The Effect of Organic Flaxseed Paste on The Colorimetric Parameters of Demineralized Tooth Surface

Marlene Azlia Abd Raffur, Izzati Mohd Shaharuddin, Luay Thanoon Younis

Abstract— Objective: The aim of the study is to evaluate the efficacy of flaxseed paste on the surface lightness (L), chroma (C) and hue (h) of extracted teeth. Materials and Methods: Thirty extracted human teeth (incisors and canines) were selected and grouped into control (no treatment), flaxseed (treatment) and fluoride (positive control) groups. Teeth in control group were placed in saline throughout the experiment, whilst the teeth in flaxseed and fluoride groups were immersed in a carbonated drink for two days to induce demineralisation prior to the treatment using fluoride toothpaste and 1gm/ml of freshly prepared flaxseed paste respectively. The values L, C and h were measured at 3 stages (initial, during treatment, after treatment) using a spectrophotometer. Computerized digital imaging analyses were performed using Photoshop CS6 software (Adobe, San Jose, CA, USA). Statistical analyses were performed using repeated measures ANOVA. Results: At the final reading, spectrophotometric analysis showed decrease in L in both positive control and test groups. Fluoride was more effective, but not significant, in reducing the lightness (L=55.69 \pm 5.38, p=0.070) than flaxseed (L=67.60 \pm 5.34, p=0.070). The mean C value was higher in flaxseed group (C=2.75 \pm 2.73, p=0.010) compared to fluoride group (C= 2.78 ± 2.73 , p=0.010). In contrast, the mean h value for flaxseed group was much lower (h=121.50 \pm 10.53, p=0.000) when compared to fluoride group (h= 260.02 ± 10.53 , p=0.000). Conclusion: Flaxseed paste has shown to be effective in reducing L. Although fluoride toothpaste produced obvious decrease in L, organic flaxseed paste shows promising effect in alleviating surface lightness, hence enhancing tooth surface mineralisation that may be explored further in future research.

Index Terms— Flaxseed, digital imaging analysis, lightness, mineralisation, spectrophotometer.

I. INTRODUCTION

The first clinically observable stage of caries initiation is the development of white spot lesion (WSL) (Hallgren, Akyalcin, English, Tufekci, & Paravina, 2016). Besides being an aesthetic challenge, WSLs are regions of demineralized enamel which are porous and structurally weaker (Figure 1). The mechanism of action is the dissolution of mainly calcium and phosphorus in the enamel matrix by acids.



Figure 1: White spot lesions developed after orthodontic treatment due to poor oral hygiene

Under optimal circumstances, salivary constituents have a natural buffering system that maintain the pH at optimum levels, and acts as a physical mechanism to remove acids from enamel surfaces. However, as the oral cavity becomes increasingly acidic, the pH of dental biofilm will decrease as well (Igarashi, Hamada, Nishimaki, Sakurai, & Kamiyama, 1987; Wolff & Larson, 2009).

Derived from the Flax plant, flaxseed (Linum usitatissimum L.) has been touted as a superfood, owing to its high omega-3 fatty acid α -linolenic acid (ALA) and high fibre content (Shim, Gui, Arnison, Wang, & Reaney, 2014). Flaxseed, also known as linseed, boasts of several distinctive qualities. Previous study suggested that flaxseed's has polyphenol compound called lignans which possesses antibacterial activity against the cariogenic bacteria, Streptococcus mutans (Imran et al., 2015). The mucilage or soluble fiber in flaxseed is believed to be a suitable saliva replacement in dry mouth patients (Andersson et al., 1995). The mineral composition in flaxseed, per 100g, was found to be 255mg, 392mg, 642mg, and 813mg for calcium, magnesium, phosphorus, and potassium, respectively (Goyal, Sharma, Upadhyay, Gill, & Sihag, 2014). The availability of calcium, phosphorus and fluoride in the oral cavity is one of the factors which affect remineralization rates of enamel (Ramashetty Prabhakar & Arali, 2009). When salivary pH level is above the critical pH, calcium and phosphorus will be precipitate into hydroxyapatite as part of enamel remineralization (ten Cate, 2008). Furthermore, it was found that a test dentifrice which contained potassium nitrate showed significant remineralization of artificially demineralized enamel (Zero et al., 2006).

The color of enamel surface, can be objectively measured via spectrophotometric analysis (Lunardi, Correr, Rastelli, Lima, & Consani, 2014; Peskersoy, Tetik, Ozturk, & Gokay, 2014; Vieira, Arakaki, & Caneppele, 2008). Its function is based on a principle that is uniform across all



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color-measuring devices, which is the illumination of an object by an internal light source (Yang, Murakami, & Yamaguchi, 2012). The authority on the science of light and color Commission.

Internationale de l'Eclairage (CIE), has defined the L*C*h color space as a means of expressing colour attributes in numerical terms (Acton & Dawson, 2004). The spectrophotometer scans the object and translates it into color constituents represented by the three axes in the L*C*h color model (Figure 2), namely lightness (L), chroma (C), and hue (h). L sits on the vertical axis. It is the measure of an object's brightness, which ranges from 0 (no lightness/absolute black) to 100 (maximum lightness/absolute white). C represents the saturation of an object, where 0 is completely unsaturated (i.e. a neutral grey, black or white). A chroma value of 100 or more represents very high saturation. Hue is every possible saturated colour, represented by degrees, 0° (red) through 90° (yellow), 180° (green), 270° (blue) and back to 0° (Kimura, 2018).

The purpose of this study is to investigate the efficacy of flaxseed as an organic remineralizing agent through colorimetric values of L, C and h using a spectrophotometer. Supplemental analysis of the same parameters is achieved by using an image-editing computer software, Photoshop CS6 (Adobe, San Jose, CA, USA).

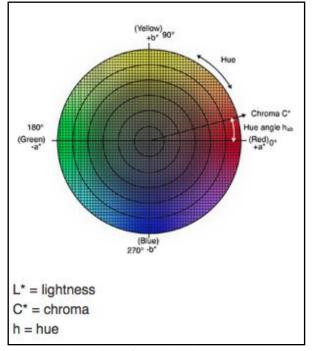


Figure 2: Illustration of L*C*h color space model (Acton & Dawson, 2004)

II. MATERIALS AND METHODS

Thirty human teeth consisting of incisors and canines were collected. The samples were grouped into control (no treatment), fluoride (positive control) and flaxseed (treatment) groups (Figure. 3). Ten teeth in the control group were placed in normal saline. The remaining ten teeth for flaxseed paste treatment and ten teeth for fluoride paste treatment were immersed in carbonated drink (Coke) for two days (Dincer, Hazar, & Sen, 2002; Kitchens & Owens, 2007; Seow & Thong, 2005) [7, 8, 32]. After two days of immersion,



spectrophotometer (Spectrophotometer CM-5, Konica Minolta, Tokyo, Japan) (Figure 4) was used to measure the L, C and h for all the groups on only the labial surfaces.

Then, 1gm/ml flaxseed paste was prepared using freshly ground flaxseed and mixed with deionised distilled water. The mixture was then properly mixed to the desired consistency. Ten teeth in the flaxseed group were then treated with the flaxseed paste for 14 days. For the positive control group, the teeth were placed in 1450ppm fluoride toothpaste (Colgate: Sugar Acid Neutralizer) for the same period of 14 days. The L, C and h were measured again. The immersion in flaxseed paste and fluoride toothpaste was repeated. The final reading was taken after another 14 days. At each stage, clinical photo of the teeth were saved as a JPEG file and computerized digital imaging analyses were performed using Photoshop CS6 software (Adobe, San Jose, CA, USA) (Table 3).

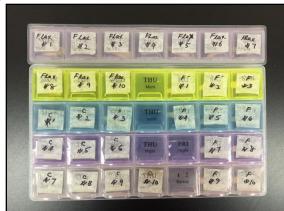


Figure 3: Thirty extracted teeth placed and labelled as control (no treatment), fluoride (positive control) and flaxseed (treatment group)



Figure 4: Spectrophotometer

The spectrophotometer (Spectrophotometer CM-5, Konica Minolta, Tokyo, Japan) was calibrated using zero calibration first, followed by white calibration. Then, each sample was placed on the target mask, ensuring the tooth is as close as possible to the specimen measuring port. Measurements were taken three times and average readings were taken. The measurements were then transferred to the computer and the data were saved.

Computerized digital imaging analyses were performed using Photoshop CS6 software (Adobe, San Jose, CA, USA). All the clinical photos that were taken and saved as JPEG format were analysed using eyedropper tool which was targeted on a point on the photo. The details of the L, C and h can be obtained from the foreground colour (Figure 5).

The results for spectrophotometric readings were analysed by statistical analysis software SPSS 24.0 (IBM, Endicott, NY, USA) using repeated-measures analysis of variance (ANOVA) followed by a multivariate Pillai test. All tests were carried out at a 5% level of significance.

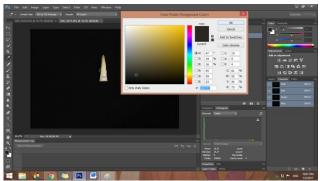


Figure 5: Computerized digital imaging analysis using Photoshop CS6 software

III. RESULTS

A. Spectrophotometric Results & Analysis

All the teeth in the two treated (flaxseed and fluoride) groups showed decrease in lightness in the final reading. In the fluoride group, the mean percentage of decrease in L is 29.62% whereas the flaxseed group showed 12.58% in mean L reduction. The percentage of changes of L, C, and h is

illustrated in Figure 6. All ten samples in the flaxseed group showed decrease in lightness at the second post-treatment reading, whereas only 90% of the fluoride group showed L reduction. As for chroma, there was only 7.95% reduction in mean C value in the fluoride group, whereas flaxseed group showed a decrease of 35.75%, which is four times this value. Overall, C decreased in both groups. It was found that there was a staggering difference between the rise in mean hue in two treatment groups. The fluoride group showed a 130% increase, but there was only 10.58% rise from the flaxseed-treated teeth.

A summary of the spectrophotometric readings is shown in Table 1.The pattern of change in lightness, chroma, and hue for all three groups is shown in Figure 7. Overall, there was a significant difference (F = 6.086, p < 0.007) of mean L value among three different groups based on time (Table 2). At the final reading, there was a difference of mean lightness value between flaxseed group and fluoride group. Fluoride had a lower mean L value (L=55.69 ± 5.38, p=0.070) than flaxseed (L=67.60 ± 5.34, p=0.070). Figure 9 shows the mean C value was higher in flaxseed group (C=2.75 ± 2.73, p=0.010) compared to fluoride group (C=2.78±2.73, p=0.010). In contrast, the mean h value for flaxseed group was much lower (h=121.50±10.53, p=0.000) when compared to fluoride group (h=260.02 ± 10.53, p=0.000) (Figure 10).

Control group showed no significant changes between the L mean values but had differences in C and h and in the initial (L: 71.09, C: 3.29, h: 116.48) and final (L:70.54, C: 7.58, h: 101.40) readings.

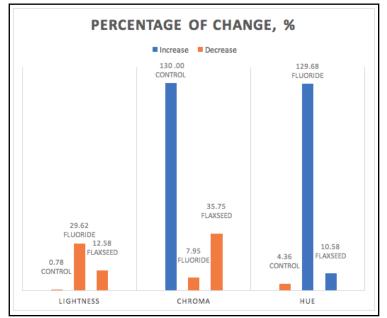


Figure 6: Percentage of changes in three groups for L*C*h parameters



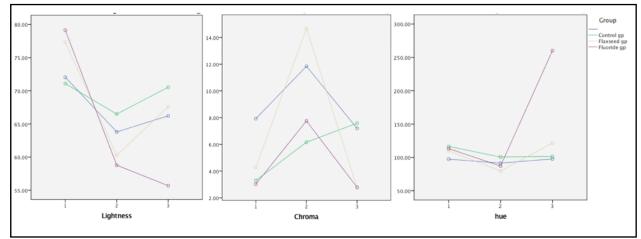


Figure 7: Line plots from mean values obtained from spectrophotometer for L, C and h.

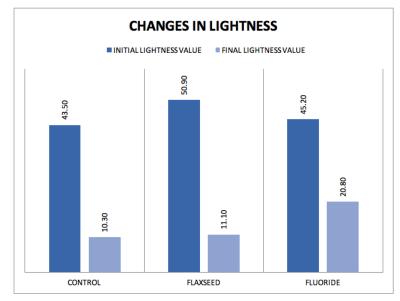


Figure 8: Mean difference in lightness when measured using computerized digital imaging analysis for control, flaxseed and fluoride

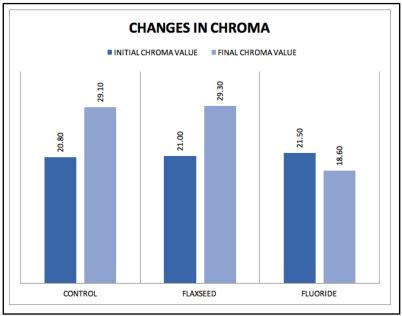


Figure 9: Mean difference in chroma when measured using computerized digital imaging analysis for control, flaxseed and fluoride



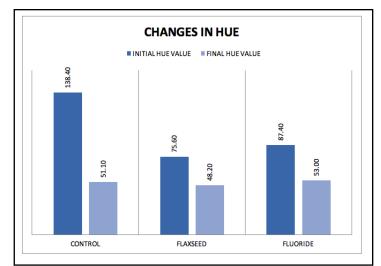


Figure 10: Mean difference in hue when measured using computerized digital imaging analysis for control, flaxseed and fluoride.

			Initial*				Post-tre	atment**		
Group	Tooth	L_0	C_0	h_0	L_1	C_1	h_1	L_2	C_2	h_2
Control	1	71.09	3.29	116.48	66.49	6.16	100.62	70.54	7.58	101.40
	2	71.56	6.30	101.35	68.96	8.23	97.74	63.38	8.66	98.97
	3	69.22	9.37	97.45	59.18	6.31	103.17	72.93	14.71	92.92
	4	55.53	5.10	90.69	64.97	9.83	88.72	68.75	9.24	90.42
	5	56.01	7.34	89.78	64.72	9.56	84.53	67.49	11.38	85.85
	6	72.88	2.62	116.49	57.12	1.71	200.75	57.40	2.41	122.09
	7	65.15	4.38	98.55	75.72	6.74	101.10	66.74	4.09	106.82
	8	74.97	7.42	99.18	66.91	6.91	101.19	70.78	7.99	97.70
	9	68.45	11.51	88.10	63.06	9.32	90.25	54.45	5.87	92.75
	10	74.23	12.65	92.57	68.79	13.00	93.26	59.14	9.23	92.67
Fluoride	1	79.13	3.02	113.21	58.78	7.75	87.10	55.69	2.78	260.02
	2	64.97	3.44	112.35	64.98	8.06	90.66	68.58	6.23	98.86
	3	75.72	11.04	97.67	64.10	18.44	79.13	71.49	8.06	96.67
	4	77.11	5.17	108.81	65.30	15.16	84.47	69.84	3.27	121.84
	5	79.32	11.76	88.55	64.92	19.41	78.67	75.24	9.94	90.87
	6	76.51	12.45	86.45	63.18	17.80	75.84	71.82	6.90	88.64
	7	75.53	9.85	95.98	68.39	12.40	86.77	68.77	7.22	94.28
	8	65.87	4.83	98.77	52.40	12.12	81.26	68.60	2.10	122.65
	9	78.06	9.89	99.24	66.05	19.23	81.38	71.24	5.79	106.86
	10	73.58	6.11	106.25	53.76	16.40	78.02	74.27	7.55	97.86
Flaxseed	1	77.33	4.28	109.88	60.27	14.67	79.63	67.60	2.75	121.50
	2	77.59	6.91	97.67	64.03	12.49	86.18	64.73	6.75	92.18
	3	71.34	4.72	102.65	66.27	7.02	93.65	64.48	3.85	98.36
	4	76.70	10.06	94.40	63.22	10.13	91.52	63.23	6.79	100.09
	5	67.54	11.60	83.11	56.79	16.82	72.66	58.90	9.88	83.81
	6	81.14	8.59	101.34	64.99	14.74	85.26	64.04	6.95	88.26
	7	78.91	9.60	98.63	57.41	10.95	86.10	59.56	7.68	91.39
	8	68.23	3.57	103.85	65.18	12.33	87.62	64.25	7.79	95.57
	9	77.94	7.73	99.46	63.44	13.59	85.26	66.30	5.78	104.20
	10	70.97	9.82	80.84	68.67	11.07	83.51	61.65	8.11	87.61
*After two da	ays of immer	sion in ca	rbonated d	lrink	1					

**14 and 28 days after treatment



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				95% Confidence Interva		
Group	Lightness	Mean	Std. Error	Lower Bound	Upper Bound	
	Lo	72.038	1.261	69.445	74.631	
	L ₁	63.797	.968	61.807	65.787	
	L ₂	66.224	1.035	64.097	68.351	
Control	Lo	71.090	6.554	57.617	84.563	
	L ₁	66.490	5.030	56.150	76.830	
	L ₂	70.540	5.377	59.488	81.592	
Flaxseed	Lo	77.330	6.554	63.857	90.803	
	L ₁	60.270	5.030	49.930	70.610	
	L ₂	67.600	5.377	56.548	78.652	
Fluoride	Lo	79.130	6.554	65.657	92.603	
	L ₁	58.780	5.030	48.440	69.120	
	L ₂	55.690	5.377	44.638	66.742	

Table 2: Results of statistical analysis for spectrophotometric L parameter

	Value	F	Hypothesis df	Error df	Sig.
Pillai's trace	.327	6.086 ^a	2.000	25.000	.007

Lightness	Group	Mean L value	95% CI
Initial lightness, Lo	Flaxseed	77.33	63.86, 90.80
	Fluoride	79.13	65.66, 92.60
	Control	71.09	57.62, 84.56
Lightness measured after 7 days, L1	Flaxseed	60.27	49.93, 70.61
	Fluoride	58.78	48.44, 69.12
	Control	66.49	56.15, 76.83
Lightness measured after 14 days. L2	Flaxseed	67.60	56.55, 78.65
	Fluoride	55.69	44.64, 66.74
	Control	70.54	59.49, 81.59

Table 3: Results of statistical analysis for spectrophotometric C parameter

					95%	Confide	nce Ir	nterval
Group	Chroma	Mean	Std.	Error	Lower B	ound	Upp	per Bound
	Co	C ₀ 7.920 .582 6.724		4	9.115			
	C1	11.843	s .e	857	10.08	2		13.605
	C ₂	7.193		526	6.11	2		8.275
Control	Co	3.290	3.	022	-2.92	2		9.502
	C1	6.160	4.	453	-2.99	2		15.312
	C2	7.580	2.	734	1.95	9		13.201
Flaxseed	Co	4.280	3.	022	-1.93	2		10.492
	C1	14.670	4.	453	5.51	8		23.822
	C2	2.750	2.	734	-2.87	1	rror df 25.000 955 -1.93 -3.19 -2.92 5.52,	8.371
Fluoride	Co	3.020	3.	022	-3.19	2		9.232
	C1	7.750	4.	453	-1.40	2	8.3 9.2 16.9	
	C ₂	2.780	2.	734	-2.84	1	5.000	8.401
		N	lultivariat	te Test				
	Va		lultivariat F		thesis df	Error	df	Sig.
Pillai's trace	Va			Нуро	thesis df 2.000			Sig. .010
	Chroma	lue	F 5.524ª	Нуро		25.	.000	-
Pillai's trace	Chroma	lue	F 5.524ª Gr	Нуро	2.000	25. alue	.000. 99	.010
	Chroma	lue	F 5.524ª Gr	Hypo roup seed	2.000 Mean C v	25. alue	.000 9! -1.9	.010 5% Cl
	Chroma	lue	F 5.524ª Gr Flax	Hypo roup seed pride	2.000 Mean C v 4.28	25. alue	.000 9! -1.9 -3.1	.010 5% CI 3, 10.49
	Chroma Co	lue .306	F 5.524ª Gr Flax Fluo Cont	Hypo roup seed pride	2.000 Mean C v 4.28 3.02	25. alue	.000 9! -1.9 -3.1 -2.9	.010 5% CI 3, 10.49 19, 9.23
Initial chroma,	Chroma Co	lue .306	F 5.524ª Gr Flax Fluo Cont	Hypo roup seed oride trol seed	2.000 Mean C v 4.28 3.02 3.29	25. alue	.000 98 -1.9 -3.1 -2.9 5.52	.010 5% CI 3, 10.49 19, 9.23 92, 9.50
Initial chroma,	Chroma Co	lue .306	F 5.524ª Gr Flax Fluo Cont Flax	Hypo seed oride trol seed oride	2.000 Mean C v 4.28 3.02 3.29 14.67	25. alue	.000 98 -1.9 -3.1 -2.9 5.52 -1.4	.010 5% Cl 3, 10.49 19, 9.23 92, 9.50 2, 23.82
Initial chroma,	Chroma Co ured after 7 da	.306	F 5.524ª Gr Flax Fluo Con Flax Fluo Con	Hypo seed oride trol seed oride	2.000 Mean C v 4.28 3.02 3.29 14.67 7.75	25. alue	.000 95 -1.9 -3.1 -2.9 5.52 -1.4 -2.9	.010 5% CI 3, 10.49 19, 9.23 92, 9.50 2, 23.82 0, 16.90
Initial chroma, Chroma measu	Chroma Co ured after 7 da	.306	F 5.524ª Gr Flax Fluo Con Flax Fluo Con	Hypo roup seed oride trol seed oride trol seed	2.000 Mean C v 4.28 3.02 3.29 14.67 7.75 6.16	25. alue	.000 98 -1.9 -3.1 -2.9 5.52 -1.4 -2.9 -2.8	.01(5% Cl 3, 10.49 19, 9.23 92, 9.50 2, 23.82 0, 16.90 9, 15.31
Initial chroma, Chroma measu	Chroma Co ured after 7 da	.306	F 5.524 ^a Gr Flax Fluo Com Flax Fluo Com Flax	Hypo roup seed bride trol seed bride trol seed bride	2.000 Mean C v 4.28 3.02 3.29 14.67 7.75 6.16 2.75	25. alue	.000 99 -1.9 -3.1 -2.9 5.52 -1.4 -2.9 -2.8 -2.8	.010 5% Cl 3, 10.49 19, 9.23 92, 9.50 2, 23.82 0, 16.90 9, 15.31 37, 8.37
Initial chroma, Chroma measu	Chroma Co ured after 7 da	uys, C ₁	F 5.524ª Flax Fluo Con Flax Fluo Con Flax Fluo Con	Hypo roup seed bride trol seed bride trol seed bride	2.000 Mean C v 4.28 3.02 3.29 14.67 7.75 6.16 2.75 2.78	25. alue	.000 99 -1.9 -3.1 -2.9 5.52 -1.4 -2.9 -2.8 -2.8	.010 5% Cl 3, 10.49 19, 9.23 92, 9.50 2, 23.82 0, 16.90 9, 15.31 37, 8.37 34, 8.40



			<u> </u>	· · · ·		r r		
					95% Co	erval		
Group	hue	Mean	Std. E	rror			r Bound	
	ho	97.414	1.60	7	94.111	10	0.717	
	h ₁	91.432	4.45	9	82.267	10	0.597	
	h ₂	97.785	2.02	7	93.618	10	1.952	
Control	ho	116.480	8.35	0	99.315	13	3.645	
	h ₁	100.620	23.10	68	52.997	14	8.243	
	h ₂	101.400	10.53	33	79.749	12	3.051	
Flaxseed	ho	109.880	8.35	0	92.715	12	7.045	
	h ₁ 79.630 23.168 32.0		32.007	12	7.253			
	h ₂	121.500	10.53	33	99.849	14	3.151	
Fluoride	ho	113.210	8.35	0	96.045	13	0.375	
	h ₁	87.100	23.10	68	39.477	13	4.723	
	h ₂	260.020	10.5	33	238.369	28	1.671	
			Multivaria					
		Value	F		othesis df	Error df	Sig.	
Pillai's trace		.824	58.397ª		2.000	25.000		
	Hue		Gr	oup	Mean h va	alue 9	95% CI	
Initial hue, h				seed	109.88		72, 127.05	
	•							
			Fluo	ride	113.21	. 96.0	96.05, 130.38	
			Con	trol	116.48	99.3	32, 133.65	
Hue measure	ed after 7 da	ys, h ₁	Flax	seed	79.63	32.0	0, 127.25	
			Fluo	ride	87.10	39.4	8, 134, 72	
			Con	trol	100.62	53.0	00, 148.24	
Hue measure	ed after 14 d	ays. h ₂	Flax	seed	121.50	99.8	35, 143.15	
			Fluo	ride	260.02	238.	37, 281.67	

Table 4: Results of statistical analysis for spectrophotometric h parameter

Table 5: Summary of L, C, and h values determined using Adobe Photoshop CS6.

Control

*Values rounded off to two decimal places

101.40

79.75, 123.05

~	_	Initial	*		Post-t	reatment**	¢			
Group	Tooth	L ₀	C_0	\mathbf{h}_0	L_1	C_1	h_1	L_2	C_2	h_2
Control	1	42	30	161	17	19	48	7	26	52
control	2	45	26	156	15	19	40	3	32	56
	3	42	20	150	20	31	41	9	32	30 44
	4	43	22	162	16	33	38	6	22	48
	5	41	18	102	16	35	39	7	29	53
	6	45	17	143	18	11	55	12	26	45
	7	47	22	164	16	17	50	12	20	53
	8	45	16	104	20	24	25	16	28	47
	9	43	18	110	18	36	41	15	44	45
	10	42	16	128	18	25	49	16	28	68
Fluoride	1	44	18	142	21	17	44	23	13	45
ridoride	2	51	10	84	21	25	42	25	24	45
	3	46	26	66	25	30	44	14	20	57
	4	45	28	62	26	24	43	28	19	41
	5	50	32	67	25	34	42	24	31	41
	6	51	13	132	19	35	42	10	19	54
	7	41	20	78	21	33	44	17	12	55
	8	40	21	92	20	30	43	25	21	46
	9	44	18	78	20	25	45	26	15	80
	10	40	22	73	23	27	43	16	12	66
Flaxseed	1	53	18	63	31	17	44	8	20	51
	2	56	27	57	35	18	44	9	26	50
	3	59	22	65	33	31	41	8	34	44



The Effect of Organic Flaxseed Paste on The Colorimetric Parameters of Demineralized Tooth Surface

4	53	19	90	30	23	41	8	23	56
5	50	33	55	26	36	41	7	35	46
6	41	15	93	31	17	45	29	26	45
7	55	26	59	29	21	45	8	24	52
8	40	10	112	25	32	39	7	26	50
9	51	19	81	32	20	45	10	29	49
10	51	21	81	27	34	40	17	50	39

*After two days of immersion in carbonated drink

**14 and 28 days after treatment

B. Computerized Digital Imaging Results & Analysis

Based on the data obtained from Table 2, a graph on changes in lightness (Figure 8) was made using the average value and reduction in L which was observed to decrease can be observed for teeth treated with flaxseed which is from 50.90 to 11.10 for teeth in flaxseed group. L kept on decreasing in value over time. The control group also showed reduction in lightness upon time. As for the fluoride group, there is a decrease in lightness although we can note the reduction in lightness after 14 days of treatment, but when the measurements were taken again in the next 14 days, half of the teeth appears to have a slight increase in L. However, when comparing the average value of L, we can note there is still reduction in L from 45.20 to 25.80. From the average value, the difference in L of teeth treated with flaxseed is 39.80 while teeth treated with fluoride is 19.4. Teeth that were treated with flaxseed exhibited greater reduction when compared with teeth that were treated with fluoride.

Results showed that C value of control group increased from 20.8 to 29.10 and C value for flaxseed group also increased from 21.00 to 29.30. The C values for fluoride group, however, decreased from 21.50 to 18.60 (Figure 9). Results also showed that h value shows reduction in all three groups (Control: from 138.40 to 51.10; Flaxseed: from 75.60 to 48.20; Fluoride: from 87.40 to 53.00) (Figure 10).

IV. DISCUSSION

The present pilot study was conducted to test the efficacy of flaxseed as an organic remineralizing agent, by measuring colorimetric parameters using a spectrophotometer and adjunctive computerized digital imaging software analysis. L, C and h were evaluated pre- and post-treatment with flaxseed and fluoridated tooth paste. Lightness parameters increased after immersion of teeth in carbonated drink, a phenomenon which indicates that demineralization cause an increase in surface lightness. As previously mentioned, demineralization is more likely to occur when the hydroxyapatite crystals in enamel become less closely packed in low pH environment, rendering the enamel more soluble and susceptible to lose its minerals (Hallgren et al., 2015).

Loss of minerals, low fluorescence radiance and white appearance are cardinal characteristics of incipient, demineralized lesions. The mineral loss causes an alteration in the refractive index of enamel and increases light scattering in the WSL (Sundararaj, Venkatachalapathy, Tandon, & Pereira, 2015). To simulate this, the samples in fluoride and flaxseed groups were immersed in carbonated drink prior to treatment to induce WSLs, or at the very least, to leach minerals from the enamel surface. The composition of Coke is carbonated water, high fructose corn syrup, caramel colouring, phosphoric acid, natural flavours and caffeine, among others. The pH recorded is 2.49 (Kitchens & Owens, 2007), which is significantly lower than the critical pH 5.5 at which demineralization starts to occur. Due to the poor aesthetics that come hand in hand with WSL (Kim, Son, Yi, Ahn, & Chang, 2016), vital bleaching is one of the methods employed to restore acceptable clinical appearance (Knosel, Attin, Becker, & Attin, 2007). However, it is important to note that bleaching is used to only lighten the surrounding background colour of the tooth for the WSL to appear indistinct (Greenwall, 2009). Despite the ability to satisfactorily camouflage inactive WSL, bleaching treatments would require supportive remineralization therapy to maintain enamel strength (Kim et al., 2016; Knosel et al., 2007). In addition, reports of dental sensitivity during and post-bleaching treatments are widely circulated (Moghadam, Majidinia, Chasteen, & Ghavamnasiri, 2013). Therefore, we believe it is imperative to find an agent that facilitates remineralization that can aesthetically improve enamel appearance without adverse effects such as sensitivity.

In this study, the decrease in L and C shown across all samples treated with flaxseed, shows that it is capable of reversing demineralization and probably restoring strength and aesthetics of demineralized enamel. This is supported by the theory that when an object's translucency decreases, the amount light passing through it will decrease as well, and the object will seem less saturated (Vieira et al., 2008) [28]. It can be postulated that the high content of calcium and potassium aids in remineralization (Knight, McIntyre, Craig, & Mulyani, 2006; Zhi, Lo, & Kwok, 2013). The spectrophotometric analysis was made the primary data measurement method because it precludes visual perception or environmental lighting as digital imaging analysis could be less accurate due to color deflections, shadows and daylight interference (Peskersoy et al., 2014).

Molecule size will affect the penetrability of the enamel (Jheon, Seidel, Biehs, & Klein, 2013) by chemicals, saliva or even bacteria; hence, it would certainly affect flaxseed paste penetration. Flaxseed paste solubility will also affect the amount of flaxseed minerals penetrating the tooth surface and subsequently influence the colorimetric parameters. In healthy enamel, the percentage of these pores is only 0.1%. In contrast to that, an initial lesion that lies above sound enamel will have 1% of these pores. This is called the translucent zone. These pores or micro-channels have a diameter of 0.5 to 1.5 μ m and a depth of 100 μ m, which eventually allow bacterial invasion (Barbosa de Sousa, Dias Soares, & Sampaio Vianna, 2013). We anticipate that using flaxseed paste for remineralizing the enamel surface would block these micro-channels and subsequently hinder bacterial invasion, therefore, saving the tooth from further mineral loss and destruction.

Due to flaxseed's remarkable mineral content, especially for calcium, potassium, phosphorus and magnesium; and its



effect on the demineralized tooth surface, it would be interesting that future investigation can be implemented to clarify the biochemical mechanism of flaxseed role on the demineralized enamel surface or mineral loss.

V. CONCLUSION

Flaxseed induces noticeable effect on the tooth surface by reducing the lightness parameters of the demineralised surface. The decreased lightness values of enamel surface after flaxseed application is attributed to the influx of high calcium and potassium minerals content of flaxseed paste into the enamel structure, rendering it less permeable to light, which makes flaxseed a promising complementary organic agent for tooth remineralization.

> CONFLICT OF INTEREST No conflict of interest existed.

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REFERENCES

- Acton, J. C., & Dawson, P. L. (2004). 25 Impact of proteins on food colour. Woodhead Publishing Series in Food Science, Technology and Nutrition, 631 - 668. doi:https://doi.org/10.1533/9781855738379.3.631
- [2] Andersson, G., Johansson, G., Attstrom, R., Edwardsson, S., Glantz, P. O., & Larsson, K. (1995). Comparison of the effect of the linseed extract Salinum and a methyl cellulose preparation on the symptoms of dry mouth. Gerodontology, 12(1), 12-17.
- [3] Barbosa de Sousa, F., Dias Soares, J., & Sampaio Vianna, S. (2013). Natural enamel caries: a comparative histological study on biochemical volumes. Caries Res, 47(3), 183-192. doi:10.1159/000345378
- [4] Dincer, B., Hazar, S., & Sen, B. H. (2002). Scanning electron microscope study of the effects of soft drinks on etched and sealed enamel. Am J Orthod Dentofacial Orthop, 122(2), 135-141.
- [5] Goyal, A., Sharma, V., Upadhyay, N., Gill, S., & Sihag, M. (2014). Flax and flaxseed oil: an ancient medicine & modern functional food. J Food Sci Technol, 51(9), 1633-1653. doi:10.1007/s13197-013-1247-9
- [6] Greenwall, L. H. (2009). Treatment considerations for bleaching and bonding white lesions in the anterior dentition. Alpha Omegan, 102(4), 121-127.
- [7] Hallgren, K., Akyalcin, S., English, J., Tufekci, E., & Paravina, R. D. (2016). Color Properties of Demineralized Enamel Surfaces Treated with a Resin Infiltration System. J Esthet Restor Dent, 28(5), 339-346. doi:10.1111/jerd.12207
- [8] Igarashi, K., Hamada, Y., Nishimaki, H., Sakurai, S., & Kamiyama, K. (1987). The acidogenic potential of plaque from sound enamel, white spot lesions, and cavities in children. Pediatr Dent, 9(3), 212-215.
- [9] Imran, M., Ahmad, N., Anjum, F. M., Khan, M. K., Mushtaq, Z., Nadeem, M., & Hussain, S. (2015). Potential protective properties of flax lignan secoisolariciresinol diglucoside. Nutr J, 14, 71. doi:10.1186/s12937-015-0059-3
- [10] Jheon, A. H., Seidel, K., Biehs, B., & Klein, O. D. (2013). From molecules to mastication: the development and evolution of teeth. Wiley Interdiscip Rev Dev Biol, 2(2), 165-182. doi:10.1002/wdev.63
- [11] Kim, Y., Son, H. H., Yi, K., Ahn, J. S., & Chang, J. (2016). Bleaching Effects on Color, Chemical, and Mechanical Properties of White Spot Lesions. Oper Dent, 41(3), 318-326. doi:10.2341/15-015-1
- [12] Kimura, E. (2018). Averaging colors of multicolor mosaics. J Opt Soc Am A Opt Image Sci Vis, 35(4), B43-b54. doi:10.1364/josaa.35.000b43
- [13] Kitchens, M., & Owens, B. M. (2007). Effect of carbonated beverages, coffee, sports and high energy drinks, and bottled water on

the in vitro erosion characteristics of dental enamel. J Clin Pediatr Dent, 31(3), 153-159.

- [14] Knight, G. M., McIntyre, J. M., Craig, G. G., & Mulyani. (2006). Ion uptake into demineralized dentine from glass ionomer cement following pretreatment with silver fluoride and potassium iodide. Aust Dent J, 51(3), 237-241.
- [15] Knosel, M., Attin, R., Becker, K., & Attin, T. (2007). External bleaching effect on the color and luminosity of inactive white-spot lesions after fixed orthodontic appliances. Angle Orthod, 77(4), 646-652. doi:10.2319/060106-224
- [16] Lunardi, N., Correr, A. B., Rastelli, A. N., Lima, D. A., & Consani, R. L. (2014). Spectrophotometric evaluation of dental bleaching under orthodontic bracket in enamel and dentin. J Clin Exp Dent, 6(4), e321-326. doi:10.4317/jced.51168
- [17] Moghadam, F. V., Majidinia, S., Chasteen, J., & Ghavamnasiri, M. (2013). The degree of color change, rebound effect and sensitivity of bleached teeth associated with at-home and power bleaching techniques: A randomized clinical trial. Eur J Dent, 7(4), 405-411. doi:10.4103/1305-7456.120655
- [18] Peskersoy, C., Tetik, A., Ozturk, V. O., & Gokay, N. (2014). Spectrophotometric and computerized evaluation of tooth bleaching employing 10 different home-bleaching procedures: In-vitro study. Eur J Dent, 8(4), 538-545. doi:10.4103/1305-7456.143639
- [19] Ramashetty Prabhakar, A., & Arali, V. (2009). Comparison of the remineralizing effects of sodium fluoride and bioactive glass using bioerodible gel systems. J Dent Res Dent Clin Dent Prospects, 3(4), 117-121. doi:10.5681/joddd.2009.029
- [20] Seow, W. K., & Thong, K. M. (2005). Erosive effects of common beverages on extracted premolar teeth. Aust Dent J, 50(3), 173-178; quiz 211.
- [21] Shim, Y., Gui, B., Arnison, P., Wang, Y., & Reaney, M. (2014). Flaxseed (Linum usitatissimum L.) bioactive compounds and peptide nomenclature: A review. Trends in Food Science & Technology, 38(1), 5 - 20.
- [22] Sundararaj, D., Venkatachalapathy, S., Tandon, A., & Pereira, A. (2015). Critical evaluation of incidence and prevalence of white spot lesions during fixed orthodontic appliance treatment: A meta-analysis. J Int Soc Prev Community Dent, 5(6), 433-439. doi:10.4103/2231-0762.167719
- [23] ten Cate, J. M. (2008). Remineralization of deep enamel dentine caries lesions. Aust Dent J, 53(3), 281-285. doi:10.1111/j.1834-7819.2008.00063.x
- [24] Vieira, G. F., Arakaki, Y., & Caneppele, T. M. (2008). Spectrophotometric assessment of the effects of 10% carbamide peroxide on enamel translucency. Braz Oral Res, 22(1), 90-95.
- [25] Wolff, M. S., & Larson, C. (2009). The cariogenic dental biofilm: good, bad or just something to control? Braz Oral Res, 23 Suppl 1, 31-38.
- [26] Yang, F., Murakami, Y., & Yamaguchi, M. (2012). Digital color management in full-color holographic three-dimensional printer. Appl Opt, 51(19), 4343-4352. doi:10.1364/ao.51.004343
- [27] Zero, D. T., Hara, A. T., Kelly, S. A., Gonzalez-Cabezas, C., Eckert, G. J., Barlow, A. P., & Mason, S. C. (2006). Evaluation of a desensitizing test dentifrice using an in situ erosion remineralization model. J Clin Dent, 17(4), 112-116.
- [28] Zhi, Q. H., Lo, E. C., & Kwok, A. C. (2013). An in vitro study of silver and fluoride ions on remineralization of demineralized enamel and dentine. Aust Dent J, 58(1), 50-56. doi:10.1111/adj.12033

