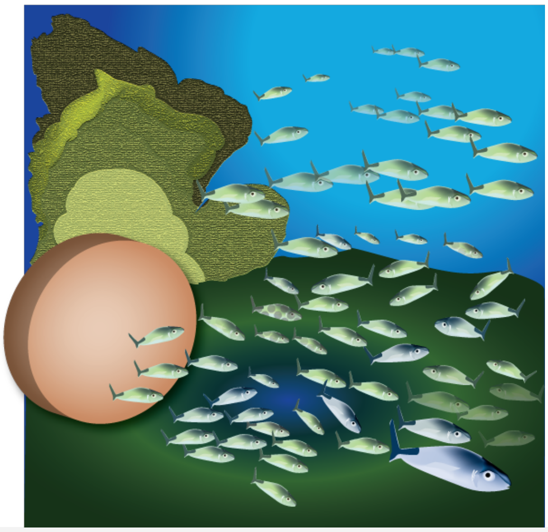
To identify all the microorganisms in a food sample is not a simple task, and it demands a lot of expertise, including the knowledge about **genomics**, which can reveal the food microbiome. The "Microbiome" refers to the collection of genes and genomes of a microbial community - it does not matter if the microorganisms are dead or alive. So, by determining the Food Microbiome, scientists can discover a lot about food authenticity, even in cases where the microbes cannot be cultivated any longer. This is part of the science of food forensics!

3. ***So what exactly do we do in food forensics?*** To determine the Food Microbiome, scientists need to extract the total DNA from the food sample. Total DNA means all the genomes – chromosomes – of all cells in the sample, both those of microbes and of any plant or animal tissue present. This DNA is used to make a DNA "library", a collection of DNA segments that can be analyzed by sequencing in a very specialized instrument - the DNA sequencer. When the total DNA from a given sample is sequenced, it generates as output a computer file with mixed DNA sequences, from all the microbiome and also from other sources. Scientists then "filter" out only the DNA sequences from the microbes using bioinformatic approaches (computer algorithms), and reconstruct "in silico" (in the computer) all the genomes of the microbial community, to determine its composition (which microorganisms were present in the sample) and to estimate the functions of the microbes (what kind of substances are those microorganisms capable of utilizing and producing).

At this point, we might ask: *Are the microorganisms we find in the food those we expect to be in it? Are microbes detected that we would not expect? Are they related to harmful microbes? Do they produce substances that have good flavor or not?* The microbiome may help in answering these questions, which are important to determine food authenticity.

To understand this more easily, let us consider some case studies:

a. *Is the food really what it is supposed to be?* Scientists are investigating the microbiome of different seafood products to detect fraudulent substitution of snapper, cod and surimi products, and so far they found out a higher abundance of bacterial DNA from the genus *Pseudomonas* in tilapia sashimi mislabelled as snapper. This is a bacterium related to food spoilage, besides being able to cause infection in debilitated persons.



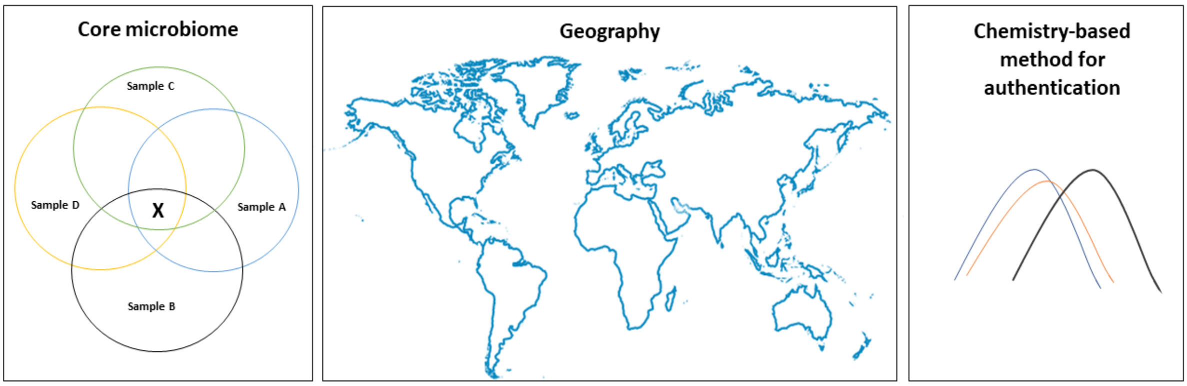
This image of School of Fish was obtained on: seekpng.com as a free image.

Tilapia is cheaper than snapper, and the former is a kind of fish typically raised in fresh-water ponds, in contrast with snapper, that lives in the ocean. Scientists also know *Pseudomonas* is commonly found in soils and aquatic environments. By analyzing the microbiome, they suggested the presence of higher amounts of DNA from *Pseudomonas* sp. might indicate the fraudulent substitution of snapper by Tilapia.

b. *Does it really come from the place indicated on the label?* There is interest in determining the geographic origin of foods for sustainability purposes - the consumer has the right to know the food production method (caught wild fish or farmed). Scientists have demonstrated for shellfish that microbial diversity indices determined by microbiome analysis can be correlated with their region of origin.

c. *Does a "Protected Designation of Origin" (PDO) item come from the specified origin?* The designation PDO assures the authenticity of a food item from a specific region, and production by traditional methods, for example the cheeses "Parmigiano Reggiano" and "Grana Padano". Having a PDO makes the product more valuable, so there is a financial gain in labelling a similar product from a different region PDO. Determination of the typical chemical composition and microbiome are important means of detecting frauds.

For each food product of interest, the scientists try to find a "core microbiome", which means the microbial taxa found in the majority of samples of a product. To illustrate this concept, in the figure below, on the left-hand side, each circle represents all the microbes from a given sample. The same microbes present in both samples, such as in A and B, are to be found where two circles overlap. The "core microbiome" of all the samples of a given food is represented by "X". This figure also reminds us about other aspects important in defining PDO products, namely geography and chemical composition.



The map file was obtained on: seekpng.com as a free image. The other figures are from the authors.

d. *Does my "probiotic" yogurt contain the right live microbe in the right amount?* A "probiotic" food is defined as a product that contains viable microorganisms that, when administered in adequate amounts, confer a health benefit on the host. To be an authentic probiotic, the name of the beneficial microorganism and the amount of viable cells present in each serving of the food/product must be written on its label. However, scientists have already identified that what is described on the label does not always correspond to reality.

One of the most common labeling errors in probiotics is the misinformation on the species of microorganism it contains (e.g. *Lactobacillus acidophilus* instead of *Lactobacillus helveticus*).

"For Patients & Caregivers" was obtained on: seekpng.com as a free image.

Microorganisms that are not indicated on the label have also been found in probiotics, in addition to errors in the amounts of microorganisms per dose of the product.

e. *Was my wine made at its specified vineyard? Does it reflect the correct "terroir"?* The transformation of grape juice into wine is a complex biotechnological process, influenced by "terroir", which can be defined as an interactive cultivated ecosystem, in a given place, including climate, soil, the underlying geology and mineralogy, the nature of available water, and the vine. These in turn influence the composition of the grape microbiome.

The microbiome of wine fermentation is key for defining sensorial attributes, and studies on DNA from microbial communities of Chardonnay grapes from 23 vineyards from four different regions in the same country, showed there were more than 200 species of fungi! This kind of knowledge may help in determining the authenticity of wines.

Wine glass Image free from seekpng.com

Relevance for Sustainable Development Goals and Grand Challenges

Food authentication is relevant to achieve the Sustainable Development Goals (SDGs) from the United Nations, including aspects related to the microbiome, such as:

- **Goal 2. End hunger, achieve food security and improved nutrition and promote sustainable agriculture** (*end hunger and malnutrition, increase agricultural productivity).* Fraudulent food must be discarded. So, instead of contributing to good health and nutrition, it leads to a waste of resources, and might even cause sickness and deaths, depending on the adulterant. From another point of view, certain microbes can improve the nutritional value of food, for example by degrading naturally occurring toxic compounds. Microbes can also help in food preservation: fermented products usually have a longer shelf life than fresh ones, and sometimes they do not even need to be kept in the fridge. You can think of fresh milk *versus* cheese or yogurt, or fresh meat compared to salami. This is very important to contribute to **food security.**
- **Goal 3. Ensure healthy lives and promote well-being for all at all ages** (*improve health, reduce preventable disease and premature deaths).* The guarantee of a food with authentic microbiome helps in delivering safe food to the consumers, and in the case of probiotics, it also helps in providing the "good bugs" that may contribute to maintaining intestinal health and reducing the risk of illness, including infectious and **chronic diseases**.
- **Goal 6. Ensure availability and sustainable management of water and sanitation for all** (*assure safe drinking water, improve water quality, reduce pollution, protect water-related ecosystems, improve water and sanitation management).* To produce food requires lots of water in several production steps on the farm and/or in the processing industry. If the food is not appropriate for consumption due to fraud, the water used in its production is also wasted. It is also important to note the need to use good quality water for food production because water may carry "bad bugs" that can contaminate our dishes.
- **Goal 7. Ensure access to affordable, reliable, sustainable and modern energy for all** (*ensure access to clean, renewable and sustainable energy, and increase energy use efficiency).* Crops are grown in agriculture for food for humans and animals (including pets), for biofuel production and for materials. Given that agricultural land and fertile soils are in limiting supply, it is essential that they are used efficiently to maximise both food and energy security. Food authentication by various methods (including the microbiome) contributes to resource

security and hence sustainability, by certifying food origin and quality and thereby reducing waste.

- **Goal 8. Promote sustained, inclusive and sustainable economic growth, full and productive employment and decent work for all** (*promote economic growth, productivity and innovation, enterprise and employment creation*). Different species of microorganisms are used for the production of fermented foods and beverages, such as dairy products, pickled cucumbers, sourdough bread and kefir, among others. The industrial use of these microorganisms, in addition to improving food quality, generate job opportunities and income for producers, including **family farming.**
- **Goal 12. Ensure sustainable consumption and production patterns** *(achieve sustainable production and use/consumption practices, reduce waste production/pollutant release into the environment, attain zero waste life cycles, inform people about sustainable development practices).* To promote a culture to guarantee food authenticity throughout the production chain contributes to ensure sustainability by supporting smart food choices for consumers and reducing waste due to discarded food.
- **Goal 13. Take urgent action to combat climate change and its impacts** *(reduce greenhouse gas emissions, mitigate consequences of global warming, develop early warning systems for global warming consequences, improve education about greenhouse gas production and global warming).* Responsible food production contributes to minimizing global warming, by saving resources to produce only food that uses sustainably produced raw materials, that has an adequate shelf life and that is not contaminated with hazardous microbes.
- **Goal 14. Conserve and sustainably use the oceans, seas and marine resources for sustainable development** *(reduce pollution of marine systems by toxic chemicals/agricultural nutrients/wastes like plastics, develop mitigation measures for acidification, enhance sustainable use of oceans and their resources).* The study of microbiome can contribute to guaranteeing the authenticity of food products that are supposed to be from one specific geographical area, or belonging to one given species (of fish, for example). This can contribute to the well-being of other organisms that are not to be caught for food consumption, to preserve biodiversity.

Potential Implications for Decisions

1. ***Individual*** (How the knowledge about Food Microbiome applied to food authenticity may affect my food choices)

a. Adulterated food may cause health harm due to the unexpected presence of food allergens, bacterial food contaminants and other toxins. Buying food from reliable suppliers is important to guarantee the authenticity of what you and your family eat.

b. Many foods may have health claims, such as those related to bioactive compounds and probiotic microorganisms, but it is very important to consume this kind of food only if they have been approved and inspected by sanitary agencies from your country.

c. If you know of any illegal commerce of food items, advise your colleagues about the dangers of selling and buying uninspected food.

2. ***Community policies***

a. Information campaigns and education informing community members of the risks of adulterated food and how to avoid it.

b. Carrying out food inspections in the supply chain.

3. ***National policies***

a. Inspections at national borders of food entering the country from abroad, and at distribution hubs of domestic food, in order to minimize public health problems from adulterated food.

b. Development of food adulteration disincentivizing policies: food adulteration is a criminal activity.

Pupil Participation

1. Class discussions

a. of the issues associated with food authentication

b. of the issues associated with smart food choice based on the importance of food authentication

c. on how the DNA of microbes present in food may help to discover if that product is truly what it says on its label

d. about what scientists have been exploring about the DNA of microbes in food to detect fraud

2. Pupil stakeholder awareness

a. Do you read the information on the packaging of some of the foods you buy? Which foods? What information are you seeking? Why? Does it have a big impact on whether or not you buy the item?

b. Which information would you like to have which is not currently provided? Why? How might it affect your choices?

c. Why is it important to have correct labelling of foods with regard to their origins?

d. Why is food authentication a safety issue for you and your Family?

3. Exercises

a. Look on the Web for different suppliers of a food item you like a lot and copy the information provided. Compare the different informations obtained and decide what is important for you and what may be missing that is also important. Discuss what could be improved in the information provided and how it might be standardized across different suppliers.

b. Imagine you lived in a village where a special type of food has been traditionally produced for a long time and that is PDO. Formulate the information you think is important (i) to define and protect its status, and (ii) for the consumer to have.

The Evidence Base, Further Reading and Teaching Aids

Microbial terroir – current research round-up. Available at: https://www.jancisrobinson.com/articles/microbial-terroir-current-research-roundup. Accessed on February 23, 2022.

SIMBA (Sustainable Innovation of Microbiome Applications in the Food System). The microbiome and the Sustainable Development Goals (SDGs). Available at: http://simbaproject.eu/the-microbiome-and-the-sustainable-development-goals-sdgs/. Accessed on December 1st, 2022.

To learn more general concepts about Metagenomics and about Food Fraud, refer to the following review papers:
Almeida, O.G.G.; De Martinis, E.C.P. Bioinformatics tools to assess metagenomic data for applied microbiology. Applied Microbiology and Biotechnology. 103: 69–82, 2019. doi: 10.1007/s00253-018-9464-9.
Manning, L.; Soon, J.M. Food Safety, Food Fraud, and Food Defense: A Fast Evolving Literature. Journal of Food Science. 81(4): R823-R834 (. doi: 10.1111/1750-3841.13256, 2016. The Figure 1 of this reference is very useful to illustrate and categorize the possible intentional and unintentional modifications of food.
Spink, J.; Moyer, D.C. Defining the Public Health Threat of Food Fraud Journal of Food Science. 76(9): R157-R163, 2011. doi: 10.1111/j.1750-3841.2011.02417.x

Specific references for case studies. Although these papers have a technical, specialized approach, the texts provide general information that can be used in the preparation of this lesson.
For case study 1, refer to:
Chen, P.Y.; Ho, C.W.; Chen, A.C.; Huang,C.Y.; Liu, T.Y.; Liang, K.H. Investigating seafood substitution problems and consequences in Taiwan using molecular barcoding and deep microbiome profiling. Scientific Reports, 10: 21997, 2020. https://doi.org/10.1038/s41598-020-79070-y

For case study 2, refer to:
Liu,X.; Teixeira, J.S.; Ner, S.; Ma, K.V.; Petronela, N.; Banerjee,S.; Ronholm, J. Exploring the Potential of the Microbiome as a Marker of the Geographic Origin of Fresh Seafood. Frontiers in Microbiology, 11:696, 2020. https://doi.org/10.3389/fmicb.2020.00696

For case study 3, refer to:
Kamilari,E.; Tomazou, M.; Antoniades, A.; Tsaltas, D. High Throughput Sequencing Technologies as a New Toolbox for Deep Analysis, Characterization and Potentially Authentication of Protection Designation of Origin Cheeses? International Journal of Food Science, ID 5837301, 2019. https://doi.org/10.1155/2019/5837301
The Figure 1 of this reference is very useful to illustrate the different steps that may affect the dynamics of cheese microbiomes.

Alexander, T.; Allen, E.E.; Roy, K. Defining and quantifying the core microbiome: Challenges and prospects. PNAS, 118 (51):e2104429118, 2021. https://doi.org/10.1073/pnas.2104429118

For case study 4, refer to:
Kim, E.; Yang, SM.; Lim, B.; Park, S.H.; Rackerby, B.; Kim, H.Y. Design of PCR assays to specifically detect and identify 37 *Lactobacillus* species in a single 96 well plate. BMC Microbiology, 20, 96, 2020. https://doi.org/10.1186/s12866-020-01781-z

For case study 5, refer to:
Taylor, M. W., Tsai, P., Anfang, N., Ross, H. A., & Goddard, M. R. (2014). Pyrosequencing reveals regional differences in fruit-associated fungal communities. Environmental Microbiology, 16(9):2848–2858, 2014. https://doi.org/10.1111/1462-2920.12456
Leeuwen, V. Terroir: the effect of the physical environment on vine growth, grape ripening and wine sensory attributes. Editor(s): Andrew G. Reynolds, *In* Woodhead Publishing Series in Food

Science, Technology and Nutrition, Managing Wine Quality,Woodhead Publishing, 273-315 , 2010. https://doi.org/10.1533/9781845699284.3.27

Additional information can also be found in the following materials, which can be helpful to demonstrate the applications to the students.

European Commission. Knowledge Centre for Food Fraud and Quality. https://knowledge4policy.ec.europa.eu/food-fraud-quality_en

European Commission. JRC Digital Media Hub. https://visitors-centre.jrc.ec.europa.eu/en/media/animations/knowledge-centre-food-fraud-and-quality

Consortium for Sequencing the Food Supply Chain. https://researcher.watson.ibm.com/researcher/view_group.php?id=9635#:~:text=The%20consortium%20is%20working%20to,to%20be%20evolved%20and%20applied

IFIS Food and Health Information. https://www.ifis.org/blog/global-food-fraud

Glossary

Adulteration: Food adulteration is the act of intentionally debasing the quality of food offered for sale either by the admixture or substitution of inferior substances or by the removal of some valuable ingredient.

Bioterrorism: The use, or threatened use of releasing viruses, bacteria, or other germs (agents) to cause illness or death in people, animals, or plants.

Chronic disease: a condition that lasts a long period (months or even years) and requires ongoing medical attention or limitation of daily activities, or both.

Family farming: a farm owned and/or operated by a family. Family farm businesses can take many forms, from smallholder farms to larger farms operated under intensive farming practices.

Food security: when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life.

Forensic: Forensic science is an element of the criminal justice system. In the food forensic investigation, forensic scientists' investigations aim to determine where and when food contamination has occurred, identify the type of contaminant and its source, and identify foreign taints and odours and their sources.

Genomics: The study of genes and their function.

Microbiome: This term refers to the entire habitat, including microorganisms (bacteria, archaea, lower and higher eukaryotes, and viruses), their genomes (i.e. genes), and the surrounding environmental conditions.

Microbiota: The set of microorganisms present in a defined environment.

DNA sequencing: Determination of the order of nucleotides (base sequences) in DNA molecule

Massive DNA sequencing: a high-throughput method used to determine a portion of the nucleotide sequence of a given DNA sample