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A Conceptual Model for Shear-Induced Phase Behavior in Crystallizing Cocoa Butter
Mazzanti, G.; Guthrie, S. E.; Marangoni, A. G.; Idziak, S. H. J.
Cryst. Growth Des.; (Article); 2007; 7(7); 1230-1241. DOI: 10.1021/cg050467r

Abstract:

We propose a conceptual model to explain the quantitative data from synchrotron X-ray diffraction experiments on the shear-induced phase behavior of cocoa butter, the main structural component of chocolate. We captured two-dimensional diffraction patterns from cocoa butter at crystallization temperatures of 17.5, 20.0, and 22.5 C under shear rates from 45 to 1440 s-1 and under static conditions. From the simultaneous analysis of the integrated intensity, correlation length, lamellar thickness, and crystalline orientation, we postulate a conceptual model to provide an explanation for the distribution of phases II, IV, V, and X and the kinetics of the process. As previously proposed in the literature, we assume that the crystallites grow layer upon layer of slightly different composition. The shear rate and temperature applied define these compositions. Simultaneously, the shear and temperature define the crystalline interface area available for secondary nucleation by promoting segregation and affecting the size distribution of the crystallites. The combination of these factors (composition, area, and size distribution) favors dramatically the early onset of phase V under shear and determines the proportions of phases II, IV, V, and X after the transition. The experimental observations, the methodology used, and the proposed explanation are of fundamental and industrial interest, since the structural properties of crystalline networks are determined by their microstructure and polymorphic crystalline state. Different proportions of the phases will thus result in different characteristics of the final material.

Determination of Cocoa Butter Equivalents in Milk Chocolate by Triacylglycerol Profiling
Buchgraber, M.; Androni, S.; Anklam, E.
Journal of Agricultural and Food Chemistry; (Article); 2007; 55(9); 3284-3291. DOI: 10.1021/jf063350z

Abstract:

An analytical approach for the detection and quantification of cocoa butter equivalents (CBEs) in milk chocolate is presented. It is based on (i) a comprehensive standardized database covering the triacylglycerol composition of a wide range of authentic milk fat (n = 310), cocoa butter (n = 75), and CBE (n = 74) samples and 947 gravimetrically prepared mixtures thereof, (ii) the availability of a certified cocoa butter reference material (IRMM-801) for calibration, (iii) an evaluation algorithm, which allows a reliable quantification of the milk fat content in chocolate fats using a simple linear regression model, (iv) a subsequent correction of triacylglycerols deriving from milk fat, (v) mathematical expressions to detect the presence of CBEs in milk chocolate, and (vi) a multivariate statistical formula to quantify the amount of CBEs in milk chocolate. The detection limit was 1% CBE in chocolate fat (0.3% CBE in milk chocolate, having a fat content of 30%). For quantification, the average error for prediction was 1.2% CBE in chocolate fat, corresponding to 0.4% in milk chocolate (fat content, 30%).

Quantification of Milk Fat in Chocolate Fats by Triacylglycerol Analysis Using Gas-Liquid Chromatography
Buchgraber, M.; Androni, S.; Anklam, E.
Journal of Agricultural and Food Chemistry; (Article); 2007; 55(9); 3275-3283. DOI: 10.1021/jf0633490

Abstract:

The development and in-house testing of a method for the quantification of milk fat in chocolate fats is described. A database consisting of the triacylglycerol profiles of 310 genuine milk fat samples from 21 European countries and 947 mixtures thereof with chocolate fats was created under a strict quality control scheme using 26 triacylglycerol reference standards for calibration purposes. Out of the individual triacylglycerol fractions obtained, 1-palmitoyl-2-stearoyl-3-butyroyl-glycerol (PSB) was selected as suitable marker compound for the determination of the proportion of milk fat in chocolate fats. By using PSB values from the standardized database, a calibration function using simple linear regression analysis was calculated to be used for future estimations of the milk fat content. A comparison with the widely used butyric acid method, which is currently used to determine the milk fat content in nonmilk fat mixtures, showed that both methods were equivalent in terms of accuracy. The advantage of the presented approach is that for further applications, i.e., determination of foreign fats in chocolate fats, just a single analysis is necessary, whereas for the same purpose, the C4 method requires two different analytical methods.

Rapid Reversed Phase Ultra-Performance Liquid Chromatography Analysis of the Major Cocoa Polyphenols and Inter-relationships of Their Concentrations in Chocolate
Cooper, K. A.; Campos-Gimenez, E.; Jimenez Alvarez, D.; Nagy, K.; Donovan, J. L.; Williamson, G.
Journal of Agricultural and Food Chemistry; (Article); 2007; 55(8); 2841-2847. DOI: 10.1021/jf063277c

Abstract:

Chocolate and other cocoa-containing products are a rich source of polyphenols. This paper describes an ultra-performance liquid chromatography (UPLC) method that can separate and quantify in 3 min six of the major chocolate polyphenols: catechin; epicatechin; B2 (epicatechin-4 -8-epicatechin); B5 (epicatechin-4 -6-epicatechin); C1 (epicatechin-4 -8-epicatechin-4 -8-epicatechin); and tetramer D (epicatechin-4 -8-epicatechin-4 -8-epicatechin-4 -8-epicatechin). A survey of 68 chocolate samples indicated that there was a strongly predictive relationship between epicatechin and the other individual polyphenols, especially procyanidin B2 (R 2 = 0.989), even though the chocolates came from varied sources and manufacturers. The relationship was less strong with catechin, and so further work to explore the reasons for this difference was performed. Chiral analysis on a subset of 23 chocolates showed that (-)-epicatechin had a predictive relationship with (+)-catechin in line with the other polyphenols, but not with (-)-catechin (the predominant form). This indicates that (-)-catechin is the most affected by manufacturing conditions, possibly formed through epimerization from (-)-epicatechin during processing. The results show that epicatechin concentrations can be used to predict the content of other polyphenols, especially B2 and C1, and total polyphenols content. Finally, the (-)-catechin content is not predictable from the epicatechin content, and it is concluded that this is the main form of polyphenol that varies according to manufacturing conditions and cocoa origin.

Confirmation of Peanut Protein Using Peptide Markers in Dark Chocolate Using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

Shefcheck, K. J.; Callahan, J. H.; Musser, S. M.

Journal of Agricultural and Food Chemistry; (Article); 2006; 54(21); 7953-7959. DOI: 10.1021/jf060714e

Abstract:

Detection of peptides from the peanut allergen Ara h 1 by liquid chromatography-mass spectrometry (LC-MS) was used to identify and estimate total peanut protein levels in dark chocolate. A comparison of enzymatic digestion subsequent to and following extraction of Ara h 1 from the food matrix revealed better limits of detection (LOD) for the pre-extraction digestion (20 ppm) than for the postextraction digestion (50 ppm). Evaluation of LC-MS instruments and scan modes showed the LOD could be further reduced to 10 ppm via a triple-quadrupole and multiple-reaction monitoring. Improvements in extraction techniques combined with an increase in the amount of chocolate extracted (1 g) improved the LOD to 2 ppm of peanut protein. This method provides an unambiguous means of confirming the presence of the peanut protein in foods using peptide markers from a major allergen, Ara h 1, and can easily be modified to detect other food allergens.

Chocolate is a Powerful ex Vivo and in Vivo Antioxidant, an Antiatherosclerotic Agent in an Animal Model, and a Significant Contributor to Antioxidants in the European and American Diets

Vinson, J. A.; Proch, J.; Bose, P.; Muchler, S.; Taffera, P.; Shuta, D.; Samman, N.; Agbor, G. A.

Journal of Agricultural and Food Chemistry; (Article); 2006; 54(21); 8071-8076. DOI: 10.1021/jf062175j

Abstract:

Chocolate today is often viewed as a food or snack with little nutritional value. The high saturated fat content of chocolate has also contributed to the belief that its consumption increases the risk of heart disease. However, recent human studies have proven that chocolate has beneficial effects on some pathogenic mechanisms of heart disease such as endothelial function and blood pressure. Although the antioxidant properties of chocolate have been known for some time, there has been no examination of its place in the U.S.
diet as a source of antioxidants. This paper demonstrates that chocolate makes a significant contribution to U.S. per capita dietary antioxidants and by inference the European Community's. In the U.S. diet chocolate is the third highest daily per capita antioxidant source. An ex vivo study shows that epicatechin, a major polyphenol in chocolate and chocolate extracts, is a powerful inhibitor of plasma lipid oxidation due to polyphenols' ability to bind to lower density lipoproteins. Conversely, the fat from chocolate alone is a pro-oxidant in this model. This is also demonstrated in an in vivo human study. After consumption of dark chocolate and cocoa powder, the lower density lipoproteins isolated from plasma were protected from oxidation compared to the lipoproteins isolated after cocoa butter consumption, which were put under oxidative stress. In an animal model of atherosclerosis, cocoa powder at a human dose equivalent of two dark chocolate bars per day significantly inhibited atherosclerosis, lowered cholesterol, low-density lipoprotein, and triglycerides, raised high-density lipoprotein, and protected the lower density lipoproteins from oxidation. Chocolate has thus been shown to have potential beneficial effects with respect to heart disease.

Procyanidin and Catechin Contents and Antioxidant Capacity of Cocoa and Chocolate Products

Gu, L.; House, S. E.; Wu, X.; Ou, B.; Prior, R. L.

Journal of Agricultural and Food Chemistry; (Article); 2006; 54(11); 4057-4061. DOI: 10.1021/jf060360r

Abstract:

Cocoa and chocolate products from major brands were analyzed blind for total antioxidant capacity (AOC) (lipophilic and hydrophilic ORACFL), catechins, and procyanidins (monomer through polymers). Accuracy of analyses was ascertained by comparing analyses on a NIST standard reference chocolate with NIST certified values. Procyanidin (PC) content was related to the nonfat cocoa solid (NFCS) content. The natural cocoa powders (average 87% of NFCS) contained the highest levels of AOC (826 ± 103 mol of TE/g) and PCs (40.8 ± 8.3 mg/g). Alkalized cocoa (Dutched powders, average 80% NFCS) contained lower AOC (402 ± 6 mol of TE /g) and PCs (8.9 ± 2.7 mg/g). Unsweetened chocolates or chocolate liquor (50% NFCS) contained 496 ± 40 mol of TE /g of AOC and 22.3 ± 2.9 mg/g of PCs. Milk chocolates, which contain the least amount of NFCS (7.1%), had the lowest concentrations of AOC (80 ± 10 mol of TE /g) and PCs (2.7 ± 0.5 mg/g). One serving of cocoa (5 g) or chocolate (15 or 40 g, depending upon the type of chocolate) provides 2000-9100 mol of TE of AOC and 45-517 mg of PCs, amounts that exceed the amount in a serving of the majority of foods consumed in America. The monomers through trimers, which are thought to be directly bioavailable, contributed 30% of the total PCs in chocolates. Hydrophilic

antioxidant capacity contributed >90% of AOC in all products. The correlation coefficient between AOC and PCs in chocolates was 0.92, suggesting that PCs are the dominant antioxidants in cocoa and chocolates. These results indicate that NFCS is correlated with AOC and PC in cocoa and chocolate products. Alkalizing dramatically decreased both the procyanidin content and antioxidant capacity, although not to the same extent.

Antioxidant Activity and Polyphenol and Procyanidin Contents of Selected Commercially Available Cocoa-Containing and Chocolate Products in the United States

Miller, K. B.; Stuart, D. A.; Smith, N. L.; Lee, C. Y.; McHale, N. L.; Flanagan, J. A.; Ou, B.; Hurst, W. J.

Journal of Agricultural and Food Chemistry; (Article); 2006; 54(11); 4062-4068. DOI: 10.1021/jf0602900

Abstract:

In the United States, commercially available foods, including cocoa and chocolate, are being marketed with statements referring to the level of antioxidant activity and polyphenols. For cocoa-containing foods, there has been no comprehensive survey of the content of these and other chemistries. A survey of cocoa and chocolate-containing products marketed in the United States was conducted to determine antioxidant activity and polyphenol and procyanidin contents. Commercially available samples consisted of the top market share products in each of the following six categories: natural cocoa, unsweetened baking chocolate, dark chocolate, semisweet baking chips, milk chocolate, and chocolate syrup. Composite samples were characterized using four different methods: oxygen radical absorbance capacity (ORAC), vitamin C equivalence antioxidant capacity (VCEAC), total polyphenols, and procyanidins. All composite lots were further characterized for percent nonfat cocoa solids (NFCS) and percent fat. Natural cocoas had the highest levels of antioxidant activities, total polyphenols, and procyanidins followed by baking chocolates, dark chocolates and baking chips, and finally milk chocolate and syrups. The results showed a strong linear correlation between NFCS and ORAC (R 2 = 0.9849), total polyphenols (R 2 = 0.9793), and procyanidins (R 2 = 0.946), respectively. On the basis of principal component analysis, 81.4% of the sample set was associated with NFCS, antioxidant activity, total polyphenols, and procyanidins. The results indicated that, regardless of the product category, NFCS were the primary factor contributing to the level of cocoa antioxidants in the products tested. Results further suggested that differences in cocoa bean blends and processing, with the possible exception of Dutching, are minor factors in determining the level of antioxidants in commercially available cocoa-containing products in the United States**.**

Development of a Gas-Liquid Chromatographic Method for the Analysis of Fatty Acid Tryptamides in Cocoa Products

Hug, B.; Golay, P.-A.; Giuffrida, F.; Dionisi, F.; Destaillats, F.

Journal of Agricultural and Food Chemistry; (Article); 2006; 54(9); 3199-3203. DOI: 10.1021/jf0527044

Abstract:

The determination of the occurrence and level of cocoa shells in cocoa products and chocolate is an important analytical issue. The recent European Union directive on cocoa and chocolate products (2000/36/EC) has not retained the former limit of a maximum amount of 5% of cocoa shells in cocoa nibs (based on fat-free dry matter), previously authorized for the elaboration of cocoa products such as cocoa mass. In the present study, we report a reliable gas-liquid chromatography procedure suitable for the determination of the occurrence of cocoa shells in cocoa products by detection of fatty acid tryptamides (FATs). The precision of the method was evaluated by analyzing nine different samples (cocoa liquors with different ranges of shells) six times (replicate repeatability). The variations of the robust coefficient of variation of the repeatability demonstrated that FATC22, FATC24, and total FATs are good markers for the detection of shells in cocoa products. The trueness of the method was evaluated by determining the FAT content in two spiked matrices (cocoa liquors and cocoa shells) at different levels (from 1 to 50 mg/100 g). A good relation was found between the results obtained and the spiking (recovery varied between 90 and 130%), and the linearity range was established between 1 and 50 mg/100 g in cocoa products. For total FAT contents of cocoa liquor containing 5% shells, the measurement uncertainty allows us to conclude that FAT is equal to 4.01 ± 0.8 mg/100 g. This validated method is perfectly suitable to determine shell contents in cocoa products using FATC22, FATC24, and total FATs as markers. The results also confirmed that cocoa shells contain FATC24 and FATC22 in a constant ratio of nearly 2:1.

Inhibition of Angiotensin Converting Enzyme Activity by Flavanol-Rich Foods

Actis-Goretta, L.; Ottaviani, J. I.; Fraga, C. G.

Journal of Agricultural and Food Chemistry; (Article); 2006; 54(1); 229-234. DOI: 10.1021/jf0522630

Abstract:

Angiotensin converting enzyme (ACE) activity was evaluated in the presence of flavanol-rich foods, i.e., wines, chocolates, and teas, and of purified flavonoids. All foods assayed inhibited ACE activity, red wines being more effective than white wine, and green tea more effective than black tea. The inhibition of ACE activity was associated with both phenolic and flavanol content in the foods. When isolated polyphenols were assayed, procyanidins (dimer and hexamer) and epigallocatechin significantly inhibited enzyme activity; similar concentrations of (+)-catechin, (-)-epicatechin, gallic acid, chlorogenic acid, caffeic acid, quercetin, kaempferol, and resveratrol were ineffective. When ACE activity was assayed in rat kidney membranes in the presence of chocolate extracts or purified procyanidins, it was observed that the inhibition depended on the chocolate content of flavanols and the number of flavanol units constituting the procyanidin. These experiments demonstrate that flavanols either isolated or present in foods could inhibit ACE activity. The occurrence of such inhibition in vivo needs to be determined, although is supported by the association between the consumption of flavanol-rich foods and reductions in blood pressure observed in several experimental models.

Evaluation of Cocoa- and Coffee-Derived Methylxanthines as Toxicants for the Control of Pest Coyotes

Johnston, J. J.

Journal of Agricultural and Food Chemistry; (Article); 2005; 53(10); 4069-4075. DOI: 10.1021/jf050166p

Abstract:

Methylxanthines were quantified in coffee, tea, and chocolate products. Tarajuilie tea from India, cocoa powder, and cocoa nibs contained the highest levels of methylxanthines. Theobromine, caffeine, and theophylline combined in the ratios observed in tea and chocolate were ingested by coyotes. Although both mixtures induced acute toxicity, the symptoms accompanying the chocolate methylxanthine mimic were preferable. Manipulation of the ratios of methylxanthines in the chocolate mimic led to the identification of a 5:1 theobromine/caffeine mixture as a promising coyote toxicant. This mixture was then administered to coyotes using the coyote lure operative device (CLOD). Mortality occurred in every coyote that ingested any portion of

the CLOD contents. These results indicate that mixtures of theobromine and caffeine have the potential to be developed into a selective, effective, and socially acceptable toxicant for the control of pest coyotes.

A Novel Approach for the Detection of Potentially Hazardous Pepsin Stable Hazelnut Proteins as Contaminants in Chocolate-Based Food

Akkerdaas, J. H.; Wensing, M.; Knulst, A. C.; Stephan, O.; Hefle, S. L.; Aalberse, R. C.; van Ree, R.
Journal of Agricultural and Food Chemistry; (Article); 2004; 52(25); 7726-7731. DOI: 10.1021/jf049278r

Abstract:

Contamination of food products with pepsin resistant allergens is generally believed to be a serious threat to patients with severe food allergy. A sandwich type enzyme-linked immunosorbent assay (ELISA) was developed to measure pepsin resistant hazelnut protein in food products. Capturing and detecting rabbit antibodies were raised against pepsin-digested hazelnut and untreated hazelnut protein, respectively. The assay showed a detection limit of 0.7 ng/mL hazelnut protein or <1 g hazelnut in 1 g food matrix and a maximum of 0.034% cross-reactivity (peanut). Chocolate samples spiked with 0.5-100 g hazelnut/g chocolate showed a mean recovery of 97.3%. In 9/12 food products labeled "may contain nuts", hazelnut was detected between 1.2 and 417 g hazelnut/g food. It can be concluded that the application of antibodies directed to pepsin-digested food extracts in ELISA can facilitate specific detection of stable proteins that have the highest potential of inducing severe food anaphylaxis.

Crystal Structures of 1,3-Distearoyl-2-oleoylglycerol and Cocoa Butter in the (V) Phase Reveal the Driving Force Behind the Occurrence of Fat Bloom on Chocolate

Peschar, R.; Pop, M. M.; De Ridder, D. J. A.; van Mechelen, J. B.; Driessen, R. A. J.; Schenk, H.
J. Phys. Chem. B.; (Letter); 2004; 108(40); 15450-15453. DOI: 10.1021/jp046723c

Abstract:

On the basis of high-resolution synchrotron powder diffraction data, crystal structures have been solved for 1,3-distearoyl-2-oleoylglycerol, a major cis-mono-unsaturated triglyceride fraction of cocoa butter, and cocoa butter itself in the (V) phase. The latter implies that in fact a crystal structure model of chocolate in the (V) phase has been obtained. The results clarify the metastability of the (V) phase and explain why fat bloom may develop on (V)-type chocolate that has been stored at temperatures that are too high.

Occurrence of Ochratoxin A in Cocoa Products and Chocolate

Serra Bonvehi, J.
Journal of Agricultural and Food Chemistry; (Article); 2004; 52(20); 6347-6352. DOI: 10.1021/jf040153w

Abstract:

In this work, the occurrence of ochratoxin A (OTA) in 170 samples of cocoa products of different geographical origins was studied. An immunoaffinity column with HPLC separation was developed to quantify low levels of OTA in cocoa bean, cocoa cake, cocoa mass, cocoa nib, cocoa powder, cocoa shell, cocoa butter, chocolate, and chocolate cream with >80% recoveries. The method was validated by performing replicate analyses of uncontaminated cocoa material spiked at three different levels of OTA (1, 2, and 5 g/kg). The data obtained were related on the acceptable safe daily exposure for OTA. The highest levels of OTA were detected in roasted cocoa shell and cocoa cake (0.1-23.1 g/kg) and only at minor levels in the other cocoa products. Twenty-six cocoa and chocolate samples were free from detectable OTA (<0.10 g/kg). In roasted cocoa powder 38.7% of the samples analyzed contained OTA at levels ranging from 0.1 to 2 g/kg, and 54.8% was contaminated at >2 g/kg (and 12 samples at >3 g/kg). Ochratoxin A was detected in cocoa bean at levels from 0.1 to 3.5 g/kg, the mean concentration being 0.45 g/kg; only one sample exceeded 2 g/kg (4.7%). In contrast, 51.2% of cocoa cake samples contained OTA at levels 2 g/kg, among which 16 exceeded 5 g/kg (range of 5-9 g/kg). These results indicate that roasted cocoa powder is not a major source of OTA in the diet.

Relationship between Procyanidin and Flavor Contents of Cocoa Liquors from Different Origins

Counet, C.; Ouwerx, C.; Rosoux, D.; Collin, S.
Journal of Agricultural and Food Chemistry; (Article); 2004; 52(20); 6243-6249. DOI: 10.1021/jf040105b

Abstract:

The flavor of eight cocoa liquors of different origins (Africa, America and Asia and different varieties (Fine grades: criollo, trinitario, and nacional. Bulk-basic grade: forastero.) was analyzed by headspace solid-phase microextraction mass spectrometry (HS-SPME-MS). Their procyanidin contents were quantified by HPLC-UV (280 nm). Fine varieties with short fermentation processes proved to contain more procyanidins, while criollo from New Guinea and forastero beans showed the highest aroma levels. The levels of cocoa aroma compounds formed during roasting are shown to vary directly with bean fermentation time and inversely with residual procyanidin content in cocoa liquor. Measurement of antioxidant activity in cocoa liquor proved to be a useful tool for assessing residual polyphenols.

Nature and Composition of Fat Bloom from Palm Kernel Stearin and Hydrogenated Palm Kernel Stearin Compound Chocolates
Smith, K. W.; Cain, F. W.; Talbot, G.
Journal of Agricultural and Food Chemistry; (Article); 2004; 52(17); 5539-5544. DOI: 10.1021/jf049401e

Abstract:

Palm kernel stearin and hydrogenated palm kernel stearin can be used to prepare compound chocolate bars or coatings. The objective of this study was to characterize the chemical composition, polymorphism, and melting behavior of the bloom that develops on bars of compound chocolate prepared using these fats. Bars were stored for 1 year at 15, 20, or 25 C. At 15 and 20 C the bloom was enriched in cocoa butter triacylglycerols, with respect to the main fat phase, whereas at 25 C the enrichment was with palm kernel triacylglycerols. The bloom consisted principally of solid fat and was sharper melting than was the fat in the chocolate. Polymorphic transitions from the initial ' phase to the phase accompanied the formation of bloom at all temperatures.

Separation of 5- and 7-Phytosterols by Adsorption Chromatography and Semipreparative Reversed Phase High-Performance Liquid Chromatography for Quantitative Analysis of Phytosterols in Foods
Zhang, X.; Cambrai, A.; Miesch, M.; Roussi, S.; Raul, F.; Aoude-Werner, D.; Marchioni, E.
Journal of Agricultural and Food Chemistry; (Article); 2006; 54(4); 1196-1202. DOI: 10.1021/jf052761x

Abstract:

A method for the separation, isolation, and identification of phytosterols was developed. A commercial phytosterols mixture, Generol 95S, was fractionated first by adsorption silica gel column chromatography and then separated by means of a semipreparative reverse phase high-performance liquid chromatography fitted with a Polaris C8-A column (250 mm × 10 mm i.d., 5 m) using isocratic acetonitrile:2-propanol:water (2:1:1, v/v/v) as the mobile phase. Milligram scales of six individual phytosterols, including citrostadienol, campesterol, -sitosterol, 7-avenasterol, 7-campesterol, and 7-sitosterol, were obtained. Purities of these isolated sterols were 85-98%. Relative response factors (RRF) of these phytosterols were calculated against cholestanol as an authentic commercial standard. These RRF values were used to quantify by gas chromatography-mass spectrometry (GC-MS) the phytosterols content in a reference material, oils, and chocolates.