Profiled tea extracts exemplifying the importance of characterizing food bioactives: opinion piece

Shiming Li¹, Alexander Gossau¹–b, Klaus Lange c and Chi-Tang Ho ab

¹Department of Food Science, Rutgers University, New Brunswick, NJ, USA
²Department of Science, City University of New York, BMCC, New York, NY, USA
³Department of Experimental Psychology, University of Regensburg, Germany
*Corresponding author: Chi-Tang Ho, Department of Food Science, Rutgers University, New Brunswick, NJ 08901, USA.
E-mail: ctho@sebs.rutgers.edu
DOI: 10.31665/JFB.2019.5172
Received: January 20, 2019; Revised received & accepted: March 20, 2019
Citation: Li, S., Gossau, A., Lange, K., and Ho, C.-T. (2019). Profiled tea extracts exemplifying the importance of characterizing food bioactives: opinion piece. J. Food Bioact. 5: 1–5.

Abstract

Natural products from food and herbs have been used as functional food and medicine for centuries, much earlier than any of the current single molecule drugs in the market. Historically, natural products are the dominant resources of current global pharmaceutical market. Examples include world’s most commonly used drugs such as aspirin, penicillin and taxol. In viewing the increasing attraction and exponentially growing need for functional foods and effective medicines, the potential for natural products to serve as safe and effective preventive and therapeutic agents is of much interest. However, the importance in the phytochemical characterization of plant origin and associated extracts containing multiple phytochemicals in research and product development in this field has been plagued by overwhelmingly focusing on their biological effects. More than often inconsistent and invalid biological results are provided without chemical component identification and validation. Hence it is vital to characterize and identify the ingredients in the plant extracts – food bioactives- that play critical roles in promoting health or having therapeutic effects. The combination of chemical identification and biological evaluation is the key to having valid and consistent results in elucidating health beneficial properties of a plant or its extracts and also a key to have a meaningful comparison among similar studies due to the use of the same standard. Herein, we use tea as examples demonstrating the importance of phytochemical profiling and associated bioactive property of functional foods.

Keywords: Characterization; Natural product; Tea biological activity; Food bioactives; Phytochemical profile.

1. Introduction

Natural products have been used globally for medicinal purposes according to historical records although the sources and applications may vary among different regions of the world. The father of modern medicine, Hippocrates, left behind historical records of pain relief medicine. From the bark and leaves of white willow tree, he made powder and used it to treat headache and other pains as well as fever. Not until 1829, by means of isolation and chemical characterization, scientists discovered that salicin in willow plants was the ingredient functioning as a pain reliever and further developed the most popular drug, aspirin by Bayer. Hippocratic phrase “let food be thy medicine and medicine thy food” presaged the modern science of therapeutic nutrition or nutritional therapy and the development of functional foods, dietary supplements and medical foods by over two millennia. The development of modern analytical chemical and instrumental methods has led the foundation for identification and characterization of nutrients and phytochemical bioactive compounds such as polyphenols in plants whose therapeutic properties have been relied upon throughout the
Traditionally, natural products have been a rich source for therapeutic discovery and have led to the development of many of the world’s most commonly used drugs, such as aspirin from white willow, penicillin from fungi, and taxol from pacific yew tree. In the area of cancer as an example, from 1940 to December 2010, among 175 available small molecular anti-cancer drugs, 131 (74.8%) of them directly came from or were inspired by natural products (Newman and Cragg, 2012). Single chemical compounds from nature can be very potent therapeutic agents, but their potential adverse side effects and limited applications must be thoroughly considered and strict usage instructions provided prior to their introduction to the market. On the other hand, consumer attention has been drawn to the use of natural bioactive products for both disease prevention and treatment in recent years. The conception and perception of health conscientious consumers is that consuming bioactive compounds as part of a complex mixture perhaps reduces the potential for adverse effects as different components may influence absorption, metabolism, and excretion of a primary bioactive. Additionally, compounds of complex natural extracts may act synergistically in ways that are difficult to reveal experimentally and produce effects that are unlikely to be replicated with a single pure compound. In addition, recent data from many laboratories suggest that natural products act more subtly but broadly than most drugs with modest effects at multiple steps in the inflammatory cascade as an example rather than exerting super potent action at a single step, as drugs are designed to do, and also more complex than previously thought with the discovery of active and multiple effects from microbiota mainly in human digestive system (van Duynhoven et al., 2013).

There are many challenges and obstacles in the identification and characterization of bioactive ingredients in natural product research and product development. Despite the potential advantages of natural products as functional foods and even therapeutic agents, this approach presents significant challenges that have slowed development and acceptance by mainstream medical enterprises. Highly specialized and costly analytical instrumentation and techniques are required to isolate, identify and characterize the bioactive compounds of a multi-component composition. For instance, thousands of studies have been performed using green and black tea and their extracts, yet a majority of these studies did not provide adequate chemical compositional data and sufficient information regarding the molecular identity of the tea extracts experimented. Some publications provided partial contents such as caffeine and total polyphenols including green tea catechins and black tea theaflavins, but replication of the study materials can be difficult without a full spectrum of phytochemicals present in the tea extracts, which consequently contributed to inconsistent biological effects of tea and tea extracts in the literature. Furthermore, bioassays are also essential in evaluating the efficacy of natural products with consistency and confidence and they can be very sensitive to chemical differences even in the absence of knowledge regarding the identity of some minor chemical components. Without a well-characterized profile of chemical entities, the challenge is to devise adequate controls for chemical and biological quality assurance of a complex mixture. Such a complex material, like a plant or plant extract, is likely to contain a large number of compounds, many of which are present at extremely low levels, and may even be unstable during analytical procedures. By the illustration of examples in this review, we advocate a combined chemical and biological evaluation method to have an adequate quality control over the characterization of natural products to achieve a level of consistency and reliability needed for use in functional foods or other therapeutic agent applications (Gosslau et al., 2011; Venskutonis, 2018).

There are several challenges facing chemical identification and characterization of natural products. The first challenge is the process of one or more mixtures. Natural products used historically as therapeutic agents usually from herbs or post fermentation were typically generated as extracts or concentrates, in liquid or dried form. These processes, even with clear documentation, are often variable and difficult to control, and can yield distinct chemical profiles due to subtle variations in processing technique. The second challenge is the multi-molecular composition of an extract. It is not uncommon that an extract from a single plant may contain hundreds or even thousands of individual components with varying stabilities and potential for interaction. However, advanced analytical methods coupled using state-of-the-art analytical instrumentation such as liquid chromatography-mass spectrometry-mass spectrometry (LC-MS-MS) and liquid chromatography-nuclear magnetic resonance (LC-NMR) are able to disentangle complex compounds, and also techniques employed for isolation and identification of virtually any chemical class of compound(s). The application of these techniques, in combination with cell and molecular bioassays, enables identification of key bioactive ingredients (Pan et al., 2012; Lai et al., 2013; Lopez-Gutierrez et al., 2015). Therefore, it is of vital importance to overcome obstacles and to accomplish meaningfully defined or standard controlled natural product research, i.e. the isolation, characterization, and biological screening of natural products and the application of these chemically defined and biologically confirmed components as standardized controls in natural product research.

Rapid development and application of computer information and material technology in recent decades has dramatically advanced the technical capability of natural product isolation and characterization. Qualitative and quantitative instruments include automated column chromatography, high performance liquid chromatography (HPLC), ultra-HPLC, LC-MS-MS, and HPLC-NMR. The LC-MS-MS exemplifies the dramatic advancement that has occurred and its application in analysis and chemical structure determination can shorten the characterization time from weeks or months to hours (Lin et al., 2012; Lopez-Gutierrez et al., 2015; Zhao et al., 2019). The challenge in development of natural products as medical foods and therapeutics having been created by the inherent variability in the starting material – whether a freshly harvested plant or a dried or partially processed plant product can be solved mostly with current modern instruments and techniques.

Tea polyphenols, usually meaning catechins and theaflavins (Fig-


2.1. Anti-inflammatory effects of tea polyphenols in characterized black tea extracts

Chronic inflammation, caused by persistent infections, immune-mediated inflammatory diseases, or prolonged exposure to toxic reagents or physical injuries, often leads to severe tissue destruction resulting from predominantly mononuclear macrophages. It has been broadly recognized that the major underlying cause of death in US, including cardiovascular disease, autoimmune disease, chronic respiratory disorders, Alzheimer’s disease, cancer, and diabetes, account for six out of top ten leading causes of death and increased human suffering (Aggarwal and Gehlot, 2009; McGeer and McGeer, 2004). Much attention has been paid to the development of pharmaceuticals, therapeutic nutraceuticals and functional foods for intervention or prevention of chronic inflammation.

In the study to control chronic inflammation, a black tea extract (BTE) containing 40% of theaflavins was characterized by HPLC analysis with well-defined standard compounds. Nine compounds have been quantified as caffeine (CF), epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), epigallocatechin gallate (EGCG), theaflavin (TF1), theaflavin-3-O-monomonogallate (TF2a), theaflavin-3′-O-monomonogallate (TF2b) and theaflavin-3-O,3′-digallate (TF3). For each batch of black tea extract (BTE), the amount of gallic acid (GA), theobromine, and theophylline as well as abnormal peaks occurring in the HPLC profile were also closely monitored. Figure 1 illustrates an HPLC trace of BTE demonstrating the contents of individual bio-polyphenols in the BTE used in the anti-inflammation study. The BTE attested by a set of cell-based assays based on a group of well-established anti-inflammatory biomarkers. For instance, among different batches of BTE and multiple commercial sources, quality control requires that CF content is limited at a maximum of 2.5%; total CA content is 20–30% and total TF content is 40 ± 5%; as well as specific requirements for EC, ECG, EGCG, TF1, TF2a, TF2b and TF3 (unpublished data).

In a human cell-based monocyte–macrophage differentiation model, the BTE significantly down-regulated the expression of COX-2, TNF-α, ICAM-1, IL-1β, IL-6, IL-8, NF-κB, C-JUN and p53, but up-regulated anti-inflammatory IL-10 as demonstrated by cDNA and oligo microarray analysis (unpublished data). Down regulation of inflammatory genes corresponds to attenuation of the canonical NF-κB pathway through IkB kinase inhibition and AP-1 activity induction by theaflavins or the synergistic effects of theaflavins and catechins (Gosslau et al., 2011). Strong antioxidative properties of polyphenols rich in tea extracts, GTEs and BTEs, also seem to have a major anti-inflammatory effect since free radical-induced cell damage is one of the hallmarks in chronic inflammation (Gosslau et al., 2011; 2018). The modulation of other pathways by tea bioactives such as G-protein signaling might add another potential anti-inflammatory mechanism (Aneja et al., 2004; Yang et al., 2009).

The theaflavin enriched BTE has been studied in multiple models. It was observed that BTE reduced edema formation in a dose-dependent manner to the levels exerted by indomethacin and aspirin using different animal models (Gosslau et al., 2011; 2018). Tissue and blood analysis after BTE treatment revealed a concomitant reduction in inflammatory mediators such as COX-2 and TNF-α (Huang et al., 2006). Moreover, a small pilot human study employing LPS-mediated irritation or exercise-induced inflammation showed prominent anti-inflammatory effects of BTE as indicated by a down-regulation of different cytokines and chemokines as well as reduced inflammatory symptoms such as reduction of reactive oxygen species (ROS) and delayed onset muscle soreness (Arent et al., 2010). The aforementioned results of the anti-inflammatory effects of BTE throughout different cell-based assays, animal and human testing indicate that cell-based models are predictive in the search for natural products and BTE to be very promising agent against inflammation associated diseases. There are many other tea related studies not covered in this opinion piece.
that emphasizes the importance of the phytochemical characterization in the study of food bioactives such as tea.

2.2. Different hypoglycemia mechanism with different profiles of tea extracts

Diabetes, one of the leading causes of death in the US, mainly has two forms, type 1 and type 2. Type 1 diabetes is insulin dependent and results from a cellular mediated autoimmune destruction of pancreatic β-cells. It can occur at any age but more common in childhood and adolescence. Type 2 diabetes (T2D), responsible for >90% of diabetic patients and previously referred to as non-insulin dependent diabetes mellitus or adult-onset diabetes, refers to individuals who have insulin resistance and usually come along with relative insulin deficiency. Age, obesity and lack of physical activity are predominant risk factors leading to the development of T2D. Obesity and T2D are closely correlated with each other and also with chronic inflammation.

Tea polyphenols exert hypoglycemic activity (Tang et al., 2013; Miao et al., 2015; Huang et al., 2015; Gosslau et al., 2018). Tea extracts in the market vary dramatically in their content and type of bioactive chemical entities, particularly polyphenols, which can be very much misleading when referring to anti-diabetic activity of tea extracts in the absence of characterization of compounds involved. By a direct comparison between green tea extracts (GTE, 70% catechins, Figure 2) and black tea extracts (TF-BTE, 70% theaflavins, Figure 3), which have been chemically well-characterized by HPLC, in T2D mouse model induced by combining low dose streptozotocin (STZ) with high fat (HF) diet, both GTE and TF-BTE in drinking water substantially lowered blood glucose levels and ameliorated glucose intolerance, but GTE was more effective than TF-BTE in lowering both the blood glucose level and body weight gain. However, their action mechanism was different. Serum insulin levels were significantly increased in the TF-BTE group but not in the GTE fed group animals, apparently indicating different pathways in the hypoglycemic effects of tea extracts due to different polyphenol profiles (Tang et al., 2013). Further exploration elucidated the possible mechanisms by homeostatic model assessment (HOMA), suggesting that the predominant mechanism for the anti-diabetic effect was due to increased insulin level in circulation in the body by reducing insulin resistance by GTE and by increasing β-cell insulin secretion by TF-BTE (Tang et al., 2013). Total catechin content in GTE is 71.5%, with 10% of EGC, 32.8% of EGCG, 24.6% of ECG and 3.9% of EC, respectively from HPLC quantification (Figure 2), whereas TF-BTE is dominant in theaflavins (68.4%) and minor catechins (15.3%). The individual contributions from theaflavins are TF1 (7.6%), TF2a (26.3%), TF2b (6.1%), and TF3 (28.4%). Therefore, catechins from green tea are responsible for the reduction of insulin resistance, while theaflavins in black tea play a critical role in stimulating β-cell insulin secretion (Tang et al., 2013), indicating different hypoglycemic mechanisms by catechins and theaflavins. Further anti-diabetic effects of TF-BTE was confirmed in an obese ZDF rat model with findings of anti-inflammatory activity of theaflavins (Gosslau et al., 2018).

3. Concluding remarks

The biological activity of a multi-component molecular extract can be assayed with available cell or animal models without difficulties. Yet, the correlation of bioactivity with individual components in an extract remains a challenge for natural product research. On the other hand, in depth research in natural products with nutritional function and therapeutic efficacy requires the identification and quantification of bioactive molecules to elucidate a relationship between chemical molecules and activity. Hence the characterization and quantification of individual phytochemicals in a complex matrix is of great importance. Adequate characterization of multi-molecular compositions can assure consistency and reproducibility in the screening and evaluation of biological activity among different prepared batches and different resources of a natural product. Not only should chemical profiles be obtained by characterization and quality control of a complex natural product mixture, but also biological assays should be performed because they may be more sensitive to subtle changes in the chemical composition of natural products than chemical methods used for quality control purposes. Thus, adequate quality control ultimately requires a combination of chemical profiling and biological screening.

References


