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Development Of Cocoa Butter Based On Coconut Extracted Oil And Its Proximate Analysis, Sensory Evaluation And Antimicrobial Activity

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ABSTRACT
Fresh coconut milk, an oil-in-water (o/w) emulsion, contained high fat content of 29 wt.%. The coconut milk was fractionated into coconut cream and coconut skim milk by a disc bowl centrifuge. Fat content in coconut cream was approximately 34 wt.%; while coconut skim milk contained no fat. Coconut cream and coconut skim milk were used as raw materials for coconut butter and coconut protein concentrate (CPC) production, respectively. Coconut butter was produced by phase inversion process using aeration. The molecular weight (MW) profiles of proteins in the co-products from coconut butter production were investigated. Use of small molecular weight surfactants, i.e. sucrose mono stearate and sucrose mono palmitate at0.05 – 1.00 wt.% of coconut cream, did not affect the yield of coconut butter (P > 0.05). Production yield of butter was at 49 wt.% of coconut cream. However, increasing the concentration of sucrose mono stearate reduced the production yield of coconut butter from 49 wt.% to 43.5 wt.% of coconut cream (P ≤ 0.05). The proteins in CPC, coconut buttermilk and coconut whey were found to have different MW profiles

Keywords: *coconut, butter, milk, protein, phase inversion, surfactant.*

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I. INTRODUCTION

Cocoa bean (CB) is the fatty seed found inside a cocoa pod, fruit of the Theobroma cacao plant. It is small evergreen tree belong to the family Malvaceae. This plant is native to the deep tropical regions of Central and South America. After harvesting the cocoa fruit, it is opened to expose the seed, then fermented for a few days to separate pulp and seed. Pulp is used in distilleries and seed is used to prepare cocoa powder or chocolate and cocoa butter. The processing method of cocoa fruit is given in Cocoa butter is obtained by pressing of mature cocoa beans. CB is a valuable by product of the cocoa industry. It is a pale yellow liquid with a characteristic odor and the flavor of chocolate. It is an important and the only continuous fat phase found in chocolate, which help in the dispersion of the other ingredients also (Wang et al., 2006). It is brittle at temperature below 25○C, soften in the hand and melts in the mouth having at a temperature of about 34C. This specific physio-chemical property makes it is an important ingredient in confectionery industry. It is not greasy to touch. Cocoa butter contains a high proportion of saturated fat, derived from starch and palmitic acid and contains trace amounts of caffeine and theobromine. It also contains fat soluble antioxidants such as vitamin E in the form of β-tocopherol, α-tocopherol and γ-tocopherol helps in its storage by increasing its therapeutic properties. CB can crystallize into several polymorphic forms, having α, γ, β' and β, crystals, with melting points of 17, 23, 26, and 35-37oC respectively. In the chocolate production, only β crystal is used because it has a high melting point. This crystal structure confers chocolate products an excellent quality in terms of sheen, snap, and smooth texture. It is also used in the formulation of cosmetics and soap due to its moisturizing and antioxidant properties which give it an anti-ageing effect. Due to the fact that about 30% of the world's coca crops are destroyed by pests and disease and is deteriorating due to climate change. With this the fat content of the cocoa bean is small in amounts as compared to the other fatty crops. It accounts for more than 50-58% of the cocoa beans. Less amount of fat content and is cultivated in few countries having a tropical climate, makes it availability unstable and expensive (Knapp, 2007). Other than this to overcome some technological problems like fat bloom, etc.

II. MATERIALS AND METHODS

Raw materials

The raw material used in the present work was dried copra. The copra was selected based on the characteristics of being the same variety, maturity index, diameter and area of cultivation. The variety of coconut used was MATAG (Cocos nucifera L.) of maturity index no 2 (12 to 14 months' cultivation) with diameters of 10 cm to 20 cm. The coconuts were cultivated in Tanjung Karang, Malaysia. Commercial cocoa butter (CB) was used as a sample reference (control), and obtained from Barry Callebaut (Port Klang, Malaysia), which was natural and deodorised with 52% fat content.

Chemicals

The chemicals used for fatty acids analysis were hexane, sodium methoxide and sodium chloride. The mix standards of methyl esters (C8 to C22).

Sample preparation

The copra was cut using commercial knife and the juice removed from the freshly opened copra. The flesh was then grated by an electric grinder into grated coconut. The grated coconut was sun-dried from 3% to 5% moisture content in order to protect the bioactive compound. The drying process was conducted during the hot climate season. The temperature was controlled between 30 to 34°C. Then, the dried grated coconut was ground, sieved into powder of sizes ranging between 0.5 to 1.0 mm, and kept in an air-tight container to keep out humidity prior to extraction**.**

Proximate analysis

Proximate analysis refers to the quantitative analysis of macromolecules in food. A Combination of different methods and techniques are used to determine protein, fat, moisture and ash levels in sample.

Moisture estimation

The moisture content was determined by oven drying method. The sample of 5gm were crushed and dried in an oven at 100°C to constant weight. After cooling in the desiccators, the sample was weighed again. The loss in weight was recorded as moisture content.

$$\text{Moisture (\%)} = \frac{W1-W2}{W1} \times 100$$

Where,

W1 = Initial weight of bottle with sample before drying.

W2 = Final weight of the sample after drying.

Fat Analysis by Soxhlet extraction methods

Soxhlet Extraction Method was used for the estimation of fat. First of all, rinse all the glass apparatus by petroleum ether and dry it in the oven at 102°C and after removing it keep in the desiccator. Weigh 5 gram of grounded and dried sample and place it in the thimble. Place the thimble in the soxhlet extractor. Take a 150ml round bottom flask and clean it and fill the flask with 90 ml petroleum ether. Place the whole setting on a heating mantle and allow the petroleum ether to boil. Continue the extraction process for several hours, almost 6 hours. Remove the condensing unit from extraction unit and allow the sample to cool down. Finally, it removes all the lipid. Collect almost all the solvent after distillation. Place the sample in the oven and after removing it place in the desiccator. Take the weight of the sample. As a result, we get a defat sample.

$$\text{Crude Fat (\%)} = \frac{\text{weight of ether soluble material}}{\text{Weight of Sample}} \times 100$$

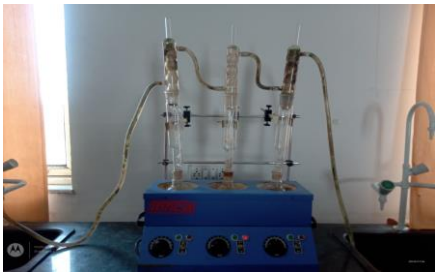

Fig.1 soxhlet method.

pH estimation pH paper strips with colour indicators were used. Three readings were taken at once and recorded. An interval of 20-25 min was given for the paper to soak thoroughly after insertion into the creams.

Fig.2 pH test

Ash estimation

Total ash content of sample was estimated by using direct-heating method of muffle furnace.

$$\text{Ash (\%)} = \frac{\text{Weight of Ash}}{\text{Weight of sample}} \times 100$$

Protein estimation

Weigh quickly about 1-2 gm of the sample and transfer to a 500 or 800 mL Kjeldahl flask taking care to see that no portion of the sample clings to the neck of the flask. Add 0.7 gm of Mercuric oxide, 15gm of Potassium Sulphate and 40mL of concentrated sulphuric acid (Mercuric oxide is added to increase the rate of organic breakdown during acid digestion. Because of environmental/safety concerns over handling and disposal of mercury, copper sulphate can be used. This is important from safety point of view as mercury vapours might escape into the environment during the distillation process.

Calculate protein as = N x 6.25 Protein on dry wt.

$$\text{basis} = \frac{\text{Protein content}}{100 - \text{Moisture content}} \times 100$$

Sensory Evaluation

Sensory evaluation in respect of colour / appearance, flavor / taste, aroma, texture, acceptability was evaluated by three trained judges using nine-point hedonic scales (Fig.3) and the result was calculated as average.

Antibacterial Activity

Ager diffusion method Source of Microorganism for Antibacterial Activity Escherichia coli, Salmonella Enteritidis and Staphylococcus Aureus were used in this study for antibacterial activity of beet root. These cultures were obtained from the laboratory of environmental microbiology, Babasaheb Bhimrao Ambedkar University, Lucknow U.P, (INDIA).

Antibacterial activity of cocoa butter

Sample for the determination of antibacterial activity of cocoa butter, which was dissolved in distilled water to a concentration of 100 mg/ml. (Markov et al., 2011). Agar well diffusion method is mainly used for the evaluation of antibacterial activity of plants or microbial extract (Mouny Balouiri et al., 2016). The determination of antibacterial activity in cocoa butter was done using the agar well diffusion method (Emoghene et al., 2014). The zone of growth was found only for Escherichia coli (ATCC 10536), Staphylococcus aureus (ATCC 11632) and Salmonella enteritidis for the well diffusion method, wells of 9mm diameter were made. Three wells are made on the surface of the agar plate. The beet root extract solution (50 and 100) microliter was then transferred into the wells of incubated agar plates (Kahkashan Perveen, Najat A. Bokahri, 2020). The plates were refrigerated at 80C for 1 h to allow the extract to diffuse into the medium, and then incubated at 37oC for 24 h. after incubation period the diameters of the inhibition zone were measured and recorded in millimetres (mm). The measurement of diameter (mm) of the zone of inhibition was done by using a transparent scale, and then evaluated (Ramesa Shafi Bhat et al., 2014).

Fig.4 microbial activity

III. RESULT AND DISCUSSION

The research paper focused on the development and analysis of cocoa butter. The proximate analysis revealed favorable nutritional composition, indicating that the product can be a valuable addition to a healthy diet. The sensory analysis demonstrated high consumer acceptance, suggesting that the cocoa butter possesses desirable taste, texture, aroma, and overall quality Moreover, the shelf life analysis using the Agar Diffusion Method confirmed that the chocolate-coated date fruit maintains microbial stability over time. These findings collectively emphasize that the developed product is a nutritious, delicious, with a satisfactory shelf life, offering a novel and enjoyable way to incorporate butter into the diet.

PROXIMATE ANALYSIS

Table no.4.1. Results of proximate analysis

Ash	77.O75%
Moisture	0.3%
Fat	0.0006%
Protein	6.03%
Carbohydrate	22.59%
pH	3.854%
Vitamin c	5.285%
Titrable acidity	0.72%

Sensory Evaluation: -

Butter was evaluated by 25 untrained members on following characteristics.

- Appearance
- Taste
- Smell
- Texture
- Overall Acceptability

Table no.2 Average score of sensory Evaluation.

S.No.	Parameters	Average Score
1	Appearance	7.5
2	Taste	8.4
3	Smell	8.4
4	Texture	5.76
5	Overall Acceptability	7.5

Methods Nutrient agar (Biotec), Nutrient broth (Lab.M), Sabouraud's dextrose broth (SDB) and Sabouraud's dextrose agar (SDA) were from Oxoid. Ps. aeruginosa ATCC 7853, E. coli

Fig.5 final product (coconut butter)

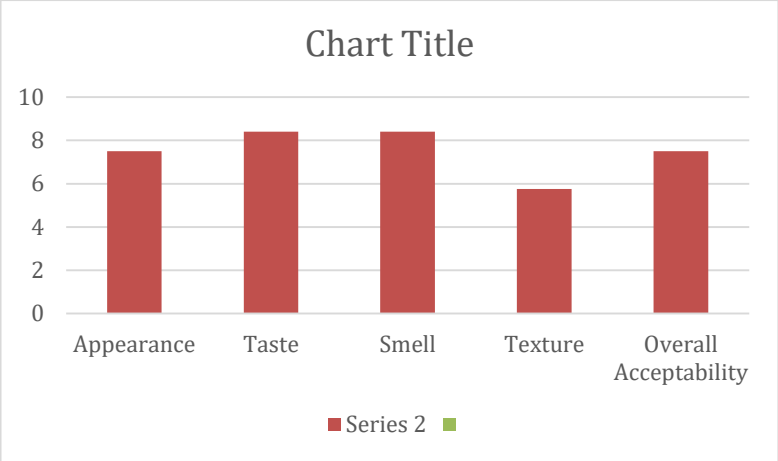

Fig. 6 sensory evaluation graph

Shelf-life

Total colonies of TPC	2337.5cfu/gm
Total colonies of coliform	Absent/gm
Salmonella	Absent/gm
Total colonies of yeast & moulds	Absent/gm

Table no. 4 Observation of shelf life

IV. CONCLUSION

This study has established that coconut oil can be formulated into an elegant cream which is active on both fungal and bacterial organisms. It also demonstrates the possibility of standardizing the quality and quantity of oil to be used therapeutically in extemporaneous preparations. CB is a unique and valuable fat. Cocoa trees can be cultivated in a few of the countries make it supply unstable and increasing demand of cocoa butter in confectionery industries increases its price. Due to some technological and chemical problems in CB made it to find cocoa butter alternatives using low cost natural fats having a closer physical property of CB. This helps in introducing fats of some non-timber forest products like mango, sal, palm, mahua seed kernel etc. CBA can be produced by either blending of these oils.

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