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Development and Evaluation of Sulfate-Free Charcoal Toothpaste from Coconut Shell and Rice husk

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Abstract

Toothpaste containing sulfate is one major concern as it is known as irritating and causes adverse effects in the oral cavity. Hence, a sulfate-free toothpaste is much more preferred nowadays. This research paper discusses on three sulfate-free toothpaste formulations that were prepared and optimized containing two types of charcoal namely activated coconut shell charcoal and rice husk charcoal. The formulations differ in the binding agents and surfactants used and they are evaluated based on 13 physiochemical characterizations including organoleptic properties, pH, foamability, spread ability, tube extrudability, moisture content, FTIR spectroscopy, cleaning test, toxicity assay, stability, abrasiveness, grittiness and morphological evaluation. One commercial toothpaste was also included in the test to be used as comparison. The objectives of this research are to prepare and optimize sulfate-free toothpaste formulations with natural charcoal from coconut shell and rice husk, evaluate the toothpaste formulations for physiochemical characterization, stability, toxicity and their effectiveness (cleaning test). Menthol crystals were dissolved in Propanediol and rice husk charcoal were grinded with mortar and pestle. All solid ingredients (including activated coconut shell charcoal, carrageenan, sodium dodecyl sulfate (SDS) and Sangelose) were then mixed together with a small amount of distilled water. After transferring to a white tile, liquid ingredients were added to the solid mixtures and mixed thoroughly using spatulas. The liquid ingredients included were dissolved menthol, liquid surfactants (including decyl glucoside (DG) and Tween-80 (T-80), glycerin, triethanolamine and coconut oil. Finally, distilled water was added gradually until a paste consistency was formed. Three toothpaste formulations have been developed namely F1, F2 and F3 of which varied in the binding agent and surfactant used. All toothpaste formulations showed results similar to commercial toothpaste in terms of appearance, odour, smoothness, tube extrudability, cleaning test and grittiness. F1 have the highest basic pH (9.78) and highest foamability (14 mL) among the toothpaste formulations. However, F1 have the lowest germination index (48.1%) indicating that it is most toxic than the others including the commercial toothpaste. In conclusion, all three toothpaste formulations were able to achieve the desired and acceptable characteristics with the physiochemical characterization. This study might provide good scope and be useful for further research as being a sulfate-free toothpaste that uses renewable waste activated coconut shell charcoal and rice husk charcoal as the abrasive ingredients and is capable of improving and maintaining oral hygiene.

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Keywords: Charcoal toothpaste, Sulfate-free toothpaste, Coconut shell, Rice husk

Introduction

Around 300 to 500 BC, toothpaste formulation designs can be found to begin in China and India where squashed bones and clamshells were used as abrasives to clean teeth (1,2). In the Middle Ages, the primary ingredients used to clean teeth in the Arab were fine sand and pumice (3). In 2020, about 307 million people in the United States have used toothpaste and this might rise to 316 million in 2024 (4). Toothpaste can be defined as a semi-solid dentifrice that cleans and maintains good oral health of teeth by removing food particles, reduce plaque formation, reduce stain, polish the tooth surface and refreshes the breath (2, 5, 6). They present in the form of paste, gel, liquid or powder dentifrices and is to be used with a toothbrush simultaneously (3, 5). Toothpaste promotes and maintains oral hygiene with the help of the ingredients in the toothpaste and brushing mechanism from the toothbrush. Brushing twice a day for 2 minutes each is the most well-known recommendation as a daily oral care routine.

Recently, a number of commercial dentifrices have been using coconut shell activated charcoal in their ingredient, either in the form of powder or paste and are used to whiten teeth. A coconut shell is the hard outer part of a coconut fruit located between the coconut husk and coconut flesh. Charcoal is produced by the process of removing water and other volatile elements from carbon-based materials such as coconut shell, coconut husk and wood. Moreover, charcoal can be activated with high temperature and gases to increase its porosity which results in activated charcoal (7).

Historically, in India, burnt rice husk in powder form have been used to clean the teeth over centuries and nowadays, activated charcoal made from rice husk was seen to be available, but not widely, as powder for teeth-cleaning. Rice husk, also referred as rice hull, is the outermost layer of the rice grain. They provide protective cover to the grain and have an elongated convex shape (8). Rice husks are the by-product of rice production milling process and are considered as agricultural wastes obtained from the milling process in large quantity. They have been recycled to be used for various purposes including as fuel to be burnt as energy, used in manufacturing, strengthening construction materials and used as raw materials such as for metal polishing agent (8,9).

Numerous common ingredients can be found in toothpaste including abrasives, binders, surfactants, humectants, preservatives, sweeteners and flavours (6). Glycerine functions as a humectant that prevents loss of moisture from the toothpaste (10). It ensures that the toothpaste remains in a moist condition preventing the toothpaste formulation from drying out (3, 11). Like thickening agents, humectants also affect the consistency and stability of the toothpaste in a long term. Having good water retention will result in glossy or shiny toothpaste formulation with an appealing texture within the mouth (12). In cosmetic industry, triethanolamine functions as preservatives, pH adjusters as well as surfactants (13). Sorbitol acts as a primary sweetening agent and humectant (10). Sweeteners work together with the flavorings by adding sweetness to the toothpaste formulation (12). One common flavouring agent is menthol that are used to refresh oral cavity and gives off cooling sensation (14). While, propanediol function as a solvent that helps in dissolving low-soluble ingredients, boosts preservative and enhance viscosity (13).

Common toothpaste usually uses Sodium Lauryl Sulfate (SLS) as the surfactant or foaming agent. However, SLS is known to be an irritating ingredient and might cause adverse effects such as oral mucosa inflammation which might develop into aphthous mouth ulcers or canker sores. It can also cause skin and eye irritation (3, 5, 15). Being a sulfate-free toothpaste has the advantage of being less irritating than those toothpaste containing Sodium Lauryl Sulfate. This is a common ingredient found in commercial toothpastes and they are known to might cause adverse effects affecting the oral mucosa and skin (3). Hence, a more natural-based and sulfate-free toothpaste is usually much preferred.

Therefore, this is the first study to combine coconut shell charcoal and rice husk charcoal to develop a natural-based, safe and effective sulfate-free toothpaste formulation. In order to determine whether the formulated toothpaste is ideal, physiochemical characterization was done. Common tests that were done in previous literature include foamability, stability test, abrasiveness and cleaning test to check toothpaste effectiveness as well as toxicity assay to ensure the safety of the toothpaste formulation. An ideal toothpaste must be non-toxic, have a good abrasive effect for cleaning, non-irritant, have a prolonged effect keeping the mouth clean and fresh, impart no stain on the teeth, stable in a long-term and is easily available at an affordable price (2). The objectives of this research are to prepare and optimize sulfate-free toothpaste formulations with natural charcoal from coconut shell and rice husk, evaluate the toothpaste formulations for physiochemical characterization, stability, toxicity and their effectiveness (cleaning test).

Materials and methods

Chemicals

Activated coconut shell charcoal (Micronized activated charcoal powder, Take it Global, Penang, Malaysia) and rice husk charcoal (prepared in UBD) were used as abrasives. Carrageenan (Sigma-Aldrich, Missouri, United States of America) and Hydroxypropyl Methylcellulose Stearoxy Ether (Sangelose 60L, Daido Chemical Corporation, Osaka, Japan) were used as binding agents. Three surfactants were included which were Sodium dodecyl sulphate (Surechem Products Ltd, Suffolk, England), Decyl glucoside (C18-C16 Glucoside, Ecosense 3000, Dow Chemicals, United States of America) and Tween-80 (Polysorbate 80, Merck KGaA, Darmstadt, Germany). Glycerin (Propane-1,2,3,triol, Surechem Products Ltd, Suffolk, England) as humectant, triethanolamine (Glentham Life Sciences, Corsham, United Kingdom) as preservative, sorbitol (D-Sorbitol, Glentham Life Sciences, Corsham, United Kingdom) as sweetening agent, Menthol (L-Menthol, Glentham Life Sciences, Corsham, United Kingdom) as flavouring agent. Propanediol (Propane-1,3-diol, Zemea, Formulator Sample Shop, Milan, Italy) was used as dissolvent to dissolve the menthol crystals. Finally, Cocos Nucifera (Coconut) oil, bought from local market, as a defoaming agent and distilled water as the carrier. Three toothpaste formulations were developed and their compositions are shown in Table 1.

Table 1 Ingredients and compositions of the toothpaste formulations

No.		Formula (%w/w)		
	Formulation code	F1	F2	F3
	Ingredients			
1	Activated coconut shell charcoal	32.3	32.3	32.3
2	Rice husk charcoal	1.0	1.0	1.0
3	Carrageenan	1.7	-	-
4	Sangelose	-	1.7	1.7
5	Sodium Dodecyl Sulphate (SDS)	6.7	-	-
6	Decyl Glucoside (DG)	-	6.7	-
7	Tween-80 (T80)	-	-	6.7
8	Glycerin	1.7	1.7	1.7
9	Triethanolamine	1.7	1.7	1.7
10	Sorbitol	2.0	2.0	2.0
11	Menthol	1.0	1.0	1.0
12	Propanediol	13.3	13.3	13.3
13	Coconut oil	3.3	-	-
14	Distilled water	q.s.	q.s.	q.s.

w/w: weight by weight; F1: Formulation 1; F2: Formulation 2; F3: Formulation 3; q.s.: quantum sufficient

Menthol crystals were dissolved in Propanediol and rice husk charcoal were grinded with mortar and pestle. All solid ingredients (sequence blue) were then mixed together with a small amount of distilled water. After transferring to a white tile, liquid ingredients were added to the solid mixtures and mixed thoroughly using spatulas. The liquid ingredients included were dissolved menthol, liquid surfactants (DG and T80), glycerin, triethanolamine and coconut oil. Finally, distilled water was added gradually until a paste consistency was formed (16).

Physiochemical characterization

The three toothpaste formulations as well as one commercial toothpaste were evaluated according to the 13 physiochemical characterizations listed below.

Organoleptic properties

The organoleptic properties evaluated were colour, appearance, odour and smoothness (texture) of the toothpaste formulations. The colour and appearance were checked visually and the odour was checked by smelling the formulation. While the smoothness was assessed by rubbing the formulation in between the fingers (1,16)

pH

The pH was measured by using a digital pH meter FiveEasy (Mettler Toledo, Greifensee, Switzerland). A suspension of the toothpaste was made by taking 1g of the formulation in a 25mL beaker with 10mL of distilled water. Then after stirring well, the pH was determined within 5 minutes (2)

Foamability

About 1g of formulated toothpaste was taken and stirred with 5 mL of distilled water in a 25 mL beaker. Taken into a 25mL measuring cylinder and V2 was recorded (volume of water only). With a gloved hand covering the top, the measuring cylinder was shaken vertically for 10 times. Foams were formed and V1 was recorded (volume of foam with water) (1,16,17)

The foamability can be determined by using the formula below:

where,

V1 – Volume of foam with water (mL)

V2 – Volume of water only (mL)

Spreadability

About 1g of the toothpaste formulation was placed on a white tile. A glass petri dish below a conical flask containing water (total weight was about 400g) was placed carefully at the centre of the formulation. Then, the diameter of the toothpaste was measured after 15 minutes have passed (18)

Tube extrudability

The formulated toothpaste was filled in a small plastic tube. Tube extrudability was determined by pressing the tube with normal force at room temperature and checking whether the toothpaste extrude homogeneously (19)

Moisture content

Moisture content was determined by using moisture analyzer MOC63U (Shimadzu Corporation, Kyoto, Japan) and the temperature was set at 105°C. About 1g of the formulated toothpaste was placed and the final moisture content was recorded (20).

Fourier transform infrared spectroscopy

Fourier transform infrared (FTIR) spectrophotometer IRSpirit (Shimadzu Corporation, Kyoto, Japan) was used to confirm the functional groups in the formulated toothpaste. They were characterized in attenuated total reflection mode over a wavenumber range from 4000 to 500 cm-1. A number of 30 scans was set and the measurement mode was in percentage transmittance (21)

Cleaning test

Eggshells were used due to their high amount of calcium, resembling tooth enamel and hence were suitable for the cleaning test. About 200ml of water were boiled in a beaker and 15 ml of vinegar as well as 20 drops of red food colour were added. A hard-boiled egg was immersed in the beaker and let sit for 5 minutes to be stained. After that, a line was drawn dividing the egg into half (one side of the egg was the control). A pea-sized (about 0.25g) formulated toothpaste was taken on to a moist toothbrush and brushed on one side of the egg with circular and back-and-forth motion for a total of 10 strokes. Then, the egg was rinsed and the red stain removal was inspected for presence or absence (19)

Toxicity assay

In order to check for the toxicity of the formulated toothpaste, a toxicity assay was done based on seed germination index. Mung beans were soaked overnight for about 12 hours. Once sterilized with distilled water, 20 seeds were placed in each container containing cotton wool pads (each container for each formulation). For a period of 5 days, about 5-10 mL of formulated toothpaste suspensions and distilled water (as control) were added to their respective containers for twice daily at room temperature to grow the seeds. Then, relative seed germination (RSG), relative root length (RRL) and germination index (GI) were determined according to the formulas below (11).

$$RSG\ \% = \frac{number\ of\ seeds\ germinated\ in\ test}{number\ of\ seeds\ germinated\ in\ control} \times 100$$

$$RRL\ \% = \frac{average\ root\ length\ in\ test}{average\ root\ length\ in\ control} \times 100$$

$$GI = \frac{RSG\ \% \times RRL\ \%}{100}$$

Stability

The formulated toothpaste was filled in Scott bottles and were stored at two different conditions which were 25°C ± 2°C with 60% ±5% relative humidity (RH), and at 40°C ± 2°C with 75% ±5% RH for a period of 30 days (Day 0 as control). The organoleptic properties, pH, foamability, spreadability, tube extrudability, moisture content and cleaning test were then evaluated for stability (16,17)

Abrasiveness

On a plastic microscopic slide, a pea-sized amount (about 0.25g to 0.5g) of formulated toothpaste was placed and 2 drops of distilled water was added. In a back-and-forth motion, the formulated toothpaste was rubbed using a cotton swab with short strokes (about 2 cm) for 30 times. The slide was rinsed carefully and dried with soft tissue. Then, the intensity of scratches on the surface of the slide was rated visually with a scale of 0 (no scratch) to 5 with 5 having a high degree of scratches (19)

Grittiness

A pea-sized amount (0.25g to 0.5g) of formulated toothpaste was placed on a piece of butter paper and was rubbed using a finger with 30 strokes in a back-and-forth motion. The grittiness was determined as present or absent (19).

Morphological evaluation

About 1-2 drops of formulated toothpaste suspension was placed on a microscope slide. After a cover slip was set, the slide was taken to the digital microscope imager (Celestron, California, United States of America) which was used to observe and capture high resolution images. Then, morphological evaluation was done.

Statistical analysis

Each test was performed in triplicates. Mean, standard deviation (SD) and one-way ANOVA (analysis of variance) statistical analysis were done using GraphPad Prism (version 8.4.3). Data were presented as mean ± SD and p-value less than 0.05 were reported as statistically significant.

Results and Discussion

The results obtained from this research are presented in the tables below. Table 2 shows 9 physiochemical characterization results. A comparison can then be made between each toothpaste formulations and the commercial toothpaste. With reference to Table 2, excluding the colour, the toothpaste formulations and commercial toothpaste have similar organoleptic properties which have a smooth texture, a paste-like appearance and gave off a pleasant smell with minty odour. They also showed good tube extrudability, good cleaning ability and absence of gritty matters. All toothpaste formulations resulted with a scale of 4 in abrasiveness test while commercial toothpaste have a scale of 2.

Table 2: Physiochemical characterization results

Formulations	F1	F2	F3	Commercial
Colour	Matte black	Glossy black	Glossy black	Glossy white-grey stripes
Appearance	Paste-like	Paste-like	Paste-like	Paste-like
Odour	Pleasant	Pleasant	Pleasant	Pleasant
Smoothness	Smooth	Smooth	Smooth	Smooth
pH	9.78 ± 0.01***	9.33 ± 0.02***	9.39 ± 0.01***	7.03 ± 0.01
Foamability (mL)	14.00 ± 1.00	3.00 ± 1.00***	3.00 ± 1.00***	16.00 ± 1.00
Spreadability (cm)	2.60 ± 0.10***	4.03 ± 0.35***	3.47 ± 0.15***	5.20 ± 0.17
Tube extrudability	Good	Good	Good	Good
Moisture content (%)	50.39 ± 0.40***	55.16 ± 1.64***	55.63 ± 0.32***	29.06 ± 0.26
Cleaning test	+	+	+	+
Abrasiveness	4.0	4.0	4.0	2.0
Grittiness	Absent	Absent	Absent	Absent

+: Presence. n=4. F1: Formulation 1; F2: Formulation 2; F3: Formulation 3. Asterisks (*) represents significant differences as compared to the commercial toothpaste (*** for p-value < 0.001). ANOVA with Tukey post-hoc test was done.

The pH of all toothpaste formulations was significantly higher than the commercial toothpaste ($p<0.001$). The pH ranges around pH 9 with F1 having the highest pH of 9.78 and F2 having the lowest pH of 9.33. While the commercial toothpaste has a lower pH with pH 7.03. Both F2 and F3 have similar foamability of 3mL which were significantly lower ($p<0.001$) than F1 and commercial toothpaste with 14mL and 16mL respectively. Furthermore, all toothpaste formulations showed significantly lower spreadability compared to commercial toothpaste ($p<0.001$). F1 gave the lowest spreadability of 2.60cm and F2 gave the highest with 4.03cm. However, commercial toothpaste gave a higher spreadability with 5.20cm. With significant results ($p<0.001$) compared to commercial toothpaste, F3 gave the highest moisture content of 55.63% and F1 have the lowest with 50.39%. On the other hand, commercial toothpaste has a much lower moisture content with 29.06%. According to Table 3, all tested samples obtained a relative seed germination of 100%. After measuring and obtaining the relative root length, germination index (GI) was calculated. Both F2 and F3 gave a high GI (100.8% and 103.2% respectively), while F1 have a significantly lower GI than the control with 48.1% ($p<0.001$) and the commercial toothpaste shows a GI of 70.6%.

Table 3: Toxicity assay results

Seed germination	F1	F2	F3	Commercial	Control
Number of seeds germinated	20	20	20	20	20
Relative seed germination (%)	100	100	100	100	-
Averageroot length (cm)	4.12 ± 2.00***	8.63 ± 3.59	8.84 ± 4.12	6.04 ± 2.17	8.56 ± 3.90
Relativeroot length (%)	48.1	100.8	103.3	70.6	-
Germination index (%)	48.1	100.8	103.2	70.6	-

n=5; F1: Formulation 1; F2: Formulation 2; F3: Formulation 3; Relative comparison with control; (distilled water); Root length assessment conducted on Day 6 (n=20); Relative Root Length was compared to the control (distilled water); Asterisks (*) represents significant differences as compared to the control (distilled water) (*** for p-value < 0.001). ANOVA with Bonferroni post-hoc test was done.

After completing the 30 days period for stability test, all formulations have shown to remain stable when tested (Table 4). ANOVA with Bonferroni post-hoc test was used for statistical analysis. The pH of all toothpaste formulation has decreased significantly (p<0.05) after 30 days in both storage conditions. The moisture content was shown too increased significantly as well in all formulations (p<0.01). On the other hand, there were no significant changes in foamability and spreadability.

Table 4 Stability results

Formulations	F1			F2			F3		
Day	0	30		0	30		0	30	
Temperature(°C)	25	25	40	25	25	40	25	25	40
Relative humidity (%)	60	60	75	60	60	75	60	60	75
Colour	Matte black	Matte black	Matte black	Glossy black	Glossy black	Glossy black	Glossy black	Glossy black	Glossy black
Appearance	Paste-like	Paste-like	Paste-like	Paste-like	Paste-like	Paste-like	Paste-like	Paste-like	Paste-like
Odour	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant
Smoothness	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
pH	9.78 ± 0.01	8.60 ± 0.01*	8.45 ± 0.02*	9.33 ± 0.02	8.53 ± 0.01*	8.32 ± 0.01*	9.39 ± 0.01	8.66 ± 0.01*	8.36 ± 0.01*
Foamability (mL)	14.00 ± 1.00	7.67 ± 0.58	11.33 ± 0.58	3.00 ± 1.00	2.67 ± 0.58	2.33 ± 0.58	3.00 ± 1.00	2.33 ± 0.58	2.00 ± 0.00
Spreadability (cm)	2.60 ± 0.10	2.90 ± 0.26	2.80 ± 0.10	4.03 ± 0.35	3.93 ± 0.06	4.37 ± 0.25	3.47 ± 0.15	4.30 ± 0.10	3.87 ± 0.12
Tube extrudability	Good	Good	Good	Good	Good	Good	Good	Good	Good
Moisture content (%)	50.39 ± 0.40	56.30 ± 1.64**	55.88 ± 3.01**	55.16 ± 1.64	59.96 ± 0.62**	61.69 ± 1.18**	55.63 ± 0.32	61.36 ± 0.38**	61.54 ± 0.28**
Cleaning test	+	+	+	+	+	+	+	+	+

+: Presence. n=6: F1: Formulation 1; F2: Formulation 2; F3: Formulation 3; Asterisks (*) represents significant differences as compared to the control, Day 0 at 25°C (* for p-value < 0.05 and ** for p-value <0.01). ANOVA with Bonferroni post-hoc test was done

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FTIR analysis were done to observe the functional groups in the formulations. In Figure 1-4, FTIR spectra of toothpaste formulations have been compared with the coconut shell charcoal, rice husk charcoal as well as their respective distinctive ingredients (binding agent and surfactants). The microscopical images of simple mixture of activated coconut shell charcoal (microparticle) and rice husk charcoal shown in the Figure 5A. It is clear that, microparticle based coconut shell charcoal uniformly distributed and appeared, whereas rice husk charcoal (non-microparticle) appeared clump mass in the images. Formulated toothpaste and commercial toothpaste microscopical photographs are Figure 5, such as B - F1, C- F2, D - F3 and E- commercial toothpaste (F1- SDS; F2- DG; F3- T80 & commercial - SLS) respectively. The images were observed from different toothpaste formulation prepared by using different surfactants showing different morphological properties.

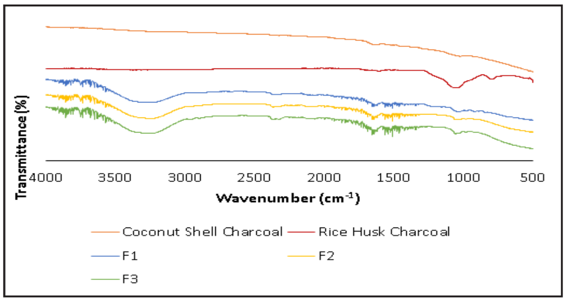

Figure 1 FTIR spectra of Coconut shell charcoal, Rice husk charcoal with the three toothpaste formulations. Abbreviations: F1 Formulation 1, F2 Formulation 2, F3 Formulation 3

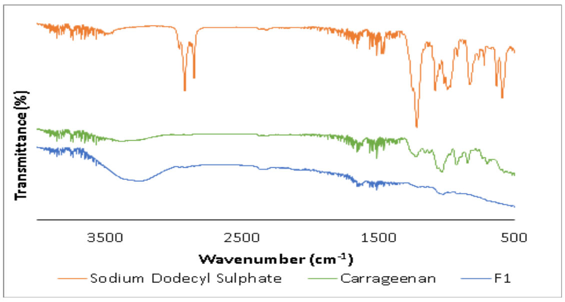

Figure 2 FTIR spectra of F1, Carrageenan and Sodium Dodecyl Sulphate (SDS). Abbreviations: F1 Formulation 1

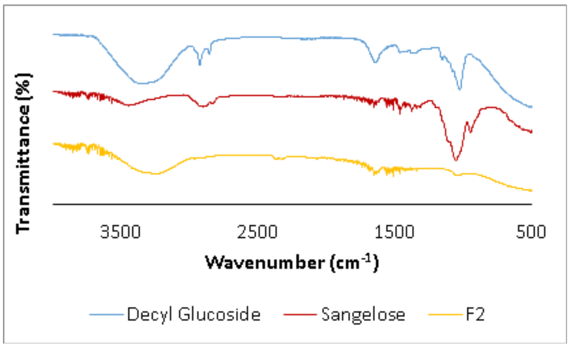

Figure 3 FTIR spectra of toothpaste F2 with Sangelose and Decyl Glucoside (DG). Abbreviations: F2 Formulation 2

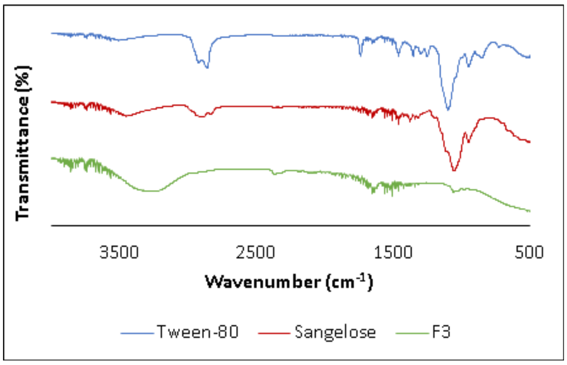

Figure 4 FTIR spectra of F3 with Sangelose and Tween-80 (T80). Abbreviations: F3 Formulation 3

Discussion

Coconut shells and rice husk are known as agricultural wastes generated in large quantities and are potential sources of carbon. They are often used as alternative energy sources by burning them, however, due to their abundant wastes, they can cause disposal problems. Hence, agricultural waste recycling should be done to solve this environmental pollution. One way is to use them as natural ingredients in producing safe and effective toothpaste.

Three different surfactants were used to formulate sulfate-free toothpaste which were Sodium Dodecyl Sulphate (SDS), Decyl Glucoside (DG) and Tween-80 (T80). Furthermore, carrageenan and Sangelose were used as the binding agents. In F1, the reason behind the

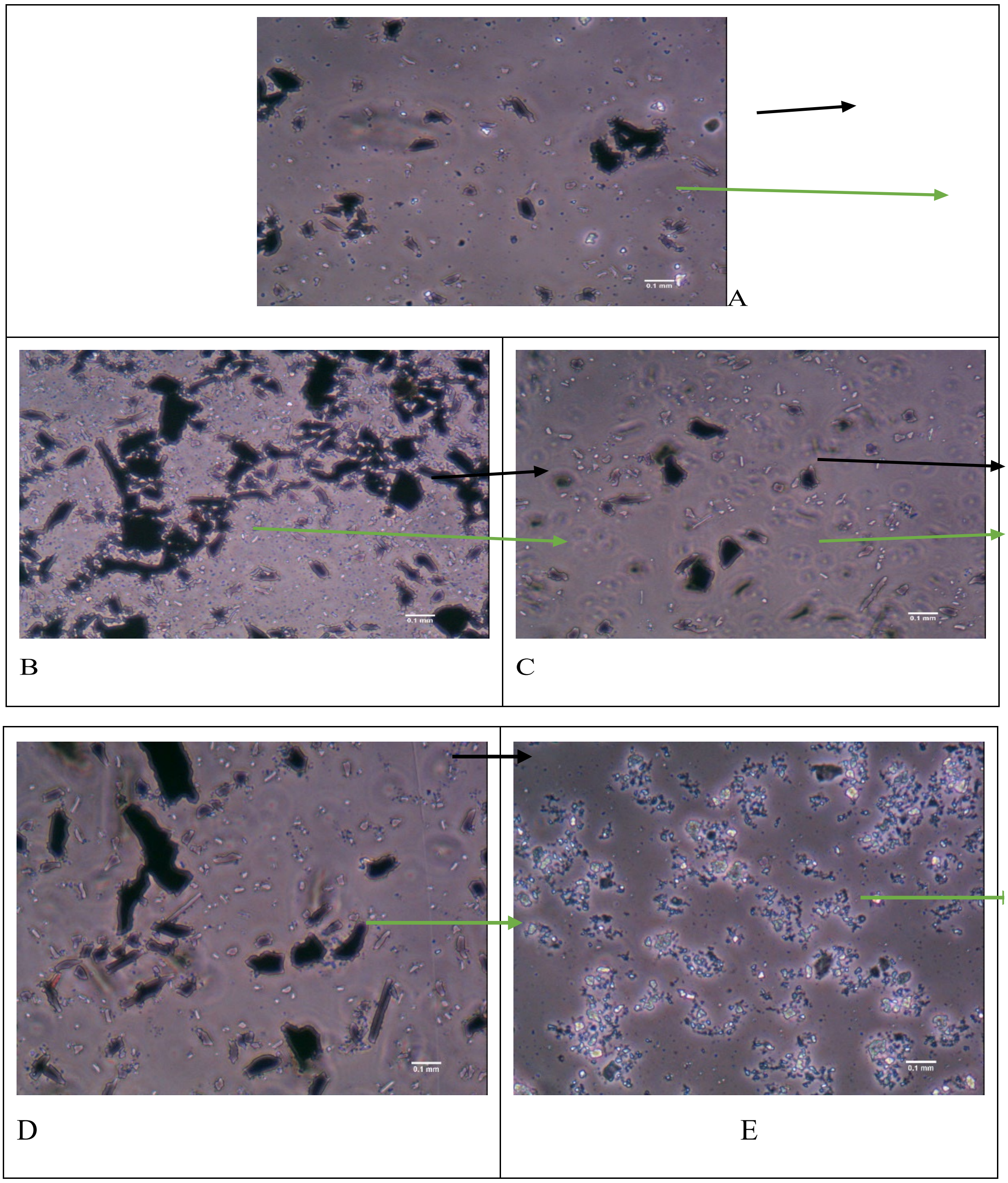

Figure 5: Microscopical photographs (20X) Figure 5A - Mixture of coconut shell charcoal and rice husk charcoal. With mixture of activated coconut shell charcoal and rice husk charcoal, toothpaste formulations were prepared by using different surfactants (Figure 5B- Sodium Dodecyl Sulphate; Figure 5C- Decyl Glucoside; Figure 5D- Tween-80). Figure 5E - Commercial toothpaste with SLS surfactant. BLACK LINE - Activated coconut shell charcoal (micro-particle); GREEN LINE - Activated rice husk charcoal (non-microparticle).

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preparation stage. Thus, coconut oil acts as a defoaming agent to control foaming.

All three formulations appeared black colour which is due to the abundant main ingredient, activated coconut shell charcoal (32.3%) which is black in nature. Although glycerin plays a role in providing glossy or shiny appearance with appealing texture (12), the matte and glossy appearance of the formulation may also be due to the surfactants used. Both DG and Tween-80 produced a glossy finish while SDS gave off a matte appearance. All formulations have similar organoleptic properties with the commercial toothpaste that have a smooth texture, paste-like appearance and pleasant smell with minty odour. The minty odour in the formulations comes from the menthol crystals added (1%). These organoleptic properties of the formulation may enhance marketability (19).

All toothpaste formulation has obtained a pH lower than 10.5 which is the maximum pH for an ideal toothpaste (22). The pH in all three toothpaste formulations were in the alkaline region that ranges between 9.33 to 9.78. Alkaline pH was shown to cause less adverse changes to the surface of the teeth (19). While, a lower pH toothpaste promotes greater alteration to the enamel surface (23). The pH of a toothpaste is important in order to maintain the basic pH of the mouth. Basic medium prevents bacteria growth in the mouth that may cause teeth damage such as cavities, gum disease and tooth decay (24). The pH values were slightly varied between each formulation and this may be due to the acidic or alkali nature of their distinctive ingredients. Among the formulation, F1 have the highest pH of 9.78 indicating that carrageenan and SDS might be more basic in nature. Both F2 and F3 resulted in having similar pH values with 9.33 and 9.39 respectively and this may be due to having the same binding agent, Sangelose.

Both F2 and F3 have a lower foamability with 3mL. On the other hand, F1 and commercial toothpaste have a foamability of 14mL and 16mL respectively. These differences suggests that the quantity of the surfactants in F2 and F3 is insufficient (6.7%). Thus, in further study, the quantity of the surfactants will need to be increased which will lead to higher foamability. Surfactant or also called as a foaming agent. It generates foam during brushing that facilitates wetting the tooth surfaces, dispersing the toothpaste in the mouth and food debris removal from the oral cavity (10,12). The surfactant used in the commercial toothpaste is the commonly used detergent, Sodium Lauryl Sulfate (SLS). High foamability suggest that there is a high concentration of SLS. High concentration of SLS causes a concern as it raises oral irritation among those who are prone to mouth ulcers or canker sores (19). Decyl glucoside, on the other hand, is gentle and less irritating. Tween-80 is also a good surfactant alternative to SLS. When using sulfate-free toothpaste, mouth ulcers may feel less painful and recovery time is faster. Sulfate-free toothpaste may also help reduce teeth sensitivity pain when compared to using SLS containing toothpaste (15).

Producing and using consumer products usually involve filling, pumping and discharge from their packaging. These processes are related with the complex rheological properties of the materials. Rheological properties that are controlled in toothpaste are ingredients such as polymer thickeners and particulate abrasives. Typical polymers include carboxymethyl cellulose, xanthan gum and carrageenan (25). Newton's law of viscosity can be defined as the relationship between shear stress and shear rate of a fluid when mechanical stress was applied. Fluids can be categorized into Newtonian fluids and non-Newtonian fluids. Toothpaste is considered as non-Newtonian fluids which does not obey Newtonian's law of viscosity where the ratio of shear stress to shear rate is not constant and depends on the shear rate (26). In other words, non-Newtonian fluids are affected by factors other than temperature, opposite with Newtonian fluids which temperature is the only factor affecting their viscosity. An example of Newtonian fluid is water. The viscosity of water will remain constant no matter how they are forced to flow through a pipe. However, when temperature is applied, the water will become less viscous and will flow easily. On the other hand, the viscosity of non-Newtonian fluids will change only when pressure, agitation or shear stress is applied. When shear stress is applied, the viscosity decreases making the fluid to flow easily and these fluids are known as shear-thinning fluids (27). Thus, toothpaste is a non-Newtonian fluid with shear-thinning behavior. In this study, there are two tests that evaluates the rheological properties of the toothpaste which are tube extrudability and spreadability.

One essential factor to consumer is the toothpaste ability to be squeezed easily from the tubes and apply on the toothbrush. In this research, all toothpaste formulations have good tube extrudability indicating that there are no difficulty obtaining the toothpaste formulation during tube extrusion (19). Spreadability can be defined as the extent of the area to which the paste readily spreads on the application area. Possessing good spreadability is an essential criterion to be an ideal toothpaste (2). F2 have the highest spreadability with 4.03cm, while F1 have the lowest spreadability of 2.60cm. The variation might be caused by the different binding agents and surfactants used. From the results, carrageenan and SDS seem to significantly decrease the spreadability of the toothpaste formulation. On the other hand, the commercial toothpaste has a higher spreadability with 5.20cm despite having carrageenan in its composition as well. This may be due to the toothpaste having a higher amount of glycerin content affecting the ability to spread. High spreadability leads to high chances of wide performance range (19).

Dry mouth can be prevented with the presence of moisture in the toothpaste. Moisture content affects the physical properties and quality of the toothpaste. As mentioned, humectants help retain moisture preventing toothpaste from drying out. Common humectants include glycerine, sorbitol as well as water (24). Among the formulations, F3 have the highest moisture content (55.63%) and F1 have the lowest with 50.39%. While, the commercial toothpaste has a much lower moisture content with 29.06%.

Toothpaste is usually stored for months before they are being used hence it is important for toothpaste to have good stability. Stability test gives a close approximate of the shelves lives of products. For example, a sample stored at 45°C for 8 weeks is equivalent to storing at room temperature for one year (19). Although there was a significant decreased in pH value ($p<0.05$) and significant increase in moisture content ($p<0.01$), all toothpaste formulations have shown to have good stability after 30 days of storing in two different conditions (25°C and 40°C).

FTIR spectroscopy helps to confirm the presence of functional groups in the toothpaste formulations. Coconut shell charcoal and rice husk charcoal peaks can be seen in all three toothpaste formulation spectra. Absorption bands found in the FTIR spectrum of coconut shell charcoal at 1644.09 cm-1 and 1030.94 cm-1 represents C=C stretching and C-O stretching respectively (28,29). While, the peak at 1050.91 cm-1 in rice husk charcoal spectrum represents secondary alcohol C-C bond or Si-O-Si stretch (30). With similar peaks in F1 spectrum, the peak at 3385.15 cm-1 and 1035.22 cm-1 shown in the carrageenan spectrum might corresponds to O-H stretching and C-OH with S=O bonds respectively (31). The peak at 1079.43 cm-1 in SDS spectrum suggests C-C band stretching (32). Three peaks in F2 spectrum can also be seen in FTIR spectrum of Sangelose and DG. Absorption band at 1050.91 cm-1 indicates the presence of glucose ring in Sangelose (33). On the other hand, in the DG spectrum shows the presence of OH groups, C-C from ring stretching and C-O-C bonds respectively (34). FTIR spectrum of F3 have two similar peaks with those in Sangelose and T80 as well. The peak seen in T80 spectrum at 1093.68 cm-1 is attributed to C-O-C stretching (35).

Presence of gritty matters in the toothpaste can cause wear to the teeth enamel and injury to skin. Therefore, test for grittiness is done by checking the presence of solid in the formulation (19). The results showed that there was absence of gritty matters in all three toothpaste formulations. All toothpaste formulations have shown to have good cleaning ability, thus the ability in cleaning tooth enamel (19). Cleaning effectiveness is another important property of an ideal toothpaste. It depends on its ability to remove stains on the teeth surface. The cleaning can be done by the abrasive ingredients contained in the toothpaste aided by the brushing mechanism of the toothbrush (36). In this study, the coconut shell charcoal (32.3%) and rice husk charcoal (1.0%) are the abrasive ingredients in the toothpaste formulations. The high compositions of these abrasives in the formulations may have caused the high abrasive potential. All toothpaste formulations have better abrasive potential than the commercial toothpaste. This can be supported by the cleaning test conducted. Based on observations, the cleaning ability of the commercial toothpaste has shown to be present but have lower cleaning ability compared to the toothpaste formulations. Upon inspecting the red stain removal after 10 brushing strokes, the toothpaste formulations were able to remove stain more than the commercial toothpaste suggesting that high abrasive potential has better stain removal (19).

Besides abrasive particles, surfactants also aid in cleaning the oral cavity. They act as foaming agents that produces foam while brushing. The foam would give an enjoyable sensation to the consumer, helps in wetting the surface of the teeth, allowing dispersion and free movement of the toothpaste as well as helping in loosening and removing debris and plaque in the oral cavity (12,14). Surfactants are amphiphilic molecules that contains hydrophilic heads and hydrophobic tails (37). When surfactants are dissolved in water, they would form spherical shapes aggregates called micelles where the hydrophilic heads are on the outside in contact with the surrounding water and hydrophobic tails are in the core of the micelles protected inside away from the water. When brushing with toothpaste, the hydrophobic tails in the surfactant molecules will attract and trap food debris into the core of the micelles. As micelles are suspended in water, they can be easily washed away from the oral cavity along with the food debris inside, cleaning the oral cavity (38).

To evaluate the abrasive effects of the formulated toothpaste, two activated charcoal mixture such as coconut shell charcoal (micro-particle) and rice husk charcoal (macro-particles) were mixed in the ratio of 32.3 is to 1. The image of toothpaste F1 in Figure 5B shows mixtures of micro and macroparticles of activated charcoal clearly appeared. This may be due to the adsorption as well as blocking the pores onto macro size particle of activated rice husk charcoal by SDS. The process of adsorption of SDS-coated RHC (rice husk charcoal) might be governed by chemisorption (39). Whereas, micrometer size activated coconut shell charcoal (CSC) has been recognized as a good candidate in adsorption process due to having a high specific surface (40). This may be due to having high number of pores that is typically acts as a better adsorbent in the application of activated charcoal in whitening toothpastes, however, Sodium dodecyl sulfate (SDS), an anionic surfactant, is used as cleaning and hygiene products (41,42). Also, adsorption layer between SDS and RHC appeared enlarged due to addition of water before observation in the microscopic evaluation. This may be due to disturbances of surfactant micelles on the coating of the microparticles. The image of toothpaste F2 in Figure 5C showed strong adsorption on the CSC where head group micelles re-enlarged with water addition before microscopical observation. Less adsorption on RHC may be the reason that clump mass was not observed in the F2 formulation. Decyl glucoside is a mild non-ionic surfactant that were used in variety of cosmetic formularies due to the safety of green surfac-

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tant. It is a biodegradable surfactant that results in products with low toxicity and friendly properties to the environment (43). The image of toothpaste F3 in Figure 5D, shows similar pattern of micelles appear like DG containing toothpaste F2, and it has been reported that activated charcoal and Tween 80 for a high wetting power. The image of commercial toothpaste in Figure 5E, which contains Sodium Lauryl Sulfate (SLS) surfactant, have shown to utilizing uniform particle size and hence, it appeared with uniform particle size without any clumpy masses compared to formulated toothpaste.

Cosmetic products usually have some level of toxicity and this could cause a major concern. Therefore, toxicity assay is vital in order to prove better performance of the toothpaste formulation. Toxicity assay was performed based toward seed germination index (11). Phytotoxicity is defined as plant growth inhibition and a delay of seed germination (44). A germination index value of more than 80% usually indicates no phytotoxicity (45). Among the formulations, only F2 and F3 have a GI of 100.8% and 103.2% respectively, surpassing the target GI%. Therefore, F2 and F3 are less toxic in nature which may be due to the toothpaste formulations being sulfate-free. F1 and commercial toothpaste, on the other hand, have a lower GI value of less than 80% with 48.1% and 70.6%. F1 are more toxic suggesting the toxicity of SDS, while SLS presence is causing the toxicity in the commercial toothpaste. This evidence has further proven the toxicity of sulfate-containing toothpaste.

In this study, all objectives have been achieved. Sulfate-free toothpaste formulations have been prepared and optimized that contains coconut shell charcoal and rice husk charcoal. Evaluation have been done that shows all toothpaste formulation of their cleaning effectiveness, toxicity, stability and other properties. A number of limitations should be acknowledged in this research study. Additional tests could have been conducted on the toothpaste formulations such as SEM-EDX analyses where particle shapes could have been observed through micrographs. Another test would be a cleaning test that uses turmeric powder to stain artificial teeth. Due to shortage of time, stability test could only be done after 30 days when in fact, stability test was usually conducted after two to three months of storage. The undesirable foamability results of F2 and F3 formulations suggest improvement should be made by increasing the composition of the surfactants in future study.

Conclusion

As expected, the toothpaste formulations were able to achieve the desired and acceptable characteristics with the physiochemical characterization. This study might provide good scope and be useful for further research as being a sulfate-free toothpaste that uses renewable waste activated coconut shell charcoal and rice husk charcoal as the abrasive ingredients and is capable of improving and maintaining oral hygiene. Upon successful formulation, the toothpaste formulations have the chance of being patented and to be produced commercially.

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Competing interests

The authors declare that they have no competing interests.

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