Session 1 Coffee and Health

Oral Session Coffee 01.01

In vitro hypocholesterolemic effect of coffee compounds

Filipe Coreta-Gomes[1, 2], Guido R. Lopes[1], Cláudia P. Passos[1], Carlos F. G. C. Geraldes[2, 3], Maria João Moreno[4], Laura Nyström[5], Manuel A. Coimbra[1]
[1]Requimte & QOPNA, University of Aveiro, Aveiro, Portugal, [2]Coimbra Chemistry Center, University of Coimbra, Coimbra, Portugal, [3]Life Sciences Department, University of Coimbra, Coimbra, Portugal, [4]Chemistry Department FCTUC, University of Coimbra, Coimbra, Portugal, [5]Department of Health Sciences and Technology, Institute of Food, Nutrition and Health, ETH Zurich, Zurich, Switzerland

Text Coffee compounds were analysed regarding their effect on cholesterol solubility in dietary micelles composed of bile salts, as measure to evaluate their hypocholesterolemic potential. Affecting both, diet and endogenous cholesterol emulsification at intestinal lumen, can promote a decrease of cholesterol absorption at gastrointestinal epithelium, lowering cholesterol blood levels.
The approach in this work was to use an *in vitro* model based in one of the most prevalent bile salt in humans, glycodeoxycholate acid, and assess cholesterol solubility dependence on the presence and absense of different chemical characterized expresso coffee samples [1] and coffee extracts [2,3] , using quantitative NMR. [4,5]
The results obtained show that both espresso coffee and correspondent coffee extracts rich in arabinogalactans and galactomannans polysacharides decrease cholesterol solubility by sequestering bile salts from solution. Coffee lipid extracts are also found to decrease cholesterol solubility, although by a co-solubilization mechanism. The effect of both polysaccharides and lipid showed to be additive, representing the overall effect observed in a typical coffee espresso.
These results are important to better understand the effect of hydrophilic matrices, such as coffee among others, and of their chemical compositions, on bioaccessibility of cholesterol, allowing the development of optimized cholesterol reducing ingredients, with commercial and industry impact.

References:
[1] Lopes, G. R.; Ferreira, A. S.; Pinto, M.; Passos, C. P.; Coelho, E.; Rodrigues, C.; Figueira, C.; Rocha, S. M.; Nunes, F. M.; Coimbra, M. A., (2016), Carbohydrate Content, Dietary Fibre and Melanoidins: Composition of Espresso from Single-Dose Coffee Capsules, Food Res. Int. , 989
[2] Passos, C. P.; Coimbra, M. A. , (2013), Microwave Superheated Water Extraction of Polysaccharides from Spent Coffee Grounds, Carbohydr. Polym. , 626
[3] Passos, C. P.; Cepeda, M. R.; Ferreira, S. S.; Nunes, F. M.; Evtuguin, D. V.; Madureira, P.; Vilanova, M.; Coimbra, M. A. , (2014), Influence of Molecular Weight on in Vitro Immunostimulatory Properties of Instant Coffee, Food Chem., 60
[4] Coreta-Gomes, F. M. F. M.; Vaz, W. L. C. W. L. C.; Wasielewski, E.; Geraldes, C. F. G.

C. C. F. G. C.; Moreno, M. J. M. J. , (2012), Quantification of Cholesterol Solubilized in Bile Salt Micellar Aqueous Solutions Using 13C Nuclear Magnetic Resonance, Anal. Biochem. , 41
[5] Coreta-Gomes, F. M.; Vaz, W. L. C.; Wasielewski, E.; Geraldes, C. F. G.; Moreno, M. J. , (2016), Quantification of Cholesterol Solubilized in Dietary Micelles: Dependence on Human Bile Salt Variability and the Presence of Dietary Food Ingredients, Langmuir , 4564

Oral Session Coffee 01.02

Interactions of Coffee Melanoidins with other Food Sourced Antioxidants

Ecem Evrim Çelik[1], Jose Manuel Amigo Rubio[2], Mogens Larsen Andersen[2], Vural Gökmen[1]
[1]Hacettepe University, Hacettepe University, Ankara, Turkey, [2]University of Copenhagen, University of Copenhagen, Copenhagen, Denmark

Text Coffee as one of the most widely consumed beverages in the world contains a wide range of antioxidants (AOX), whose composition depend on the origin of coffee beans, blending, grinding and brewing processes. Roasting also has a great impact on its AOX profile due to the degradation of native AOX and formation of new ones like melanoidins [1], which are named as AOX-dietary fibers (DF) [2]. AOX-DF can quench free radicals continuously formed in the gastrointestinal (GI) tract and they are known to be regenerated by free soluble AOX in vitro [3]. Interactions of such coffee AOX with other AOX generally consumed together is significant in GI system health since it determines the overall effect created. In this context, the interactions of coffee melanoidins (CM), with hydroxycinnamic/hydroxybenzoic acids (HCA/HBA), found in a variety of foods, containing different numbers of –OH/–OCH_3 groups localized at different positions on the aromatic ring were investigated. By doing so, mechanism of the interactions was intended to be explained with a structural approach. Experiments were performed in DPPH radical medium. Chemometric methods were used for experimental design and multivariate data analysis. Area under the curve values calculated from the plots of time versus inhibition (%) for CM and HCA/HBA derivatives were ranged between 6532±97–19,106±85 and−1678±81–22,486±119, respectively. Synergism was revealed between CM and HCA/HBA derivatives. The interactions between insoluble fractions of different coffee infusions, containing melanoidins, and major cocoa free AOX, catechins, as well as the interactions between different coffee infusions and dark chocolate were also investigated. Espresso, filtered coffee, French press and Turkish coffee were used for this purpose. AOX capacity measurements were performed by monitoring the percentage inhibition of DPPH radical. Multivariate approach was adopted for experimental design and data analysis steps. In dry basis, the AOX capacity values of infusions (mmol Trolox/kg) were ranged between 953±2.6 and 1184±11.3, while the AOX capacity values for their insoluble fractions were ranged between 45±0.0 and 105–1.3. Interactions between the insoluble fractions of coffee infusions and catechins were synergistic for espresso and additive/antagonistic for the other infusions. Interactions between coffee infusions and chocolate were synergistic for French press and Turkish coffee and additive/antagonistic for the other infusions.

Oral Session Coffee 01.03

Stability and cell metabolism limit the anti-proliferative activity of hydroxycinnamic acids

Serena Martini, Angela Conte, Davide Tagliazucchi
University of Modena and Reggio Emilia, Department of Life Sciences, Reggio Emilia, Italy

Text Hydroxycinnamic acids are widespread dietary components found, for instance, in coffee, cherries, apples and chocolate and are particularly intriguing for their potential beneficial effects in the gastro-intestinal tract where they can reach µmolar concentrations (1-3). In this study, we investigated the anti-proliferative activity, the stability in cell media and the cell metabolism of the main dietary hydroxycinnamates, using two colonic adenocarcinoma cell models (Caco-2 and SW480) and LC-ESI-IT-MS/MS. Di-hydro-caffeic (DHC) and di-hydro-ferulic (DHF) acids were the most effective against cell proliferation in both cell lines with IC_{50} values of 71.7 ± 1.1 and 83.1 ± 1.1 µmol/L, respectively, in Caco-2. At 200 µmol/L, caffeic and ferulic acids inhibited SW480 proliferation by 40.8 ± 1.6 and 59.9 ± 1.3%, respectively. Hydroxycinnamic acids with a catechol-type structure were degraded in DMEM resulting in the production of H_2O_2. Hydrogenation of the C_7═C_8 double bond also decreased the stability of the compounds. In contrast, hydroxycinnamic acids were stable in the SW480 cell medium (L-15). The instability of some hydroxycinnamic acids in DMEM may partially explain the observed results regarding the anti-proliferative activity. For example, caffeic acid was drastically degraded in DMEM hindering the detection of any biological activities. Furthermore, caffeic acid was stable in L-15 and able to reduce SW480 cells proliferation. Intracellular Caco-2 UDP-glucuronosyltransferases and catechol-*O*-methyltransferases were able to form glucuronide and methyl conjugates. However, only lower amount of sulphate conjugates were detected after incubation with SW480. Ferulic acid was stable in both the cell media but active only against SW480 proliferation. During incubation with Caco-2 ferulic acid was extensively glucuronidated and sulphated. The Caco-2 metabolism of ferulic acid may have hampered its anti-proliferative activity. Our results highlighted that the drastic biotransformations occurring in cell cultures should be carefully taken into account in order to fully understand the real compounds exerting the observed bioactivities. Indeed, the remarkable effect of DHC and DHF against cell proliferation is of paramount importance since these compounds are the main metabolites detectable at colonic level.

References:
[1] Clifford, M. N.; Jaganath, I. B.; Ludwig, I. A.; Crozier, A., (2017), Chlorogenic acids and the acyl-quinic acids: discovery, biosynthesis, bioavailability and bioactivity, Natural Product Report, 1391-1421, 34
[2] Halliwell, B.; Rafter, J.; Jenner, A., (2005), Health promotion by flavonoids, tocopherols, tocotrienols, and other phenols: direct or indirect effects? Antioxidant or not? , American Journal of Clinical Nutrition, 268S-276S, 81
[3] Vitaglione, P.; Fogliano, V.; Pellegrini, N. , (2012), Coffee, colon function and colorectal cancer, Food and Function, 916-922, 3

Short Oral Communications

Session 2 Cocoa composition and chemistry

Oral Session Cocoa 02.01

Linking cocoa polyphenol composition to chocolate quality with Average-Mass-Spectra fingerprints

Noémie Fayeulle[1], Emmanuelle Meudec[1], Jean-Claude Boulet[1], Clotilde Hue[2], Renaud Boulanger[3], Véronique Cheynier[1], Nicolas Sommerer[1, 4]
[1]INRA - SPO - plateforme polyphenols, Montpellier, France, [2]Valrhona, Tain l'Hermitage, France, [3]CIRAD - QualiSud, Montpellier, France, [4]Univ. Montpellier, Montpellier, France

Text Approaches enabling prediction of chocolate quality from cocoa composition would avoid time- and money-consuming steps to chocolate makers. Average mass spectra of cocoa-polyphenol-extracts led to fingerprints used to select the molecules that discriminate chocolate sensory groups.
16 worldwide cocoa samples were processed into chocolates which were characterized by sensory analysis, allowing sorting of the samples into four sensory groups.
The cocoa polyphenol extracts were analyzed by liquid chromatography−low-resolution mass spectrometry. Averaging each mass spectrum provided polyphenolic fingerprints, which were combined into a matrix and processed with chemometrics (PCA, PLS-DA) to select the most meaningful molecules for discrimination of the chocolate sensory groups[1]. A larger set af 44 cocoa samples was used to validate the previous results. 29 mass signals of known and unknown molecules, mainly flavan-3-ols, were finally targeted, including 2 newly described ethyl-bridged flavan-3-ols[2], enabling sensory-group discrimination.
Average mass spectra fingerprints of cocoa-polyphenol-extracts proved to be quick and efficient to select the molecules that discriminate chocolate sensory groups.
A targeted MRM (Multiple Reaction Monitoring) mass spectrometry method was then developed and validated to routinely analyse large series of cocoa samples.

References:
[1] Fayeulle N, Meudec E, Boulet JC, Vallverdú-Queralt A, Hue C, Boulanger R, Cheynier V, Sommerer N, (2019), Fast Discrimination of Chocolate Quality Based On Average Mass Spectra Fingerprints of Cocoa Polyphenols, Journal of Agricultural and Food Chemistry, 2723-2731, 67, https://doi.org/10.1021/acs.jafc.8b06456
[2] Fayeulle N, Vallverdú-Queralt A, Meudec E, Hue C, Boulanger R, Cheynier V, Sommerer N, (2018), Characterization of new flavan-3-ol derivatives in fermented cocoa beans, Food Chemistry, 207-212, 259, https://doi.org/10.1016/j.foodchem.2018.03.133

Oral Session Cocoa 02.02

Biochemical fate of vicilin storage protein during fermentation and drying of cocoa beans

Kumari Neha, Warren John, Nikolai Kuhnert, Matthias Ullrich
Jacobs University Bremen, Jacobs University Bremen, Bremen, Germany

Text Key cocoa-specific aroma precursors are generated during the fermentation of cocoa beans via the proteolysis of the vicilin-like globulin. Previous studies had shown that degradation of this particular 566 amino acid-long storage protein leads to three distinct subunits with different molecular masses. Although oligopeptides generated from the proteolysis of vicilin-like globulin have been studied previously, changes occurring to vicilin at different stages of fermentation have not yet been explored in detail. The aim of this study was to investigate the fate of vicilin protein from the non-fermented stage up to the dried cocoa beans and to perform a comparative analysis of the peptides generated from the degradation of vicilin protein in a commercial fermentation with that of the artificial fermentation system, free from microbial activity. The protein profile was analyzed using SDS-PAGE and 2D-PAGE analytical methods. All major protein spots obtained were subjected to proteolytic digestion and MALDI-MS analysis in order to assign them to respective cocoa proteins. Our results showed a remarkable shift in the electrophoretic mobility of vicilin towards higher pI during the onset of fermentation. The pI-shifted subunit was found susceptible to further degradation into a lower-molecular-weight vicilin subunit. The observed pI shift correlated with, but did not depend on protein phosphorylation. Glycosylation of some but not all vicilin subunits occurred at different stages of the fermentation process. Peptides generated from vicilin throughout fermentation (commercial as well as artificial) were analyzed by UHPLC-ESI-MS/MS. Peptides generated from the two different fermentation methods showed nearly ninety percent peptide similarity and revealed an initial increase and subsequent decrease in their diversity with an increasing degree of fermentation. We furthermore describe the rate of degradation of different vicilin subunits. The detected diversity and dynamics of vicilin peptides will help to define biochemical markers of distinct steps of the fermentation process.

Oral Session Cocoa 02.03

The effect of cocoa processing and alternative sweeteners on the volatile organic compound profile of dark chocolate

Burce Atac Mogol, Evy Vera Yasmijn Bögels, Sine Yener, Fabiola Pena Correa, Vincenzo Fogliano
Wageningen University & Research, Wageningen, Netherlands

Text Volatile organic compounds (VOCs) forms the aroma of a particular food, which is a significant attribute of chocolate because it is considered as a pleasure food. Roasting of the cocoa beans is the most critical step in the formation of VOCs in chocolate due to

Maillard reaction. Pyrazines and aldehydes, together with ketones, alcohols, and esters are responsible for indicating the roasting process in terms of aroma compounds[1]. Conching which is performed with added sugar and/or other ingredients also plays an important role to reach the final desired aroma of the chocolate[2]. Among these ingredients, sugar is one of the main ingredients in chocolate and fulfils multiple functional properties such as sweetness, bulking agent, particle size distribution and mouthfeel. With the increasing consciousness in a healthy lifestyle, consumers tend to consume lower glycemic index foods and reduce their calorie and sugar intake. However, replacing the sugar in chocolate should be handled carefully due to its possible effect on the final aroma. Therefore, this study aimed to investigate the effect of roasting, conching, and different sweeteners on the VOC profile of dark chocolate. The beans were roasted at 125°C and 145°C to represent the low and high roasting, respectively. Ground beans were mixed with different sweeteners (maltitol, trehalose, stevia-inulin) or sucrose (reference) and cocoa butter in concher to produce the chocolates. Proton-Transfer-Reaction Time-Of-Flight Mass-Spectrometry and Gas-Chromatography Mass-Spectrometry were used to analyze the changes in VOCs profiles during roasting and conching of the cocoa beans. Chocolates with different roasting degrees and different sweeteners or sucrose were analyzed for their VOCs before and after conching. Clear differences were found between the chocolates roasted at low and high temperature as well as in samples before and after conching as analysed by Principal Component Analysis. High roasting led to reducing the esters (which mainly have a fruity odour) while it increased pyrazines with higher roasting. The volatiles responsible for fruity flavour, were more abundant before conching compared to after conching. Different VOC profiles were formed by using maltitol, trehalose, and stevia as sweeteners. Among them, high roasted chocolate made with maltitol had the most similar VOC profile to the chocolate made with sucrose.

References:
[1] Ramli, N., Hassan, O., Said, M., Samsudin, W., & Idris, N. A., (2006), Influence of roasting conditions on volatile flavor of roasted Malaysian cocoa beans., Journal of Food Processing and Preservation, 280-298, 30(3)
[2] Afoakwa, E. O., Paterson, A., Fowler, M., & Ryan, A., (2008), Flavor formation and character in cocoa and chocolate: A critical review., Critical Reviews in Food Science and Nutrition, 840-857, 48(9)

Oral Session Cocoa 02.04

Formation of Amadori-type flavor precursors and 2,5-diketopiperazines from dipeptides in cocoa

Pawel J. Andruszkiewicz[1], Roy N. D'Souza[1], Irem Altun[1], Marcello Corno[2], Nikolai Kuhnert[1]
[1]Jacobs University, Bremen, Germany, [2]Barry Callebaut AG, Zurich, Switzerland

Text Amadori compounds and 2,5-diketopiperazines (DKPs) are important compounds that have an influence on the organoleptic properties of heated foods. Amadori compounds are conjugates of amino acids and sugars, which form during Maillard reaction, and their further chemical transformations yield flavor and colored species. 2,5-

diketopiperazines, which could also be produced from Amadori compounds, are bitter cyclic dipeptides. These compounds are known to be present in cocoa, but many more derivatives possibly exist. Here we present a mass spectrometric characterization of novel chemical compounds forming from dipeptides during fermentation (Amadori compounds) and roasting of cocoa (DKPs). Additionally, both novel and known 2,5-diketopiperazines' kinetics were studied, and their quantities correlated to their putative precursors. The results show that most of the 2,5-diketopiperazines originate from a single peptide, and their relative concentrations correlate positively with their putative precursors. Therefore, bitterness resulted from the presence of DKPs could be modulated via manipulation of the fermentation process. The new findings imply greater variety in flavor precursors, which in turn suggests the formation of more diverse Maillard reaction products in cocoa.

Session 3 - Tea composition

Oral Session Tea 03.01

Comparative investigation of chemical profile and beneficial effect of green teas made by three varieties of Camellia sinensis

Zhutong Wang, Huimin Guo, Daxiang Li, Zhongwen Xie
State Key Laboratory of Tea, Anhui Agricultural University, Hefei, China

Text Tea is widely consumed around the world. There are three tea varieties of tea plants , which are Camellia sinensis var. sinensis, Camellia sinensis var. assamica, Camellia sinensis var. kucha. Upon today, there are very few reports on comparative investigation of chemical profile and beneficial effects of different varieties of Camellia senensis. Multi-platform-based metabolomics was employed to analyze metabolic profiles of green tea made with three varieties of Camellia senensis. By comparing the human metabolome database, two hundred and twenty unique tea metabolites were identified in these three different teas. Moreover, a partial least squares discriminate analysis (PLS-DA) model clearly discriminated three different teas. According to the PCA score chart, the samples of three varieties of tea showed significant separation, indicating that there were significant differences in the composition of total metabolites among the three varieties of teas. Furthermore, the high-fat-diet-(HFD) induced C57BL/6 obese mice were used as an animal model to compare the preventive effects of three tea varieties (Fuding tea , FD, C.sinensis var. sinensis, Yunkakng10 , YK, C. sinensis var. assamica, and Kucha, C.sinensis var. kucha) on metabolic syndrome (MetS). We found that all three varieties of tea intervention ameliorated MetS of HFD C57BL/6 mice. The three varieties teas significantly prevented body weight gain, body fat ratio increase, blood glucose and serum lipid profiles elevation. And the three varieties teas obviously reduced the expression of lipid synthesis genes, Acaca and Srebf1 expression, and inhibited the overexpression of inflammatory cytokines (IL-6, TNF-α etc.). Over all, the YK green tea had the best preventive effect, followed by FD, KC was the less effective one. In conclusion, the three varieties tea had distinct metabolites profiles, and had slightly different preventive effects on MetS of HFD induced C57BL/6 mice.

Oral Session Tea 03.02

Characterization and chemometric investigation of processed tea carbohydrates

Anastasiia Shevchuk, Nikolai Kuhnert
Jacobs University Bremen, Bremen, Germany

Text This work aims to show an approach to perform the characterization of commercial teas through the analysis of low molecular weight carbohydrates content (LMWC). Samples of six types of tea produced from the leaves of *Camellia Sinensis* were analysed by HILIC mass spectrometry for the LMWC. Quantities of sucrose, glucose, fructose, *myo*-inositol, maltose, mannitol, raffinose, galactinol, and stachyose were determined in samples of white, yellow, green, black, oolong, and dark tea.
The use of chemometric tools (HCA, PCA, correlation analysis) allowed to establish differences and similarities between samples and correlate chemical parameters to the tea quality [1].
The analysis of the low molecular weight carbohydrates (LMWC) was performed using a validated hydrophilic interaction liquid chromatography-electrospray ionization-time of flight mass spectrometry (HILIC-ESI-TOF MS) method.
To the best of our knowledge, this is the first time that the main LMWC content has been reported in a high number of tea samples representing a wide selection of origins and types of commercial teas. Among the LMWC identified and quantified in commercial teas were: monosaccharides (glucose, fructose); disaccharides (sucrose, maltose); α-galactooligosaccharides (α-GOS) such as raffinose and stachyose; sugar alcohols such as mannitol and inositols (*myo*-inositol, galactinol, 2-O-(β-L-arabinopyranosyl)-*myo*-inositol). *Myo*-inositol has been reported to have physiological and therapeutical role in human reproductive health [2]. The data on the LMWC compositions, specifically on the inositols and α-GOS content, would provide important information for consumers and professionals in the tea industry.

References:
[1] Yang C, Hu Z, Lu M, Li P, Tan J, Chen M, et al, (2018), Application of metabolomics profiling in the analysis of metabolites and taste quality in different subtypes of white tea, Food Research International, 909-919, 106
[2] Papaleo E, Unfer V, Baillargeon JP, Chiu TT, (2009), Contribution of myo-inositol to reproduction, European Journal of Obstetrics & Gynecology and Reproductive Biology, 120-123, 147(2)

Oral Session Tea 03.03

Global dissection of alternative splicing uncovers transcriptional diversity in tissues and associateswith the flavonoid pathway in tea plant (Camellia sinensis)

Junyan Zhu[1], Chaoling Wei[1], Xuewen Wang[2], Qingshan Xu[1], Shiqi Zhao[1]
[1]State Key Laboratory of Tea Plant Biology and Utilization/Key Laboratory of Tea Biology

and Processing, Anhui Agricultural University, Hefei, China, [2]Kunming Institute of Botany, Chinese Academy of Sciences, Yunnan, China

Text Alternative splicing (AS) regulates mRNA at the post-transcriptional level to change gene function in organisms. However, little is known about the AS and its roles in tea plant (Camellia sinensis), widely cultivated for making a popular beverage tea.In our study, the AS landscape and dynamics were characterized in eight tissues (bud, young leaf, summer mature leaf, winter old leaf, stem, root, flower, fruit) of tea plant by Illumina RNA-Seq and confirmed by Iso-Seq. The most abundant AS (20%) was intron retention and involved in RNA processes. The somealternative splicings were found to be tissue specific in stem and root etc. Thirteen co-expressed modules of AS transcripts were identified, which revealed a similar pattern between the bud and young leaves as well as a distinct pattern between seasons. AS events of structural genes including anthocyanidin reductase and MYB transcription factors were involved in biosynthesis of flavonoid, especially in vegetative tissues. The AS isoforms rather than the full-length ones were the major transcripts involved in flavonoid synthesis pathway, and is positively correlated with the catechins content conferring the tea taste. We propose that the AS is an important functional mechanism in regulating flavonoid metabolites.Our study provides the insight into the AS events underlying tea plant's uniquely different developmental process and highlights the important contribution and efficacy of alternative splicing regulatory function to biosynthesis of flavonoids.

Oral Session Tea 03.04

Gas Detection and Analysis for the Operational Timing of Oolong Tea Manufacturing Process

Tseng, T.-S.1, Chen, P.-A.2, Lin, S.-Y.3, Yao, D.-J.1
1. Institute of NanoEngineering and MicroSystems, National Tsing Hua University, Hsinchu City, Taiwan
2. Department of Plant Technology Laboratories, Agricultural Technology Research Institute, Hsinchu City, Taiwan
3. Department of Horticulture and Landscape Architecture, National Taiwan University, Taipei City, Taiwan

Text
The manufacturing of oolong tea will make tea leaves wither if four rounds of shaking and setting (for oxidation) process cannot be well controlled. During this process, tea practitioners need to smell tea aroma personally to determine time interval for shaking and setting. Some chemical variation happens over volatile organic compounds (VOCs) of tea leaves after shaking tea leaves. Thus, the smell changes after shaking process [1]. Our goal is to use intelligent gas sensing array to monitor tea aroma, then determine when we should shake the leaves or stop.
For the detecting system setup, metal oxide semiconductor (MOS) gas sensor arrays were installed. MOS gas sensor uses metal oxide as sensing material, whose resistance will vary with different surrounding gases as well as their concentration [2]. By observing

variation of resistance, we can identify the type and concentration of surrounding gases. During adsorption process (blue arrays in Fig 1.) for gas sensors, tea leaves samples were placed inside a gas generator (purchased from Molecular Analysis LLC.). A carrier gas (synthetic air) was passed towards gas generator which generates a gas from the sample tea leaves. Then, synthetic air will be mixed with generated sample gas in a mixing chamber for complete blending. After blending of both gases, the gas will pass through the sensor arrays, so that the sensors can detect the VOCs of tea leaves. After 25 minutes, the sensors will achieve the saturation level. Then we can desorb the VOCs from the surface of sensors. So, during the desorption process (red arrays in Fig 1.), synthetic air was passed through the surface of sensors for making the VOCs desorbed.

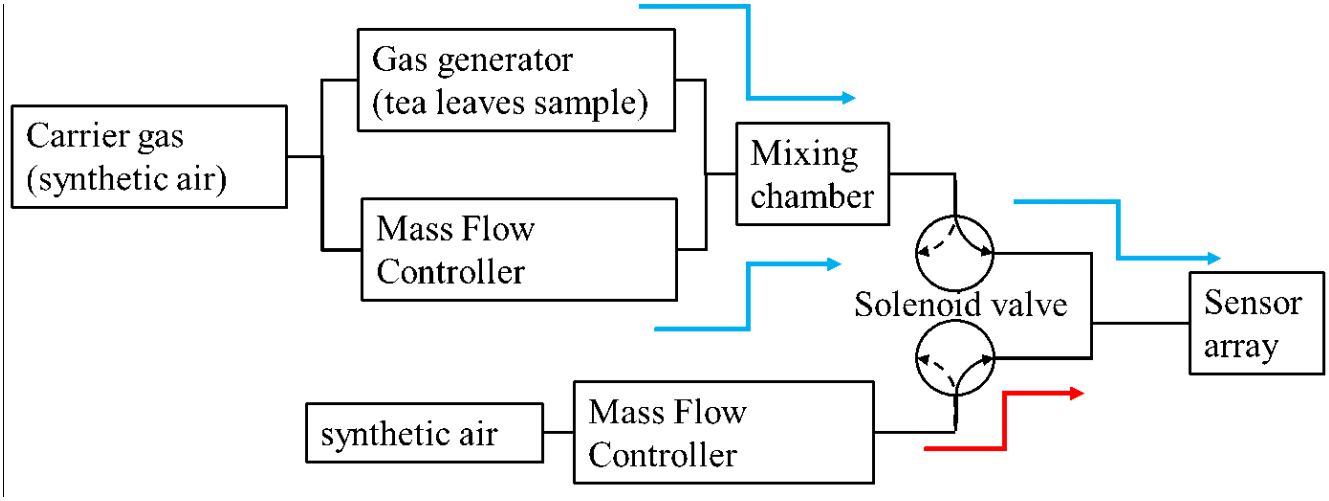


Fig. 1. Experimental apparatus.

We have successfully used linear discriminant analysis (LDA) to distinguish the samples before and after shaking (fig 2.). In the future, we will monitor the tea aroma during the setting process, and then determining the initiation time for the shaking or tossing.

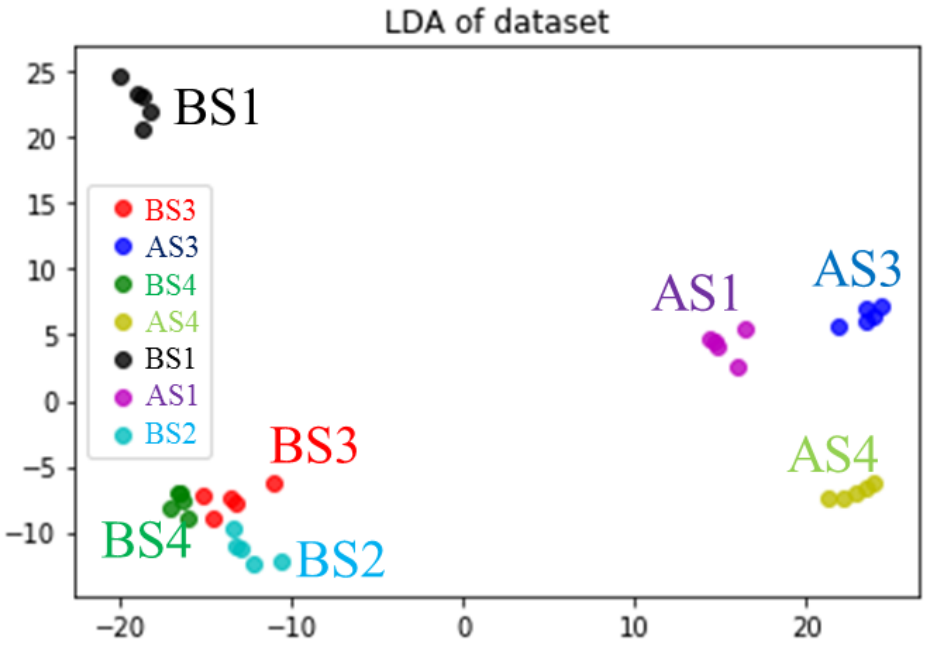


Fig 2. Result of LDA.(BSn=sample before nst shaking. ASn=sample after nst shaking.)

References
[1] Shu-Yen Lin, Li-Chiao Lo, Iou-Zen Chen, Po-An Chen, (2016), Effect of shaking process on correlations between catechins and volatiles in oolong tea., Journal of Food and Drug Analysis , Taipei, 500-507, Volume 24, Issue 3, https://www.ncbi.nlm.nih.gov/pubmed/28911555, Department of Horticulture and Landscape Architecture, National Taiwan University
[2] N Bârsan and U Weimar, (2003), Understanding the fundamental principles of metal oxide based gas sensors; the example of CO sensing with SnO2 sensors in the presence of humidity, Journal of Physics: Condensed Matter, Tübingen, R813–R839, https://iopscience.iop.org/article/10.1088/0953-8984/15/20/201, Institute of Physical and Theoretical Chemistry, University of Tübingen

Session 4 Novel Analytical Methods

Oral Session Coffee 04.01

Development of a Flavour Prediction Tool Using Single Photon Ionization for Coffee Roasting

Courtney Benson
Universität Rostock, Rostock, Germany

Text Courtney Benson, Zach McCarthy, Kristen Boyko, Jessalin Howell – The J. M. Smucker Company
Sven Ehlert – Photonion GmbH
Ralf Zimmermann, Hendryk Czech – University of Rostock[SC1] [HJ2]

Coffee roasting is a process that creates the rich flavor and aroma of coffee enjoyed every day by millions of people across the globe. Coffee roasting can be carried out in small batches using fire pits, air poppers, in-home roasters, or in large batches using pilot and commercial scale roasters. Regardless of roaster type, understanding the development of flavor during roasting is key to producing an excellent cup of coffee for consumption. The influence of roasting on the formation of flavor attributes in the finished cup is the focus of this study.
In this experiment, a Single Photon Ionization - Time of Flight Mass Spectrometer was connected to a lab scale roaster as a means for measuring the formation of coffee volatiles generated during the roasting process. Coffee beans were removed at various stages within the roasting process beginning with lightly roasted beans to beans that were burnt and over roasted. The beans were quickly cooled, canned and stored at 40° F to create a set of samples for sensory evaluation. A trained panel of 10-14 members rated the coffee samples using a universal scale (1-10) for specific attributes related to roasted coffee. Three to six samples of coffee of similar colors were evaluated during each panel.
As expected, many sensory attributes such as ashy, bitter, dark and coffee [SC3]

increased throughout the roasting process. Likewise, green vegetative attributes were reduced during roasting. Congruency plots of roasting gases and sensory attributes were also very well correlated. The outcome of this study was the development of a model to predict flavor attributes during roasting.

Oral Session Coffee 04.02

FTIR, HPLC and chemometrics to discriminate specialty coffee

Verônica Belchior
Burgeon Specialty Coffee, Burgeon Specialty Coffee, Poços de Caldas, Brazil

Text Verônica Belchior[1] × Bruno Gonçalves Botelho[2] × Susana Casal[3] × Leandro S. Oliveira[1,4] × Adriana S. Franca[1,4]
[1]PPGCA, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, 31270-901, Belo Horizonte, MG, Brazil. veronica@burgeon.com.br
[2] DQ, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, 31270-901, Belo Horizonte, MG, Brazil.
[3]LAQV/REQUIMTE, Faculdade de Farmácia, Universidade do Porto, R. Jorge de Viterbo Ferreira 228, 4050-313 Porto, Portugal.
[4]DEMEC, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, 31270-901, Belo Horizonte, MG, Brazil

A small portion of coffee production achieves the highest quality standards, being considered “specially coffee”, increasingly seeked by consumers. The *Specialty Coffee Association* (SCA) sensory analysis protocol is the official methodology to classify specialty coffee. However, because sensory analysis is sensitive to the taster’s training and physiology, among other parameters, the feasibility of an instrumental approach has been studied as an alternative. The present study proposes the application of spectroscopy (FTIR), physicochemical (pH and titratable acidity), and HPLC (organic acids) to distinguish different qualities and sensorial characteristics within specialty coffees. Samples of specialty coffee were provided by the Federação dos Cafeicultores do Cerrado Mineiro and Fazenda Barinas (n=28), roasted in a IKAWA coffee roaster (in duplicate, n=56), tasted by a group of Q-graders, and the beverage, prepared in accordance with the SCA protocol was submitted to the analysis above.
Only pH showed significant difference between beverages. The organic acids analyzed (quinic, oxalic, malic, citric, acetic, succinic, fumaric and tarataric) showed no difference between samples. PLS results were built for all the analysis, but the FTIR results were the best to predict quality, providing consistent models for predicting the quality previous given by the cuppers, with low values of RMSEC and RMSEP (0.23 both). Also, the models showed high values of Rc (0.99) and Rv (0.97).
The results showed that the analysis of coffee beverage full spectra is more efficient and interesting from an industrial and scientific point of view than the analysis of single compounds or properties. These results are encouraging for the definition of spectral limits that could be associated with sensorial attributes and quality, enabling to distinguish

quality even within high-quality coffees.

Oral Session Coffee 04.03

PAs in Honey, Tea and Herbs – A Challenge for Routine Laboratories

Arne Duebecke[1], Gudrun Beckh[2], Cord Luellmann[3]
[1]Quality Services International GmbH/Tentamus Center for Food Fraud (TCF2), Bremen, Germany, [2]Quality Services International GmbH, Bremen, Germany, [3]Tentamus Analytics GmbH, Berlin, Germany

Text During the last few years pyrrolizidine alkaloids (PAs) in food and feed turned out to be permanent hot topic in Europe. After discovery of parts of PA-containing plants in a mixed salad, interest increased quickly. In 2009, leaves of the PA-plant Senecio vulgaris were found in rucola (Eruca sativa). In August 2011 the German Federal Institute for Risk Assessment (BfR, Bundesinstitut für Risikobewertung) published a statement on PAs in honey, followed by an opinion of the European Food Safety Authority (EFSA) about PAs in Food and Feed in the same year. The occurrence of PAs in tea was reported in 2013 by the BfR and just last year a report on the “Occurrence of Pyrrolizidine Alkaloids in food” was published by the EFSA. Research activities targeting PAs in food and feed also increased as can be seen by the number of papers published on that topic.

In the past years several thousand samples of honey, tea and herbs of different origin have been analyzed. Analysis was done by means of LC-MS/MS after a cleanup step using solid phase extraction. Data evaluation revealed certain patterns of PAs found in honey as well as (herbal) tea. Since then, measures have been taken to reduce the PA level, e.g. by means as proposed by the Codex Alimentarius in the code of practice for weed control to prevent and reduce pyrrolizidine alkaloid contamination in food and feed”.

The challenge for routine laboratories consists in the huge number of structurally closely related PAs. Overlapping with other PAs may happen which complicates quantification. Reference material is expensive and availability is variable.

Not all laboratories use the same method, which may lead to different results, depending which of the isomers are overlapping. Furthermore, labs take different approaches to take the isomers into account when calculating there concentration.

Short Oral Communications

Session 5 - Cocoa fermentation

Oral Session Cocoa 05.01

Inhibition of growth and ochratoxin A production of *Aspergillus carbonarius* by B*acillus* strains isolated from cocoa bean fermentation.

Adobi Christian Kadjo[1], Corinne Guézéré Beugré[1], Kra Brou Didier Kédjébo[1], Renaud Boulanger[2], Angelique Fontana-Tachon[2], Noel Durand[2], Tagro Simplice Guéhi[1]
[1]University Nangui Abrogoua, University Nangui Abrogoua, Abidjan, Côte d'Ivoire, [2]CIRAD, UMR Qualisud, 34398 Montpellier, France, University Montpellier, CIRAD, Montpellier Sup Agro, Université d'Avignon, Université de La Réunion, Montpellier, France, Montpellier, France

Text Ivorian raw cocoa beans are recurrently subject to ochratoxin A (OTA) contamination. The use of chemical and physical means to reduce or prevent the OTA production in cocoa beans is prohibited or inefficient. The present study aimed to improve the sanitary quality of raw cocoa bean by determining the potential for biological control of fungal growth and OTA production of *Aspergillus carbonarius* using *Bacillus* sp strains isolated from fermented cocoa beans. Results of both direct and indirect tests carried out using the double layer agar technique showed seven (7) *Bacillus* strains with *A. carbonarius* growth inhibition abilities at up 50 %. In addition, inhibition of fungal growth tests in a liquid culture medium have revealed OTA production inhibition abilities of tested *Bacillus* strains, whether by the culture supernatant or the cell suspensions. The cell suspensions of strains BC35, BC46, BC52, BC53 and BC54 showed an important antagonistic effect to OTA production ranging from 78.7 to 95.8 %. However, only liquid culture supernatants of strains BC35, BC54 and BC46 recorded the best activities about 6.4, 48.4 and 70.0 % respectively against to OTA production. Results suggest a direct or an indirect action via metabolites produced by tested *Bacillus* strains on *A. carbonarius* growth coupled probably with consumption and / or OTA binding. We could hope tested Bacillus strains could be a promising agent for biological control of growth and OTA production of *A. carbonarius* in raw cocoa beans during the post-harvest processing.

Oral Session Cocoa 05.02

Influence of specific *Saccharomyces cerevisiae* yeasts on cocoa beans flavor and final chocolate quality

Anne Julien-Ortiz[1], Pierre Poirot[1], Othilie Vian[2], Sabine Quintana[2], Jean-Marc Laurens[2], Christelle Kouamé[3], Joel Grabulos[3], Marie-Christine Lahon[3], Marc Lebrun[3], Renaud Boulanger[3]
[1]Lallemand, Blagnac, France, [2]Cemoi, Perpignan, France, [3]CIRAD, Montpellier, France

Text Abstract
Chocolate is a "pleasure" food whose organoleptic quality is essential to the product's value. The aromatic quality of chocolate comes from many parameters, some are intrinsic to the raw material, and others are driven by the manufacturing process. In the frame of a

research project dealing with the management of cocoa fermentation process, we investigated the impact of specific yeasts inoculation on the quality of cocoa beans after fermentation and on the final sensory quality of the chocolate
Two different yeasts have been inoculated and fermentations were compared to a spontaneous fermentation (standard cocoa). Then, the beans were transformed into chocolate via a standard process and the sensory characterization of these samples was carried out. The analysis of the volatile fraction and the biochemical composition have also been done on beans and chocolate.
The results of the beans fermented by selected yeasts show levels of sugars (sucrose, glucose and fructose) lower than in standard cocoa. The polyphenol contents are also impacted by depending on the fermentation process.
The analysis of the volatile compounds were also performed on all fermented beans, and important differences were detected. Among the various chemical families measured, the alcohols and esters are more abundantly present in the fermentations carried out with the yeast starters. The sensory tests also confirm that the quality of these beans are also considered better. In conclusion, these studies confirmed the high interest to better control the fermentation step with specific yeast inoculation, in order to improve the final quality of chocolate.

Keywords : cocoa beans, yeast, fermentation, organoleptic quality

Oral Session Cocoa 05.03

Polycyclic Aromatic Hydrocarbons (PAH) Contamination of Raw Cocoa and Degradation by Bacterial Strains

Axel-Didier Sess-Tchotch[1], Adobi Christian Kadjo[1], Kra Brou Didier Kédjébo[1], Joel Grabulos[2], Benjamin Guilbert[3], Renaud Boulanger[3], Noel Durand[3], Angelique Fontana-Tachon[3], Tagro Simplice Guéhi[1]
[1]University Nangui Abrogoua, University Nangui Abrogoua, Abidjan, Côte d'Ivoire, [2]University Nangui Abrogoua, University Montpellier, CIRAD, Montpellier Sup Agro, Université d'Avignon, Université de La Réunion, Montpellier, France, Montpellier, France, [3]CIRAD, UMR Qualisud, 34398 Montpellier, France, University Montpellier, CIRAD, Montpellier Sup Agro, Université d'Avignon, Université de La Réunion, Montpellier, France, Montpellier, France

Text Polycyclic Aromatic Hydrocarbons (PAH) contamination in raw cocoa beans and derivative products has become more and more alarming. The European Union has set maximum rates in PAHs for cocoa beans and derived products (Regulation 835/2011). No chemical or physical method to reduce or prevent PAH occurrence in cocoa beans appears to be efficient. Thus, this study aimed to test the capacity of bacterial strains isolated from cocoa beans to detoxify cocoa beans contaminated by PAH. Firstly bacterial strains were selected from cocoa bean samples for their ability to grow in PAH synthetic media. Secondly the strains were isolated after successive cultures using mineral liquid media containing PAHs as sole carbon source. Results shows that ten (10) different bacterial isolates were pre-identified as belonging to the *Bacillaceae* family. Using 16S

rDNA sequencing, 5 species, which exhibited detoxifying abilities against major PAHs such as (Benzo(a)Anthracene (BaA), Chrysène (Chr), Benzo(a)Pyrene (BaP), Benzo(b)Fluoranthène (BbF), were identified as *Bacillus cereus*, *Bacillus anthracis*, *Lysinibacillus sphaericus*, *Brevibacillus brevis* and*Brevibacillus laterosporus.* Results suggested a direct action of the bacterial isolates by PAHs degradation, consumption and / or binding. These observations allows believing that these bacterial strains could be promising agents for the biological control of PAHs contamination of raw cocoa.

Session 6

Oral Session Tea 06.01

Yellow tea ameliorates metabolic syndrome in db/db mice

Qingshuang Cai, Yun Teng, Da Xiang Li, Zhongwen Xie
State Key Laboratory of Tea Plant Biology and Utilization, Anhui Agricultural University, Hefei, China

Text There are approximately a quarter of adults suffering from Metabolic syndrome (MetS), and obesity is the most primary and direct cause. Notably, green tea, black tea and secondary metabolite of tea have health functions on MetS. However, whether yellow tea plays a role in regulating MetS remains largely unknown. The current study aims to investigate the molecular mechanisms of dietary supplements of large yellow tea (LYT), small yellow tea (SYT), fresh-leaves of large yellow tea (F-LYT) and extracts of large yellow tea (water extract of large yellow tea, LWE, ethanol extract of large yellow tea, LEE) on controlling MetS and T2D (type 2 diabetes) in male db/db mice. Db/db mice, which manifest morbid obesity, chronic hyperglycemia, hypoinulinemic, and hypertension, are widely used for the study of MetS and T2D. The main findings are as following.
1. LYT, SYT and LWE significantly reduced blood glucose, improved the insulin sensitivity, and prevented the glucose metabolic disorder in db/db mice by inhibiting the mRNA expression of *gckr* and *gck*.
2. LYT and SYT significantly decreased blood lipid and the hepatic fatty infiltration, protected the integrity of hepatic structure in db/db mice via reduction of the lipid synthesis genes including *acca*, *fasn*, and *srebf1*.
3. In the study of energy metabolism in mice, the overall exercise and energy consumption of all groups of db/db mice were significantly lower than those of the control mice, and db/db control mice had no rhythms in exercise, oxygen consumption and RER values. Although LYT did not increase the amount of exercises, it significantly improve the circadian rhythm in the energy expenditure of db/db mice.
4. LYT, SYT, and LEE had significant protective effects on the hepatic injuries and kidney damages in db/db mice.
5. In the vasoconstrictive study of mesenteric artery, only LYT and SYT had significant vasodilatation effects in db/db mice.
In conclusion, LYT and SYT, as dietary supplements, play significant preventive roles in the occurrence and development of MetS in db/db mice. The different effects of health function between LYT and F-LYT suggest that chemical changes in the processing old leaves of yellow tea may increase the effects on MetS in db/db mice. Furthermore, the

different effects of LWE and LEE on glucose and lipid metabolism, as well as liver and kidney injury in db/db mice suggested that some other chemicals may also play important roles in the MetS of db/db mice.

References:
[01] SaMetSon SL, Garber AJ., (2014), Metabolic syndrome, Endocrinology and Metabolism Clinics of North America, 43: 1-23
[02] Engin A., (2017), The Definition and Prevalence of Obesity and Metabolic Syndrome, Advances in Experimental Medicine and Biology, 960: 1-17
[03] Latrino R, Manunta P, Zagato L., (2015), Salt sensitivity: challenging and controversial phenotype of primary hypertension, Current hypertension reports, 18: 70-84.
[04] Sandouk Z, Lansang MC. , (2017), Diabetes with obesity- Is there an ideal diet?, Cleveland Clinic Journal of Medicine, 84:S4-S14.
[05] Lee Y, Berglund ED, Yu X, et al. , (2014), Hyperglycemia in rodent models of type 2 diabetes requires insulin-resistant alpha cells, Proceedings of the National Academy of Sciences of the United States of America, 111: 13217-13222.
[06] Geng F, Cheng X, Kim SH, et al. , (2016), Inhibition of SOAT1 suppresses glioblastoma growth via blocking SREBP-1-mediated lipogenesis, Clinic Cancer Research, 22: 5337-5348.
[07] María BM, Daiana F, Verónica W, et al. , (2016), Maternal saturated-fat-rich diet promotes leptin resistance in fetal liver lipid catabolism and prograMetS lipid homeostasis impairments in the liver of rat offspring, The Journal of Nutritional Boichemistry, 27: 61-69.
[08] Kazue H, Michiko K, Toshinao G, et al. , (2017), Insulin-induced inhibition of gluconeogenesis genes, including glutamic pyruvic transaminase 2, is associated with reduced histone acetylation in a human liver cell line, Metabolism Clinical and Experimental, 71: 118-124.
[09] David C, Søren SL, Bruce AP, et al. , (2016), The effect of sodium glucose contransporter 2 inhibition with empagliflozin on microalbuminuria and macroalbuminuria in patients with type 2 diabetes, Diabetologia, 59: 1860-1870.
[10] Xiabin C, Hayashi S, Nishiguchi S, et al. , (2017), Pu'erh tea extract-mediated protection against hepatosteatosis and insulin resistance in mice with diet-induced obesity is associated with the induction of de novo lipogenesis in visceral adipose tissue, Journal of Gastroenterology, 52: 1240-1251.

Oral Session Tea 06.02

Supplement of Zijuan and Yunkang10 Green tea ameliorates high-fat-diet induced metabolic syndrome in C57BL/6J mice

Ru Li, Ying Shen, Zhongwen Xie
State Key laboratory of Tea Plant Biology and Utilization, Anhui Agricultural University, Hefei, Anhui Province, China

Text Abstract: Metabolic syndrome (MetS) is defined as at least has three of insulin resistant, center obese, hypertention, hyperglycemia and dyslipidemia. Both genetic factors and lifestyle, including dietary habits and physical activity, contribute to the pathogenesis of MetS. The prevalence of MetS has dramatically increased in recent

decades around the world. Existing drugs against MetS have some side effects. Therefore, researches on finding natural herbs to preventive MetS are always critical. This study aimed to investigate the prevention of different doses of Zijuan and Yunkang10 Green tea on MetS induced by high fat diet. The underlying mechanisms are also investigated.
Six week old C57BL/6J mice were randomly separated into six groups: low-fat diet group (LFD), high-fat diet group (HFD), high-fat diet plus 2.5% Zijuan green tea (HFD+2.5%ZJ), high-fat diet plus 5% Zijuan green tea (HFD+5%ZJ), high-fat diet plus 2.5% Yunkang10 green tea (HFD+2.5% YK) and high-fat diet plus 5% Yunkang10 green tea (HFD+5%YK). HFD C57BL/6J mice exhibited stable MetS symptoms, yet mice in the tea intervention groups ameliorated these symptoms in the dose-dependent manner. The obese animals increased the lipogenic genes *Fasn* and *Srebf1* expression, and reduced the fat oxidative genes *Cpt1a* and *Ppara* expression. In addition, the obese mice presented higher levels of proinflammatory cytokines (*TNF-α*, *MCP-1*, *IL-6* and *IL-1β*) and glycometabolic cytokines (*GCKR*). However, the intervention groups all reversed these syndromes. Over all, intervention with 5% green tea has better preventive effects than that of 2.5% green tea supplement. And Yunkang10 showed better preventive effects than that of Zijuan. Our data suggested that the YK and ZJ green tea may improve the glucose and lipid metabolism, reduced lipid accumulation, and relieved inflammatory response, thereby alleviating the Met S of HFD induced MetS of mice.

Oral Session Tea 06.03

Ameliorating metabolic syndrome and alleviating renal injury of db/db mice by yellow tea intervention

Su Peng, Yun Teng, DaXiang Li, ZhongWen Xie
State Key Laboratory of Tea Plant Biology and Utilization, Anhui Agricultural University, HeFei, China

Text Abstract Yellow tea has been widely recognized for its health benefits. Based on its original fresh leaves tenderness, yellow tea can be classified large yellow tea (LYT) and small yellow tea (SYT). Our previous studies discovered that LYT significantly reduced blood glucose, improved serum lipid profile and prevented fatty liver formation of db/db mice. Two questions are remained for further investigation. Do functional compounds come from LYT? Does manufacture process produce functional compounds? Comparative investigation of LYT, SYT and Fresh Large Yellow Tea (F-LYT) on ameliorating metabolic syndrome of db/db mice has been conducted to answer these questions.
Our results showed that all three tea supplements ameliorating metabolic syndrome of db/db mice. However, LYT performed the better effective and F-LYT less effective on reducing blood lipid and lowering blood glucose and reducing blood pressure and functions not remarkable. Mechanistic studies showed that three types of yellow tea regulating hepatic glycometabolism mainly through increasing *Gck* and reducing *Gckr* gene expression, and regulating hepatic fat metabolism mainly through decreasing the expression of hepatic lipogenic gene *Fasn*, *Acaca* and *Srebf1* and decreasing blood pressure through suppressing arteriole contractility. In addition, all of teas protected renal injury significantly through TGF-β/Smads signaling pathway. This study indicated that

substrates produced in the manufacture processing contribute to ameliorating metabolic syndrome of db/db mice.

Session 7 coffee byproducts and sustainibility

Oral Session Coffee 07.01

Rarely-Explored Grounds – Process-Engineering Aspects of Coffee Brewing

Michael Kuhn, Verena Hargarten, Heiko Briesen
Chair of Process Systems Engineering, Technical University of Munich, Freising, Germany

Text Coffee beverages are significantly influenced by the brewing process. Nevertheless, coffee extraction is among the scientifically least-studied steps in the whole processing chain of coffee. Therefore, we introduce different findings regarding this deceive step, mainly the influence of particle size, packing structure, and tamping pressure. Results are shown for two different hand-brewing methods, gaining again importance in the third wave of coffee, as well as for espresso extraction.

First, we report about particle size measurement strategies, providing a concise comparison of dry and wet measurement of the particle size distribution of ground coffee by laser diffraction and dynamic imaging. A particular emphasis is laid on the swelling of coffee particles. Second, an immersion and a percolation (pour-over) hand brewing method are compared with respect to overall extraction, as quantified by the refractometrically-determined TDS value (TDS = total dissolved solids). Both brewing methods are mimicked in a reproducible way using a laboratory filter cell. The influence of particle size and pressure on extraction as well as the influence of tamping pressure, in case of the percolation method, is studied. In a third step, we show how the packing of coffee particles for percolation can be influenced by mixing the coffee powder with random packings, known from thermal process engineering. By this measure, the flow resistance of the particle packing can be controlled in a targeted way, thus, decoupling residence time of the water from the coffee's particle size. Lastly, the influence of particle size and tamping pressure on espresso extraction is analyzed by high-performance liquid chromatography (HPLC).

It is found that swelling of coffee particles is usually overrated. For hand brewing as well as espresso extraction, particle sizes strongly influence overall extraction as well as extraction kinetics. Tamping, on the other hand, plays no significant role. By using random packings, overall extraction can be increased as well as decreased, offering a completely new control variable for the coffee brewing process which could also foster production innovations, e.g., concerning capsule systems. In case of espresso, the dynamic extraction of caffeine and trigonelline is studied. We found a strong influence of particle size and overall coffee volume on the coffee composition. In an outlook, further relevant investigations along similar lines are presented.

Short Oral Communications

Oral Session Coffee 07.02

Potential use of coffee as high value carbohydrates resource to new applications

Claudia Passos[1], Guido R. Lopes[1], Cláudia P.A. Ferreira[1], Sofia F. Antunes[1], Ana Bastos[2], Margarida M. Almeida[2], Manuel A. Coimbra[1]
[1]QOPNA & LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, Aveiro, Portugal, [2]CICECO, Department of Materials and Ceramic Engineering, University of Aveiro, Aveiro, Portugal

Text There is an increasing demand for natural polymers, including polysaccharides, that can be used as carriers in food and drug delivery applications by preparation of microparticles, allowing storage stability and safe delivery processes of active compounds. Coffee is a rich source of polysaccharides, namely galactomannans (GM) and type II arabinogalactans (AG), but also chlorogenic acids and melanoidins which can confer charge and potentiate additional stability during microparticles preparation. Optimization of hot water extractions can be addressed to prepare soluble coffee with different composition. But, the majority of the polysaccharides are not even extracted, remaining an underused resource of carbohydrates in spent coffee grounds. Pressurized water conditions can be applied to favour their extractability[1].
Ground coffees were used for optimization of hot water extraction conditions, namely: time, temperature, and coffee/water ratios. The remaining unextracted material was submitted to pressurized conditions evaluating the impact of temperature, time, and use of dilute alkali conditions. Commercial instant coffee, as well as hot water extracted, and pressurized coffee samples were afterwards spray-dried to evaluate microparticles formation and characteristics.
An optimized hot water extraction achieved the exact 1.2 mannose/galactose ratio existent in the espresso composition. Additional extraction of carbohydrates was achievable under pressurized conditions and correlated with increasing temperature. The extraction conditions favoured GM at <170ºC, while an abundant extraction of AG occurred at higher temperatures (η_{AG}/η_{GM} >1). The different compositions obtained allowed a rich portefolio of carbohydrates to be evaluated as delivery agents. Spray-dried powders contained shrivelled, raisin-like shape microparticles with theoretical average aerodynamic diameters of 3-5 µm. Micronization of extracts was possible, highlighting the potential use of coffee resources as high value carbohydrate carriers to food and drug delivery applications.

Acknowledgements
The authors thank FCT (FCT/POCI - Compete2020 - Portugal 2020 – FEDER/EU) Nº POCI-01-0145-FEDER-029560, project “PulManCar”. QOPNA (FCT UID/QUI/00062/2019) thanks UA and FCT/MCT; CICECO (POCI-01-0145-FEDER-007679, FCT Ref. UID /CTM /50011/2013) thanks FCT/MEC. C. Passos (CEECIND/00813/2017) and G.Lopes (SFRH/BD/104855/2014) were supported by FCT.

References:
[1] Passos, C.P., Rudnitskaya A., Neves J.M.M.G.C., Lopes G.R., Evtuguin D.V., Coimbra M.A. , (2019), Structural features of spent coffee grounds water-soluble polysaccharides:

Towards tailor-made microwave assisted extraction. , Elsevier, Journal of Carbohydrate Polymers, 53-61, 214, https://doi.org/10.1016/j.carbpol.2019.02.094

Oral Session Coffee 07.03

Hacia La Sostenibilidad Del Café En Colombia, Un Modelo En Espiral

Juan Leonardo Garzón
CAFEEXCOL S.A.S., Envigado, Colombia

Text La caficultura está en crisis por bajos precios y baja rentabilidad para el productor. La producción no es sostenible.

El objetivo de este trabajo es determinar un nuevo enfoque y un cambio profundo para la producción del café bajo criterios de sostenibilidad, un modelo en espiral para lograr un desarrollo sostenible del café en Colombia.

1. Pasar de la producción intensiva hacia una producción sostenible bajo criterios de agricultura de precisión.

2. Cambios en la política de selección de variedades, con oferta de cafés diferenciados.

3. Establecimiento de micro centrales de proceso para micro lotes de café diferenciados; pasar del modelo actual de venta de cafés indiferenciados a venta de café diferenciado por microlotes.

4. Transformación en el enfoque de mercado mediante un cambio en el modelo de asociatividad actual (relación de comprador proveedor). Nuevo modelo basado en la unión de esfuerzos de productores empoderados para mejorar su productividad y aumentar su competitividad accediendo directamente al mercado (Cooperativas Agrarias).

5. Producción del café tostado al origen (eliminación de impuestos y aranceles en países compradores, al café tostado directamente por los países productores).

The coffee industry is in a crisis due to low prices and low profitability for the producer. Production is not sustainable.

The objective of this work is to determine a new approach and a profound change to the production of coffee under criteria of sustainability, a spiral model to achieve a sustainable coffee development in Colombia.

1. Moving from intensive production to sustainable production under criteria of agriculture of precision.

2. Creating changes in the policy of variety selection, with a supply of differentiated coffee.

3. Establishment of micro-processing centers for differentiated micro-lotes of coffee; move from the current model of selling undifferentiated coffees to sell differentiated coffee by micro-lotes.

4. Transformation in the market approach through a change in the current associativity model (supplier-buyer relationship). A new model based on the union of efforts of empowered producers to improve their productivity and increase their competitiveness by accessing the market directly (Coffee Cooperatives).

5. Production of roasted coffee in the place of origin (reduction of taxes and tariffs in purchasing countries, to roasted coffee directly by producing countries).

Oral Session Coffee 07.04

Use of microwave-assisted extraction for the production of instant coffee-like extracts from roasted coffee

Guido R. Lopes[1], Cláudia P. Passos[1], Carla Rodrigues[2], José A. Teixeira[3], Manuel A. Coimbra[1]
[1]QOPNA & LAQV-REQUIMTE, University of Aveiro, Aveiro, Portugal, [2]Diverge, Grupo Nabeiro Innovation Center, Lisboa, Portugal, [3]Center of Biological Engineering, University of Minho, Braga, Portugal

Text The application of Microwave-assisted extraction (MAE) to roasted coffee would be a potential method for obtaining instant coffee-like extracts, providing reduced extraction times and/or improved yields as main advantages. This is possible by the use of closed-vessel systems, allowing the use of extraction temperatures higher than those possible to achieve by atmospheric boiling.
Box-Behnken design (3 factors) was used to study and optimize the coffee MAE process through response surface methodology. After filtration and freeze-drying, the coffee extracts were analysed. It was studied the influence of time of extraction (1-10 min), temperature (120-180 ºC), and mass-to-volume ratio (2-6 g/ 60 mL) on the overall extraction yield, carbohydrate content and sugar composition (GC-FID), caffeine and chlorogenic acids content (HPLC-DAD), and colour of the extracts ($K_{mix,\ 405\ nm}$). A commercial instant coffee product was used as reference concerning instant coffee properties.
MAE methodology allows to quickly obtain up to 47% w/w of coffee compounds present in the coffee powder. Temperature was clearly the main factor for differentiation of extracts content and properties, although time and m/V ratio also exerted effect on the responses. The colour of the extracts became less brown (more yellowish) with increasing extraction temperatures. The increase in extraction yield verified at higher temperatures was associated to an increase in arabinogalactan extraction, structures distinctive of instant coffee samples.
The use of pressurized conditions allows an increase of the amount of compounds extracted when compared with extractions performed at atmospheric conditions, approaching instant coffee composition. The models developed allowed to modulate the composition of the extracts, while the comparison to an commercial instant coffee sample

enables to define the conditions to quickly obtain coffee extracts resembling instant coffee chemical properties (sugar content and composition, caffeine and 5-CQA content), which can be used in food/brew formulations.

Acknowledgements
Thanks are due to University of Aveiro and FCT/MCT for the financial support of QOPNA (FCT UID/QUI/00062/2019) through national founds and, where applicable, co-financed by the FEDER, within the PT2020 Partnership Agreement, and to the Portuguese NMR Network. GR Lopes and CP Passos were respectively supported by individual doc (SFRH/BD/104855/2014) and post-doc (SFRH/BDP/107881/2015) grants by FCT.

Session 8 Tea and health 2

Oral Session Tea 08.01

Comparative evaluation of different grades of LGGT on preventing metabolic syndrome in high-fat diet Induced C57BL/6J Mice

Mengwan Li, Zhutong Wang, Ru Li, Daxiang Li, Zhongwen Xie
State Key Laboratory of Tea Plant Biology and Utilization, Anhui Agricultural University, Hefei Anhui, China

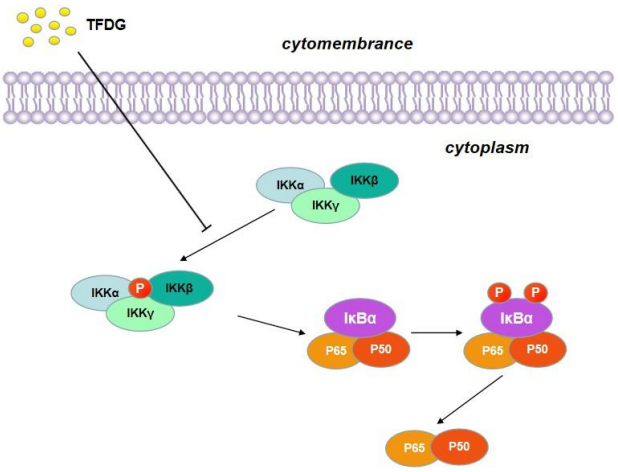
Text Lu'an guapian green tea (LGGT) is one of the most famous green tea in China. The unique character of this tea is that made with only leaves, without buds and young stem of *Camelia sinensis*. Comparative investigation of health effects of different grades of this tea have not been evaluated yet. The aim of the study was to establish a health-based evaluation system to justify different grades of LGGT, and to provide healthy information to tea consumers. Non-targeted ultra-performance liquid chromatography Orbitrap mass spectrometry (UPLC-Orbitrap-MS) was used to analyze chemical profiles of six different grades of LGGT. Hierarchical clustering analysis of chemical profiles showed that premium grades clustered together first, and then do first and second grades, and the third grade and summer LGGT grouped last. High-fat-diet (HFD) induced metabolic syndrome of C57BL/6J mice were used to evaluate health benefits of the six grade LGGT. The C57BL/6J mice were assigned for following groups to feed different diets for 12 weeks: chow diet (C), HFD or HFD supplement with the premium LGGT (HFD+PG), HFD with first LGGT (HFD+FG), HFD with third LGGT (HFD+TG) and HFD with the summer LGGT (HFD+SumG). Our data showed that dietary supplement of different grades of LGGT reduced the body weight, body fat mass and preventive fatty liver formation, improved blood lipid panel induced by high-fat diet; and effectively decreased the blood glucose elevation and improved glucose tolerance and insulin sensitivity in HFD induced C57BL/6J mice. Furthermore, the dietary supplement of LGGT obviously reduced the expression of lipid synthesis related genes fatty acid synthase, the sterol regulatory element-binding transcription factor 1 and acetyl-CoA carboxylaseα, while the lipid catabolic genes were not altered in the liver of HFD induced C57BL/6J mice. Over all, the premium grade LGGT has the best effect on prevention of obesity and metabolic abnormalities in HFD induced C57BL/6J mice compared to the other group mice.

Oral Session Tea 08.02

Cardioprotection of theaflavin-3,3'- digallate on myocardial ischemia reperfusion injury of isolated Rat heart

Feng Zhu[1], Yiyao Qi[1], Siyu Deng[1], Xiaochun Wan[1], Zijian Xie[2], Zhongwen Xie[1], Daxiang Li[1]
[1]State Key Laboratory of Tea Plant Biology and Utilization, and International Joint Laboratory on Tea Chemistry and Health Effects of Ministry of Education, Anhui Agricultural University, Hefei, Anhui, China, [2]Marshall Institute for Interdisciplinary Research, Marshall University, Huntington, United States

Text Myocardial ischemia-reperfusion injury (MIRI) is a serious complication of acute myocardial infarction (AMI). It has a serious adverse effect on the patients. The interventional protection of MIRI has become a hot topic in clinical research. Inflammatory signaling pathways not only urge MIRI, but also influence the clinical consequences of the post-infarction remodeling and heart failure. Theaflavin-3, 3' - digallate (TFDG),an antioxidant natural polyphenol found in black tea, has been shown the inhibition on inflammatory factors in DBA/1J mice and BALB/c mice. This study aimed to explore whether TFDG improved MIRI via suppression of inflammation.
Isolated male SD rat hearts underwent 30 min of global ischemia and 30 min of reperfusion using Langendorff perfusion system. Pre-treatment of TFDG was in sub-micromolar concentrations reduced infarct size by more than 45%, decreased lactate dehydrogenase release, and improved the recovery of cardiac function. Moreover, TFDG remarkably inhibited the activation of NF-κB p65 subunit translocation and IκB-α phosphorylation induced by MIRI.
This findings proveed that TFDG has cardioprotective effect in improving cardiac function and relieving myocardial ischemia reperfusion through mediating inflammatory NF-κB signal pathway in the isolated rat heart. This suggested that TFDG may contribute to the positive impact of black tea consumption on cardiovascular health.



Oral Session Tea 08.03

Supplementation of Keemun Black Tea modulates microbiota, permeability,and inflammation of gut in high-fat diet induced obese mice

Sen Zheng, ZhongWen Xie
Anhui agricultural university, HeFei, China

Text Black tea is one of the most consumed beverages and accounts for a significant part of polyphenol intake in the world population. Keemun black tea is one of most famous black tea, which has unique flavor. However, the health effect against metabolic syndrome remains to be evaluated. This study was planned to explore the supplementation of Keemun black tea modulating microbiota, permeability, and inflammation of gut in high-fat diet induced obese C57BL/6J mice.
Forty eight specific pathogen-free male C57BL/6J mice (5 weeks old) were divided into four groups (n=12) as follows: normal diet (ND), high-fat diet (HF), high-fat diet plus green tea (HF+GT), high-fat diet plus Keemun black tea (HF+KBT). On completion of 13 weeks administration, half of the animals from each group were fasting over night and sacrificed under aesthesia, and all the biological samples were harvested for further studies. The remaining mice were provided ND and water ad libitum as wash out period. At the end of 23 weeks (9 weeks of wash out period) all the animals sacrificed under aesthesia upon overnight fasting and all the biological samples were harvested for further studies. Compared to HFD group, the body weight gain, blood glucose, body fat ratio, plasma LDL,T-CHO,AST,ALT, endotoxin and gut permeability in the tea infusion intervention groups were significantly suppressed at the end of tea intervention. However, after nine weeks of wash out period, there is no significant difference in body weight , blood glucose, body fat ratio, plasma LDL,T-CHO,AST,ALT,endotoxin and gut permeability between the tea intervention groups and HFD group. Our preliminary data showed that tea intervention for 13 weeks significantly prevented obesity of HFD C57BL/6J mice and the nine weeks wash out time reversed these preventive effects.

Oral Session Tea 08.04

EGCG Alters Transcriptional Rhythm and Circadian Gens Expression in Vascular Smooth Muscle Cells Induced by AngⅡ

Xie Zhongwen, Wang Ruru, Xiao Mengchao, Zheng Sen, Peng Su, Li Ru
State Key Laboratory of Tea Plant Biology and Utilization, Anhui Agricultural University, Hefei, China

Text Circadian clock system plays a critical role in human health. Rhythmic blood pressure during day and night is one of important biomarkers for healthy human. Vascular smooth muscle manages the contraction and relaxation of blood vessels, and thus regulates blood pressure. Therefore, investigating the biological rhythm of vascular smooth muscle cells (VSMCs) is important for homeostasis of blood pressure and health.

First, circadian gene expression model of VSMCs induced by AngII was successfully set up. Transcriptome sequencing, qPCR, western blot, and biorhythm fluorescence detection were employed to investigate the EGCG, a main catechins from tea, modulating circadian genes expression in VSMCs induced by AngII. Based on the transcriptome sequencing data, analysis tof KEGG pathway of DEGs revealed that EGCG down-regulated an array of differentially genes expression in circadian rhythm signal pathways, whereas <*i*>*Dec1*</i>and <*i*>*Dec2*</i> were up-regulated. The qPCR results demonstrated that EGCG significantly reduced the rhythmic amplitude of the <*i*>*Bmal1*</i>, <*i*>*Per2*</i> and <*i*>*Rev-erbα*</i>. Meanwhile, EGCG prolonged the periods and delayed the phases of the <*i*>*Bmal1*</i>, <*i*>*Per2*</i> and <*i*>*Rev-erbα*</i>. In addion, western blotting results showed that EGCG significantly prolonged the cycle, down-regulated the amplitudes and delayed the phases.of BMAL1 and REV-ERBα protein rhythmic expression. The results from fluorescence detection showed that EGCG reduced the amplitude and also delayed the rhythm phase of the PER2 expression in the aorta and mesenteric artery isolated from the PER2::LUC mice.
In conclusion, our results are first time to find that EGCG down regulated an array of circadian gene expression and decreased the rhythmic amplitude induced by AngII in VSMCs. The potential mechanisms of EGCG-regulated rhythm of circadian genes is to stimulate the over expression of <*i*>*Dec1*</i> and <*i*>*Dec2*</i>, to down-regulate of the transcriptional activation of the BMAL1-CLOCK heterodimer, and consequently down regulated other circadian genes expression. The pathophysiological significance of EGCG down regulated circadian gene expression and decreased the rhythmic amplitude induced by AngII in VSMCs is under investigation.

Session 9 Cocoa - Byproducts and sustainibility

Oral Session Cocoa 09.01

Effects of raw and roasted cocoa bean extracts supplementation on intestinal enzyme activity, hematological parameters and antioxidant status in rats fed a high-fat diet

Dorota Żyżelewicz[1], Joanna Oracz[1], Małgorzata Bojczuk[1], Grażyna Budryn[1], Adam Jurgoński[2], Jerzy Juśkiewicz[2], Zenon Zduńczyk[2]
[1]Institute of Food Technology and Analysis, Faculty of Biotechnology and Food Sciences, Lodz University of Technology, Lodz, Poland, [2]Department of Biological Functions of Food, Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Olsztyn, Poland

Text The aim of the study was to analyze the influence of diet enriched with the polyphenol-rich material on intestinal enzyme activity, hematological parameters and antioxidant status of laboratory rats. The animals were fed high-fat diet supplemented with freeze-dried water extracts of raw and roasted cocoa beans of Forastero variety. The influence of different cocoa extracts on oxidative stress, hyperglycemia, and lipid metabolism was studied during long-term feeding of laboratory rats with a high-fat diet supplemented with cocoa extracts. After the 4-week experimental feeding biological samples were collected i.e. intestines content, blood, and organs retrieved individually

from each rat. The observed changes indicate the biological activity of polyphenol extracts and other components of cocoa beans present in the prepared extracts. The differences in the results obtained for the analyzed parameters of the gastrointestinal tract revealed that the freeze-dried cocoa bean extracts being the subject of this investigation differently affected the physicochemical properties of rats' intestinal content, including intestinal microflora. The presence of raw and roasted cocoa bean extracts in the diets diversified the activity of the enzymes of the cecal microflora of rats.. Apart from the glucose, no statistically significant differences were found in the analyzed biochemical and enzymatic blood plasma indicators between control groups and groups fed supplemented diets. The experimental cocoa bean preparations showed no significant effect on the mass of rats' liver, heart, and kidneys, but varied some parameters of the antioxidant status of their organisms. Moreover, supplementation with roasted cocoa bean extract significantly increased the blood HDL cholesterol concentration. Results of this study contribute to the evidence that dietary supplementation with raw and roasted cocoa bean extracts can exert health-promoting effects, however further studies are necessary.

Oral Session Cocoa 09.02

Understanding the genetic background of Vietnamese cocoa cultivars (*Theobroma cacao* L.*)* using SSRs and SNPs

Helena Everaert[1, 2], Jocelyn De Wever[1, 3], Steve Lefever[3], Kim Hong Tang[4], An Lam Vu[5], Hayley Rottiers[1, 2], Kevin Maebe[6], Guy Smagghe[6], Koen Dewettinck[2], Kathy Messens[1]
[1]Research unit Molecular Biology, Department of Biotechnology, Faculty of Bioscience engineering, Ghent University, Ghent, Belgium, [2]Laboratory of Food Technology and Engineering, Department of Food Technology, Safety and Health, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium, [3]Center for Medical Genetics Ghent (CMGG), Department of Pediatrics and Medical Genetics, Ghent University, Ghent, Belgium, [4]Center for Forestry Research and Technology Transfer, Nong Lam University, Ho Chi Minh City, Viet Nam, [5]Department of Food Microbiology, Nong Lam University, Ho Chi Minh City, Viet Nam, [6]Laboratory of Agrozoology, Department of Plants and crops, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium

Text Increase in global chocolate consumption may cause a future cocoa shortage and subsequently an elevation of cocoa bean price. In order to accommodate the market's demand, cocoa trees were planted in Southern Vietnam. Thanks to the appropriate climate, soil and humidity, Vietnam is currently growing as a cocoa-producing country. In general, the cocoa quality depends on various factors, among others the genotype. Hence, to guarantee cocoa quality, there is a need for genetic characterization methods to identify promising cultivars, to eliminate low quality cocoa cultivars early in the process and to conserve valuable genetic material. Previous research [2], using 14 microsatellite markers (SSRs), has shown that Vietnamese cocoa is genetically diverse, but further research is required to obtain a better insight. Therefore, in this study, the dataset was enlarged and 42 single nucleotide polymorphism (SNP) assays [1] were applied in addition to the SSRs. The objective was twofold, namely (1) comparing the individual and synergetic strength of the applied markers to obtain the most suited marker set for (mis)classification and (2) examining the genetic diversity and population structure of 80 Vietnamese cocoa cultivars

using this marker set. 53 International cultivars, representing ten genetic groups of [3], were analyzed using Bayesian clustering to compare the three marker sets. The ten reference groups were only confirmed when using the combination of SSRs and SNPs, which makes this marker set a suitable classification tool for unknown cocoa cultivars (Fig 1). Thus, this marker set was used to assess the population structure and genetic diversity of the Vietnamese cultivars using Bayesian clustering and principal coordinate analysis, respectively. The obtained results provide a strong genetic basis for the Vietnamese cocoa industry which could also be useful in other countries for future breeding programs in terms of propagation, dissemination and production of single origin chocolate.

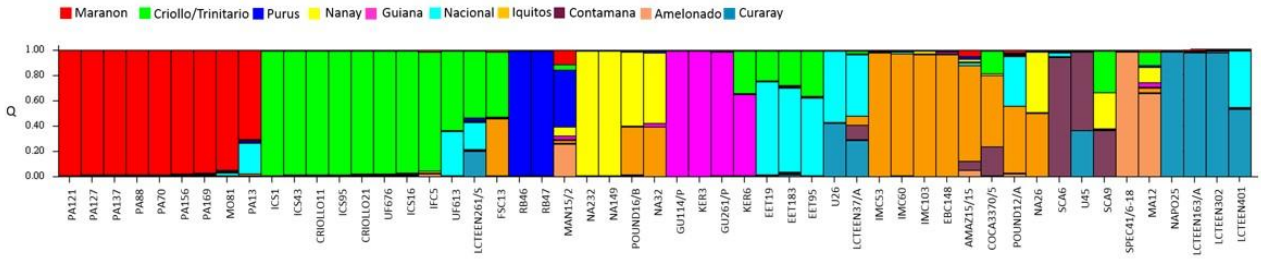


Fig 1: Bayesian clustering of 53 international cocoa cultivars, representing ten genetic groups of Motamayor et al. (2008), using the combination of SSRs and SNPs.

References:

[1] De Wever J, Everaert H, Coppieters F, Rottiers H, Dewettinck K, Lefever S, Messens K, (2019), The development of a novel SNP genotyping assay to differentiate cacao clones, Scientific reports, Submitted

[2] Everaert H, Rottiers H, Pham PHD, Ha LTV, Nguyen TPD, Tran PD, De Wever J, Maebe K, Smagghe G, Dewettinck K, Messens K , (2017), Molecular characterization of Vietnamese cocoa genotypes (Theobroma cacao L.) using microsatellite markers., Tree genetics & genomes, 13:99

[3] Motamayor JC, Lachenaud P, da Silva EMJW, Loor R, Kuhn DN, Brown JS, Schnell RJ , (2008), Geographic and genetic population differentiation of the Amazonian chocolate tree (Theobroma cacao L.), PLoS One, 3(10):e3311

Oral Session Cocoa 09.03

Establishment of some health benefits in consumption of cocoa powder among Secondary Schools Students in Southwest Nigeria.

Christina Olayinka Jayeola, Samsedeen Oluwasegun Aroyeun, Semiu Ogunwolu, Lateef Eugene Yahaya, Busayo Solomon Famuyiwa, Justina Oluyemisi Lawal, Olaide Williams, Olayiwola Olubamiwa
Cocoa Research Institute of Nigeria, Cocoa Research Institute of Nigeria, Ibadan, Nigeria

Text There are increasing literature evidence and anecdotal reports worldwide on the health benefits of cocoa powder consumption. Regular consumption of cocoa powder beverage (95-100% cocoa powder) has been shown to combat malaria, diabetes and increase wellness. The study specifically established reports of some health benefits of cocoa consumption such as include; increase in wellness, educational performance and

alertness through consumption of cocoa powder. The study was carried out in 4 states; Ogun, Oyo, Ekiti and Osun in Southwest Nigeria. Two thousand four hundred respondents; between the ages of 10-15 years were randomly selected from the class register of 12 secondary schools and where parent negated inclusion; students were substituted. They were included for daily consumption of cocoa drink for 8 weeks. Permissions were obtained from parents and the ministries of education and health. Information was solicited using key interpretative methods; Participant observers, unstructured interview, recorded video and individual class results before and after. The study revealed that 50.17% were male while 49.83% were female, the mean scores of educational performance before was 4.70 + 1.39, and 6.8 + 2.17 after. Reports from participants' observers claimed improvement in class attendance, student educational performances; increase in wellness while 62.17% liked the taste, 64% preferred it to other drink and 81.65% like it as a daily drink. Responses were also documented from their Teachers and some parents who were persuaded for their wards inclusion wished the program be emulated by the government. It was concluded that consumption of cocoa powder and cocoa products will increase cocoa market and consequently achieve better price for cocoa farmers and combat some health challenges.

Keywords: Cocoa powder, Health benefit, government, Ekiti

Oral Session Cocoa 09.04

Extrusion as an alternative method for cocoa alkalization

Damián Valverde García, Virginia Sánchez Jiménez, Édgar Pérez Esteve, José Manuel Barat Baviera
Universidad Politècnica de València, Valencia, Spain

Text The production of cocoa comprises a succession of steps that leads from the ripe pods to the powder. Natural cocoa powder is characterized by its acidity, its astringent taste and its light color, features modified by the industry by employing a method called alkalization. It consists of treating cocoa with an alkaline solution at high temperatures and pressures, leading to a darker, less acidic and less astringent product [1]. From an industrial point of view, traditional alkalization has two important drawbacks: it is realized in batches and it is a slow process needing up to three hours [2], [3], [4]. Thus, cocoa industry is looking for alternative techniques able to darken the samples in the same way than the traditional method, but in a faster and continuous fashion. In this work, extrusion has been tested as an alternative technology for alkalizing natural cocoa powders. The objectives are determining if extrusion darkens the samples as much as the traditional method and its impact over the functional properties. The different conditions employed in this work were: two different alkalis (sodium hydroxide and potassium carbonate), different temperatures (ranging from 80 to 160ºC), water contents (20, 25 and 30%) and concentrations of alkali (0, 1, 3,5 and 6%). Once produced, the extruded samples were analyzed and compared to commercial cocoas in terms of their color, pH, moisture and functional properties (antioxidant activity, total phenol content and the concentrations of catechin and epicatechin). The results show that the extruded samples classified as moderately and strongly alkalize have values of color coordinates similar (and even lower,

meaning darker and more reddish samples) than the commercial references. In relation to the evaluated functional characteristics, the extruded cocoas have similar or lower levels in the different parameters than the commercial powders, being an exception the strongly alkalized samples, which have a higher antioxidant activity than their commercial references. This shows that, in harsh conditions, extrusion is less aggressive than the traditional method. All the results point out that extrusion is a fast and continuous technology able to darken the samples even more than the conventional process and that the impact on functional properties is similar to the traditional one.

References:
[1] De Zaan cocoa, (2013), Cocoa & Chocolate manual, ADM Cocoa
[2] Brandon, M. J., Terink, J. L. , (1984), Alkalized cocoa powders and foodstuffs containing such powders, patent: US4435436A
[3] Ellis, L. D. , (1992), Process for making dark cocoa, patent: US5114730A
[4] Wissgott, U. , (1998), Process of alkalization of cocoa in aqueous phase, patent: US4784866A

Oral Session Cocoa 09.05

Optimization of microwave-assisted extraction of bioactive compounds from cocoa bean shells

Ana Cristina Mellinas Ciller, Alfonso Jiménez, Maria Carmen Garrigós
Analytical Chemistry, Nutrition & Food Sciences Department, University of Alicante, Alicante, Spain

Text 1.Introduction

Cocoa bean shell (CBS) is the major by-product of the cocoa bean obtained during processing. Tons of CBS residues are necessary to be disposed of after processing into chocolate or other cocoa derivatives[1][2]. The aim of this work is to optimize an extraction method for the active compounds present in CBS by microwave-assisted extraction by using response surface methodology. The obtained extracts have been fully characterized and functional properties were also evaluated.

2.Methods

The effect of four different factors (pH, time, temperature, solid/liquid ratio (S/L)) on the extraction process was evaluated by a Box-Behnken design (BBD) at 3 response levels. This design consists of 29 experimental runs. The responses obtained from the BBD were evaluated by determining the extraction yield, uronic acid content, total polyphenols content (TPC) and antioxidant capacity by the Ferric Reducing Antioxidant Power assay (FRAP). A regression analysis was performed from all experimental data and a second order polynomial model was used for fitting to obtain optimal synthesis conditions.

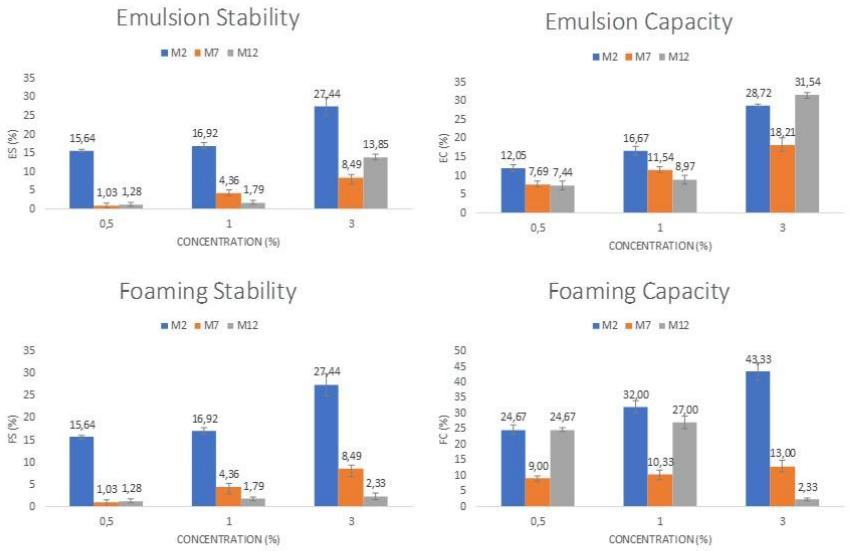
3.Results and discussion

The obtained model was able to explain the different results showing >90 % correlation in all cases (Table 1). Optimum extraction conditions for CBS included high pH values (12), low extraction times (5 minutes), high temperatures (100 ºC) and 0.04 g mL^{-1} S/L. The MAE process was highly influenced by pH. For this reason, pH was varied at 2, 7 and 12 (M2, M7 and M12 samples, respectively) while the others factors were fixed under optimal

conditions. The obtained extracts are rich in active compounds, such as polysaccharides, proteins and polyphenols, with high antioxidant properties. CBS extracts showed improved functional properties (Figure 1) and they can be considered as interesting materials for active packaging applications within the food manufacturing and agricultural sectors.

	R2	Desirability function (di)	Observed* (mean ± SD)	Predicted** (value ± SD)
Yield (%)	0.94	0.952407	34.2 ± 0.2	35.8 ± 5.3
Uronic acid (mg AGlc/g CBS)	0.98	0.956524	115.2 ± 10.0	103.6 ± 11.9
TPC (mg AG/g CBS)	0.95	0.932567	35.9 ± 0.9	30.2 ± 5.1
FRAP (mg Trolox/g CBS)	0.99	0.965928	35.5 ± 0.4	33.8 ± 2.7

Results under optimal conditions for each variable and desirability functions obtained in the BBD (*n=3, ** confidence interval at 95% level)



Functional properties of CBS extracts obtained under optimal conditions.

References:

[1] Martínez, R., Torres, P., Meneses, M. A., Figueroa, J. G., Pérez-Álvarez, J. A., & Viuda-Martos, M, (2012), Chemical, technological and in vitro antioxidant properties of cocoa (Theobroma cacao L.) co-products, Food Research International, 39-45

[2] Panak Balentić, J., Ačkar, Đ., Jokić, S., Jozinović, A., Babić, J., Miličević, B., … Pavlović, N, (2018), Cocoa Shell: A By-Product with Great Potential for Wide Application, Molecules, 23 (6)

Oral Session Cocoa 09.06

Sustainable recovery of cocoa bean shell for the production of a novel functional beverage with antioxidant and antidiabetic properties

Olga Rojo-Poveda[1, 2], Letricia Barbosa-Pereira[1], Livia Mateus-Reguengo[1], Marta Bertolino[1], Caroline Stévigny[2], Giuseppe Zeppa[1]
[1]Department of Agriculture, Forestry and Food Sciences (DISAFA), University of Turin, Turin, Italy, [2]RD3 - Pharmacognosy, Bioanalysis & Drug Discovery Unit, Université Libre de Bruxelles, Brussels, Belgium

Text The reuse of food by-products within the frame of a circular economy is becoming crucial due to economical and environmental reasons. Cocoa bean shell (CBS) is a main by-product of cocoa industry, representing approximately 12% of the total bean[1]. CBS discard could be expensive and produce environmental problems. Besides, CBS represents a source of polyphenols and dietary fiber that could make it useful as food ingredient/additive. The high polyphenol content of CBS could also give it antidiabetic properties, which is of big interest considering that the WHO estimates that 422 million people worldwide were living with diabetes in 2014, and this number will double by 2030[2].
The purpose of this work was to develop and to optimize preparations for a new home-made functional beverage based on CBS.
Different types of beverage production techniques (Moka pot, Neapolitan flip coffee pot, French press, Espresso, Capsule and American coffee maker) used with CBS grinded at different degrees (ranging between 250 µm and 4 mm) were studied. The influence of these factors on the sensory characteristics and chemical composition of beverages was defined. The antioxidant capacity and the total phenolic, tannin and flavonoid content of the obtained beverages were determined with colorimetric assays and the polyphenols characterization was performed by HPLC-PDA analysis. Antidiabetic properties were determined with the α-glucosidase inhibition assay.
The various techniques and grinding degrees allowed to obtain several beverages with different chemical and sensory characteristics. Several compounds were identified and quantified by HPLC-PDA (phenolic acids, flavan-3-ols, quercetin-3-O-glycosides, catechin-3-O-glycosides and procyanidins). These compounds showed high correlation with the total phenolic content values (up to 1803.83 mg gallic acid equivalents/L), total flavonoid content (up to 566.42 mg catechin equivalents/L), total tannin content (up to 334.64 mg catechin equivalents/L), antioxidant capacity (up to 7.29 mmol Trolox equivalents/L) and antidiabetic properties (up to 52.0% of α-glucosidase inhibition) displayed.
The CBS could therefore represent an optimal ingredient for the production of functional beverages with potential health benefits for the consumers, reducing the environmental and economic impact of the by-product disposal.

References:
[1] EFSA, (2008), 1-66
[2] World Health Organization, Global Report on Diabetes 2016

Oral Session Cocoa 09.07

German cacao of Cameroon - new facts on a traditional variety fallen into oblivion

Nicolas Niemenak[1], Lina Stoll[2], Reinhard Lieberei[2], Bernward Bisping[2]
[1]00237698195641, Yaounde I, Yaounde, Cameroon, [2]Biozentrum Klein Flottbek, University of Hamburg, Hamburg, Germany

Text “German” cacao cultivated in Cameroon has emerged from a mixture of different gene pools with a large proportion of Trinitario and with a pronounced content of polyphenols. In order to characterize this old genotype, polyphenols and polyphenol oxidase were compared with hybrid selected genotypes. Epicatechin (25 mg/g - 52 mg/g fat free dm) and catechin (0.5 - 1.9 mg/g fat free dm) content of German cacao seeds were of similar range with hybrid investigated samples. German cacao is characterized by its high content of anthocyanins especially cyanidine-3-arabinoside which ranges from 8.84 mg/g to 17.51 mg/g fat free dm. Hybrid genotypes displayed 1 mg/g to 6.4 mg/g fat free dm of cyanidine-3-arabinoside. PPO activity was 10 to 20-fold higher in German cacao seeds compared to hybrid. Anthocyanin and PPO through the oxidation of phenols to quinone are involved in colour development and pests and diseases resistance. Pigment is one of the most important factors for the colour of cocoa powder. We discuss the high content of anthocyanin and PPO activity in German cacao in relation with the reddish colour of cocoa powder derived from Cameroonian cacao.
Key words: anthocyanin, polyphenol oxidase, polyphenols, cacao, Cameroon

Oral Session Cocoa 09.08

Quality attributes and consumer acceptability of single-origin cocoa liquors from highly-recommended cacao clones in the Philippines

Joel Juvinal[1, 2], Hans De Steur[1], Sofie Lagast[1], Joachim Schouteten[1], Alma de Leon[2], Koen Dewettinck[1], Xavier Gellynck[1]
[1]Ghent University, Ghent, Belgium, [2]Central Luzon State University, Science City of Munoz, Philippines

Text Preliminary genetic analysis and productivity experiments on Philippine cocoa varieties conducted by the country’s National Seed Industry Council determined highly recommended clones. However, information on bean quality and flavor is still lacking. Therefore, this study was conducted to determine the quality attributes and key volatile compounds in the cocoa liquor samples and to assess the acceptability of cocoa liquor beverage (*tablea*) among consumers.

Selected cocoa bean cultivars (UF18, BR25, and W10) were fermented, dried and sorted. Fermentative quality of cocoa beans was assessed using the cut test and fermentation index. Quality parameters (moisture content, pH, titratable acidity, total fat content were also determined. Fingerprinting of aroma volatiles was done using an electronic nose based on ultra-fast gas chromatography. Next, a locally popular hot beverage made from

solid cocoa liquor with sugar and non-dairy creamer was prepared for consumer testing. A total of 50 consumers (26 females, 24 males, mean age 19.2 (±0.7) evaluated the overall liking (9-point hedonic scale), sensory attributes (colour, mouthfeel, aroma, taste, aftertaste (just-about-right scale) and purchase intention (5-point Likert scale) of the samples.

Results showed significant differences ($p < 0,05$) among the three samples in all the quality parameters. UF18 had highest pH (6,56) and W10 the lowest (4,78). W10 had highest titratable acidity (6,30 meq NaOH g^{-1}) while UF18 had the lowest (1,75) and the highest fat content (52,1%). Aroma fingerprinting of volatiles revealed that W10 had highest peak area in terms of acidity and other desirable volatile compounds such as 3-Methylbutanal, pyrazine (chocolate), and acetophenone (fruity) followed by BR25 and UF18. Significant differences were found in the consumer's overall liking for the samples ($p < 0,05$). W10 was the most liked (8,2 ±0,3) followed by BR25 (7,4 ±0,5) and UF18 (5,9 ±0,3). Just-about-right (JAR) scores for the sensory attributes of the three cocoa liquor beverage samples indicated that W10 had the highest scores (>50%). Purchase intention was highest for W10 (4.1 ±0,2) followed by BR25 and UF18.

This is the first study that explored the quality characteristics and consumer acceptability of highly recommended cocoa beans from the Philippines. The results can guide processors in harnessing the flavor potential of local cocoa varieties as well as direct government efforts in pushing for a more robust cacao industry.

Oral Session Cocoa 09.09

Supervised and Unsupervised Classification of Cocoa Bean Origin and Processing using LCMS

Santhust, S.[1, 2], D'Souza, R. N.[2], Behrends, B.[2,] Kuhnert, N.[2], Ullrich, M.[2], Hütt, M.-T.[2]
1. Jacobs University, Jacobs University, Bremen, Germany
2. Jacobs University, Bremen, Germany

Text
Identifying the country-of-origin of cocoa beans (*Theobroma cacao*) based upon their chemical composition holds potential for multiple applications in the chocolate industry: from development of single origin chocolates to fine tuning of desired flavors [1,2]. Here we use a large data set comprising of 213 samples of fermented (113) and unfermented (77) cocoa beans, as well as selected cocoa liquors (23), sourced from various countries (8) and belonging to different stages of biochemical transformation in the cocoa processing pipeline. We study the classification of bean origin using an unsupervised and a supervised method of learning, PCA (Principal Component Analysis) and LDA (Linear Discriminant Analysis), respectively. We observe that while PCA can only provide a limited separation in bean origin (see [1]), the separation becomes better using LDA as a supervised method, as expected. In this case, a simple filtering criterion of compounds

based on a Gaussian distribution of intensities (Gaussian Feature Stability Requirement) dramatically enhances the classification of the samples according to bean origin. Essentially this method is capable of removing conflicting signals from the LCMS data, which limit the classification. In this form, the supervised learning holds the possibility to identify potential markers of a specific origin.
Going from classification to prediction, we show how well the country can be predicted from the LCMS data for a sample. Furthermore, we employ a range of methods for extracting the minimal set of compounds required for such a prediction.
On a more theoretical level, we test the classification using a binarized version of LC-MS profile representing presence (1) or absence (0) of compounds rather than their actual intensity values. We observe that the mere presence or absence of compounds can still provide a good amount of classification, which suggests alternative strategies of biomarker search.

References:

[1] D'Souza, R. N., Grimbs, S., Behrends, B., Bernaert, H., Ullrich, M. S., & Kuhnert, N. (2017). Origin-based polyphenolic fingerprinting of Theobroma cacao in unfermented and fermented beans. Food research international, 99, 550-559.

[2] Kumari, N., Grimbs, A., D'Souza, R. N., Verma, S. K., Corno, M., Kuhnert, N., & Ullrich, M. S. (2018). Origin and varietal based proteomic and peptidomic fingerprinting of Theobroma cacao in non-fermented and fermented cocoa beans. Food research international, 111, 137-147.

Session 10 Coffee processing and quality

Oral Session Coffee 10.01

Quality & physiological markers in coffee breeding

Jwanro Husson, Charles Lambot, Stéphane Michaux, Eric Goulois, Nathaly Vial, Jérôme Spiral, Fabrizio Arigoni
Nestle Research - Plant Science Research Unit, 37097 Tours, France

Text Coffee is a popular drink worldwide and is enjoyed everyday by millions of people. The coffee drink quality and green coffee supply chain sustainability need continuous improvement in order to satisfy consumer demands. There are two coffee species (*Coffea arabica* and *C. canephora*) represented by a number of coffee varieties with different quality criteria and agricultural performances. Biochemistry is used in breeding programs for different purposes and could be particularly interesting to select new varieties for

quality but also for some agricultural traits like drought tolerance. Sensory quality parameters are influenced by different factors (genetic, environment, process) and their interactions making the selection process complicate. Biochemistry can afford reliable and practical solutions with markers for quality and appropriate techniques of analyses which will help the breeders to select the best varieties for the appropriate market.
Our study shows the role and contribution of biochemistry in coffee breeding programs by providing valuable information and biomarkers for cup quality. Several biochemical markers are identified and quantified in coffee beans or leaves: quality markers such as caffeine and lipids are used to select coffee varieties for sensory attributes. The same approach can be used for traits related to the agricultural performance like drought tolerance. Other biochemical markers are identified and associated to physiological traits for drought tolerance selection. The combination of Near Infra-red spectroscopy (NIR) and analytical tools such as HPLC and GCMS, sensory analyses, and physiological measurements with appropriate equipments is powerful to identify biochemical markers and build predictive models for the benefit of coffee breeders.

Oral Session Coffee 10.02

On-line coffee roast control using photoionization mass spectrometry: prediction of bean color and antioxidant capacity.

Jan Heide[1], Hendryk Czech[1], Patrick Martens[1], Michael Wendler[1], Sven Ehlert[2], Andreas Walte[2], Thomas Koziorowski[3], Ralf Zimmermann[1]
[1]Universität Rostock, Rostock, Germany, [2]Photonion GmbH, Schwerin, Germany, [3]PROBAT-Werke von Gimborn Maschinenfabrik GmbH, Emmerich, Germany

Text Being one of the most popular beverages, coffee has a high economic value. Coffee roasting is a chemically highly complex process. On-line analysis of the coffee roasting off-gas enables monitoring of the roasting phase [1], its color [2] and health-promoting properties such as antioxidant capacity [3].
We applied laser-based single-photon ionization (SPI) at 118 nm and resonance-enhanced multi-photon ionization (REMPI) at 248 nm combined with time-of-flight mass spectrometry (Photonion GmbH, Schwerin/Germany), which was coupled to a drum roaster of 100 g capacity (Probat Werke, Emmerich, Germany). While REMPI refers to a selective ionization technique for aromatic compounds, SPI denotes a more universal technique which ionizes compounds with ionization energies below the photon energy of 10.5 eV. The roasted Colombian Arabica beans were analyzed regarding color (Colorette 3b, Probat Werke Emmerich, Germany) and antioxidant capacity (total phenolic content, TPC) by Folin–Ciocalteu assay. Color and TPC were taken as response variable in a PLS regression with mass spectra at the coffee drop temperature as predictor.
PLS regression as performed with the full mass spectra with the exclusion of m/z related to caffeine because it does not arise from chemical reactions rather than evaporation. Analyses of target projection loadings and selectivity ratios of the PLS loadings revealed that substituted furans, such as furfural and hydroxy-furfural had the highest influence on both models. Those furans mainly result from caramelization reactions of monosaccharides and higher sugars, which are partially responsible for the darkening of the bean color. Although bean color and TPC are correlated, TPC cannot be derived with

sufficient precision.
Coffee bean color and TPC are predicted from roasting off-gas with 1 s time resolution by photoionization mass spectrometry (PIMS).

References:
[1] Czech, Hendryk, (2016), Resolving Coffee Roasting-Degree Phases Based on the Analysis of Volatile Compounds in the Roasting Off-Gas by Photoionization Time-of-Flight Mass Spectrometry (PI-TOFMS) and Statistical Data Analysis: Toward a PI-TOFMS Roasting Model, Journal of agricultural and food chemistry, 5223–5231, 25
[2] Wieland, Flurin, (2012), Online monitoring of coffee roasting by proton transfer reaction time-of-flight mass spectrometry (PTR-ToF-MS): towards a real-time process control for a consistent roast profile, Analytical and bioanalytical chemistry, 2531–2543, 8
[3] Smrke, Samo, (2013), How does roasting affect the antioxidants of a coffee brew? Exploring the antioxidant capacity of coffee via on-line antioxidant assays coupled with size exclusion chromatography, Food & function, 1082–1092, 7

Oral Session Coffee 10.03

Do coffee particles swell?

Verena Hargarten, Michael Kuhn, Heiko Briesen
Chair of Process Systems Engineering, Technical University of Munich, Freising, Germany

Text Coffee brewing is significantly influenced by the coffee particles' size distribution. It affects the flow rate through the particle packing as well as the diffusive transport from the inside of the grains. The initial wetting of coffee particles and its impact on their size is therefore crucial for accessing the following extraction. Several authors [1], [2] have described that water ingress led to swelling and hence to an increase in the particles' volume by more than 20 %, whereas others [3], [4] did observe no or a distinctly lower volume increase.
This study shall provide clarification about the effect of water on the dimensions of coffee particles covering the entire particle size distribution of differently ground coffee beans. Particles are analysed by laser diffraction analysis and microscopy. We have discovered that a slight increase in size of coarse particles with a mean diameter of above 1.4 mm is visible under the microscope. Laser diffraction measurements of sieved particle fractions revealed an average increase in size by around 15 %, independent of the initial particle diameter, which corresponds to a volume increase of about 52 %. Coupling force/displacement measurements with pressure filtration, effects of percolation on the volume and compressibility of the whole packing of coffee particles are additionally examined.
Our work enables coffee researchers to better understand the impact of wetting on the coffee cake structure during the brewing process. It will prevent false assumptions when designing processing strategies and models.

References:
[1] Spiro, M., Toumi, R., & Mangayetkarasy, K., (1989), The Kinetics and Mechanism of Caffeine Infusion from Coffee: The Hindrance Factor in Intra-bean Diffusion, Journal of the

Science of Food and Agriculture, 349-356,
https://onlinelibrary.wiley.com/doi/abs/10.1002/jsfa.2740460313
[2] Mateus, M.-L., Rouvet, M., Gumy, J.-C., & Liardon, R., (2007), Interactions of Water with Roasted and Ground Coffee in the Wetting Process Investigated by a Combination of Physical Determinations, Journal of Agricultural and Food Chemistry, 2979-2984,
https://pubs.acs.org/doi/10.1021/jf062841g
[3] Hinz, T., Steer, A., Waldmann, C., Cammenga, H.K., & Eggers, R., (1997), Röstkaffee-Extraktion: Einflußparameter und Modellierung, Chemie Ingenieur Technik, 685-690,
https://onlinelibrary.wiley.com/doi/abs/10.1002/cite.330690517
[4] Corrochano, B.J., (2015), Advancing the Engineering Understanding of Coffee Extraction, https://etheses.bham.ac.uk/id/eprint/7176/, University of Birmingham Research Archive, School of Chemical Engineering, University of Birmingham

Oral Session Coffee 10.04

Investigation of coffee roast gas composition and on-line process control in real-time using Photoionization MS

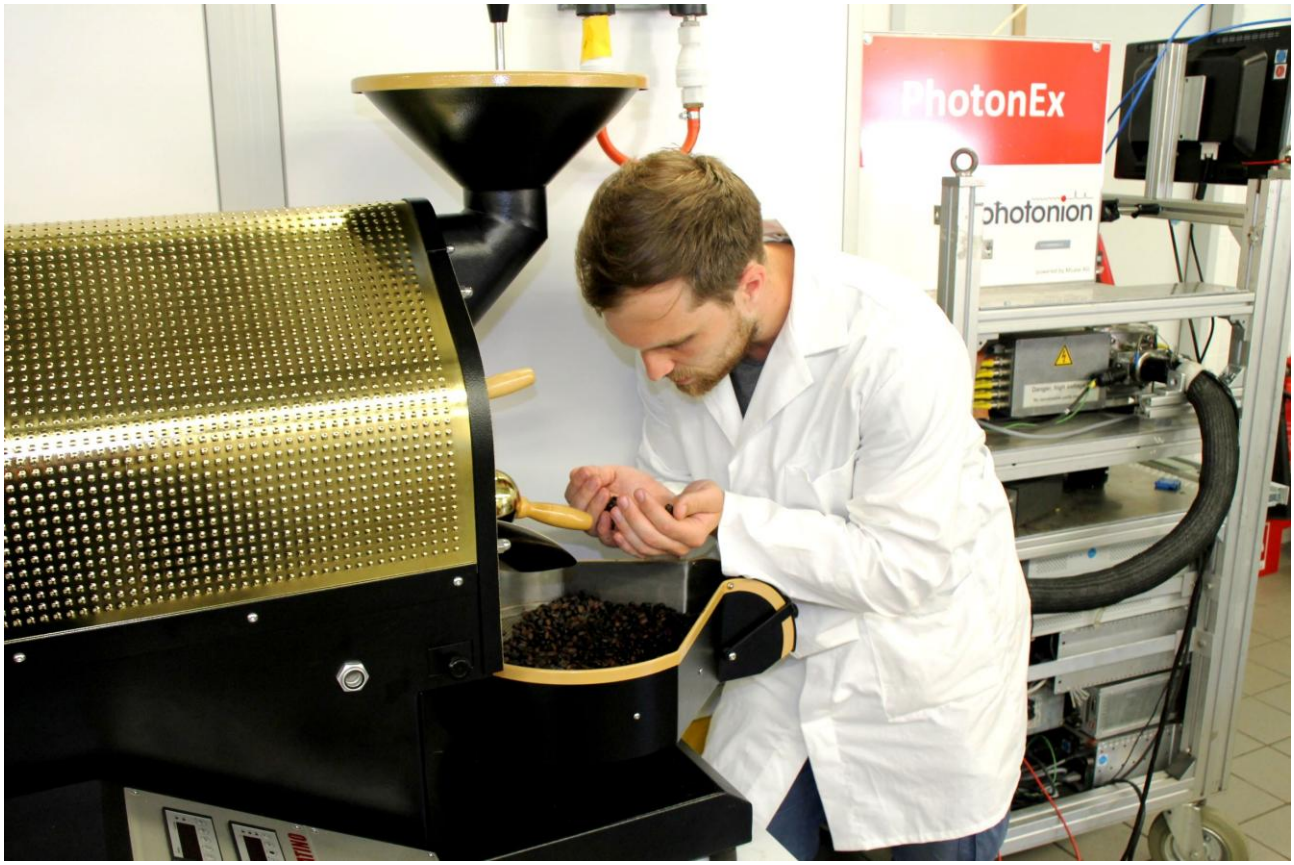
Sven Ehlert[1, 2], Jan Heide[2], Andreas Walte[1], Thomas Koziorowski[3], Ralf Zimmermann[2, 4]
[1]Photonion GmbH, Schwerin, Germany, [2]Dept. of Analytical Chemistry, University of Rostock, Rostock, Germany, [3]PROBAT-Werke von Gimborn Maschinenfabrik GmbH, Emmerich am Rhein, Germany, [4]Comprehensive Molecular Analytics, Helmholtz Centre Munich, Munich, Germany

Text The steps required to turn green coffee into a popular beverage are highly complex. Especially the roast process decides upon the quality and flavor of the coffee in the final cup. Photoionization MS (PIMS) allows to monitor and to control this roasting process on-line and in real time, whereby time resolutions in the range of seconds enable the investigation of fast and dynamic processes. [Czech] On the basis of the obtained mass spectra a prediction of actual properties such as bean color is possible as well as a targeted manipulation of the roast process.

Process overview

While the temporal resolution of one second is typically sufficient for process monitoring,

for specific scientific aspects a faster sampling rate is required. A new 248 nm Excimer Laser light source is used for vacuum ionization (REMPI – resonance enhanced multi photon ionization). A second immanent problem is the limited sensitivity caused by the suppression of low abundant species by main compounds such as caffeine. A promising new approach is the usage of a fast-pulsed ion source in combination with an oa-ToF MS (orthogonal acceleration – Time of Flight mass spectrometer). In principle, oa-ToF systems can only be used with continuously working ion sources. With a 1kHz Excimer laser for REMPI and the oa-ToF with advanced timing a precise selection of monitored mass ranges without a loss in temporal resolution can be obtained.
These developments can be transferred to other applications requiring a fast, selective investigation of complex gas mixtures without a time-consuming pre-separation.



Sensorical check of roasted coffee beans

References:
[Czech] Hendryk Czech et al., (2016), Resolving Coffee Roasting-Degree Phases Based on the Analysis of Volatile Compounds in the Roasting Off-Gas by Photoionization Time-of-Flight Mass Spectrometry (PI-TOFMS) and Statistical Data Analysis: Toward a PI-TOFMS Roasting Model, ACS Publications, J. Agric. Food Chem., 5223-5231, 64

Short Oral Communications

Oral Session Coffee 10.05

Transfer of volatiles and aroma precursors into the coffee seeds during fermentation, a reality?

Fatma Hadj Salem[1, 2], Marc Lebrun[1], Nathalie Sieczkowski[2], Antoine Collignan[3], Renaud Boulanger[1]
[1]CIRAD, Montpellier, France, [2]Lallemand SAS, Toulouse, France, [3]CIRAD, Montpellier SupAgro, Montpellier, France

Text For the consumer, the flavor is arguably the most important aspect of coffee. Thereby, the coffee industry has dedicated efforts in improving and controlling the final beverage quality using roasting and brewing steps [1]. Moreover, recent research studies have highlighted that the postharvest processing can have a direct impact on the quality and value of the final product, and they showed that wet processing offers a coffee with higher acidity and more aroma than dry and semi-dry processing [2]. Furthermore, de Melo Pereira et al., [3], Lee et al., [4] and D. de Carvalho Neto et al., [5] assumed that it might exist a diffusion process of microbial metabolites into the coffee beans during the fermentation, enhancing the final coffee quality. However, one question remains: are these molecules of interest (volatiles and aroma precursors) able to cross the different layers (mucilage and parchment) surrounding the coffee beans?

To answer this question, 7 labelled molecules were chosen to follow their transfer into coffee beans (extracted from frozen coffee cherries – unfermented) during simulated fermentation. The molecules transfer was studied in 4 media following a progressive experimental approach to evaluate the resistance of the different layers. The first medium contained green coffee beans without their mucilage and parchment, the second with green coffee beans and parchment, the third with depulped coffee, and the fourth with depulped coffee and yeast. 10g of coffee samples were submerged in distilled water concentrated in marked compounds; they were maintained at 25°C and under agitation (120 rpm) for 12h. Then, the labelled volatiles (butanal, 2-phenylethnaol, isoamyl acetate) were analyzed by SPME-GCMS, and the labelled aroma precursors (fructose, glutamic acid, alanine and lactic acid) were analyzed by GC-MS after extraction and derivation.

Results showed that all studied compounds (volatiles and precursors) could diffuse from water to the coffee beans in the 4 media. The comparison of the transferred molecules' amounts in the 4 media revealed that the parchment, with its fibrous structure, acts as a molecular filter. Related to the molecule conformation, only the 2-phenylethanol amount decreased significantly in coffee beans with parchment. Furthermore, during fermentation, it was shown that some molecules could interact with yeasts.

References:
[1] de Carvalho Neto, D. P., de Melo Pereira, G. V., Finco, A. M. O., Letti, L. A. J., da Silva, B. J. G., Vandenberghe, L. P. S., & Soccol, C. R, (2018), Efficient coffee beans mucilage layer removal using lactic acid fermentation in a stirred-tank bioreactor: Kinetic, metabolic and sensorial studies, Elsevier, Food Bioscience, 26 , 80-87
[2] Pereira, G. V. de M., Soccol, V. T., Brar, S. K., Neto, E., & Soccol, C. R, (2017), Microbial ecology and starter culture technology in coffee processing, Elsevier, Critical

Reviews in Food Science and Nutrition, 57(13), , 2775–2788
[3] de Melo Pereira, G. V., Neto, E., Soccol, V. T., Medeiros, A. B. P., Woiciechowski, A. L., & Soccol, C. R, (2015), Conducting starter culture-controlled fermentations of coffee beans during on-farm wet processing: Growth, metabolic analyses and sensorial effects. , Elsevier, Food Research International, 75, 348–356.
[4] Lee, L. W., Tay, G. Y., Cheong, M. W., Curran, P., Yu, B., & Liu, S. Q, (2017), Modulation of the volatile and non-volatile profiles of coffee fermented with Yarrowia lipolytica : II. , Elsevier, LWT, 80, 32–42
[5] de Carvalho Neto, D., de Melo Pereira, G., Tanobe, V., Thomaz Soccol, V., G. da Silva, B., Rodrigues, C., & Soccol, C , (2017), Yeast Diversity and Physicochemical Characteristics Associated with Coffee Bean Fermentation from the Brazilian Cerrado Mineiro Region. , Elsevier, Fermentation, 3(1), 11

Oral Session Coffee 10.06

Metabolomics Fingerprint of Philippine Coffee by SPME-GCMS for Geographical and Varietal Classification

Emelda Ongo[1], Veronica Sberveglieri[2], Giuiseppe Montevecchi[3], Fortunato Sevilla III[4]
[1]Industrial Technology Development Institute, Department of Science and Technology, Taguig City, Philippines, [2]CNR-IBBR, Institute of Biosciences and Bioresources,, Via Madonna del Piano 10, 50019 Sesto Fiorentino (FI), Italy, Italy, [3]bDipartimento di Scienze Agrarie e degli Alimenti, Universitá di Modena e Reggio Emilia,, Via G. Amendola 2, 42122 Reggio Emilia, Italy, Italy, [4]dUniversity of Santo Tomas, Graduate School,, Espana Blvd., Sampaloc, Manila, 1008, Philippines

Headspace metabolites of Philippine Arabica and Robusta coffees grown from different geographical origins were identified using solid-phase microextraction gas chromatography mass spectrometry (SPME-GCMS). A great number of metabolites with a wide variety of functional groups were extracted from two different coffee varieties. About forty prominent metabolites were identified in reference to the NIST spectral database (MS library) and twenty seven of which were confirmed using reference standards. The metabolomics fingerprint of Arabica coffee considerably differs with Robusta coffee and geographical origin slightly alters the fingerprint profile of coffee samples. Chemometric analysis such as principal component analysis (PCA) displays a good classification between Arabica and Robusta coffee samples. Although, Arabica coffee samples from different geographical origins were clustered separately from each other, the proximity of clusters between Arabica coffee samples which can be classified into one large group, indicated their close similarity of headspace metabolites. PCA also identified several key metabolites for the distinction of this group from Robusta coffees which is attributed to the higher amount of maltol, acetic acid, 2-furancarboxaldehyde, 1-H-pyrrole-2-carboxaldehyde and lower concentration of phenol and 4-ethyl-2-methoxyphenol in all Arabica samples. These discriminating compounds could be useful quality markers to differentiate Arabica with Robusta coffee. Results revealed that the headspace metabolites in coffee provides significant information on its inherent aroma quality. Also, the findings suggested that the overall quality of Philippine coffee is variety and region specific.

Keywords: headspace metabolites, Arabica, Robusta, geographical origin, SPME-GCMS, PCA,

Oral Session Coffee 10.07

Biological control of coffee leaf rust *Hemileia vastatrix* Using Mycoparasites Collected from Coffee Arabica Leaves, in Ethiopia

Kifle Belachew Bekele
Ethiopian Institute of Agricultural Research, Jimma Ethiopia, Jimma University, Jimma, Ethiopia

Text Abstract
Coffee leaf rust (CLR), caused by *Hemileia vastatrix* is the main coffee disease, occurring in the main coffee producing countries World wide. CLR management has relied on escaping the disease through highland plantation in the tropics, breeding for resistance and fungicide spraying. The increasing losses caused by CLR, because of failures of the predominant methods and climate change have led to the search for novel approaches for the old methods and to the exploration of new or neglected alternatives. This is the case of biological control. A search for fungal antagonists of *H. vastatrix* started in late 2014 led to the discovery of diversity of mycoparasites fungi for use in biological control. In this study, an analysis was made of the possible reduction of CLR severity and germination inhibition of *H. vastatrix* using anatagonst mycoparasites. A combination of disease severity evaluation on leave discs and germination inhibition was performed. The CLR severity was significantly different and higher on untreated rust-inoculated coffee leave discs. As the result indicate that from tested 92 isolates seven of the Isolates siginificuntly decrease the germination of *H. vastatrix.* Moreover, five of the isolates effectively reduce the growth of *H. vastatrix* on the coffee leaf disc. There for we have great potential to obtain biocontrol agents to control coffee leaf rust in the world. These results indicated that *five of the isolates namely Simplicillium, Pleurodesmospora, Cladosporium, Lecanicillium* and *Paraphaeosphaeria* species has good potential for use as a biocontrol agent against CLR, as observed during 50 days of evaluation, through a putative induction of resistance and a direct effect on *H. vastatrix.*

References

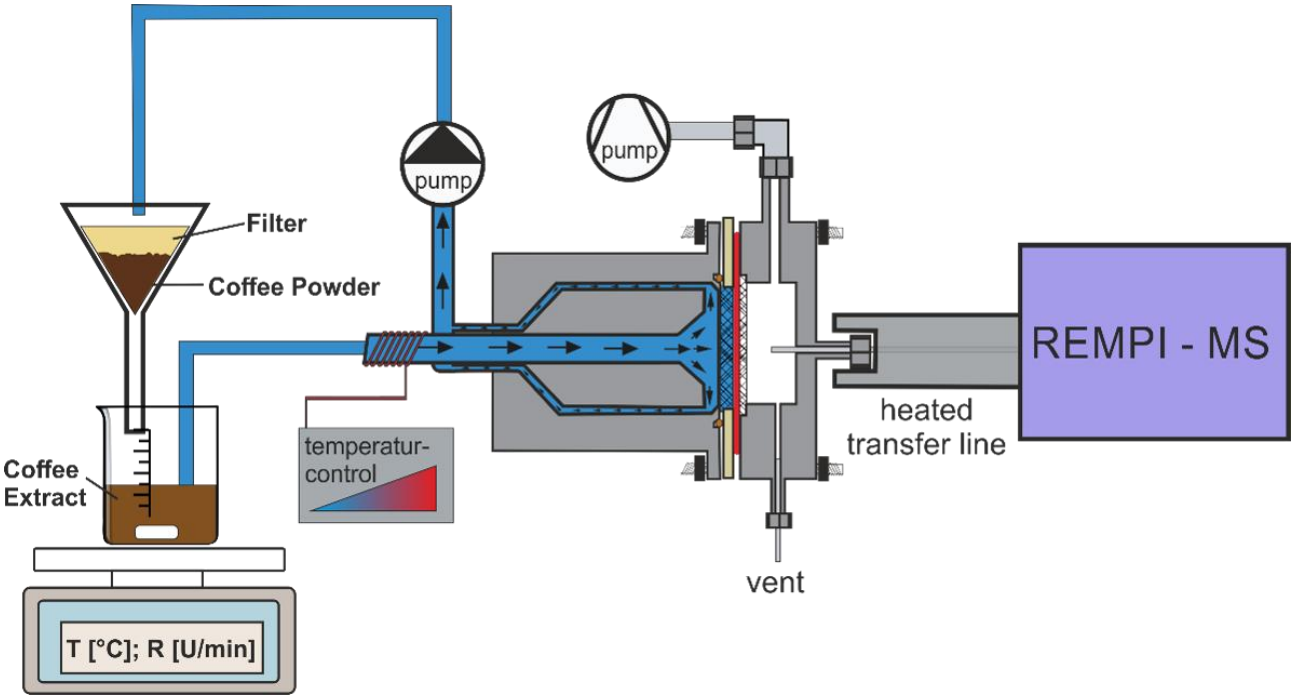
Carrión G, Rico-Gray V. (2002). Mycoparasites on the coffee rust in Mexico. Fungal Diversity 11: 49–60.
Chen, WH, Han YF, Liang ZQ, Jin DC. (2016). *Lecanicillium araneogenum* sp. nov., a new araneogenous fungus. Phytotaxa 305(1): 029–034.
Edgar RC. (2004). MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32: 1792-1797.
Gams W. (1971). Cephalosporium-artige Schimmelpilze (Hyphomycetes). Gustav Fischer Verlag, Stuttgart. 262 pp.
Gomes AAM, Pinho DB, Cardeal ZL, Menezes HC, Queiroz MV, Pereira OL. (2018). *Simplicillium coffeanum*, a new endophytic species from Brazilian coffee plants, emitting

Oral Session Coffee 10.08

MIMS for Coffee – Membrane Inlet Photoionization MS as an on-line investigation tool for liquid coffee samples

Sven Ehlert[1, 2], Christian Gehm[2], Jan Heide[2], Andreas Walte[1], Ralf Zimmermann[2, 3]
[1]Photonion GmbH, Schwerin, Germany, [2]Dept. of Analytical Chemistry, University of Rostock, Rostock, Germany, [3]Comprehensive Molecular Analytics, Helmholtz Centre Munich, Munich, Germany

Text Coffee is one of the worldwide most popular beverages. Accordingly, there is also an intense scientific activity around the brown drink. The most used techniques are either LC-MS or solid phase extraction or related methods coupled to GC-MS. Vacuum PI is already known as a powerful tool for the investigation of complex gas samples and is used in the coffee related field for the on-line analysis of coffee roast gases [1], [2] . However, coffee is typically served as a liquid extract of the roasted and grounded beans. The most reasonable approach is therefore to have a direct look into the cup. Membrane inlet coupled to a photoionization (PI) mass spectrometer enables a new view on coffee by extracting dissolved analytes directly into the vacuum of the MS without using pre-separation techniques.
For the presented experiments, a polydimethylsiloxane (PDMS) flat sheet membrane was used. The coffee extract was pumped with 150 ml/min along the membrane. The applied 266 nm REMPI (Resonance Enhanced Multi Photon Ionization) 10 Hz ion source was used to achieve highly sensitive and selective results for aromatic structures, with the positive side effect to suppress small highly abundant molecules with low informational content.
Varying the membrane material enable a selective view on specific substance classes and potential target compounds. A further advantage of using the membrane is the capability of substance accumulation and triggered release by a heating of the membrane.



Shematic of MIMS photoionization setup for liquid coffee extract

References:
[1] Hertz-Schünemann et al., (2013), Looking into individual coffee beans during the roasting process: Direct micro-probe sampling on-line photo-ionisation mass spectrometric analysis of coffee roasting gases, Springer, Analytical and Bioanalytical Chemistry, 7083-7096, 22
[2] Hendryk Czech et al., (2016), Resolving Coffee Roasting-Degree Phases Based on the Analysis of Volatile Compounds in the Roasting Off-Gas by Photoionization Time-of-Flight Mass Spectrometry (PI-TOFMS) and Statistical Data Analysis: Toward a PI-TOFMS Roasting Model, ACS Publications, J. Agric. Food Chem., 5223-5231, 64

Session 11 coffee quality and origin

Oral Session Coffee 11 .01

Simplified Detection of 16-O-Methylcafestol by NMR Analysis

Annika Hoffmann, Jane van der Meulen, Gudrun Beckh
Quality Services International GmbH, Bremen, Germany

Coffee is one of the most consumed drinks in Germany. The two main species in world trade are *Coffea arabica* (Arabica) and *Coffea canephora* (Robusta) with Arabica having the bigger market share. According to Regulation (EU) No. 1169/2011 Article 7 "Food information shall not be misleading, particularly: as to the characteristics of the food and, in particular as to its nature, identity, properties, composition […]". Therefore a product labelled as "100 % Arabica" must contain 100 % Arabica coffee and shall not have added any Robusta coffee. Arabica and Robusta coffee beans look different in shape and size, so they can theoretically be distinguished visually. But due to natural variances differentiation can be quite difficult and is not possible for ground coffee. Therefore the

visual identification is not always possible / reliable and is very time consuming. 16-O-Methylcafestol has been identified as a marker substance in the past. This substance is only present in Robusta coffee and not in Arabica coffee. DIN 10779 describes a standardized method for the determination of 16-O-Methylcafestol in roasted coffee. This method is based on a HPLC method. Prior to analysis the sample has to be prepared by a fat extraction, saponification of the coffee lipids, after which the unsaponifiable substances can be isolated. This procedure is time consuming (approximately 7 hours for sample preparation only) and has been modified into a simplified LC-MS/MS method which gives results within 2 hours and is cheaper in material used.
In order to make analysis of 16-O-Methylcafestol faster, we have further simplified the method into an NMR method, which gives results within an hour as the sample preparation only takes about 30 minutes because no fat extraction and saponification are necessary. The sample is only weighed in and extracted in a solvent, which is then analysed with a Bruker Avance III HD 400 MHz equipped with a 5 mm PA BBI 400SI H-BB-D-05 Z probe. The method can be applied to raw and roasted coffee and has shown correlating results to the LC-MS/MS method. The method has been proven a good alternative to the extensive DIN 10779 method and by the modifications performed the analysis time in routine has been reduced from three working days to less than one working day and less material use.

Oral Session Coffee 11 .02

Tracing the geographical origin of roasted and green coffee using isotope fingerprints by EA-IRMS

Oliver Kracht, Christopher Brodie, Andreas Hilkert, Harald Hertle
Thermo Fisher Scientific (Bremen) GmbH, Bremen, Germany

Text Coffee is one of the most popular beverages worldwide, sourced from different geographical regions and exported through a commercial chain that usually involves several intermediates. To ensure that coffee beans come from labelled locations, laboratories need an analytical solution, enabling to discriminate geographical origin, with a special emphasis on the country of origin.

Detecting food and beverage fraud can be achieved using stable isotope measurements because stable isotopes can differentiate between food and beverage samples which otherwise share identical chemical composition. Roasted and green coffee beans have a fingerprint, a unique chemical signature that allows them to be identified: isotope fingerprints of carbon, nitrogen, sulfur, hydrogen and oxygen have been reliably used for origin, authenticity and product label claim verification.

In this presentation, an overview of the interpretation of isotope fingerprints and the technology used is provided. We report data that show how stable isotopes offer conclusive answers on questions associated with origin, adulteration and correct labeling of food and beverage products. Isotope measurements from green and roasted coffee beans measured using the Thermo Scientific™ EA IsoLink™ IRMS System illustrate how isotope fingerprints can determine the origin of coffee beans. Consequently, it is evident

that isotope fingerprint approach helps support legislation on food integrity and labelling (EC Reg. No. 1169/2011) and product geographical indication/origin (EC Reg. No. 510/2006) and therefore, protect consumers and brands.

Oral Session Coffee 11 .03

Chlorogenic acids as biomarkers for coffee quality

Sabur Badmos, Nikolai Kuhnert
Jacobs University Bremen, Jacobs University Bremen, Bremen, Germany

Text Background: *Coffea arabica* and *Coffea canephora* (Robusta coffee) are the most commonly consumed coffee varieties globally. In this study, NMR and LC-ESI-MS^n techniques were employed to profile and quantify the most abundant chlorogenic acid in 54 different samples of the two coffee varieties from diverse origins of the world.
Results: Mono-caffeoyl quinic acids were found to show no variations if the two coffee varieties were compared. Significant variations were observed for feruloyl quinic acids, dicaffeoyl quinic acids and 5-sinapoylquinic acid. Additionally isomer ratios were explored as a potential marker for coffee authenticity along with a thorough statistical evaluation of rather extensive data set. Using key markers a LDA model was established for 100 % differentiation.

Oral Session Coffee 11 .04

GC-MS Profiling of Fatty Acids in Green Coffee (*Coffea arabica* L.) Beans and Chemometric Modeling for Tracing Geographical Origins from Ethiopia

Bewketu Mehari Workneh[1], Mesfin Redi-Abshiro[2], Bhagwan Singh Chandravanshi[2], Sandra Combrinck[3], Rob McCrindled[4], Minaleshewa Atlabachewe[5]
[1]Department of Chemistry, University of gondar, Gondar, Ethiopia, [2]Department of Chemistry, Addis Ababa University, Addis Ababa, Ethiopia, [3]Department of Pharmaceutical Sciences, Tshwane University of Technology, Pretoria, South Africa, [4]Department of Chemistry, Tshwane University of Technology, Pretoria, South Africa, [5]Department of Chemistry, Bahir Dar University, Bahir Dar, Ethiopia

Text Background: This study was aimed at the development of objective analytical method capable of verifying the production region of the coffee beans. One hundred samples of green coffee (*Coffea arabica* L.) beans from the major producing regions, comprising various sub-regional types, were studied for variations in their fatty acid compositions by using gas chromatography coupled with mass spectrometry. Principal component analysis (PCA) was used to visualize data trends. Linear discriminant analysis (LDA) was used to construct classification models.
Results: Twenty-one different fatty acids were detected in all of the samples. The total fatty acid content varied from 83 to 204 g kg−1 across the regions. Oleic, linoleic, palmitic, stearic and arachidic acids were identified as the most discriminating compounds among the production regions. The recognition and prediction abilities of the LDA model for

classification at regional level were 95% and 92%, respectively, and 92% and 85%, respectively, at sub-regional level.
Conclusion: Fatty acids contain adequate information for use as descriptors of the cultivation region of coffee beans. Chemometric methods based on fatty acid composition can be used to detect fraudulently labeled coffees, with regard to the production region. These can benefit the coffee production market by providing consumers with products of the expected quality.

Session 12 New products

Oral Session Tea 12.01

Understanding the carbohydrate composition of kale tea

Roberto Megias Perez[1], Christoph Hahn[2], Ana Isabel Ruiz Matute[3], Dirk Albach[2], Nikolai Kuhnert[1]
[1]Jacobs University Bremen, Bremen, Germany, [2]Carl von Ossietzky University of Oldemburg, Oldemburg, Germany, [3]Instituto de Quimica Organica CSIC, Madrid, Spain

Text The high interest in kale *(Brassica oleracea* var. *sabellica)* consumption is based on the different health-promoting benefits reported for its regular intake such as anticancerogenic activity and protection of the cardiovascular system (1). Apart from its use in cuisine, kale leaves are also widely employed in the elaboration of new products such as commercial teas.
There is scarce information on carbohydrate composition in kale teas or the raw material. Thus, in this work, a comprehensive characterization (qualitative and quantitative) of the low molecular weight carbohydrate (LMWC) was performed by the use of different chromatographic techniques (gas chromatography and hydrophilic interaction liquid chromatography) coupled to mass spectrometry.
Leaves from different kale genotypes and different farming conditions (25°C and 2°C) were freeze-dried to obtain kale powder. The carbohydrates were extracted using 100 mg of kale powder and 10 mL of water during 1 hour. GC-MS analyses were performed on a ZB-5 (5% phenylmethylsiloxane) capillary column (25 m × 0.25 mm, 0.25 µm). Previous to the GC-MS analysis, LMWC were submitted to derivatization. LC-MS analyses were carried out on a BEH X-Bridge column, with a trifunctionally-bonded amide phase and the following characteristics: 150 mm × 3.0 mm; 3.5 µm particle size and 135 Å pore size.
Different LMWC were identified and quantified in kale leaves such as: monosaccharides (glucose, galactose, fructose), disaccharides (sucrose, maltose, melibiose); α-galactooligosaccharides (α-GOS) such as raffinose; sugar alcohols such as sorbitol and inositols (*myo*-Inositol and galactinol). In some of the identified LMWC (*myo*-Inositol and raffinose), different bioactive properties have been reported (2)(3).
The data on the LMWC composition would provide important information for consumers and professionals in the tea industry.
References
(1) Hahn C. et al Diversity of Kale (Brassica oleracea var. sabellica): Glucosinolate Content and Phylogenetic Relationships
(2) Papaleo E, et al. Contribution of myo-inositol to reproduction. European Journal of

Obstetrics & Gynecology and Reproductive Biology. 2009 December 2009;147(2):120-3.
(3). Gibson GR et al. The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nat Rev Gastroenterol Hepatol. 2017;14(8):491-502.

Oral Session Tea 12.02

Identification of Composition Kinetics of Traditional Korean Green Tea Producing Method by LC-MS

Seung-Hun Lee[1], Inamullah Hakeem Said[1], Anastasiia Shevchuk[1], Un-Jae Lee[2], Nikolai Kuhnert[1]
[1]Jacobs University Bremen, Bremen, Germany, [2]Keumro Handmade Tea, Jeollabuk-do, South Korea

Text Number nine meant ‘complete’ in ancient oriental countries. In the same context, nine times repeating method was regarded as the utmost efforts by human being. Nine repetitive parching and drying process, which is one of the Korean traditional green tea production methods, has been adapted from nine repetitive steaming and drying process used in Korean red ginseng production. In the Korean red ginseng method, an increase of health promoting saponins during repetition of the parching process has already been observed.[1] This nine times repeating method is known to the public to increase the value of the product.

In this research, we tried to provide scientific grounds to common conception whether the traditional 9 times repeating method really increase the value or not. We analyzed sequential samples by 9 times repetitive parching and drying of green tea leaves with two different sampling methods; extracting with organic solvent by sonication of finely ground tea leaves in order to analyze all the components in the leaves, and aqueous brewing following the traditional Korean tea ceremony in order to analyze the content of tea infusions consumed. The two different extracted teas were analyzed by LC-high resolution ESI-MS and LC-tandem ESI-MS to identify and quantify polyphenols and caffeine, following the published plant analysis methods.[2,3] It could be shown that repeated steaming increases the extractability of catechins.

Keywords: Korean traditional green tea; Nine times steaming and drying; Polyphenol; Korean tea ceremony

References

1. Do Wan Kim et al., Korean J. Oriental Physiology & Pathology 22(6):1557 ~ 1561, 2008
2. AnastasiiaShevchuk et al., Food Research International, Volume 109, July 2018, Pages 387-402
3. Inamullah Hakeem Said et al., Phytochem. Anal.2017,28, 454–464

Short Oral Communications

Oral Session Tea 12.03

Evaluation of Quality and Intensity of Astringent Taste of Green Tea Based on Mass Spectrometry-Based Targeted Metabolic Profiling of Phenolic Compounds

Zhuang, J.1, Gao, L.2, Xia, T.1
1. State Key Laboratory of Tea Plant Biology and Utilization, Anhui Agricultural University, Hefei, China
2. School of Life Science, Anhui Agricultural University, Anhui Agricultural University, Hefei, China

Text

Abstract
It is well known that the phenolic compounds are the predominant factors for astringent taste of tea beverage. However, the correlation between astringent taste and phenolic compounds of tea beverage remains limited understanding. Here, the aforementioned correlation was investigated via twice partial least-squares (PLS) analyses using 47 green teas as samples. On the basis of the first round of PLS analysis, the samples were assigned to four groups for different astringent qualities. Targeted metabolic profiling analysis enabled by the MRM mode of UPLC-QQQ-MS/MS revealed that compared with the samples in the fourth sample group (i.e., little astringent), the following metabolic pathways were upregulated: in the first sample group (i.e., coarse and astringent), proanthocyanidin condensations, myricetin, and quercetin glycosylations; in the second sample group (i.e., grassy and astringent), phenolic compound acylations and kaempferol glycosylation; in the third sample group (i.e., bitter and astringency), myricetin and quercetin glucosylations. Based on the second PLS analysis of each group sample, the key metabolic pathways contributing to the astringent intensity of the first, second and fourth groups samples were confirmed as the phenolic acids acylations. In the third group sample, several metabolic pathways together affect the astringent taste intensity. In this study, the biochemistry basis of astringency qualities and intensity of green tea were elucidated.

Keywords: Astringency; Green teas; Mass spectrometry-based targeted metabolic profiling; phenolic compounds.

Oral Session Tea 12.04

Assessment of tea plots across agro-ecologies in Nigeria for cultivation expansion

Rotimi Ipinmoroti
Cocoa Research Institute of Nigeria, Ibadan, Nigeria

Text Tea production and expansion across agro–ecological areas is the main thrust in Nigeria to resolve limited land for tea plantation expansion on the Mambilla Plateau. Soils

under tea cultivation and the tea leaves from three commercial tea growing areas in Kusuku, Ngoroje, Kakara (highland agro-ecology) and three adaptive trial areas in Mayo-Selbe, Ikom amd Ibadan (Lowland agro-ecology) were assessed for their nutrient contents for proper nutrient management and possible expansion of tea cultivation across the agr-ecologies. Ten tea plants per location were tagged; leaf samples and soil samples at 0–30cm were collected at the same points. The soils and tea leaves were analyzed for N, P, K, Ca, Mg and Zn contents in addition to soil pH and organic OC. The Ikom soil was strongly acidic but slightly acidic for other locations. The OC was below critical value of 3.0g kg^{-1} soil for the lowland locations but higher in the highlands. The 0.08g N kg^{-1} for Mayo–Selbe, 9.82mg P kg^{-1} for Ngoroje, and 0.2cmol K.kg^{-1} for Karkara were below their critical values of 0.1g N /kg^{-1}, 10mg P /kg^{-1} and 0.3cmol K kg^{-1} soil respectively while the Ca and Mg contents were higher than their critical levels. Tea leaves nutrient composition indicated no significant difference in Ca, Mg and Zn contents but were significant in the N, P and K contents. Generally, tea nutrient contents for the lowland ecology compared favorably with the highland areas. This indicated that tea cultivation could be advised for all the locations. However, there is need for tea harvests from the different ecologies to be processed and used as a tea blend. For profitable tea production in the locations, there is need to improve and sustain the soil OC and N contents for the lowland locations while, Kakara and Ikom locations should be supplied 78.2kg K, and 8.72kg P for Ngoroje.

Session 13

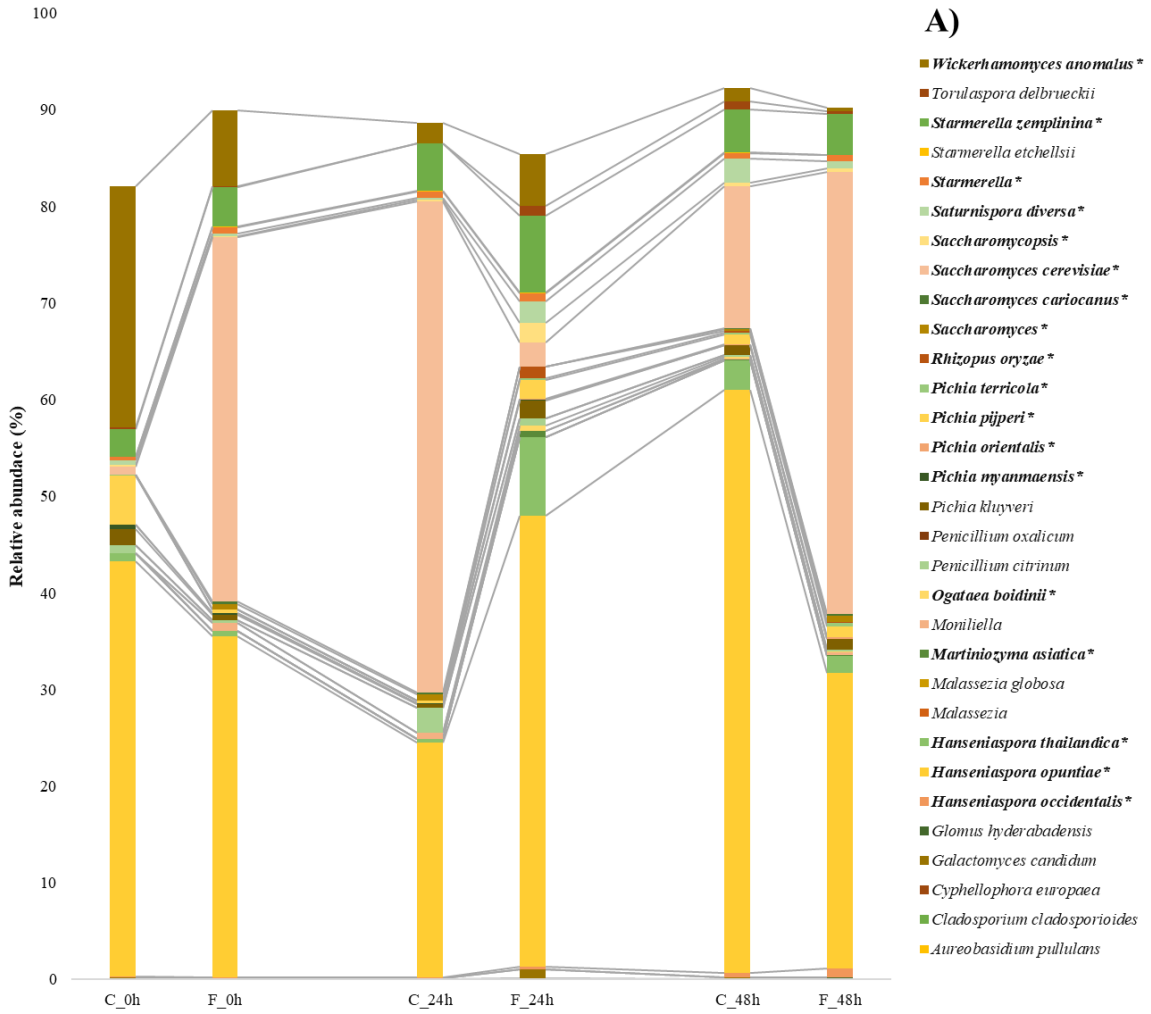
Oral Session Tea 13.01

A matter of chocolate aroma: What microbial communities can do on the formation of sensorial attributes in the early stages of the cocoa fermentation

Jatziri Mota-Gutierrez[1], Mirna Leonor Suarez-Quiroz[2], Oscar Gonzalez-Rios[2], Ilario Ferrocino[1], Luca Simone Cocolin[1]
[1]University of Turin, Grugliasco, Turin, Italy, [2]Unidad de Investigacion y Desarrollo en Alimentos, Instituto Tecnologico de Veracruz, Veracruz, Mexico

Text A desirable chocolate aroma is considered a marker for high-quality [1]. However, the flavor characteristic depends on the variety's genetics and post-harvesting methods of cocoa beans [3][4]. The aroma development starts during fermentation were the microbiota plays an important role in the generation of the cocoa flavor [2][5]. This work aimed to identify the microbes that can contribute to the aroma generation in two different Mexican cocoa varieties (*Criollo* and *Forastero*) during the first two days of spontaneous fermentation. To evaluate the microbial communities of fermented cocoa beans we performed an amplicon-based sequencing on the microbiota and mycobiota by targeting 16S and the 26S rRNA respectively using the Illumina MiSeq platform. Microbial richness analysis indicated a higher level of complexity across fermentation time for fungi ($P < 0.05$), while the bacterial community remarkably indicated a higher level of complexity in *Criollo* varieties compared to *Forastero* ($P < 0.05$). In general, we observed that the fermentation was driven by *Hanseniaspora opuntiae, Saccharomyces cerevisiae, Acetobacter pasteurianus, Lactobacillus cacaonum,* and *Lactobacillus plantarum* group

(Fig. 1).


A)
Relative abundance (%)
100
90
80
70
60
50
40
30
20
10
0
C_0h
F_0h
C_24h
F_24h
C_48h
F_48h
Wickerhamomyces anomalus*
Torulaspora delbrueckii
Starmerella zemplinina*
Starmerella etchellsii
Starmerella*
Saturnispora diversa*
Saccharomycopsis*
Saccharomyces cerevisiae*
Saccharomyces cariocanus*
Saccharomyces*
Rhizopus oryzae*
Pichia terricola*
Pichia pijperi*
Pichia orientalis*
Pichia nyanmaensis*
Pichia kluyveri
Penicillium oxalicum
Penicillium citrinum
Ogataea boidinii*
Moniliella
Martiniozyma asiatica*
Malassezia globosa
Malassezia
Hanseniaspora thailandica*
Hanseniaspora opuntiae*
Hanseniaspora occidentalis*
Glomus hyderabadensis
Galactomyces candidum
Cyphellophora europaea
Cladosporium cladosporioides
Aureobasidium pullulans

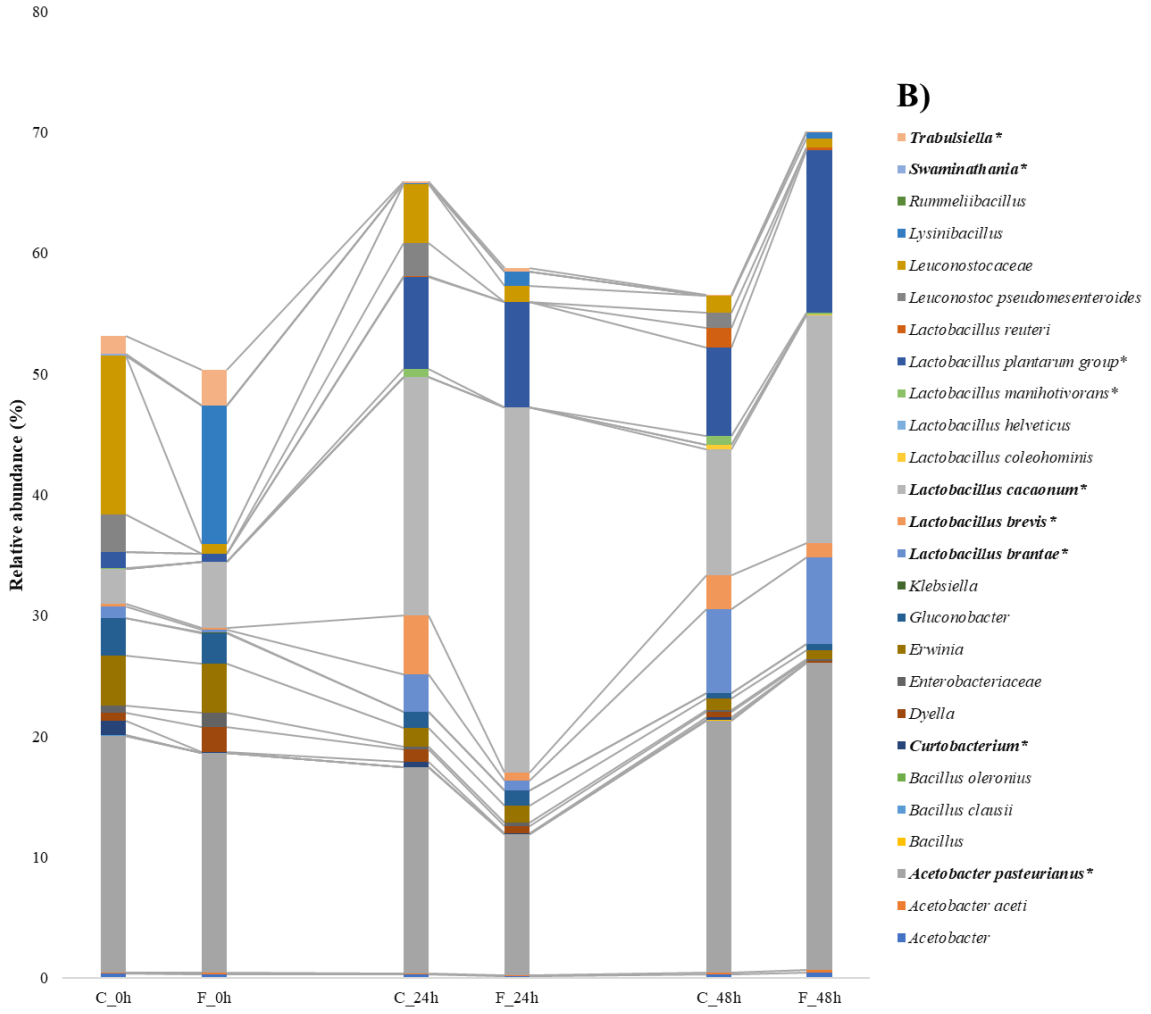

80
B)
Relative abundance (%)
70
60
50
40
30
20
10
0
C_0h
F_0h
C_24h
F_24h
C_48h
F_48h
Trabulsiella*
Swaminathania*
Rummeliibacillus
Lysinibacillus
Leuconostocaceae
Leuconostoc pseudomesenteroides
Lactobacillus reuteri
Lactobacillus plantarum group*
Lactobacillus manihotivorans*
Lactobacillus helveticus
Lactobacillus coleohominis
Lactobacillus cacaonum*
Lactobacillus brevis*
Lactobacillus brantae*
Klebsiella
Gluconobacter
Erwinia
Enterobacteriaceae
Dyella
Curtobacterium*
Bacillus oleronius
Bacillus clausii
Bacillus
Acetobacter pasteurianus*
Acetobacter aceti
Acetobacter

Figure 1. Relative abundance of fungi (A) and bacterial (B) communities in fermented cocoa beans. Abbreviations: Criollo (C) and Forastero (F).

A quantitative descriptive analysis was also performed using a trained panel and the frequency data obtained was subjected to correspondence analysis. Figure 2 showed that the sensorial perception of fermented cocoa beans changes over fermentation time as a function of the microbial metabolic activity and development, while no influences were observed between cocoa varieties. However, the roasting process had a positive influence on the sensorial profile of the cocoa beans.

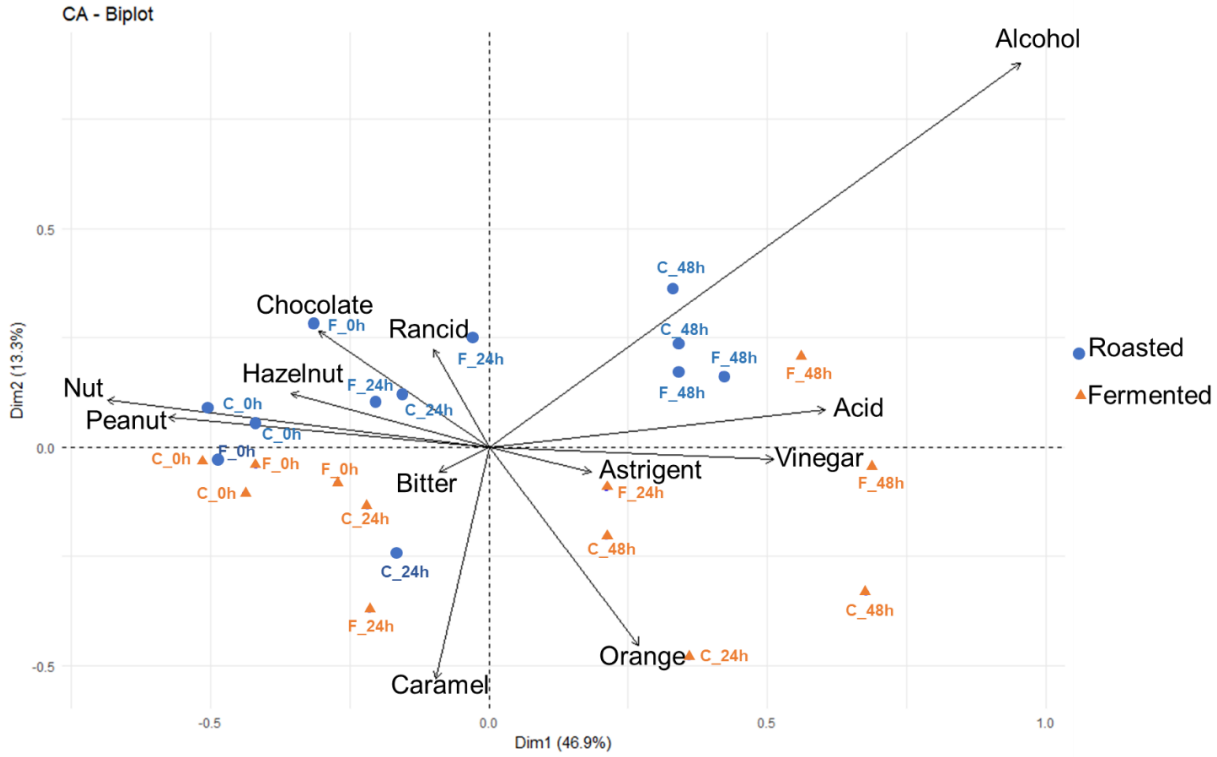


Figure 2. Correspondence analysis factor (CA) map showing the position of the taste and aroma attributes of fermented and roasted cocoa beans obtained from two different cocoa varieties

These results provide useful information on the microbial composition and aroma compounds of fermented cocoa beans that can be used to improve the aroma and flavor characteristics of chocolate.

References:

[1] Magagna, F., Guglielmetti, A., Liberto, E., Reichenbach, S. E., Allegrucci, E., Gobino, G., and Cordero, C. , (2017), Comprehensive chemical fingerprinting of high-quality cocoa at early stages of processing: Effectiveness of combined untargeted and targeted approaches for classification and discrimination., Journal of agricultural and food chemistry

[2] Chetschik, I., Kneubühl, M., Chatelain, K., Schlüter, A., Bernath, K., and Hühn, T. , (2017), Investigations on the aroma of cocoa pulp (Theobroma cacao L.) and its influence on the odor of fermented cocoa beans, Journal of agricultural and food chemistry

[3] Mota-Gutierrez, J., Botta, C., Ferrocino, I., Giordano, M., Bertolino, M., Dolci, P., M. Cannoni and Cocolin, L. , (2018), Dynamics and biodiversity of bacterial and yeast communities during fermentation of cocoa beans. , Applied Environmental Microbiology

[4] Cevallos-Cevallos, J. M., Gysel, L., Maridueña-Zavala, M. G., and Molina-Miranda, M. J. , (2018), Time-related changes in volatile compounds during fermentation of bulk and

fine-flavor cocoa (Theobroma cacao) beans. , Journal of Food Quality
[5] Magalhães da Veiga Moreira, I., Gabriela da Cruz Pedrozo Miguel, M., Lacerda Ramos, C., Ferreira Duarte, W., Efraim, P., and Freitas Schwan, R. , (2016), Influence of cocoa hybrids on volatile compounds of fermented beans, microbial diversity during fermentation and sensory characteristics and acceptance of chocolates., Journal of food quality

Oral Session Tea 13.02

Key Volatiles of Grassy and Floral Smell before Firing in Bao-Chung Oolong Tea Processing

Shu-Yen Lin[1], Ya-Hsin Hsiao[1], I-Ling Kuo[1], Po-An Chen[2]
[1]Department of Horticulture and Landscape Architecture, National Taiwan University, Taipei, Taiwan, [2]Plant Technology Laboratories, Agricultural Technology Research Institute, Hsin-Chu, Taiwan

Text Bao-Chung oolong tea is a well-known partial-fermented Taiwan tea and famous for the delicate aroma and smooth flavor. The quality depends on the control of the oxidation and the suitable firing timing. The smell at the end of the oxidation was defined in 3 olfactory groups: the strong moderate, and weak green odor groups. The oxidation was also divided into 3 levels by 3, 4, or 5 shaking times. Total nine combinations (3 shaking times × 3 olfactory groups) were included in this study. The results indicated that the major green type odor, trans-2-hexenal, showed a significant drop with the green note decreasing in all three levels of oxidation status (FIG01). Most floral scent compounds showed a similar pattern in which the peak areas performed higher with the strong floral smell while the oxidation finished. Among all detected aroma compounds, methyl salicylate showed a significant difference between each oxidation groups (FIG02). Linalool, geraniol and 1-nonanol increase with more oxidation. Phenylethyl alcohol and nonanal have higher amounts of floral smell but reached a similar amount between the different oxidation groups. Geraniol, 1-nonanol, beta-ocimene, and indole had different changing patterns in the treatments, but all showed more stable and consistency when the smell with the weakest green odor at the end of oxidation. In this study, we showed the relationships between the levels of oxidation, the smell at the end of oxidation, and the major aromatic compounds. The fresh and grassy smell related to the high peak areas of green odor compounds especially the changing trend in trans-2-hexenal. After the green note reducing, the floral scent comes out with the detection of the significant high peak areas of methyl salicylate and other aromatic compounds. The changing patterns of trans-2-hexenal and methyl salicylate could be the suitable indicators for the firing timing index in oolong tea processing.

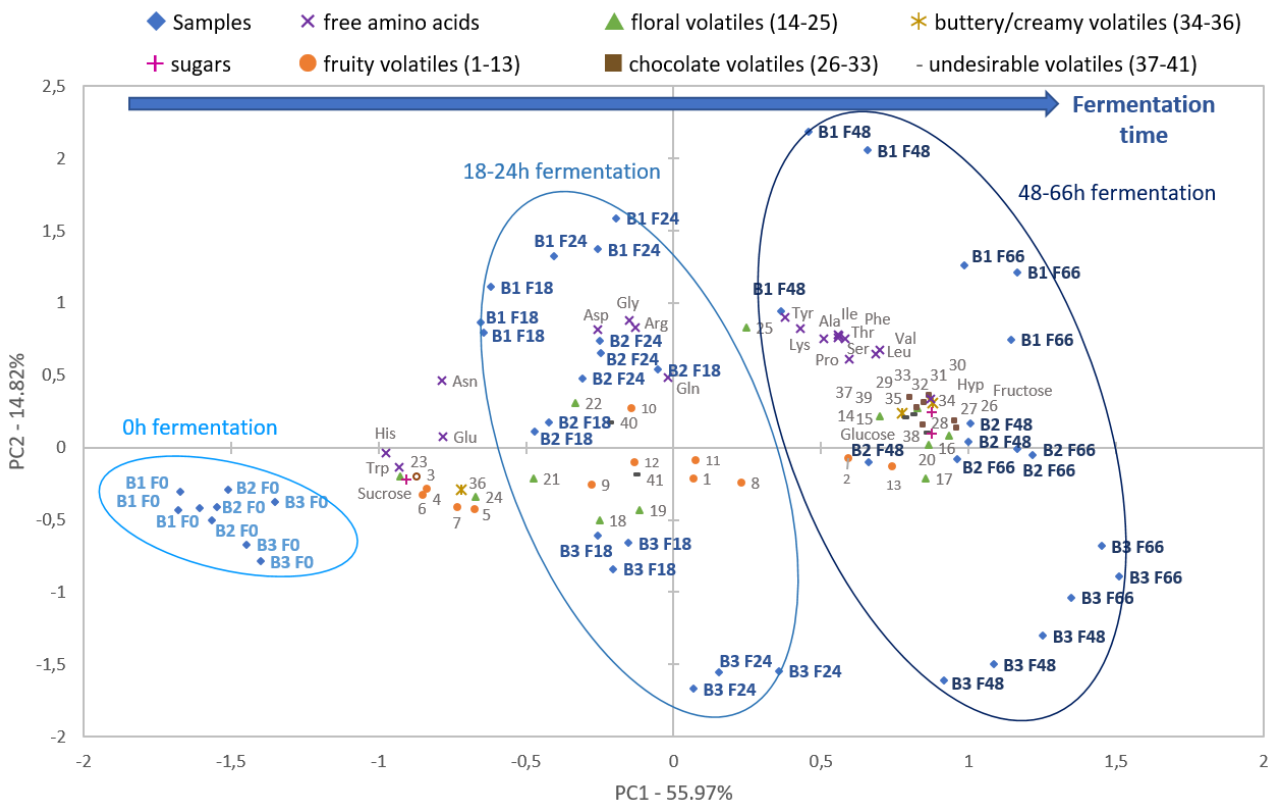
References:
[1] S.-Y. Lin, L.-C. Loa, I.-Z. Chen, P.-A. Chen, (2016), Effect of shaking process on correlations between catechins and volatiles in oolong tea, Journal of Food and Drug Analysis, p. 500-507, Volume 24, Issue 3, https://doi.org/10.1016/j.jfda.2016.01.011

Oral Session Tea 13.03

Fine flavor dynamics during fermentation of Ecuadorian Trinitario cocoa beans

Hayley Rottiers[1, 2], Daylan Amelia Tzompa Sosa[1], Ann De Winne[3], Jenny Ruales[4], Jessika De Clippeleer[5, 6], Ilse De Leersnyder[7], Jocelyn De Wever[2], Helena Everaert[1, 2], Kathy Messens[2], Koen Dewettinck[1]
[1]Department of Food Technology, Safety and Health; Laboratory Food Technology Engineering, Ghent University, Ghent, Belgium, [2]Department of Biotechnology, Research Group Molecular Biotechnology, Ghent University, Ghent, Belgium, [3]Department of Microbial and Molecular Systems, Center for Aroma & Flavour Technology, KU Leuven, Ghent, Belgium, [4]Department of Food Science and Biotechnology, Escuela Politécnica Nacional, Quito, Ecuador, [5]Department of Biotechnology, Laboratory for Brewing and Fermentation Science & Technology, Ghent University, Ghent, Belgium, [6]Department of Biosciences and Food Sciences, University College Ghent, Ghent, Belgium, [7]Department of Green Chemistry and Technology, Laboratory of Chemical Analysis, Ghent University, Ghent, Belgium

Text Fine flavor cocoa is worldwide renowned to produce origin chocolates as it harbors special aroma notes described as e.g. fruity or floral, in addition to its chocolate aroma. This research aims to elucidate flavor generation during fermentation by analyzing the sugar, free amino acid, volatile and final sensory profile, with focus on the dynamics of fine aroma notes. Trinitario cocoa beans (Sacha Gold), cultivated in the Ecuadorian Amazon, were fermented and samples were taken after 0, 18, 24, 48 and 66 h. The unfermented beans contained significant sucrose, glutamic acid and asparagine amounts, while the fermented beans contained more flavor precursors, e.g. glucose, fructose, hydrophobic and other free amino acids. A total of 41 volatiles were identified, including 13 fruity- and 12 floral-like compounds, derived from various sources and metabolic pathways. Whereas the level of fatty acid-derived fruity volatiles decreased, the amount of amino acid-derived volatiles increased and floral terpenes remained stable. Fine volatiles were assumed to be pulp-derived, intrinsic to the bean, or microbially synthesized during fermentation. Multivariate analysis clustered samples according to fermentation time and quality.



PCA bi-plot showing sugars, free amino acids and volatile profile as affected by fermentation time (F0, F18, F24, F48, F66) over the three cocoa bean batches (B1, B2, B3).

Moreover, the sensory profile was described by trained panelists using a 10-point scale, and showed high scores for tropical, dried fruit with some floral earthy notes, in addition to the basic cocoa, acid, bitter and astringent tastes. These findings demonstrate that cocoa fermentation is not only essential for the formation of flavor precursors, but also for the development or preservation of important fine aroma compounds. Ecuador is the top fine flavor producer, having different cocoa genotypes with fine flavor potential, and hence, care should be taken during post-harvest to fully exploit this fine flavor character and deliver high-quality cocoa beans with fine organoleptic characteristics.

Oral Session Tea 13.04

Coffee – aroma coding at the receptor level

Franziska Haag, Sandra Hoffmann, Dietmar Krautwurst
Leibniz-Institute for Food Systems Biology at the Technical University of Munich, Freising, Germany

Text The aroma profile of roasted coffee can be simulated with 27 aroma compounds. These key food odorants (KFOs) can be divided into 5 groups with respect to their main odor qualities sweet/caramel-like, earthy, sulfur/roasted and smoky. Specific concentration ratios of potent KFOs, such as 2-Furfurylthiol, 4-Hydroxy-2,5-dimethyl-3(2H)furanone and E-(β)-Damascenone, which target odorant receptors (OR) of our chemical sense olfaction, are causative for coffee's complex hedonic percept. The identification of molecular targets and sensors for KFO compounds of roasted coffee, such as 2-Furfurylthiol, is crucial for an

understanding of coffee's hedonic properties. Here we identified distinct receptor activity patterns elicited by single coffee KFOs or their combination, by expressing 391 recombinant human ORs together with olfactory signaling molecules in test cell systems, by means of odorant/receptor-induced cAMP signaling and GloSensor® luminescence measurements. An aroma-induced receptor activity pattern may serve as an objectified quality control parameter for coffee's complex hedonic percept. Further, we observed that individual OR haplotypes, defined by coding single nucleotide polymorphisms, could be activated by KFOs with different efficacies and potencies. A genetically encoded, individual odor perception, e.g. a specific anosmia, may underlie individual consumer food preferences.

Oral Session Tea 13.05

Classification of cocoa beans based on their fluorescent fingerprint to predict sensory poles of chocolates?

Karine Alary[1], Sebastien Preys[2], Clotilde Hue[3], Adriana Descalzo[4], Isabelle Maraval[1], Fabrice Davrieux[5], Renaud Boulanger[1, 6]
[1]CIRAD, Montpellier, France, [2]Ondalys, Montpellier, France, [3]Valrhona, Tain l'Hermitage, France, [4]INTA, Buenos Aeres, Argentina, [5]CIRAD, Saint Denis, France, [6]cirad, Montpellier, France

Text Natures and quantities of aroma compounds present in chocolate vary according to several criteria such as the origin and the variety of cocoa beans, the cocoa post-harvest treatment and the process of manufacturing chocolate. These organoleptic qualities are evaluated through sensory evaluation. This method enable to define the sensory profiles of chocolates and then their belonging to a sensory pole. Could a classification of merchantable cocoa beans based on their fluorescent fingerprint be an alternative to predict sensory poles of chocolate? The objective of our study was to develop a chemometric model obtain with fluorescent fingerprint. To do this, 3D spectral analyses were performed at 20°C by Front Face Fluorescence Spectroscopy (FFFS) on refined cocoa powder samples (N=208). All of them were analyzed following similar operating conditions. At the same time, a sensory analysis was performed on the corresponding dark chocolates, prepared by and standardized and controlled fabrication process. The prediction model was developed on the 208 samples divided into the four sensory poles, and validated by a set of 50 samples. The prediction error was around 30%.
To interpret the data, preprocessing of signals and cleaning of non-informative areas (Rayleigh scattering) was carried out. Subsequently, a multiway exploratory analysis (PARAFAC) was carried out to determine the discriminant wavelengths in the distribution of classes. Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) were performed on spectral data to identify sensory pole separation and to elaborate chemometric model. As a result, analysis of fluorescent fingerprints enabled to reach a reliable distribution of cocoa beans according to the sensory pole of chocolate.

Oral Session Tea 13.06

Flavour Characterisation of Chocolate & Cocoa Products Produced by Novel Processing Techniques.

Irene Chetschik, Lisa Ullrich, Ansgar Schlüter, Karin Chatalain, Tilo Hühn
Institute of Food and Beverage Innovation, Zurich University of Applied Sciences, Wädenswil, Switzerland

Text Chocolate making and cocoa processing can be regarded as a traditional craft, which was established and refined through many decades of practice [1]. A lot of research has been done to understand the impact of the classical post-harvest treatment and technological processing on the evolution of the cocoa aroma from bean to the final product chocolate [2]. Cocoa processing along the cocoa value is a very traditional process, and alternative cocoa processing techniques such as cold extraction [3] are till now quite exceptional. Novel technological cocoa processing methodologies result in products with flavour properties that are different from those in traditional products, as it was demonstrated by a recent study on chocolates produced by decanter technology [4]. Our conference contribution will highlight the results of the characterisation of the flavour properties of dark chocolates manufactured by different processing technologies. In addition to that, the influence of further alternative post-harvest treatment techniques such as cocoa incubation [5] on the flavour profile of cocoa beans will be discussed, as characterised by instrumental analysis combined with human odour perception.

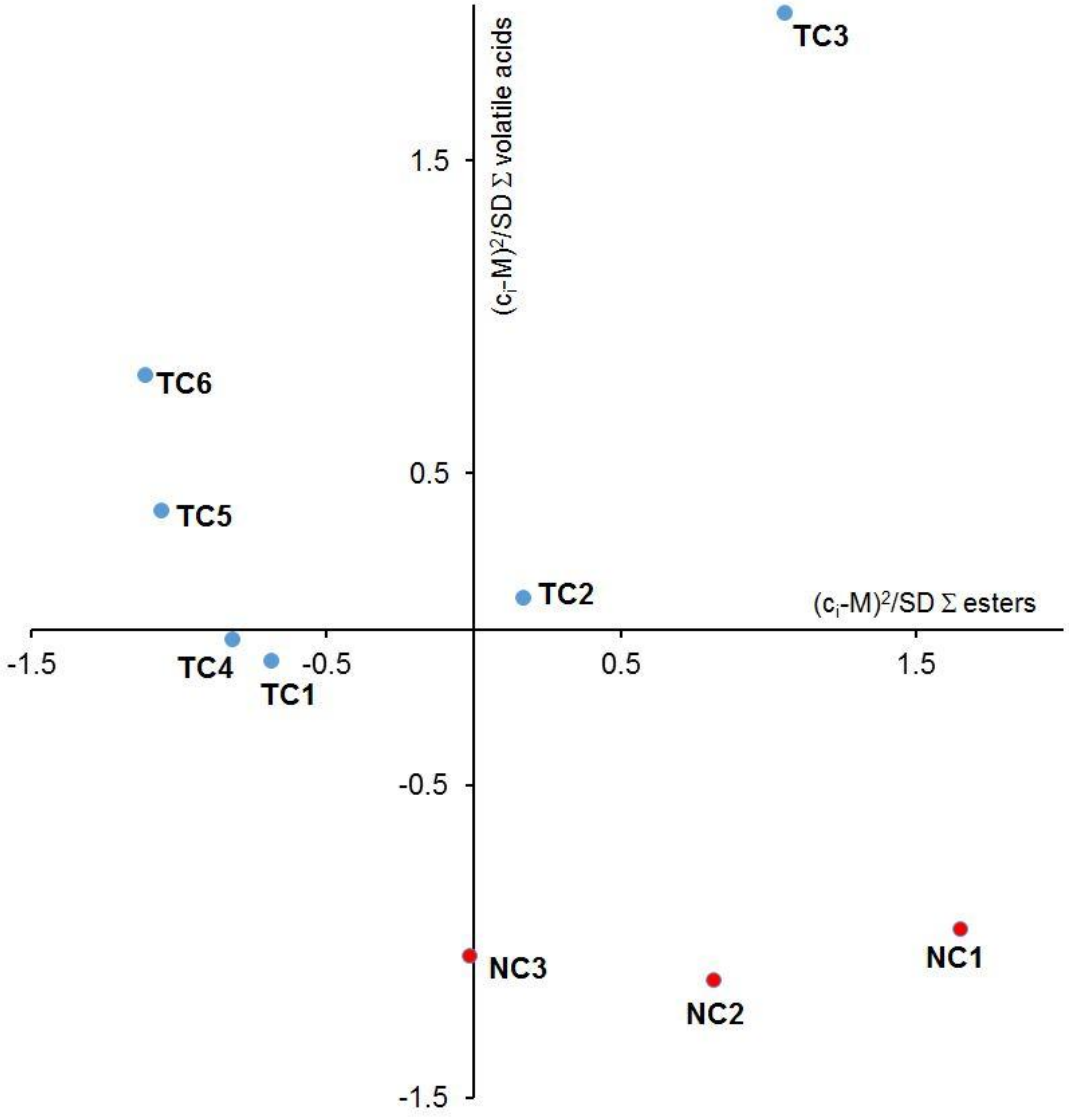


Figure 1. Relationship between odor active esters and odor active volatile acids in dark chocolates processed with a novel processing technique (NC) and traditionally produced chocolates 5

(1): Beckett, S. T. The Science of Chocolate, (2nd ed.); RCS Publishing: London, 2008.
(2): Afokawa, E. O.; Paterson, A.; Fowler, M.; Ryan, A. Flavor formation and character in cocoa and chocolate: A criticial review. In *Critical Reviews in Food Science and Nutrition*. **2008**, 48, 840-57.
(3): Hühn, T.; Laux, R.; inventors. Cocoa bean processing methods and techniques, PCT Int. Appl. 2010.
(4): Chetschik, I.; Pedan, V.; Chatelain, K.; Kneubühl, M.; Hühn, T. Characterization of the Flavor Properties of Dark Chocolates Produced by a Novel Technological Approach & Comparison with Traditionally Produced Dark Chocolates. *J Agric Food Chemistry,* **2019**, (**DOI:** 10.1021/acs.jafc.8b06800, publication date March 20, 2019)
(5): Kadow, D.; Niemenak, N.; Rohn, S.; Lieberei, R. Fermentation-like incubation of cocoa seeds (Theobroma cacao L.) - Reconstruction and guidance of the fermentation process. *LWT - Food Sci Technol*. **2015**; 62(1):357-361.