

ESTIMATION OF PROTEIN CONTENT AND PHYTOCHEMICALS STUDIES IN COCOA FRUIT OUTER COVERING

Dr.D.Sailaja, P.Srilakshmi*, K. Puneeth and C. Ramya Krishna

Corresponding author: P.Srilakshmi*, Gokarajurangaraju institute of engineering and technology, Kukatpally, Hyderabad, Telangana State, India

ABSTRACT: This paper reports the concentration of proteins and phytochemical evaluation on cocoa fruit outer covering. The amount of protein was estimated by Lowry method. Phytochemical screening was done with various successive extracts such as methanol, ethanol and petroleum ether for confirmation of the presence of alkaloids, tannins, flavonoids, steroids, glycosides and carbohydrates.

Key words: Cocoa, proteins, phytochemical studies, secondary metabolites.

INTRODUCTION

Cocoa or cacao, is an ancient crop having been harvested and used by the indigenous people of Central and South America for thousands of year. Cocoa was introduced to Europe during the 16th century. Cocoa (*Theobroma cacao* L) belongs to the family Sterculiaceae is worldwide known for its beans used in the manufacture of chocolate. For a long time the production and commercialization of cocoa has been the basis for the economy. The fruits look like fat cucumbers and are 15-20cm big. It is filled with sweet, mucilaginous pulp enclosing 30-50 large seeds that are fairly soft and white to a pale lavender color. Cocoa pulp is a substrate rich in nutrients, by-product manufacture [1] &[2]. The cocoa also called cacapoor simply cocoa is the dried and fully fermented fatty bean of *Theobroma cacao*, from which cocoa solids and cocoa butter are extracted. They are the basis of chocolate. Chocolate is derived from the seeds (or pods) of the cacao. These fruits grow on the cacao tree. The development of chocolate flavor requires that seeds are fermented and dried (also known as cacao beans) which are essential for obtaining flavor precursors, which are fully expressed later during the roasting process [4].

While seeds are usually white, they become violet or reddish brown during drying process. Cocoa fruit (pod) varies in size, shape, external color and appearance. *Theobroma cacao* L seeds are unique source of cocoa butter and solids, essential raw material for chocolate production [3]. The tree produces pods that contain 40 cocoa beans surrounded by a sweet tasting mucilaginous pulp. In Nigeria, it is a plant grown under bean remnant forest by mostly small holders accounting for about 90 percent of aggregate hectarage but who employ cheap labour to achieve harvest. Cocoa pulp can be readily fermented by yeasts such as *Saccharomyces cerevisiae*, producing and alcoholic beverage. *Saccharomyces cerevisiae* has been used in fermentative processes for thousands of years, according to the first historical stories of the production of beer and wine [13],[14],[15]&[16]. Raw cocoa beans have an astringent and unpleasant taste and have to be fermented, dried and roasted to obtain the characteristic chocolate taste and flavor. The fermentation process generates flavor precursors namely free amino acids and peptides from enzymatic degradation of cocoa proteins and reducing sugars form enzymatic degradation of sucrose in cocoa [17] &[18]. Cacao bean is surrounded by and aromatic mucilaginous pulp, the outer seed coat together with the mucilaginous pulp surrounding it and an inner embryo or cotyledons contained within [5] &[6].

The seed pulp represents around 40% of the seed fresh weight and is composed of a parenchymatous layer containing cell sap, which is rich in sugars (10-13% glucose and fructose, 0.7% sucrose) salts (8-10%), pentose's (2-3% pectin), organic acids (1-2%) and 0-6% proteins [7],[8],[9]&[10]. Traditionally, cacao seed fermentation is a non-controlled process initiated by microorganisms naturally occurring at fermentation sites including yeasts, lactic and acetic bacteria, Bacilli and filamentous fungi [11]&[12]. M.J. Abbe (2009) studied microbial activity on cocoa powder. He determined that high concentration of Theobromine and caffeine. Moreover, dimer, trimers are identified and catechin, epicatechin was stable during the fermentation process. He mentioned that polyphenols are used for health promoting activities. Cocoa pulp also contains methyl xanthenes which are responsible for antioxidant capacity. Investigations conducted on polyphenol extracts from the lower quantity cocoa beans: antioxidant, antibacterial and food coloring. Polyphenol extracts from unfermented dry cocoa beans have higher polyphenol contents and stronger antioxidant activity than partially fermented cocoa beans have higher polyphenol contents and stronger antioxidant activity than partially fermented cocoa bean, can be used at 3% as natural functional food colors. In recent years, cocoa and cocoa products, namely cocoa powder, dark chocolate and cocoa liquor have been shown to suppress the development of atherosclerotic lesions [21], decreased platelet function [22], increased dermal blood flow [23] and inhibit the proliferation of human breast cancer cells [24] and exerted hypoglycemic properties [25]. [26]. Cacao bean contains 10-15% protein [19] with albumin and globulin being the predominant fractions [20]. The studies of cocoa and their related products have become an area of interest owing to their health promoting properties. The husk is known to have medicinal properties having been traditionally applied to treat the pains of pregnancy, fevers, and coughs. Theobromine relaxes the smooth muscle in the digestive tract.



Fig-1: Cocoa Fruit

Scientific classification:

Kingdom - Plantae
Order - Malvales
Family - Malvaceae
Genus - Theobroma
Species - T. cacao

Binomial Name: Theobroma Cacao

Several studies have been shown the beneficial effects of Cocoa fruit against chronically diseases such as cancer, ulcer, kidney stones, intestinal infections, liver diseases. An aqueous layer made from the cocoa fruit, is used in the Moroccan traditional medicine for the treatment of diabetes, as well as a number of other diseases. Overall review of literature explained that most of the study is done on cocoa fruit by performing fermentation process. Various studies were done, as the bioactive compounds by comparing them before and after fermentation process. All the results revealed that cocoa fruit is having increased amounts of proteins, polyphenols. Which show antioxidant activity and used as health promoting factors. However biochemical estimations like protein estimation and phytochemical evaluation are not reported so far. So we estimated the biochemical compounds and screened the secondary metabolites in cocoa fruit outer covering and compared.

MATERIALS AND METHODS

Cocoa fruit was collected from local areas of Hyderabad, Telanga state, India during March 2014. Cocoa fruits were screened. They were cut into 3 different parts: Outer covering, soft middle part and seeds with their covering. These different parts of the fruit were shade dried and coarsely powdered for use in tests and analyses.

Biochemical estimation:**Estimation of protein by Lowry method:**

Quantitative estimation of protein was carried out by the methods of Lowry et al (1951) [27]. 1g cocoa pulp material was homogenized with 10ml of 80% ethanol. The extract was centrifuged at 5000rpm for 5 minutes and the supernatant was discarded. 5% 10 ml TCA (Trichloro acetic acid) or perchloric acid was added to the residue and incubated at 80C for 20minutes. The pellet was centrifuged and the supernatant was discarded. Residue was washed with 10ml distilled water and again centrifuged. The supernatant was discarded. 2% 10ml sodium carbonate in 0.1N NaOH was added to the residue and incubated for an hour at 30C. Again it was centrifuged and residue was discarded. To 1ml of sample extract 5ml of alkaline Copper reagent was added and then was allowed to stand for 10minutes. The tubes were agitated gently during the addition of 0.5ml of phenol reagent and incubated at room temperature in the dark for 30minutes till blue color was developed. The OD of the characteristic blue was measured at 660nm in a spectro photometer after setting for 100% transmission against the blank, standard curve was prepared by using known concentration of protein.

Phytochemical screening

The powdered plant material was subjected to successively soxhlet extraction with various solvents such as ethyl acetate and methanol. After concentration and drying of each extract identification of phytoconstitents was carried out using chemical tests. Standard methods were used for phytochemical screening of the different extracts to know the nature of phytoconstitents present within them [28] [29].

RESULTS

Protein content in cocoa fruit outer covering was estimated by Lowry method and showed high protein content when compared to Soybean extract and BSA. The phytochemical investigation of various solvent extract of cocoa fruit outer covering showed they contain proteins, amino acids, carbohydrate, fats and oils, steroids, alkaloids, tannins and glycosides. The results were shown in graph and in Table-1.

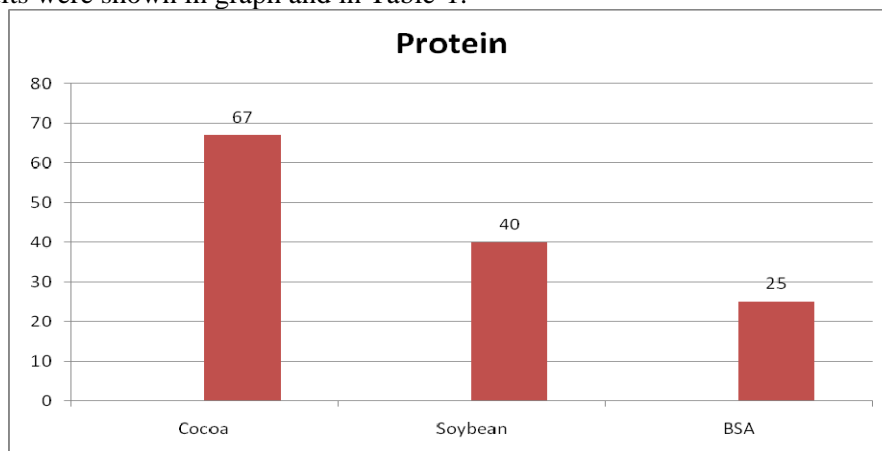


Table 1. Phytochemical constituents of coca fruit outer shell

S.No.	Secondary Metabolite	Presence/ Absence
1.	Fats and oils	+
2.	Steroids	++
3.	Alkaloids	+
4.	Tannins	+++
5.	Phenolic compounds	+++
6.	Cardiac Glycoside	+
7.	Antheraquinone	-
8.	Saponins	++
9.	Flavonoids	+++
10.	Proteins	+++
11.	Tyrosine	+
12.	Carbohydrates	+
13.	Reducing sugars	+
14.	Tannic acids	++
15.	Gums	+++

“+” indicates presence; “++” indicates good presence; “+++” indicates strong presence “-” indicates absence;

DISCUSSION

Phytochemical constituents such as alkaloids, flavonoids, Tannins, phenols, saponins and several other aromatic compounds. Secondary metabolites of plants that serve a defense mechanism against predation by many microorganisms, insects and other herbivores. The present study carried out on fruit samples revealed the presence of active constituents. Fruits are a source of large amount of drugs comprising to different groups such as antispasmodics, emetics, anti-cancer, antimicrobials etc. A large number of Fruits are claimed to possess the antibiotic properties in the traditional system and are also used extensively by the tribal people worldwide. It is now believed that nature has given the cure every disease in one way or another. Fruits have been known to relieve various diseases in Ayurveda. Therefore, the researchers today are emphasizing on evaluation and characterization of various Fruits and Fruits constituents against a number of diseases based on their traditional claims of the plants given in Ayurveda. Extraction of the bioactive Fruits constituents has always been a challenging task for the researchers. In this present review, an attempt has been made to give an overview of certain extracts and extraction processes with their advantages. The result of the present study showed that the extract of cocoa fruit outer covering is rich in protein and contains many photochemical components. Generally Cocoa contains phenol that acts as antioxidants. The current study has proved that coca fruit outer covering also rich source of phenols. They may help to protect against the development of coronary heart and cardiovascular diseases. So, these studies are useful for further investigations.

REFERENCES

- [1] Freire, ES. Schwan, R.F. and Mororo, R.C 1999. The cocoa pulp agroindustry and the use of its residues in Bahia: progress achieved in the last ten years. In: proceedings of the 12th International cocoa research conference. Pp.1013-1020. Kuala Lumpur: cocoa producers Alliance.
- [2] Schwan R.F and Wheals, A.E. 2014. The microbiology of cocoa fermentation and its role in chocolate quality. *Critical reviews of Food science and Nutrition* 44, 205-222.
- [3] Pires JL, Cascardo JCM, Lambert SV and Figueria A 1998. Increasing cocoa butter yield through genetic improvement of *Theobroma cacao* L: seed fat content variability; inheritance and association with seed yield *Euphytica* 103:115-121.
- [4] Lopez AS and Dimick PS 1995. Cocoa fermentation BT, vol.9 (Reed G and Nagodawithana TW, eds), pp-561-577. VCH Publishers, weinheim, Basel.
- [5] Thompson SS, Miller KB and Lopez AS 2001. Cocoa and coffee. *Food microbiology- Fundamentals and Frontiers* (Doyle M Beuchat LR and Montville TJ, eds), pp 721-733. ASM press, Washington DC.

- [6] Figueira A, Janick J and Bemiller JN 1993. New product from Theobromacacac: seed pulp and podgum. New crops (Janick J and Simon JE,eds)pp-475-478. Willey, Newyork.
- [7] Biehl B. and Ziegleder, G.2003. cocoa: chemistry of processing. Encyclopedia of food sciences and nutrition.2nd Edition pp-1436-1448.
- [8] Roelofsen PA 1958. Fermentation, drying and storage of cocoa beans, Adv Food Res 8: 225-296.
- [9] Lopez AS 1986. Chemical changes occurring during the processing of cocoa. Proceedings of the symposium cacao BT (Dimick.ps.ed) pp-19-54. The Pennsylvania state university, University park.
- [10] Ostovar k and Keeney PG 1973. Isolation and characterization of microorganisms involved in the fermentation of Trinidad's cacao beans, J. food Sci 38:611-617.
- [11] Schwan RF 1998. Cocoa fermentation conducted with defined microbial cock tail inoculums. Appl Environ Microbiol 64: 1477-1483.
- [12] Rose AH 1977. History and scientific basis of alcoholic beverage production. In. Alcoholic beverages (edited by A.H.rose(vol 1/pp 1-37. London: Academic press.
- [13] Hansen J and Kielland- Brandit, M.C. 1996. Modification of biochem pathways in industrial Yeast.Jou of Bioch, 49,1-12.
- [14] Demin AL. 2000. Microbial Biotechnology Trends in BiotechnolOgy, 18,26-31.
- [15] Ostergaard, S. Olsson, L and Nielsen, J. (2000) Metabolic engineering of S.cerevisae. Microbiology and M.B. Reviews 64, 34-50.
- [16] Misnawi S.2008. physic-chemical changes during cocaoa fermentation and key enzymes involved. Review penelitankopidankakao 24 (1): 54-71.
- [17] Afoakwa EO. Quao j, Takrama J, Budu AS and Saalia, FK 2011b.Effect of pulp preconditioning on acidification, proteolysis of sugars and free fatty acids concentration during fermentation of cocoa beans.Intr J. of Food science and nutrition 62 (7): 755-764.
- [18] Bertazzo A, Comaei, S, Brunato, I, Zancato,M. and Costa, C.V.1-2011. The content of protein and non-protein (free and protein-bound) tryptophan in Theobroma cacao beans. Food chem. 124:93-96.
- [19] Voigt, J. Biehl, B and Kamaruddin, S.1993. The major seed proteins of Theobromacacac.L. Food cahem 47:145-151.
- [20] Kurosawa, T, ITOH, F, Nozaki.a, Nakano, Y, Katsuda, s,I, Osakabe , N. Tubone, H, Konodo K and Itakura, H.2005. Suppressive effect of cocoa powder on atherosclerosis in Kurosawa and Kusanagi-hypercholesterolemia rabbits. J.atheroscler. Thromb.12, 20-28.
- [21] Murphy, KJ, Chronopoulos, AK singh, I. Francis MA. Moriarty,H, Pike, MJ, Turner, A.H, Mann, NJ and Sinclair, Aj.2003. Dietary flavonols and procyanidin oligomers from cocoa inhibit platelet function. Ame.J.clin. Nutr.77,1466-1474.
- [22] Neukan, K Stahl ,W, Tronnier, HSies,H and Heinric U.2007. Consumption of flavonol-rich cocoa acutely increases microcirculation in human skin. Eur.J. nutr.46,53-56.
- [23] Ramljak,D, Romanczyk K, LJ Methney-Barlow LJ Thompson,N, Knezevic,V, Galperin,M, Ramesh,A and Dockson RB,2005. Pentamericprocynadin from Theobroma cacao selectively inhibits growth f human breast cancer cells. Mol.Cancer Ther.4 (4), 537-546.
- [24] Tomaru M, Takano H, Osakabe N, Yasuda a, InouseK, I,Yangisawa R, Ohwatari I and uematsu, H.2007. Dietary supplementation with cacao liquor proanthocyanidins prevents elevation of blood glucose levels in diabetic obese micr. Nutrition 23,351-355.
- [25] Prayoga, RD, Murwani, R and Anwar, S.2013. Polyphenol extracts from low quality cocoa beans: antioxidant, antimicrobial and food coloring properties.
- [26] Abbe-Maleyki, MJ and A. Ismail, 2001. Antioxidant properties of cocoa powder.J.Food Biochem, 34:111-128.
- [27] Lowry, OH, Rosebrough, NJ, Farr, A L and Randall, RJ 1951. J. Biolchen 193-265
- [28] Khandelwal KR 2001. Preliminary phytochemical screening, in: Practical Pharmacognosy Techniques and Experiments. 8thedn.Nirali Publication, Pune, pp. 149-156.
- [29] Indian pharmacopoeia, Vol (1) & (2): published by the controller of publication, Delhi, 1996, A67-76.