



Brunswick Labs Ingredient Corner

July 2015 Feature: Cacao fruit

Cacao Fruit (*Theobroma cacao*): An Interesting Source of Functional Components with Anti-inflammatory Potential

Cacao plants (*Theobroma cacao*) thrive in the understory of tropical forests. These petite, delicate, evergreen trees may reach up to 60 feet in height in the wild, although most grow only 20 to 40 feet high. In translation, the botanical name *Theobroma*, given to the cacao plant by the Swedish scientist Linnaeus, simply means “food of the gods”.¹



Theobroma fruit. Image: Depositphotos

The cacao plant is restricted to a geographical zone that spans from approximately 20° North to 20° South of the Equator.¹ Today, cacao trees are primarily cultivated in the equatorial region of West Africa, as well as Central and South America, the West Indies, and tropical regions of Asia.² *Theobroma cacao* plants are mostly grown and appreciated for their seeds which are used worldwide for the production of sweet delicacies. Cacao grows best where temperatures range from 20 °C to 30 °C and humidity levels are not excessive, according to Rafael Parducci of Ecuador Produce S.A., the leading producer of commercially available cacao puree. Since the early cultivation period (from 250-900 AD), the uses of cacao have ranged from medicine to currency.² With more than 200

documented medicinal uses in Europe and New Spain, as well numerous recently published research studies, cacao has justified its spot on the list of functional food ingredients.

The berry-like, ovoid fruit of the cacao plant, filled with sweet and mucilaginous pulp in which the seeds are contained, has received less attention in the research community.¹ The cacao pod is typically yellow-orange, red, or green in color, 5.9–11.8 in long, and 3.1–3.9 in wide. Each pod contains between 25-75 large white seeds, which turn violet or reddish brown after drying.¹ In certain areas, says Chris Boudinet, COO of [Agro Innova](#), the pulp is used to [prepare refreshing juices, smoothies, and jelly](#).³ Cacao pods may also be used as fertilizers and animal feed, however, they are largely considered as agricultural waste and are discarded after separation from cacao beans.⁴ Cacao pod waste makes the ground susceptible to growth of fungi under hot and humid conditions, which may pose an agricultural problem.

Although numerous investigations have focused on the phytochemical composition and potential health benefits of cacao beans, very limited data is available about the biological activity of cacao fruit pulp and its constituents. The cacao pod extract has recently been identified as a potent cosmetic ingredient, due to its anti-wrinkle, skin whitening, and sunscreen effects.⁴ In this study, collagen longevity in the skin layer was attributed to the presence of various phenolic compounds, especially flavonoids and resveratrol, in the cacao pod extract. The cacao bean extracts are especially rich in phenolic compounds (flavonoids and phenolic acids) and their derivatives;⁵ flavonoids constitute the largest and most diverse group of phenolic compounds in cacao beans.⁶

In order to fill the existing knowledge gap on cacao fruit, Brunswick Labs has recently completed a series of analyses and biological functionality tests on cacao fruit pulp. This article will give a brief overview of testing results to date.

Functional components. The following functional constituents were identified in cacao fruit pulp: flavonoids, phenolic acids, isoflavones, anthocyanins, proanthocyanidins and alkaloids. Epicatechin and gallic acid were the most abundant (23.01 µg/g and 11.17 µg/g, respectively) flavonoids, while sinapinic and ferulic acids were the most concentrated phenolic acids (20.82 and 9.04 µg/g). Theobromine, a bitter alkaloid similar in structure and properties to caffeine, was the most notable alkaloid constituent of cacao fruit pulp. Theophylline, theobromine and caffeine were found in the following concentrations: 0.63 µg/g, 0.83 µg/g and 0.3 µg/g, respectively. The content of organic acids (tartaric acid, malic acid, lactic acid, acetic acid, citric acid and propionic acid) in cacao fruit pulp ranged from 0.26 mg/g – 7.06 mg/g, with citric acid being most abundant. The total content of proanthocyanidins was 238.54 µg/g.

Biological activity. A range of biological effects has been attributed to cacao bean extracts and preparations in multiple studies, such as antioxidant,⁷ antimicrobial,⁸ and anti-inflammatory⁹ activities. However, similar studies on cacao fruit extracts are lacking in literature. Brunswick Labs recently subjected cacao fruit puree to antioxidant and anti-inflammatory testing. The ORAC 5.0 assay score of the cacao fruit pulp was determined as 92 µmol TE/g fw. The tested puree was most effective against the super oxide anion (36 µmol TE/g fw) and singlet oxygen species (32 µmol TE/g fw). Since free radicals are directly involved in many inflammatory processes, antioxidant activity is a good indicator of a sample's anti-inflammatory potential.

The anti-inflammatory properties of cacao fruit pulp were subsequently tested in the tumor necrosis factor (TNF)-alpha inhibition assay. The TNF-alpha is a proinflammatory cytokine, which has been identified as a trigger and precursor of inflammation. Recent research has focused on identifying plant natural products, such as flavonoids, as sources of TNF-alpha inhibitors potentially useful in the treatment of inflammatory conditions. The production of TNF-alpha was correlated with the treatment doses of cacao puree applied to human THP-1 macrophages. A dose-dependent curve was derived, reaching 100% inhibition at a dose of 33 µg/mL of cacao puree in cell media. The half maximal inhibitory concentration, IC₅₀, was 16.03 µg/mL indicating potentially high TNF-alpha inhibition capability of cacao fruit puree in biological systems.

The outlined pioneering testing results indicate that cacao fruit pulp abounds in functional constituents, which are responsible for its proven antioxidant and anti-inflammatory effects. Although the cacao fruit pulp has been largely treated as a by-product of the more popular and sought-after cacao bean, the biological potential of this plant material should not be disregarded. Brunswick Labs is hopeful that this initial testing will lead to more comprehensive studies of particular active constituents, their potential biological functions and mechanisms of action. The observed *in vitro* anti-inflammatory effects ideally will be confirmed using additional biomarkers and *in vivo* testing, in the scope of clinical studies or trials.

It will be interesting to see what the future holds for the cacao fruit!

References

1. Kim J, Lee KW, Lee HJ. Cacao (*Theobroma cacao*) seeds and phytochemicals in human health. In Preedy V, Watson EE, Patel VB, eds. Nuts and Seeds in Health and Disease Prevention. London, UK: Academic Press; 2011: 351-360.
2. Dillinger TL, Barriga P, Escarcega S, Jimenez M, Salazar LD, Grivetti, LE (2000). Food of the gods: cure for humanity? A cultural history of the medicinal and ritual use of chocolate. *J Nutr.* 2000;130: 2057S-2072S.

3. Figueira A, Janick J, BeMiller JN. New Products from Theobroma cacao: Seed Pulp and Pod Gum. *New crops*, p. 475-478, Wiley, New York 1993. Accessed 2014-06-15.
4. Karim AA, Azlan A, Ismail A, et al. Phenolic composition, antioxidant, anti-wrinkles and tyrosinase inhibitory activities of cacao pod extract. *BMC Comp. Altern. Med.* 2014;14:381.
5. Latif R. Chocolate/cacao and human health: a review. *Neth. J. Med.* 2013;71(2):63-8.
6. Oracz J, Zyzelewicz D, Nebesny E. The content of polyphenolic compounds in cacao beans (*Theobroma cacao* L.), depending on variety, growing region, and processing operations: A Review. *Crit. Rev. Food Sci. Nutr.* 2015;55:1176-1192.
7. Miller KB, Stuart DA, Smith NL, Lee CY, McHale NL, Flanagan JA, Ou B, Hurst WJ. Antioxidant activity and polyphenol and procyanidin contents of selected commercially available cacao-containing and chocolate products in the United States. *J Agric. Food Chem.* 2006;54(11):4062-8.
8. Summa C, McCourt J, Cämmerer B, Fiala A, Probst M, Kun S, Anklam E, Wagner KH. Radical scavenging activity, anti-bacterial and mutagenic effects of cacao bean Maillard reaction products with degree of roasting. *Mol. Nutr. Food Res.* 2008;52(3):342-51.
9. Gu Y, Lambert JD. Modulation of metabolic syndrome-related inflammation by cacao. *Mol. Nutr. Food Res.* 2013;57(6):948-61.