Food Research International 111 (2018) 361–370

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Variation of triacylglycerol profiles in unfermented and dried fermented cocoa beans of different origins

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ARTICLE INFO

Keywords:
Fermentation
Drying
Cocoa
Origin
Triacylglycerol
Soft butter
Lipid
Profile

ABSTRACT

Fermentation and drying are the two crucial processing steps required to produce cocoa beans with desired properties, especially taste and flavor. To understand their impact on the lipid profile of cocoa, the lipid composition of unfermented raw and fermented dried beans from six different origins was investigated using high-performance liquid chromatography-mass spectrometry methods. While the comparison of triacylglycerol profiles across the different origins showed only small variations in individual compound concentrations, the comparison along the fermentation status showed major differences regarding the occurrence of polar lipids. These compounds may serve as biomarkers for the fermentation status of the beans and a simple analytical method suitable for field trials is proposed. Finally, a hypothesis identifying key unsaturated triacylglycerols contributing to the hardness and softness of cocoa butter is presented.

1. Introduction

The production and marketing of origin chocolate constitute one of the latest trends in the cocoa industry, applying winemakers' concept of terroir to chocolate. Therefore chocolate manufactures spend significant effort on sourcing raw material with specific characteristics form a particular country. Even that might be the trend, price, trade balances, politics, and the volume of cocoa beans available determine the choice of bean origin for lower value cocoa products. The main cocoa bean producing countries are African countries supplying 73.3% of the global production in the last year, and the leading cocoa bean processing countries are European countries grinding 38.7% of the 4.6 million tons of cocoa harvested in 2016 ("The International Cocoa Organization (ICCO) | Cocoa Producing and Cocoa Consuming Countries, 2018") The origin can have an impact for several reasons: first, different countries cultivate botanically different subspecies of *Theobroma cacao*. There are four main cultivars of cocoa: Criollo, Forastero, Trinitario, and Nacional (Lima, Almeida, Rob, & Zwietering, 2011; Rusconi & Conti, 2010). Secondly, the practice of producing dried beans, the first stage of the complex production process of chocolate, cocoa powder and butter, can occur in different ways. Cocoa beans are submitted to an extensive production chain, starting from harvesting the fruits on farms and plantations followed by pod opening and bean removal from the pod. Subsequently, beans are fermented and then dried to < 8% (Badrie, Bekele, Sikora, & Sikora, 2015; Beckett, Harding, & Freedman, 2008). Drying is essential, both for stopping the fermentation process and reducing moisture thus minimizing microbial growth during transport and storage. Climate conditions at the geographical areas of cultivation have been shown to significantly influence bean chemical composition and quality (Chaiseri & Dimick, 1989; Lehrian, Keeney, & Butler, 1980). Finally, different storage and shipping methods have their contribution to the quality of the dried beans (Beckett, Fowler, & Ziegler, 2017).

Lipids represent approximately half of cocoa beans' chemical constituents. As in many other plant seed oils, triacylglycerols (TAGs) are the predominant species, defining the physical characteristic of cocoa butter (CB). Despite the extensive work made on studying the formation of lipid-related flavor precursors during the fermentation process (Afoakwa, Paterson, Fowler, & Ryan, 2008; Kadow, Bohlmann, Phillips, & Lieberei, 2013) and ample progress on understanding the chemistry of CB, there are few attempts on describing the TAGs in unfermented (please note that the terms wet beans and raw beans are used as well) cocoa beans and changes of lipids occurring during fermentation (Liendo, Padilla, & Quintana, 1997; Lima et al., 2011). Studies on CB classify cocoa butter as soft and hard butter on origin basis with each butter used in dedicated product applications. It was previously suggested that soft CBs are characterized by higher 1-palmitoyl-2,3-dioleoyl-glycerol (POO), 1-stearoyl-2,3-dioleoyl-glycerol (SOO) content (Chaiseri & Dimick, 1989; Lipp & Anklam, 1998) whereas hard CBs are characterized by increased saturated fatty acid content (Chin, 1989).

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https://doi.org/10.1016/j.foodres.2018.05.025
Received 23 February 2018; Received in revised form 25 April 2018; Accepted 11 May 2018

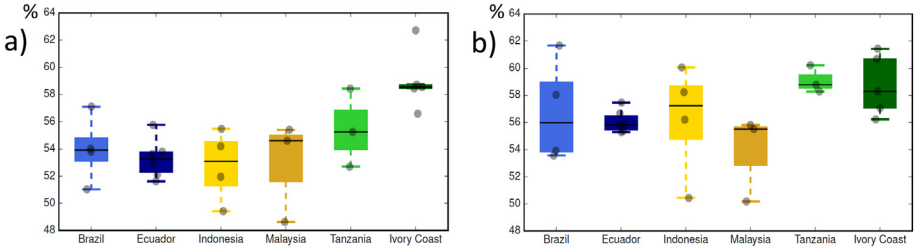

Fig. 1. Box plots of total lipid content determined gravimetrically following Soxhlet extraction for (a) unfermented raw and (b) fermented dried cocoa beans from different countries of origin.

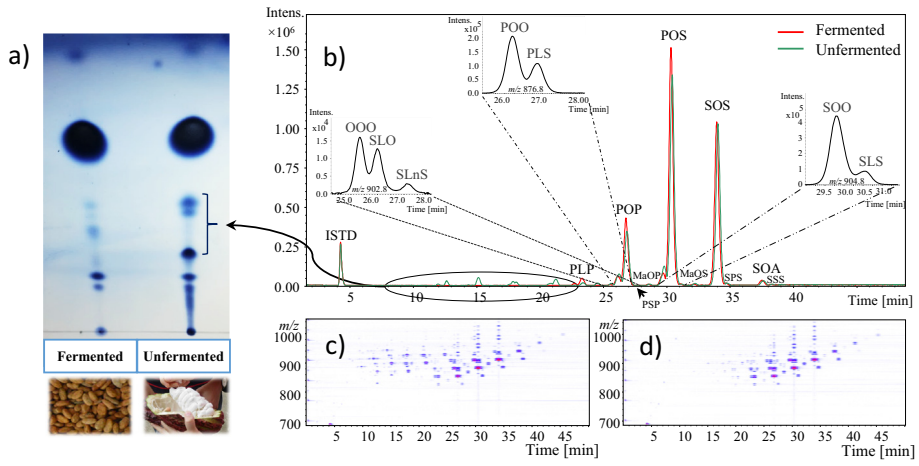

Fig. 2. Representative chromatography results for fermented dried and unfermented raw cocoa lipid extract of Ivory Coast origin. (a) TLC plate image and the parallel corresponding (b) HPLC-MS chromatograms in positive ion mode (P – palmitic acid, O - oleic acid, S – stearic acid, L – linoleic acid, Ln – linolenic acid, Ma – margaric acid, A – arachidic acid). (c) Two-dimensional (2D) map of reverse-phase high-resolution MS of unfermented raw and (d) fermented dried cocoa bean samples from Ivory Coast. The colormap of the 2D map represents the peak intensity with red being the highest and blue the lowest. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Therefore, Brazilian and Malaysian CBs are generally considered as extremes since Brazilian cocoa butter is rich in di- and tri-unsaturated TAGs (di- and tri- are referred to the number of double bonds in the fatty acid chain), while Malaysian cocoa butter was reported to contain a lower concentration of polyunsaturated TAGs and a higher concentration of saturated TAGs (Figueira et al., 1997; Marty & Marangoni, 2009).

For the description of chemical differences between cocoa beans from varying origin, several different analytical techniques have been employed to profile the typical metabolite classes in cocoa beans including polyphenol profiling, protein profiling, and lipid profiling (Caligiani, Palla, Acquotti, Marseglia, & Palla, 2014; Dsouza et al., 2017; Kumari et al., 2016; Marseglia et al., 2016). HPLC-MS provides most chemical information (Hernandez, Castellote, & Permanyer, 1991; Lísa, Holčapek, & Boháč, 2009) to profile the chemical composition of CB including minor lipids and species co-eluting with each other.

Previously we presented data concerning the TAGs diversity in wet unfermented cocoa beans, identifying 83 TAG species in these beans. To achieve this, we have developed a suitable analytical procedure, which employs HPLC coupled to mass spectrometry (MS) (Sirbu, Corno, Ullrich, & Kuhnert, 2018). We now apply this method to assess the differences in the lipid profile specific to geographic origin and the process status of the samples. In this manuscript, we profile TAGs in unfermented wet beans and fermented dried beans from six different origins including Brazil, Ecuador, Ivory Coast, Tanzania, Malaysia, and Indonesia. These countries were chosen mostly based on their global cocoa production, as well as sourcing opportunities and geographical diversity. Multivariate statistical analysis has been employed to confirm the key compositional difference in TAG profiles when comparing unfermented and fermented beans, as well as to visualize the chemical differences according to origins of the seeds identifying in the process biomarkers allowing a clear distinction between sample groups. This is the first study examining the TAG profile in unfermented raw and fermented dried cocoa beans.

2. Material and methods

2.1. Chemicals and reagents

Ethanol gradient grade was purchased from Merck (Darmstadt, Germany), isopropanol (Rotisolv® HPLC grade), acetonitrile (Rotisolv® HPLC ultra gradient grade), chloroform (Rotisolv® HPLC grade) and Tetra-dodecylammonium bromide was purchased from Carl Roth (Karlsruhe, Germany), dichloromethane 99,8% stabilized with amylene for synthesis was purchased from Panreac AppliChem (Darmstadt, Germany), ammonium formate LC-MS Ultra and formic acid (puriss., ≥98% (T) for mass spectrometry) were purchased from Sigma-Aldrich Chemie (Steinheim, Germany). Ethanol was subjected to distillation prior use.

2.2. Sample preparation

Frozen fresh unfermented cocoa beans samples and dried fermented cocoa beans samples from 6 different origins were received in several sets from Barry Callebaut. A total of 24 pairs of samples coming from Ivory Coast, Indonesia, Ecuador, Tanzania, Malaysia, and Brazil were analyzed. Firstly, the seed material was deshelled and ground using a grinder with the purpose of making homogenous powder. Henceforth, the samples were extracted employing an overnight Soxhlet (Buchi Extraction System B811 instrument, Flawil, Switzerland) method using dichloromethane as an extraction solvent, 150 mL and 5 g of powdered beans. Extracted lipids were quantified gravimetrically after evaporation to dryness in a rotavapor. The dry residue was then stored at −20 °C until further analysis. For HPLC analysis, a concentration of 0.045 mg/mL in chloroform/ethanol (50/50) of cocoa lipid extract was prepared.