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Determination Antioxidant and Sensory Evaluation of Fermented Tamarind Seed Lotion

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Abstract

Antioxidant is any substance that delays, prevents, or removes oxidative damage to a target molecule. Antioxidants are used as cosmetic additives to help against skin deterioration which is exposure to radiation UV sunlight. This factors that lead to produce synthesis melanin and an enzymatic antioxidant towards skin. Therefore, an antioxidant agent which are can be in two types, synthetic or natural antioxidant agents. The synthetic antioxidants such as propyl gallate, tertiary butylhydroquinone, butylated hydroxyanisole, butylated hydroxytoluene, vitamin E, BHA (E-320) and BHT (E-321) are used in cosmetic industries as an oxidation process on skin occurred and can extend the shelf life of the cosmetic into its ingredient lotion. In this study, Tamarindus indica seed oil contained compounds that exhibit antioxidant activity based on the several previous studies. Some improvement had been done to increase an antioxidant by fermented Tamarindus indica seed for 24 hours, 48 hours and 72 hours. To obtain an antioxidant compounds from the samples, the solvent extraction method is needed to extract the desired compounds. The solvent extraction is indeed the efficient and reliable method to extract and preserve the antioxidant properties of the samples. Then, oil extraction from fermented Tamarindus indica seed added in lotion. Different antioxidant shown its own properties and polarities that may certain solvent can extract it. To evaluate the effectiveness of the oil extract of the samples the chemical analysis is needed to strengthen the data validation which are DPPH assay, TPC assay and HPLC to prove the antioxidant content from the samples used. Analysis for lotion by using sensory evaluation in products.

Key-word: - Antioxidant, tamarind seed, tamarind seed powder, fermented tamarind seed powder, lotion,sensory evaluation

1. Introduction

Nowadays, cosmetic product is one of the important products to the people either man or woman. The cosmetic product has high potential and largest market in the industry. The eager to be beauty promotes the daily use of the cosmetic to cleanse, deodorize, face and body products. Millions of peoples in this world are using cosmetic daily (Engasser et al, 2007). According to FDA, cosmetic is referring to “their intended to use, likes to be rubbed, poured, sprinkled, or sprayed on, or other applied to the human body. Cosmetics are for cleansing, beautifying, or altering the appearance” (Federal Food Drug, 2010).

Furthermore, many consumers are facing skin aging problem was making colour skin has changes that cause the radiation of UV sunlight penetration. This can cause the production of synthesis melanin and an enzymatic antioxidant. The addition of antioxidant in the cosmetic product may help to improve skin aging. Skin become more moisturizes and less wrinkle on surface skin. It is intuitive to hypothesize that the topical application of antioxidants may neutralize some of the resulting free radicals, and consequently lessen or prevent the signs of aging skin. At present, topical antioxidants are marketed to prevent aging and UV-induced skin damage, as well as to treat wrinkles and erythema due to inflammation (e.g., post laser resurfacing).

Fermented plant products are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found in vitro to have antimicrobial properties. Antimicrobial ingredients are materials that protect against the growth of microorganisms in cosmetics and skin, including bacteria, viruses and fungi. Antimicrobial ingredients can also kill organisms that may be present in ingredients that may be used to make cosmetics. Antimicrobial ingredients play an important role in making sure that cosmetics are free of microorganisms during storage and after they are opened. They are effective at low levels so that it does not take much of the ingredient to work. There are many different types of materials that can be used in products and they are selected based on the specific type of product. A water-based product may use a different type of ingredient (or combination of ingredients) than an oil-based product.

Lotion is one of cosmetic or personal care product that is used as the skin moisturizer. Income lotion has been applied since ancient times. Lotion are aqueous emulsions containing oil, surfactants, pH balancing ingredient and moisturizing that studied by (Adam et al., 2009). Lotion defined semi-solid emulsion of oil in water and mostly in the market lotion is can be water based. A lotion is droplets of oil mixed in the water and have a high water and low oil content. Generally, lotion is intended as moisturizes, softens and water in the outermost layers of the skin or body. Lotion contains special ingredients that are used to protect against moisture loss from the skin and replace the oil contained in the skin. Some individual want to protect the skin from becoming too dry skin with sun exposure of UV light. So, this product lotion helps the dried skin so that the skin is always in moisture. Lotion can be hydrated the high-water content helps the skin absorb lotion quickly and also the moisturizing effect of lotion is shorter-lived. In addition, this project research develop lotion with addition of Fermented Tamarind Indica seed oil as an additive antioxidant.

Tamarind (Tamarindus indica) is a member of the dicotyledonous family Fabaccae (Leguminose) (Chant, 1993). It is growing in more than 50 countries of the world. The major areas of production are in Asia countries like India, Bangladesh, Sri Lanka, Thailand, Indonesia, and in the African and the American continents. In Tamarind indica, it is containing a crude lipid which is 6.94% to 11.43% (Folch et al., 1957). Also, Tamarindus indica has a large quantity of oil and fats, for human consumption or for the industrial purposes that are presently derived from plant sources. Tamarindus indica seeds are important sources in nutritional of oils, industrial and pharmaceutical significance (Rice-Evans et al., 1997).

Tamarind Indica seed was fermented to enrich the content nutritional value. Tamarindus indica seed fermented in 24 hours, 48 hours and 72 hours to increase the content an antioxidant value. An antioxidant is a molecule that inhibits the oxidation happen in another molecule. Oxidation is chemical reaction that can produce free radicals that leads to chain reactions that can be damage the cells due to the oxygen. Antioxidants that include enzyme and other substances such as vitamin C, vitamin E, and beta carotene, which are can capable to counteracting the damaging effects of oxidation. This an antioxidant agent oil was extracted from fermented Tamarindus Indica seed added in the lotion product.

1.1 The problem statement of this research is about Fermented Tamarindus indica seed oil as antioxidant agent.

1) Fermented Tamarindus indica seed oil contain compound antioxidant properties.
2) Sensory evaluation in production of fermented tamarind seed lotion

1.2 This research is to describe physical characteristics of Fermented Tamarindus indica seed lotion. The purpose of this research is:

1) To determine Fermented Tamarindus seed oil as antioxidant agent.

2) To determine the sensory evaluation of the Fermented Tamarind seed lotion.

2. Methodology

2.1 Sample preparation

Firstly, sample of Tamarindus indica seed will be extracted must be crushed and powdered (fine) and must be free from all possible foreign particle and adulteration. Then, prepared fermentation of Tamarind indica seed process by using yeast and water. Ratio of sample tamarind indica seed : water is 1:1 which is 325g of tamarind indica seed: 325g of water addition with 0.4% yeast from mass powdered of fermentation tamarind indica seed . After that, powdered fermentation tamarind indica seed was placed under the sunlight following the set time (A) 24 hours, (B) 48 hours and (C) 72 hours. All the fermentation was freshly prepared then keep it in bottle and stored in refrigerator. Then, samples were weighted for 50g for each batch for extracting oil. Next, we used the sample of Tamarindus indica seed to grinded until it fine. Then, we stored the sample of powdered Tamarindus indica seed in the glass bottle.

i) Dried powder tamarind seed

ii) Fermentation powder tamarind seed

Figure 1 Figure shows the sample preparation for fermented tamarind seed powder

2.2 Extraction oil from fermented tamarind seed powder

Soxhlet extraction is an especially useful tool for preparative purposes in which the analyte is concentrated from the matrix as a whole or separated from interfering substances. The sample preparation of environmental samples has been developed for decades using a wide variety of techniques. For the sample of Fermented Tamarindus indica seed, the sample has been taken for 50g and put it into the thimble. Then, the thimble that contained the sample was placed into the extraction chamber. The solvent that was used is N-Hexane. N-Hexane was added for 200ml as solvent into the solvent flask. The soxhlet extractor was left for 8 hours and 60 ºC.

2.3 Total Phenolic Compounds (TPC)

The Folin-Ciocalteu reagent assay was used to determine the TPC (Singleton & Rossi,1965). An aliquot of the samples (30 ml) was introduced into 96 well ELISA plate followed by 150 ml FolinCiocalteu reagent, which was previously diluted with distilled water (1:10) and 120 ml sodium carbonate (75 g/l). The ELISAplates were vortexed, covered with parafilm and allowed to stand for 30 min in ELISA reader. Absorbance at 765 nm was recorded in an ELISA reader (SpectroMax M2 e, Molecular Devices, Sunnyvale, CA, United States). TPC was expressed in gallic acid equivalents (GAE mg/100 g). The calibration equation for gallic acid was y ¼ 0.0104x - 0.0589 (R^2 ¼ 0.9978) where y is the absorbance at 765 nm and x is the concentration of gallic acid in mg/l (Girish Korekar et al., 2014).

2.4 DPPH Radical Scavenging Activity

The DPPH radical cation method was modified to evaluate the free radical-scavenging effect of one hundred pure chemical compounds. The DPPH reagent was DPPH (8mg) dissolved in MeOH (100mL) for a solution concentration of 80µL/mL. To determine the scavenging activity, 100µL DPPH reagent was mixed with 100µL of sample in a 96- well microplate and was incubated at room temperature for 30min. After incubation, the absorbance was measured 514nm using an ELISA reader (TECAN, Gröding, Austria), and 100% methanol was used as a control. The DPPH scavenging effect was measured using the following formula:

$$\text{Scavenging effect (\%)} = \left[1 - \frac{(\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}})}{\text{Abs}_{\text{control}}}\right] \times 100\%$$

The IC_{50} DPPH values (the concentration of sample required for inhibition of 50% of DPPH radicals) were obtained through extrapolation from regression analysis. The antioxidant was evaluated based on this IC_{50} value (K.J Lee et al., 2015).

2.5 Determination of rutin, quercitrin and quercetin Concentration in Fermented Tamarind indica seed extracts using HPLC

Based on studies from Sharifuldin,M.M.A., et al., (2016) gave this method by using HPLC instrumentation. Instrumentation and high-performance liquid chromatography conditions: A Dionex-Ultimate® 3000 Rapid Separation LC system (Dionex, Seri Kembangan, Selangor, Malaysia) was used. The instrument is equipped with auto sampler, quaternary. pump, degasser, column oven, and a DAD-3000RS diode array detector (DAD) detector. The chromatographic analysis was performed on a reverse phase Acclaim Polar Advantage II column (150 × 4.6 mm × 5.0 µm; Dionex). The column temperature was set at 40 °C, the mobile phase was consisting of A (0.3% formic acid in water) and B (acetonitrile), the elution program was gradient for 20 min, the flow rate was maintained at 1 ml/min, and the injection volume was 10 µl. The spectral data from the DAD was collected at 254 nm and data acquisition was performed by Chromeleon software version 6.8 (Dionex). 10 µl of Fermented Tamarind indica seed extracts were injected at 1000 µg/ml, and the peak area corresponding to rutin, quercitrin and quercetin was recorded. The linear regression equations of the standard calibration curves were applied to in order calculate the concentration of the marker compounds in the samples, and the results are presented as a %w/w using the formula:

%w/w = (the found concentration / 1000 µg/ml) × 10 (n=3)

2.6 Sensory evaluation of fermented tamarind seed lotion

The nine-point hedonic scale was developed at the Quartermaster Food and Container Institute of the US Armed Forces, expressly to measure the food preferences of US soldiers (Peryam and Girardot, 1952; Jones et al., 1955; Peryam and Pilgrim, 1957). The nine-point hedonic scale is a bipolar scale with like and dislike on each end and a neutral point in the middle. The scale quickly spread as the preferred hedonic scaling instrument because it works well in showing differences in degree of liking among products and is still likely the most used scale for this purpose This happened despite acknowledged weaknesses, such as lack of linearity of the adjectives (Peryam and Pilgrim, 1957), the likely use of the scale as ordinal rather than interval by the consumers (Moskowitz and Sidel, 1971), the lack of equivalent use of the adjectives among the consumers, and so on. A few researchers have proposed alternatives to the nine-point hedonic scale, for example, the labeled affective magnitude scale (Schutz and Cardello, 2001), the best/worst method (Marley and Louviere, 2005), the semantically labeled hedonic scale (Lim et al., 2009), the hedonic general labeled magnitude scale (Bartoshuk et al., 2012), and so on. However, in nearly all cases the results of comparison studies showed that the nine-point hedonic scale performed equally well (Kalva et al., 2014; Lim, 2011; Lawless et al., 2009). The one exception to this seems to be best/worst scaling where the nine-point hedonic scale clearly performed better than the best/worst scaling (Mueller et al., 2009; Mielby et al., 2012). For these reasons we suggest that sensory practitioners use the nine-point hedonic scale, since Lim (2011) stated “when the primary concern of a study is measuring hedonic differences among foods, beverages, and consumer products and predicting their acceptance, the nine-point hedonic scale has proven itself to be a simple and effective measuring device.” This sensory evaluation had been done at IBD UTM, Skudai Johor. It was conducted by 30 panelist from IBD, UTM who is familiarized with lotion sample. Penalist were given two sample in the market. Panelist were asked to score the sensory properties of sample (using 9 terms). Each attribute was scored by using like dislike sacle (9 hedonic scales). The result and and data was analysed using Statistical Package for Social Sciences (SPSS) version 22.0.

Table 2 Table Of Sensory Evaluation Testing Lotion for Attributes and 9 Hedonic Scales

ATTRIBUTES	9 HEDONIC SCALES
1. Appearance	1- Dislike Extremely
2. Fragrance	2- Dislike very much
3. Color	3- Dislike moderately
4. Consistency	4- Dislike slightly
5. Smoothing	5- Neither like nor dislike
6. Spreadibility	6- Like slightly
7. Oiliness	7- Like moderately
8. Adhesiveness	8- Like very much
9. Overall Acceptance	9- Like extremely

3.0 Discussion analysis

3.1 Percentage of Oil Yield Extraction

Table 3.1 Result of Oil Yield Percentage (n-Hexane)

			Soxhlet Extractor	
Sample	Mass of empty jar (g)	Mass of jar + extract (g)	Mass of extract (g)	Percentage of yield (%)
Fresh TMS	20.3448	25.5080	5.1632	10.3
A 24 H TMS	21.1079	24.3131	3.2052	6.4
B 48 H TMS	20.7374	22.4751	1.7377	3.5
C 72 H TMS	20.8868	22.3442	1.4574	2.9

3.2 Determination Of Total Phenolics

Table 3.2 shows Total phenolic content present in different extracts of aerial part of fresh Tamarindus indica seed (TMS) and fermented Tamarindus indica seed (TMS)

Sample	Total Phenolics (mg GAE/100 g Sample)
Fresh TMS	656.09 $\pm$ 1.78
(A)24H TMS	914.50 $\pm$ 2.79
(B)48H TMS	668.70 $\pm$ 2.71
(C)72H TMS	879.80 $\pm$ 2.00

Table 3.2 shows that the total phenolic content found in the extracts of fresh Tamarindus indica seed was 656.09 ± 1.78 mg GAE/100g sample, 24 hours of fermented Tamarindus indica seed was 914.50 ± 2.79 mg GAE/100g sample and 72 hours of fermented Tamarindus indica seed was 879.80 ± 2.00 mg GAE/100g sample respectively. Higher phenolic concentrations were found in between 24 hours and 72 hours of fermented Tamarindus indica seed oil as compared to fresh ones ($p < 2.50$).

Evaluation Of Antioxidant Activity: Dpph• Radical Scavenging Activity Method

Table 3.3 of DPPH Analysis

Sample	Results IC_{50} (mg/L)
Standard: Ascorbic acid	1.21 ± 0.07
Fresh TMS oil	5.75 ± 0.43
(A) 24 H TMS oil	16.75 ± 0.43
(B) 48 H TMS oil	9.92 ± 0.38
(C) 72 H TMS oil	8.92 ± 0.29

Table 3.3 shows that all extracts exhibited significantly higher antioxidant activity than the control, standard ascorbic acid was 1.21 ± 0.07 mg/ L ($p < 0.05$) meanwhile fresh Tamarindus indica seed was 5.75 ± 0.43 mg/ L and 72 hours of fermented Tamarindus indica seed was 8.92 ± 0.29 mg/ L respectively. It shows that 72 hours of fermented Tamarindus indica seed oil has a good potential high antioxidants activity.

3.3 Evaluation Of Antioxidant Activity By Using High Performance Liquid Chromatography (HPLC)

Based on the table 4.1.3.3 evaluation of antioxidant activity by using High Performance Liquid Chromatography (HPLC), fresh Tamarind seed oil was 0.2249 ± 0.02 g/100 compared with 72 hours of fermented Tamarind seed oil was 0.5660 ± 0.01 g/100. This shows that higher Epicatechin concentrations were found in between 24 hours and 72 hours of fermented Tamarindus indica seed oil as compared to fresh ones ($p < 0.05$).

3.4 Table of evaluation of antioxidant activity by using High Performance Liquid Chromatography (HPLC)

Sample	Results G / 100g
Fresh TMS oil	0.2449 ± 0.02
(A) 24 H TMS oil	0.5220 ± 0.02
(B) 48 H TMS oil	0.4520 ± 0.01
(C) 72 H TMS oil	0.5660 ± 0.01

3.5 Sensory Evaluation Testing Lotion

Based on the table above shows that, the sensory of formulation lotion Tamarindus indica seed. This means formulation was nearer formulation compared with the commercial formulation. This lotion can meet consumer acceptance because of the overall acceptance value nearer compared commercial formulation.

Table 3.5.1 Sensory Evaluation Testing Lotion

SAMPLE	ATTRIBUTES								
	APPEARANCE	FRAGRANCE	COLOR	CONSISTENCY	SMOOTHING	SPREADIBILITY	OILINESS	ADHESIVENESS	OVERALL ACCEPTIBILITY
LOTION	5.87	5.20	6.83	5.77	5.83	6.20	6.17	6.13	5.9
COMMERCIAL LOTION	7.17	6.90	7.27	7.00	7.43	7.33	6.47	6.8	7.1

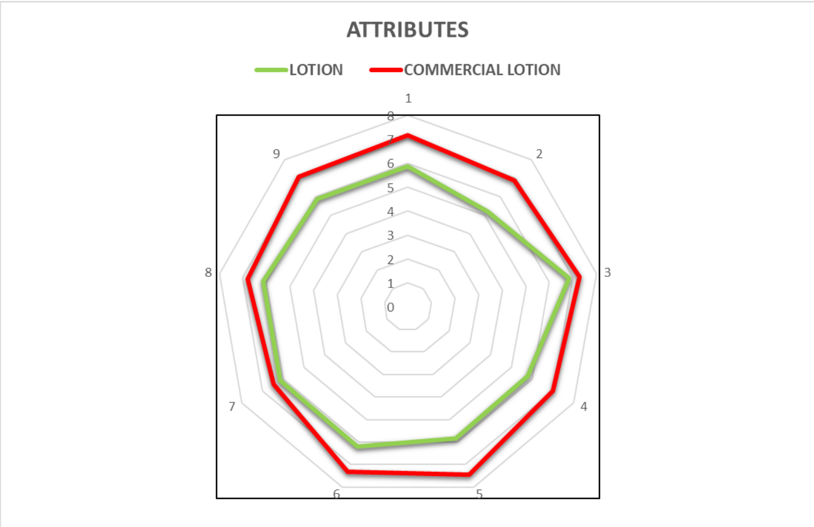

Figure 3.5.5 Shows Spider Web of The Lotion Formulation Prepared Comparison Between Commercial Formulation

Based on the table above shows that, the sensory of formulation lotion Tamarindus indica seed. This means formulation 5 was nearer formulation compared with the commercial formulation. This lotion can meet consumer acceptance because of the overall acceptance value nearer compared commercial formulation.

4.0 Conclusion

The test also to find a formulation that is resistant than microorganism on the lotion. Based on research found that, fermentation 72 hours of Tamarindus indica seed lotion was acceptable compared to others. This is because of the long-time fermenting gave the best effectiveness of the lotion. Moreover, the 72 hours fermentation Tamarindus indica seed oil can increase to produce a lot of an antioxidant according to DPPH assay, TPC assay and HPLC analysis. When a longtime fermenting give more effective to produce more good compound antioxidants agents and others good compounds inside the raw materials. Raw material Tamarindus indica seed can be proves as antioxidants agents substitutes to synthethic antioxidants agents. So this can save environment from synthethic or chemical antioxidants agents**.** These results were also seen in terms of characteristics such as texture corresponding with the lotions, smell, and colour in order to produce the best formulation of this lotion. This is important to ensure that the features should be available on the same lotion with features on new formulation selected in order to meet the quality which is better for the lotion. The quality of lotion is important in order it will also help the absorption of lotion on the skin.

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Rusyidah binti Mat Zin Boestami was born in Teluk Intan, Perak on 27 September 1990. She was awarded with the Bachelor of Science with Honours (Oleochemistry) degree by Universiti Kebangsaan Malaysia (UKM) on the year 2012. She is a lecturer in Politeknik Tun Syed Nasir Syed Ismail (PTSN), Pagoh, Johor from 2015 until present.

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