Cocoa Beans

Subjects: Food Science & Technology

Contributor: Elodie JEAN-MARIE

Cocoa beans are the basic material occurring in the most consumed product in the world, namely chocolate. Their composition includes polyphenols, methylxanthines, lipids and other compounds that may vary qualitatively and quantitatively according to criteria such as variety or culture area. Polyphenols and methylxanthines are known as being responsible for many health benefits, particularly by preventing cardiovascular and neurodegenerative diseases. Recent studies emphasized their positive role in dietary metabolic disorders, such as diabetes and weight gain.

cocoa polyphenol methylxanthines intestinal immunity microbiota diabetes

1. Introduction

Theobroma cacao Linnaeus 1753 is a food plant that has been domesticated by Mesoamericans and propagated to European countries in 1502 [1]. Originating from neotropical rainforests, its fruits are called “pods” and contain “cocoa beans” with a surrounding muscillagineous sweet pulp. To become one of the most widely consumed products in the world, namely chocolate, cocoa seeds must undergo several processes such as fermentation, drying and roasting [2]. Consumed for its flavor, raw cocoa and cocoa-derived products revealed human health benefits as protective against cardiovascular and neurodegenerative diseases, antioxidant and anti-inflammatory activities [3][4]. Bioactive compounds, particularly polyphenols and methylxanthines, are thought to be responsible for these properties [5][6]. Nowadays, dietary metabolic disorders, such as diabetes, are on the rise, and recent in vitro and in vivo studies on cocoa compounds revealed preventive actions such as anti-diabetic and anti-obesity activities [7][8][9]. In addition, cocoa modulated the profile of intestinal microbiota, which can provide health benefits to the host [10].

2. Health Benefits of Polyphenols and Methylxanthines from Cocoa

2.1. Cocoa and Intestinal Inflammation

During food allergy, allergens engender the differentiation of T cell into T-helper 2 cells which can produce cytokines (IL-4, IL-5, IL-10, and IL-13), resulting of the production of Immunoglobulin E (IgE) antibodies by B cells [11]. IgE is recognized by mast cell receptors named FcεRI and induces the release of histamine, protease and cytokines that revealed anaphylactic response. Polyphenols from cocoa, especially flavonoids, upregulated the gene expression of IgE receptor FcεRI and decrease in rat mast cell protease II (RMCP-II) levels. This marker of

fast degranulation would be the consequence of the cocoa-inducing lower rate of IgE. Interestingly, the nature of polyphenol contained in the sample could induce different mechanisms. Indeed, even if both cocoa samples inhibited IL-5 and IL-13 that promote Th2 response, only fermented cocoa inhibits the IL-4 release, which acts in the synthesis of IgE. This explained why fermented cocoa prevented the synthesis of anti-ovalbumin IgE, whereas unfermented cocoa decreased these antibodies only after the end of the diet. Moreover, cocoa intake reduced the release of Th1-cytokines IL-1α, IL-1β and IFN-γ from mesenteric lymph nodes cells and specific IgG antibodies associated with Th2-immune response [12].

To go further, abnormal level in lipid and glucose are associated with inflammation process that has occurred in adipose tissue. Activation of the TLR4 receptor by free fatty acid can trigger c-Jun N-terminal kinase (JNK) and induce a crucial inflammatory transcription factor called NF-κB. Pro-inflammatory cytokine genes and protein levels such as TNF-α and IL-6 decreased with cocoa powder and cocoa extracts in white adipose tissue, suggesting an anti-inflammatory action of cocoa [7].

2.2. Cocoa and Obesity

Cocoa has already shown anti-obesity properties by acting in various ways. Obesity is characterized by abnormal fat and glucose levels. After daily intake of flavonoid-rich cocoa products, total blood cholesterol, triglycerides, and LDL-cholesterol were significantly decreased and blood pressure was improved [7][13]. Moreover, (−)-epicatechin, cocoa powder and cocoa extract have caused a significant decrease in body weight gain, fat mass accumulation, dyslipidemia, hyperglycemia, and insulin resistance induced by a high-fat diet intake [7].

A strategy to reduce adiposity and dyslipidemia is based on the upregulation of peroxisome proliferator-activated receptor (PPARγ), peroxisome proliferator-activated receptor γ coactivator 1 α (PGC1α), and Sirtuin 1 (SIRT1). The PPARγ ligand plays a key role because of its ability of lower plasma triglyceride levels and increase HDL-cholesterol [14]. Cocoa enhanced the expression of PPARγ levels related to the high-fat group, in association with a diminution of serum triglycerides and FFA levels [15]. The latter are PGC1α, which can bind to and co-activate PPARγ to contribute to the transports and use of fatty acids [16]. PGC1α could interact with SIRT1 and they regulated energy homeostasis and increase energy expenditure over energy storage in WAT. Cocoa powder, cocoa extract and epicatechin modulated the expression of PGC1α, SIRT1 and PPARγ in WAT, suggesting that one of the possible mechanisms involved in body weight gain and fat mass accumulation would be to increase energy expenditure in the case of hypercaloric diet-induced [7]. PPARγ and C/EBPα act as transcription factors in adipogenesis. In a recent study, theobromine has suppressed the accumulation of lipids and the differentiation of adipocytes by decreasing the expression of PPARγ and C/EBPα (involved in the latter stage of adipogenesis) and by degrading the C/EBPβ (involved in the early stage of adipogenesis) [17][18].

Another strategy is based on the AMP-activated protein kinase (AMPK) pathway. After consuming cocoa in high-fat diet, the expression of AMPK was superior to that of the standard group, suggesting that the AMPK pathway could be activated by cocoa [15]. This enzyme is related to the promotion of β-oxidation, lipogenesis of FFA and lipogenic and fatty oxidation enzymes activities. Indeed, in another study, cocoa polyphenols supplementation activated

again the AMPK pathway phosphorylation but also downregulated the expression of Acaca (Acetyl-coenzyme A carboxylase alpha) and Mcat (Malonyl coa ACP acyltransferase) genes in the liver, and Fasn (Fatty acid synthase) and Scd1 (Stearoyl-coenzyme A desaturase) genes in the WAT [19].

Another important pathway is based on the existence of leptin, which is a hormone secreted by WAT and has a key role in regulating body weight. This is due to its ability to influence satiety and energy expenditure. Hyperleptinemia is present in obese humans and is thought to engender a leptin-resistance resulting from a suppressed action in WAT metabolic function. Cocoa revealed a positive correlation between serum leptin levels body weight gain/fat mass accumulation for treatment with cocoa extracts, cocoa powder and epicatechin in high-fat diet. These treatments have also been associated with high downregulation of leptin gene expression in WAT, suggesting that cocoa would reduce hypercaloric diet induced-leptin resistance in WAT [7]. Flavanol-rich powder supplementation in athletes also showed a decrease in leptin levels and a reduction in fat body mass [20].

2.3. Cocoa and Diabetes

A potential antidiabetic action of cocoa could result from a protective action on β-cells against death-inducing factors. Indeed, Fernández-Millán and collaborators have shown that cocoa-rich diet induced an antioxidative action (mainly glutathione peroxidase) that could protect β-cell against oxidative injuries induced by pre-diabetic conditions. As a result, they suggested that cocoa polyphenols would reduce apoptosis of β-cell mass and delay the progression of T2D [21]. This prediabetic phase characterized by insulin resistance and glucose tolerance and its progression could be delayed by changes in lifestyle or treatments. Sucrose or fructose-rich diet could enhance endocrine-metabolic disturbance in rats and induced a prediabetes. A daily uptake of polyphenol-enriched cocoa extract has prevented the increase in homeostasis model assessment-insulin resistance index (HOMA-IR index) in sucrose-rich diet for rats, suggesting that polyphenols of cocoa have a protective effect on insulin resistance [22]. In this study, polyphenols also induced the decrease of P-Akt/Akt and P-eNOS/eNOS ratios. In the literature, the Akt-dependent phosphorylation of Ser is involved in the activation of nitric oxide synthesis by eNOS. Insulin would be involved in Akt-modulated mechanism and would be able to release oxide nitric [23]. Changes in the previous ratios would show that cocoa would be able to prevent insulin resistance. Moreover, cocoa showed an involvement in improving insulin resistance by decreasing insulin resistance receptor phosphorylation (IRS-1, Ser 307, and Ser 636/639) and activating the Glycogen Synthase Kinase 3/Glycogen Synthase (GSK3/GS) pathway. Indeed, this could be a good antidiabetic strategy because GSK3-β is a capital substrate of the phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) signaling involved regulating glycogen synthesis, and GS plays an important role in insulin resistance [24].

Hyperglycemia may be managed by the ability of cocoa (−) epicatechin and other flavonoids to increase GLUT-2 levels [25]. GLUTs maintain the inter- and extracellular balance of glucose and may be affected in diabetes state. Although Bowser and collaborators have inhibited GLUT-4 activity, a significant reduction in glucose uptake in human skeletal muscle was noted in the presence of cocoa extract and procyanidin fractions. This behavior suggested that GLUT-4 activity would be involved in the antidiabetic mechanism cocoa extracts [26]. A cocoa-rich diet has also induced a reduction in postprandial plasma glucose elevation. This is believed to be due to increased

early insulin secretion and the activity of the glucagon-like peptide-1(GLP-1) activity [27]. These antidiabetic actions would not be visible for all cocoa polyphenols because only the catechin cocoa fraction showed an enhancement in glucose-stimulated insulin secretion, whereas treatments with crude cocoa extract or procyanidin fraction showed no improvement [4].

2.4. Cocoa and Gut Microbiota

Even if cocoa has already shown antimicrobial activities, it could be appropriated to maintain the integrity of GM while it also could engender the emergence of new microorganism. Indeed, the daily consumption of rich flavanol cocoa drink increased the growth of *Lactobacillus* spp. and *Bifidobacterium* spp. and decreased the *clostridium pefringens* in comparison with a control group. This modulation of cocoa presented benefits because *Lactobacillus* spp. and *Bifidobacterium* spp prevent the apparition of pathogenic species and *Clostridium pefringens* is involved in colonic cancer and onset of inflammatory bowel disease [28].

In cocoa-enriched diet, some compounds could influence the emergence of new bacteria species of GM. Indeed, Martin-Pelaez and collaborators noted that a cocoa diet containing 0.4% polyphenols and 0.25% theobromine induced the emergence of seven fecal-detected bacteria species belonging to the *Actinobacteria*, *Cyanobacteria*, *Firmicutes,* and *Proteobacteria* phyla. However, consumed without polyphenols, the cocoa-diet containing 0.25% theobromine was the only one that allowed the emergence of *Candidatus Arthromitus* (*Firmicutes* phylum). In addition, theobromine was the only one that reduced the counts of *Bifidobacterium* spp., and *Firmicutes* phylum (*Streptococcus* spp., and *C. histolyticum–C. perfringens)* [29]. This suggests possible interferences in activities between several cocoa compounds. Indeed, 0.25% theobromine diet increased almost fourfold *Tenericutes* phylum in gut microbia, whereas cocoa-diet did not, suggesting the possible delay interference of others cocoa compounds [29]. An interesting hypothesis is based on the possibility that methylxanthines and polyphenols may have opposite actions. Indeed, theobromine, taken individually, induced the reduction of Ruminococcus flavefaciens, (cellulolytic bacteria) and the increase of Erysipelotrichaceae while flavonoids induced opposite actions [29][30][31].

3. Conclusions

Cocoa has many varieties that can distinguished by morpho-geographical and genetic factors. These factors influence the composition of each part of the plant, including cocoa beans. In recent studies, two groups of compounds were of great interest, including polyphenols and methylxanthines. As mentioned, these compounds are thought to be responsible for many health benefits, particularly antioxidant and anti-inflammatory capacities in the digestive tract. Indeed, this review focused on the influence of polyphenols and methylxanthines on dietary metabolic disorders as diabetes, obesity, and intestinal inflammations, which are constantly increasing. Our work has shown that all these disorders are related and can interfere with each other, especially with the gut microbiota (GM). Recently, several studies have presented the GM contribution in metabolic disorders. The Dabke and collaborators model suggest that a high-fat, low-fiber diet could lead to dysbiosis (unbalanced GM conditions) which could lead to altered TJ integrity. The dysfunction of the permeability of intestinal epithelial could lead to chronic inflammation of liver and adipose tissue by certain threats to the body (LPS, metabolites…). As a result of

insulin resistance, increased FFA levels and dyslipidemia, this chain of response could result in a high risk of obesity, diabetes, and cardiovascular diseases [32]. This was supported by the fact that infusion of GM from lean volunteers to subjects with metabolic disorders demonstrated an increase in insulin sensitivity [33].

In this paper, we discussed the dual action of cocoa compounds (internal and external) on dietary metabolic disorders and GM. Cocoa polyphenols and methylxanthines modulated the GM itself leading to the emergence of 11 new bacterial species such as *Bacteroides*, *Firmicutes* and *Proteobacteria* phyla. In addition, an external action also exists because these compounds have affected more than the gut. For example, the influence of these compounds on the abundance of *Lactobacilli* and *Bifidobacteria* phyla leads to an increase in pro-inflammatory cytokine IL-10 levels. This rise could delay obesity and body weight by increasing thermogenesis and increasing insulin-sensitivity, a key parameter of diabetes. Our work has also shown that polyphenol and methylxanthine in cocoa can have opposites actions.

This suggests that cocoa is a complex matrix, and its composition is the masterpiece of its effectiveness. Indeed, it is well-known that not all the native compounds reach the bloodstream. Only a few compounds, especially small ones such as (−)-epicatechin, can surround the entire digestive process. Thus, all parameters influencing the composition, such as variety, geographic origins and fermentation process, must be managed. Knowing that the latter engenders a loss of 90% of (−)-epicatechin content, one may wonder if alternatives could be found, such as raw chocolate that is made with unfermented and/or unroasted cocoa beans. Even if the raw material is crucial, the extraction procedure is not negligible because the choice of extraction solvent or/and the extraction method could play a key role. For example, 70% acetone results in a higher total polyphenol content in cocoa pod husk extract than 70% ethanol (94.92 and 49.92 mg GAE/g extract, respectively). Water and ethanol provide a partial extraction of oligomeric polyphenols, but high polymers are not extracted at all [5][34]. Moreover, ultrasound (2h-60Hz) provided extracts with higher total polyphenol content than those obtained by agitation (6h-200rpm) [35].

Cocoa can be considered a “Swiss knife” because it offers many possibilities for use in various forms in the agri-food, cosmetic and other sectors. For instance, the cocoa shell, incorporated into ice cream, showed prebiotic activities, while the cocoa incorporated in mouth products showed anticariogenic and antibacterial capacities [36][37]. As a conclusion, further studies are needed to fully characterize the potential actions of cocoa compounds on dietary metabolic disorders and GM.

References

1. Lachenaud, P.; Sounigo, O.; Sallée, B. Les Cacaoyers Spontanés de Guyane Française: État Des Recherches. Acta Bot. Gall. 2005, 152, 325–346.
2. Beg, M.S.; Ahmad, S.; Jan, K.; Bashir, K. Status, Supply Chain and Processing of Cocoa—A Review. Trends Food Sci. Technol. 2017, 66, 108–116.
3. Andújar, I.; Recio, M.C.; Giner, R.M.; Ríos, J.L. Cocoa Polyphenols and Their Potential Benefits for Human Health. Oxid. Med. Cell Longev. 2012, 2012, 906252.

4. Jean-Marie, E.; Bereau, D.; Poucheret, P.; Guzman, C.; Boudard, F.; Robinson, J.-C. Antioxidative and Immunomodulatory Potential of the Endemic French Guiana Wild Cocoa “Guiana”. Foods 2021, 10, 522.
5. Wollgast, J.; Anklam, E. Review on Polyphenols in Theobroma cacao: Changes in Composition during the Manufacture of Chocolate and Methodology for Identification and Quantification. Food Res. Int. 2000, 33, 423–447.
6. Latif, R. Chocolate/Cocoa and Human Health: A Review. Neth. J. Med. 2013, 71, 63–68.
7. Rabadan-Chávez, G.; Quevedo-Corona, L.; Garcia, A.M.; Reyes-Maldonado, E.; Jaramillo-Flores, M.E. Cocoa Powder, Cocoa Extract and Epicatechin Attenuate Hypercaloric Diet-Induced Obesity through Enhanced β-Oxidation and Energy Expenditure in White Adipose Tissue. J. Funct. Foods 2016, 20, 54–67.
8. Crichton, G.E.; Elias, M.F.; Dearborn, P.; Robbins, M. Habitual Chocolate Intake and Type 2 Diabetes Mellitus in the Maine-Syracuse Longitudinal Study: (1975–2010): Prospective Observations. Appetite 2017, 108, 263–269.
9. Żyżelewicz, D.; Zakłos-Szyda, M.; Juśkiewicz, J.; Bojczuk, M.; Oracz, J.; Budryn, G.; Miśkiewicz, K.; Krysiak, W.; Zduńczyk, Z.; Jurgoński, A. Cocoa Bean (Theobroma cacao L.) Phenolic Extracts as PTP1B Inhibitors, Hepatic HepG2 and Pancreatic β-TC3 Cell Cytoprotective Agents and Their Influence on Oxidative Stress in Rats. Food Res. Int. 2016, 89 Pt 2, 946–957.
10. Mirpuri, J.; Raetz, M.; Sturge, C.R.; Wilhelm, C.L.; Benson, A.; Savani, R.C.; Hooper, L.V.; Yarovinsky, F. Proteobacteria-Specific IgA Regulates Maturation of the Intestinal Microbiota. Gut Microbes 2014, 5, 28–39.
11. Larché, M.; Akdis, C.A.; Valenta, R. Immunological Mechanisms of Allergen-Specific Immunotherapy. Nat. Rev. Immunol. 2006, 6, 761–771.
12. Abril-Gil, M.; Pérez-Cano, F.J.; Franch, À.; Castell, M. Effect of a Cocoa-Enriched Diet on Immune Response and Anaphylaxis in a Food Allergy Model in Brown Norway Rats. J. Nutr. Biochem. 2016, 27, 317–326.
13. Leyva-Soto, A.; Chavez-Santoscoy, R.A.; Lara-Jacobo, L.R.; Chavez-Santoscoy, A.V.; Gonzalez-Cobian, L.N. Daily Consumption of Chocolate Rich in Flavonoids Decreases Cellular Genotoxicity and Improves Biochemical Parameters of Lipid and Glucose Metabolism. Molecules 2018, 23, 2220.
14. Yoon, M. The Role of PPARα in Lipid Metabolism and Obesity: Focusing on the Effects of Estrogen on PPARα Actions. Pharmacol. Res. 2009, 60, 151–159.
15. Coronado-Cáceres, L.J.; Rabadán-Chávez, G.; Quevedo-Corona, L.; Hernández-Ledesma, B.; Garcia, A.M.; Mojica, L.; Lugo-Cervantes, E. Anti-Obesity Effect of Cocoa Proteins (Theobroma

cacao L.) Variety “Criollo” and the Expression of Genes Related to the Dysfunction of White Adipose Tissue in High-Fat Diet-Induced Obese Rats. J. Funct. Foods 2019, 62, 103519.

16. Cheng, C.-F.; Ku, H.-C.; Lin, H. PGC-1α as a Pivotal Factor in Lipid and Metabolic Regulation. Int. J. Mol. Sci. 2018, 19, 3447.
17. Jang, Y.J.; Koo, H.J.; Sohn, E.-H.; Kang, S.C.; Rhee, D.-K.; Pyo, S. Theobromine Inhibits Differentiation of 3T3-L1 Cells during the Early Stage of Adipogenesis via AMPK and MAPK Signaling Pathways. Food Funct. 2015, 6, 2365–2374.
18. Mitani, T.; Watanabe, S.; Yoshioka, Y.; Katayama, S.; Nakamura, S.; Ashida, H. Theobromine Suppresses Adipogenesis through Enhancement of CCAAT-Enhancer-Binding Protein β Degradation by Adenosine Receptor A1. Biochim. Biophys. Acta (BBA)—Mol. Cell Res. 2017, 1864, 2438–2448.
19. Ali, F.; Ismail, A.; Esa, N.M.; Pei, C. Cocoa Polyphenols Treatment Ameliorates Visceral Obesity by Reduction Lipogenesis and Promoting Fatty Acid Oxidation Genes in Obese Rats through Interfering with AMPK Pathway. Eur. J. Lipid Sci. Technol. 2016, 118, 564–575.
20. García-Merino, J.Á.; Moreno-Pérez, D.; de Lucas, B.; Montalvo-Lominchar, M.G.; Muñoz, E.; Sánchez, L.; Naclerio, F.; Herrera-Rocha, K.M.; Moreno-Jiménez, M.R.; Rocha-Guzmán, N.E.; et al. Chronic Flavanol-Rich Cocoa Powder Supplementation Reduces Body Fat Mass in Endurance Athletes by Modifying the Follistatin/Myostatin Ratio and Leptin Levels. Food Funct. 2020, 11, 3441–3450.
21. Fernández-Millán, E.; Cordero-Herrera, I.; Ramos, S.; Escrivá, F.; Alvarez, C.; Goya, L.; Martín, M.A. Cocoa-Rich Diet Attenuates Beta Cell Mass Loss and Function in Young Zucker Diabetic Fatty Rats by Preventing Oxidative Stress and Beta Cell Apoptosis. Mol. Nutr. Food Res. 2015, 59, 820–824.
22. Castro, M.C.; Villagarcía, H.; Nazar, A.; Arbeláez, L.G.; Massa, M.L.; Del Zotto, H.; Ríos, J.L.; Schinella, G.R.; Francini, F. Cacao Extract Enriched in Polyphenols Prevents Endocrine-Metabolic Disturbances in a Rat Model of Prediabetes Triggered by a Sucrose Rich Diet. J. Ethnopharmacol. 2020, 247, 112263.
23. Muniyappa, R.; Montagnani, M.; Koh, K.K.; Quon, M.J. Cardiovascular Actions of Insulin. Endocr. Rev. 2007, 28, 463–491.
24. Klover, P.J.; Mooney, R.A. Hepatocytes: Critical for Glucose Homeostasis. Int. J. Biochem. Cell Biol. 2004, 36, 753–758.
25. Cordero-Herrera, I.; Martín, M.A.; Bravo, L.; Goya, L.; Ramos, S. Cocoa Flavonoids Improve Insulin Signalling and Modulate Glucose Production via AKT and AMPK in HepG2 Cells. Mol. Nutr. Food Res. 2013, 57, 974–985.

26. Bowser, S.M.; Moore, W.T.; McMillan, R.P.; Dorenkott, M.R.; Goodrich, K.M.; Ye, L.; O'Keefe, S.F.; Hulver, M.W.; Neilson, A.P. High-Molecular-Weight Cocoa Procyanidins Possess Enhanced Insulin-Enhancing and Insulin Mimetic Activities in Human Primary Skeletal Muscle Cells Compared to Smaller Procyanidins. J. Nutr. Biochem. 2017, 39, 48–58.
27. Kawakami, Y.; Watanabe, Y.; Mazuka, M.; Yagi, N.; Sawazaki, A.; Koganei, M.; Natsume, M.; Kuriki, K.; Morimoto, T.; Asai, T.; et al. Effect of Cacao Polyphenol-Rich Chocolate on Postprandial Glycemia, Insulin, and Incretin Secretion in Healthy Participants. Nutrition 2021, 85, 111128.
28. Guarner, F.; Malagelada, J.-R. Gut Flora in Health and Disease. Lancet 2003, 361, 512–519.
29. Martín-Peláez, S.; Camps-Bossacoma, M.; Massot-Cladera, M.; Rigo-Adrover, M.; Franch, À.; Pérez-Cano, F.J.; Castell, M. Effect of Cocoa's Theobromine on Intestinal Microbiota of Rats. Mol. Nutr. Food Res. 2017, 61, 1700238.
30. Klinder, A.; Shen, Q.; Heppel, S.; Lovegrove, J.A.; Rowland, I.; Tuohy, K.M. Impact of Increasing Fruit and Vegetables and Flavonoid Intake on the Human Gut Microbiota. Food Funct. 2016, 7, 1788–1796.
31. Etxeberria, U.; Arias, N.; Boqué, N.; Macarulla, M.T.; Portillo, M.P.; Martínez, J.A.; Milagro, F.I. Reshaping Faecal Gut Microbiota Composition by the Intake of Trans-Resveratrol and Quercetin in High-Fat Sucrose Diet-Fed Rats. J. Nutr. Biochem. 2015, 26, 651–660.
32. Dabke, K.; Hendrick, G.; Devkota, S. The Gut Microbiome and Metabolic Syndrome. J. Clin. Investig. 2019, 129, 4050–4057.
33. Vrieze, A.; Van Nood, E.; Holleman, F.; Salojärvi, J.; Kootte, R.S.; Bartelsman, J.F.W.M.; Dallinga-Thie, G.M.; Ackermans, M.T.; Serlie, M.J.; Oozeer, R.; et al. Transfer of Intestinal Microbiota from Lean Donors Increases Insulin Sensitivity in Individuals with Metabolic Syndrome. Gastroenterology 2012, 143, 913–916.e7.
34. Rachmawaty; Mu'nisa, A.; Hasri; Pagarra, H.; Hartati; Maulana, Z. Active Compounds Extraction of Cocoa Pod Husk (Thebroma Cacaol.) and Potential as Fungicides. J. Phys. Conf. Ser. 2018, 1028, 012013.
35. Sotelo, C.L.; Alvis, B.A.; Arrázola, P.G. Evaluation of Epicatechin, Theobromine and Caffeine in Cacao Husks (Theobroma cacao L.), Determination of the Antioxidant Capacity. Rev. Colomb. Cienc. Hortíc. 2015, 9, 124–134.
36. Rossin, D.; Barbosa-Pereira, L.; Iaia, N.; Sottero, B.; Danzero, A.C.; Poli, G.; Zeppa, G.; Biasi, F. Protective Effect of Cocoa Bean Shell against Intestinal Damage: An Example of Byproduct Valorization. Antioxidants 2021, 10, 280.
37. Shashikiran, N.; Subba Reddy, V.; Srikanth, R. Chocolate Mouth Rinse: Effect on Plaque Accumulation and Mutans Streptococci Counts When Used by Children. J. Indian Soc. Pedod. Prev. Dent. 2008, 26, 67.