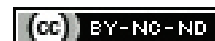


Colour Stability of Aligners in Distilled Water, Mouthwash and Carbonated Drinks: An In-vitro Study

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ABSTRACT

Introduction: Clear aligners have gained immense popularity in orthodontics due to their aesthetic appeal and convenience. However, colour stability is a crucial factor that can affect patient satisfaction and treatment outcomes. Commonly consumed mouthwashes and carbonated beverages may cause discolouration of aligners, necessitating an investigation into their effects.

Aim: To assess the impact of various mouthwashes and carbonated drinks on the colour stability of orthodontic aligners over a prolonged exposure period.

Materials and Methods: An in-vitro experimental study was conducted in the Department of Biochemistry, SRM Medical College Hospital and Research Centre, Kattankulathur, Chennai, Tamil Nadu, India, in September 2024 using 45 clear aligner samples (SmartTrack). The samples were immersed in control solutions (distilled water), mouthwash (chlorhexidine), and carbonated drinks for two weeks. Each sample underwent simulated daily exposure at 37°C, and colour assessments were performed using a Ultraviolet-visible (UV-Vis) spectrophotometer

at the end of the study. Optical density values were obtained and converted to Commission Internationale de l'Éclairage Laboratory* colour system (CIELAB) values. Statistical analysis included descriptive statistics (mean, median, standard deviation, and range), intragroup analysis (Fisher's-exact test), and intergroup analysis (Mann-Whitney U test).

Results: Significant colour changes were observed in aligners immersed in carbonated drinks, which demonstrated the highest ΔE values, the standard measurement of colour difference given by CIE. A p-value <0.05 indicated statistical significance, particularly at the T1 timepoint. Mouthwashes also contributed to noticeable discolouration, though to a lesser extent than carbonated drinks.

Conclusion: Both carbonated beverages and mouthwashes negatively impact the colour stability of clear aligners, with carbonated drinks causing more pronounced discolouration. Clinicians should inform patients about these effects and recommend appropriate care routines to maintain the aesthetic quality of aligners. Further research is warranted to explore methods for enhancing the stain resistance of aligner materials.

Keywords: Aesthetics, Chlorhexidine, Orthodontics, Orthodontic appliances

INTRODUCTION

The increasing the number of adult orthodontic patients has prompted an upsurge in the demand for aesthetic and comfortable alternatives to conventional fixed appliances [1,2]. Clear aligners that satisfy this demand are also prone to rapid technological improvements in materials and production techniques [3,4]. They have revolutionised orthodontic treatment by offering a discreet alternative to traditional braces [5]. Their transparent appearance and removable nature provide patients with greater comfort and aesthetic freedom [6,7].

However, the long-term aesthetic appeal of aligners is closely tied to their colour stability. While it is advisable to remove the aligners in course of food and beverages intake [8]. But it has been stated in various studies that the compliance regarding removable appliance is insufficient, and patients often continue wearing them during consumption of food [9,10]. Hence, the pigments from the staining agents accumulate, leading to discolouration and an unaesthetic appearance [11].

Discolouration can occur due to various factors, including exposure to dietary substances, oral hygiene products, and salivary components. Mouthwashes are widely recommended for oral hygiene maintenance, but certain formulations may inadvertently contribute to staining [12]. Similarly, carbonated beverages, especially those high in sugar and acidity, are prevalent in many diets and pose a potential risk to the integrity of orthodontic materials.

To effectively care for patients and improve treatment satisfaction, it is essential to comprehend how these substances impact the colour stability of clear aligners [13,14]. By tackling the relatively unexplored problem of colour stability in clear aligners when exposed to mouthwashes and carbonated drinks, this study offers a distinctive and noteworthy contribution to orthodontics. Specifically, it examines the effects of two commonly used, but frequently disregarded, substances mouthwash (chlorhexidine) and carbonated beverages on the aesthetic integrity of clear aligners, whereas earlier research has concentrated on the general susceptibility of aligner materials to staining from food and beverages.

The present study's main goal is to use a strict in-vitro methodology to assess how mouthwash containing chlorhexidine mouthwash and carbonated beverages affect the colour stability of clear aligners.

MATERIALS AND METHODS

An in-vitro experimental study was conducted in September 2024 in the Department of Biochemistry, SRM Medical College Hospital and Research Centre, Kattankulathur, Chennai, Tamil Nadu, India. The present study was done in accordance with the ethical standards of the responsible committee on human experimentation and the Helsinki Declaration of 1975, revised in 2013. The present study was presented and approved by the SRM Medical College Hospital and Research Centre Institutional Ethics Committee Ethics Clearance Number: SRMIEC-ST0424-1058.

Sample size calculation: Using G*Power statistics with the effect size of 1, a proportional error (α) of 0.05, and a power of 95%, the minimum sample size required for each group was 13. For the present study, 15 samples were included in each group, resulting in a total of 45 samples.

1. Clear aligner samples: A total of 45 clear aligner samples (SmartTrack) were selected. such as SmartTrack, which utilises thermoplastic polyurethane or similar polymers to ensure optimal transparency and comfort.
2. Test solutions (15 Aligners in each solution):
Control: Distilled water served as the baseline control to assess any changes without external influences.
Mouthwash: An alcohol-based mouthwash (0.2% chlorhexidine) was selected, reflecting common usage patterns among patients.
Carbonated drinks: Well-known sugary drinks were selected to symbolise typical dietary choices.
3. Instruments: Optical Density (OD) measurements, which enable the quantification of colour changes, were obtained using a UV-Vis spectrophotometer (Thermo Scientific Evolution 201 UV-Vis Spectrophotometer) for accurate colour analysis. To replicate intraoral conditions [15]. Additional laboratory equipment was utilised, such as beakers, tweezers, and timers, ensured consistent sample handling.
4. To guarantee consistent sample handling, extra laboratory equipment was utilised, such as beakers, tweezers, and timers.

Study Procedure

1. Sample preparation: In accordance with the solutions, aligners were split into three groups of 15, corresponding to the three test solutions. Each aligner was set at 1 cm² to guarantee consistent exposure for every sample. In order to reduce results' variability, this standardisation was essential [1,16].
2. Exposure protocol: To replicate the exposure that patients might experience in the real world, samples were submerged in their corresponding test solutions for two weeks [17,18]. Every day, the solutions were updated. The samples were rinsed with distilled water after every exposure (every 24 hours) and kept at 37°C to maintain their integrity [19,20].
3. Colour measurement: Using the UV-Vis spectrophotometer, colour evaluations were carried out at baseline and after 2 weeks. The ΔE value was used to quantify colour changes were quantified using the ΔE value, with $\Delta E > 3.3$ indicating noticeable discolouration [13,21].
4. Control measures: Aligners were rinsed with distilled water before exposure to remove any residual impurities [22].

Colour change evaluation: The CIELAB colour system was used to evaluate the colour shift. The International Commission on Illumination (CIE) established the CIELAB colour space (Laboratory*) in 1976. It expresses colour as three values: L* for perceptual lightness and a* and b* for the four unique colours of human vision: red, green, blue and yellow [1].

L*: Lightness (0=black, 100=white)

a*: Red-green axis (positive=red, negative=green)

b*: Yellow-blue axis (positive=yellow, negative=blue)

The values and parameters that help assess the colour changes has been depicted in [Table/Fig-1-3].

S. No.	Sample ID	470 nm (Abs) Optical density	Reflectance (R)	RGB (Red, Green, Blue)	CIELAB
1	Water 1	0.523	0.298	(0.298, 0.268, 0.238)	L*=60.21, a*=-8.12, b*=4.63

2	Water 2	0.859	0.139	(0.139, 0.125, 0.111)	L*=38.55, a*=-15.32, b*=22.11
3	Water 3	0.981	0.010	(0.010, 0.009, 0.008)	L*=20.02, a*=-17.15, b*=4.21
4	Water 4	0.793	0.160	(0.160, 0.144, 0.128)	L*=39.40, a*=-9.29, b*=13.25
5	Water 5	0.797	0.159	(0.159, 0.143, 0.127)	L*=39.28, a*=-9.24, b*=13.14
6	Water 6	0.571	0.267	(0.267, 0.240, 0.214)	L*=48.93, a*=-9.51, b*=21.09
7	Water 7	1.306	0.050	(0.050, 0.045, 0.040)	L*=29.48, a*=-13.56, b*=14.22
8	Water 8	1.393	0.040	(0.040, 0.036, 0.032)	L*=25.84, a*=-14.21, b*=16.08
9	Water 9	0.806	0.158	(0.158, 0.142, 0.126)	L*=39.56, a*=-9.16, b*=12.89
10	Water 10	0.592	0.257	(0.257, 0.231, 0.205)	L*=48.06, a*=-8.74, b*=17.45
11	Water 11	0.810	0.157	(0.157, 0.141, 0.125)	L*=39.43, a*=-9.12, b*=12.76
12	Water 12	0.746	0.179	(0.179, 0.161, 0.143)	L*=41.96, a*=-8.73, b*=15.35
13	Water 13	1.167	0.068	(0.068, 0.061, 0.054)	L*=32.55, a*=-11.45, b*=18.05
14	Water 14	0.351	0.447	(0.447, 0.402, 0.358)	L*=58.72, a*=-5.98, b*=18.94
15	Water 15	0.652	0.224	(0.224, 0.202, 0.179)	L*=42.47, a*=-5.72, b*=14.61

[Table/Fig-1]: The optical density (absorbance at 470 nm), corresponding reflectance values, RGB colour values (Red, Green, Blue), and CIELAB coordinates (L*, a*, b*) for aligner samples immersed in distilled water. These parameters were used to assess changes in translucency and colour perception of the aligner materials.

S. No.	Sample ID	470 nm (Abs) Optical density	Reflectance (R)	RGB	CIELAB
1	Mouthwash 1	1.117	0.076	(0.076, 0.068, 0.061)	L*=41.56, a*=-14.98, b*=16.88
2	Mouthwash 2	1.174	0.067	(0.067, 0.060, 0.054)	L*=40.09, a*=-14.56, b*=15.88
3	Mouthwash 3	0.876	0.134	(0.134, 0.121, 0.107)	L*=48.16, a*=-6.58, b*=10.28
4	Mouthwash 4	0.614	0.243	(0.243, 0.219, 0.194)	L*=58.12, a*=-5.81, b*=19.92
5	Mouthwash 5	0.615	0.242	(0.242, 0.218, 0.194)	L*=58.08, a*=-5.80, b*=19.90
6	Mouthwash 6	0.838	0.144	(0.144, 0.130, 0.115)	L*=46.58, a*=-6.27, b*=13.83
7	Mouthwash 7	1.400	0.040	(0.040, 0.036, 0.032)	L*=28.73, a*=-14.41, b*=16.10
8	Mouthwash 8	1.255	0.056	(0.056, 0.050, 0.045)	L*=31.99, a*=-13.81, b*=16.18
9	Mouthwash 9	1.363	0.043	(0.043, 0.039, 0.034)	L*=30.91, a*=-13.61, b*=15.89
10	Mouthwash 10	0.894	0.127	(0.127, 0.114, 0.102)	L*=45.29, a*=-7.94, b*=12.45
11	Mouthwash 11	0.456	0.352	(0.352, 0.317, 0.281)	L*=61.38, a*=-4.78, b*=25.91
12	Mouthwash 12	0.500	0.316	(0.316, 0.285, 0.253)	L*=57.63, a*=-5.32, b*=22.10

13	Mouthwash 13	1.218	0.060	(0.060, 0.054, 0.048)	L*=30.02, a*=-14.20, b*=15.56
14	Mouthwash 14	0.868	0.135	(0.135, 0.121, 0.108)	L*=45.85, a*=-7.79, b*=12.25
15	Mouthwash 15	0.715	0.193	(0.193, 0.174, 0.154)	L*=48.97, a*=-6.63, b*=16.52

[Table/Fig-2]: The absorbance at 470 nm (optical density), calculated reflectance (R), RGB values, and CIELAB colour coordinates for aligner samples after immersion in mouthwash. These parameters reflect the extent of optical and colour changes caused by the solution.

S. No.	Sample ID	470 nm (Abs) Optical density	Reflectance (R)	RGB	CIELAB
1	Carbonated drink 1	1.115	0.077	(0.077, 0.069, 0.061)	L*=41.62, a*=-12.82, b*=16.33
2	Carbonated drink 2	0.553	0.281	(0.281, 0.253, 0.225)	L*=55.16, a*=-5.46, b*=19.99
3	Carbonated drink 3	1.662	0.022	(0.022, 0.020, 0.018)	L*=27.60, a*=-16.79, b*=13.74
4	Carbonated drink 4	0.702	0.199	(0.199, 0.179, 0.159)	L*=47.34, a*=-7.57, b*=14.01
5	Carbonated drink 5	0.730	0.188	(0.188, 0.169, 0.150)	L*=46.49, a*=-7.53, b*=13.33
6	Carbonated drink 6	0.343	0.451	(0.451, 0.406, 0.361)	L*=61.57, a*=-4.11, b*=22.90
7	Carbonated drink 7	0.578	0.265	(0.265, 0.239, 0.212)	L*=50.88, a*=-5.81, b*=17.68
8	Carbonated drink 8	1.378	0.042	(0.042, 0.038, 0.034)	L*=28.04, a*=-15.75, b*=12.56
9	Carbonated drink 9	1.242	0.057	(0.057, 0.051, 0.046)	L*=29.37, a*=-14.50, b*=13.29
10	Carbonated drink 10	0.780	0.166	(0.166, 0.150, 0.133)	L*=47.10, a*=-6.49, b*=13.79
11	Carbonated drink 11	0.554	0.281	(0.281, 0.253, 0.225)	L*=55.16, a*=-5.46, b*=19.99
12	Carbonated drink 12	0.531	0.296	(0.296, 0.266, 0.237)	L*=54.36, a*=-5.10, b*=19.41
13	Carbonated drink 13	1.000	0.100	(0.100, 0.090, 0.080)	L*=34.62, a*=-13.55, b*=12.91
14	Carbonated drink 14	1.075	0.084	(0.084, 0.076, 0.067)	L*=32.16, a*=-14.14, b*=12.58
15	Carbonated drink 15	0.631	0.234	(0.234, 0.211, 0.187)	L*=49.88, a*=-6.26, b*=15.92

[Table/Fig-3]: The optical properties of aligner samples immersed in a carbonated drink. Measurements include absorbance at 470 nm, calculated reflectance, Red, Green and Blue (RGB) values, and CIELAB coordinates to assess staining and colour alteration.

STATISTICAL ANALYSIS

Descriptive statistics the mean, median, SD and range of Optical Density (OD) for each group are presented in [Table/Fig-4]. The values indicate the overall colour stability trends among water, mouthwash, and carbonated drink samples. The relationship between ΔE values and human perception of colour differences are presented in [Table/Fig-5] [23]. Intergroup analysis (Mann-Whitney U Test): Mann-Whitney U test was performed to compare differences

between groups. The U values, Z scores, and p-values are provided in [Table/Fig-6]. Effect size (r) for intergroup comparisons was calculated using the formula: $r = \frac{Z}{\sqrt{N}}$, where Z is the standard score from the Mann-Whitney test and N is the total sample size [Table/Fig-7].

Set	Mean OD	Median OD	Standard Deviation (SD)	Range
Water	0.784	0.839	0.309	1.042
Mouthwash	0.957	0.797	0.309	0.944
Carbonated drink	0.833	0.730	0.390	1.640

[Table/Fig-4] The mean, median, SD, and range of OD values for the three test solutions: water, mouthwash, and carbonated drink. These values reflect the central tendency and variability of OD measurements after exposure to the respective solutions.

ΔE value [24]	Perception
0-1	Imperceptible to the human eye
1-2	Perceptible only to trained eyes
2-3	Slightly perceptible
3-5	Noticeable difference
5-10	Large difference
>10	Extremely different colours

[Table/Fig-5] The relationship between ΔE values and human perception of colour differences. Lower ΔE values (0-1) represent imperceptible colour changes, while values above 10 indicate extremely different colours, making them noticeable even to the untrained eye [24].

Time point	Group comparison	U-value	p-value	Interpretation
T0	Water vs. mouthwash	12.5	0.278	Not significant
	Water vs. carbonated drink	9.0	0.067	Not significant
	Mouthwash vs. carbonated drink	11.0	0.192	Not significant
T1	Water vs. mouthwash	6.0	0.008*	Significant
	Water vs. carbonated drink	5.5	0.006*	Significant
	Mouthwash vs. carbonated drink	8.5	0.045*	Significant

[Table/Fig-6] The results of intergroup comparisons between different solutions (water, mouthwash, and carbonated drink) at two time points (T0 and T1). Note: The U-values and p-values from the Mann-Whitney test are provided, along with their statistical significance. Asterisks (*) indicate significant differences (p<0.05) between groups. p<0.05 indicates statistical significance

Time point	Group comparison	Z-score	Effect size (r)	Interpretation
T1	Water vs mouthwash	-2.45	0.55	Large effect
	Water vs carbonated drink	-2.12	0.47	Medium effect
	Mouthwash vs carbonated drink	-2.01	0.45	Medium effect
T2	Water vs mouthwash	-2.89	0.64	Large effect
	Water vs carbonated drink	-3.10	0.69	Large effect
	Mouthwash vs carbonated drink	-2.01	0.45	Medium effect

[Table/Fig-7] The effect sizes (r) for intergroup comparisons between different solutions (water, mouthwash, and carbonated drink) at two time points (T1 and T2). The effect sizes were calculated based on Z-scores from the Mann-Whitney test.

RESULTS

Analysis of the three sets (After 2 weeks): The baseline values were set to zero and compared with the values obtained for the aligners after two weeks of immersion in the respective solutions.

Set 1: Average L*=44.96, a*=-10.68, b*=12.99

Medium lightness with a slightly greenish-yellow hue. The negative a* value indicates a slight green tint, while the positive b* value suggests a slight yellow tone.

Set 2: Average $L^*=50.28$, $a^*=-10.65$, $b^*=14.10$

Slightly lighter than Set 1 with a neutral green-yellow hue. The higher b^* value indicates a more pronounced yellowish tone, while the negative a^* value still show a slight green tint.

Set 3: Average $L^*=44.05$, $a^*=-11.46$, $b^*=14.32$

Similar lightness to Set 1 but with a more pronounced greenish-yellow hue. The lower a^* value indicates a stronger green tint, while the higher b^* value suggests a stronger yellow tone than both Set 1 and Set 2. [21,24].

$$\Delta E = \sqrt{(L_1^* - L_2^*)^2 + (a_1^* - a_2^*)^2 + (b_1^* - b_2^*)^2}$$

The classification of spread (i.e., low, moderate, or high) was based on two statistical measures of variability: Standard Deviation (SD) and range, both considered relative to the mean of the dataset.

Low spread: was characterised by a small SD and narrow range relative to the mean, indicating that data points are tightly clustered around the average.

Moderate spread: Assigned when the SD and range are neither very small nor very large compared to the mean, suggesting a reasonable level of dispersion.

High spread: Identified when the SD and/or range are relatively large compared to the mean, indicating greater variability among data points.

- Set 1: Shows a moderate spread, based on an analysis of how much the data values deviate from the mean (through SD) and how widely the values range. The term “moderate” suggests that the variation in Set 1 is not extreme, indicating a reasonable degree of spread) with an average of 0.784.
- Set 2: Has a slightly higher average of 0.957, but with a similar SD and range, also showing a moderate spread.
- Set 3: Exhibits the largest range (1.640), a mean of 0.833, and the highest SD of 0.390, indicating the highest variability among the three sets and a high spread.

Intragroup comparisons: Fisher's-exact test revealed significant changes within all groups: water ($p=0.017$), mouthwash ($p=0.002$), and carbonated drink ($p=0.032$). These results indicate statistically significant colour changes over time.

The relationship between ΔE values and human perception of colour differences. Lower ΔE values (0-1) represent imperceptible colour changes, while values above 10 indicate extremely different colours, making them noticeable differences, even to the untrained eye has been depicted in [Table/Fig-5].

Intergroup comparisons: The intergroup comparisons of the different solutions (water, mouthwash, and carbonated drink) at two time points (T0 and T1) has been depicted in [Table/Fig-6].

- T0 (Baseline): No statistically significant differences between groups, indicating similar initial colour stability.
- T1: Significant differences were observed across all comparisons, demonstrating distinct effects of each solution on colour stability over time.

The effect sizes (r) for intergroup comparisons between different solutions (water, mouthwash, and carbonated drink) at two time points (T1 and T2) has been depicted in [Table/Fig-7]. The effect sizes were calculated based on Z-scores obtained via the Mann-Whitney test. Effect size interpretation is as follows:

Effect size interpretation:

- Small effect: $r < 0.3$
- Medium effect: $0.3 \leq r < 0.5$
- Large effect: $r \geq 0.5$

At T1, the effect sizes were moderate, suggesting initial colour changes, except for water vs. mouthwash, which showed a large effect size, suggesting a major difference in colour stability. At T2,

large effect sizes were observed, particularly between water vs. mouthwash and water vs. carbonated drink, indicating substantial differences in colour stability.

The Mann-Whitney U test confirmed significant changes in colour stability over time, with notable differences between water, mouthwash, and carbonated drinks. Effect size calculations reinforced these findings, showing the most pronounced changes at T2. These results suggests that exposure to mouthwash and carbonated drinks causes measurable and significant discolouration over time.

Colour differences (ΔE analysis): The ΔE values calculated between the three sets reveal the perceptibility of colour differences. Colour interpretations has been provided in [Table/Fig-8-10]:



[Table/Fig-8]: Aligners after immersion in carbonated drinks for 2 weeks.



[Table/Fig-9]: Aligners after immersion in mouthwash for 2 weeks.



[Table/Fig-10]: Aligners after immersion in distilled water for 2 weeks.

Set 1 vs. Set 2: $\Delta E \approx 5.43$ (Large difference)

This represents a moderate colour difference that is likely noticeable to the human eye. Set 1 and 2 have similar lightness but differ in their yellowish tint, with Set 2 appearing slightly lighter and more

yellow. This ΔE value suggests that these sets would be perceived as distinct when viewed together, as shown in [Table/Fig-9,10].

Set 2 vs. Set 3: $\Delta E \approx 6.29$ (Large difference)

This difference is more pronounced than that between Set 1 and Set 2 and likely easily perceptible to most individuals. The lower lightness and a slightly stronger yellow tint, contributes to a more noticeable difference. This suggests that Set 3 may represent a product or material that is darker and more yellowish compared to Set 2, which could be important in applications requiring consistent colour representation, as seen in [Table/Fig-8,9].

Set 1 vs. Set 3: $\Delta E \approx 1.79$ (Perceptible only to trained eyes)

This is the smallest ΔE value among the comparisons, but still indicates a slightly noticeable difference. Although the colour difference between Set 1 and Set 3 is 2 or Set 2 and Set 3 less pronounced than between the other sets, the subtle shift toward a greener and more yellowish hue in Set 3 would still be perceptible under close inspection, as seen in [Figure 8,10]. Such slight colour changes are important, like in product batches that need to have a high level of colour consistency, this could be important.

DISCUSSION

The current study offers important new information about how mouthwash and carbonated drinks affect the colour stability of clear aligners. The results are in line with earlier studies showing that exposure to acidic, pigmented, and alcohol-based substances can cause thermoplastic orthodontic materials to stain and degrade. This section discusses the broader clinical and product-development is provided in this section, along with a comparison with previous research.

The notable discolouration brought on by carbonated drinks is consistent with earlier research showing how acidic and sugary drinks can jeopardise the structural and visual integrity of polymeric dental appliances. According to Venkatasubramanian P et al., (2022), pigments from the stains agents accumulate, resulting in discolouration and an unaesthetic appearance of the aligners [1]. Liu CL et al., (2016) aligners' ability to absorb water is improved by extended exposure to carbonated beverages increases aligners' water absorption, exacerbating colour change [15]. Even brief exposure to such beverages can have a cumulative effect on aligner aesthetics, highlighting the significance of patient education regarding beverage choices. Patients should be encouraged to rinse their aligners immediately after consuming such beverages to minimise staining risk.

The study's conclusions regarding alcohol-based mouthwashes causing aligner discolouration are consistent with earlier investigations examining the effects of oral hygiene products on polymeric dental materials. Concerns regarding prolonged exposure to chemical agents in oral care solutions are heightened by the finding by Checchi V et al., (2024) reported that long-term chlorhexidine use includes taste alteration, oral mucosal irritation, and discolouration of teeth and dental restoration (composites and cements) discolouration [12]. Additionally, as noted by Sharma R et al., (2018) noted that alcohol-containing mouthwashes may also contribute to staining of clear aligners, potentially affecting their aesthetic longevity [8].

Based on these results, orthodontic patients using clear aligners should either remove them before washing or switch to non alcoholic alternatives to minimise aesthetic compromise. Additionally, manufacturers should consider developing more stain-resistant polymeric materials to enhance the long-term visual quality of aligners.

The findings are consistent with earlier research showing that distilled water has little effect on the colour stability of aligners. According

to Bernard G et al., (2020), unlike mouthwashes and pigmented beverages, exposure to pure water does not change the chromatic characteristics of aligners. Additionally, non carbonated, sugar-free fluids were the least likely to alter the colour of aligner materials, making them the best choice for patients who are worried about maintaining the aesthetic longevity of their aligners [21]. These results suggest that orthodontic patients should be encouraged to consume more water rather than sugary or acidic drinks to preserve the clarity and integrity of their aligners.

Clinically, a ΔE threshold of 3.0 is generally considered perceptible to the human eye. Values above this threshold, as observed in aligners exposed to mouthwash and carbonated beverages, signify a colour shift that is clinically significant. Similar ΔE thresholds were reported by Paravina RD et al., (2020) when evaluating colour stability in dental composites and polymeric materials, indicating that rigorous dietary and oral hygiene adjustments are necessary to maintain aligner aesthetics [25]. Consequently, dietary counselling should be a regular part of clear aligner therapy, advising patients on appropriate beverages and emphasising the importance of cleaning aligners right away after contact with staining agents.

These results are in line with material science studies, as reported by Liu CL et al., (2016) and Bernard G et al., (2020), which underscore the importance of colour consistency for polymeric dental materials [15,21]. Additionally, manufacturers looking to create better polymer blends with increased stain resistance may be impacted by changes in colour stability brought on by beverage exposure impacts colour stability.

Comparisons with previous research indicate that oral hygiene practices and dietary choices are critical for preserving the aesthetic qualities of clear aligners. Future studies should examine the long-term effects of repeated exposure to staining agents and assess whether specific polymer compositions provide better resistance to discolouration. Additionally, research on surface coatings and sophisticated cleaning agents may shed more light on ways to minimise aligner staining [26]. Implementing standardised guidelines for dietary and hygiene practices among aligner users could improve patient outcomes and overall satisfaction with clear aligner therapy [27,28].

Implications for product development and quality control:

1. Colour consistency: The calculated ΔE values underscore the importance of maintaining colour consistency in industries where colour is a key product attribute. For products requiring precise colour matching, such as cosmetics, food and beverages, or pharmaceutical formulations, even small ΔE differences, like those between Set 1 and Set 3 ($\Delta E \approx 1.79$), can impact product quality. Minor colour changes can influence brand perception or consumer trust, especially when the colour plays a role in the expected appearance of a product [29].
2. Quality control: The ΔE values also suggest that Set 2 and Set 3 exhibit more noticeable colour differences than Set 1, indicating that even moderate colour differences between batches may require closer monitoring during quality control. A ΔE of 5 or more is typically noticeable to most consumers, and even small colour shifts might be deemed unacceptable in competitive markets or high-end products where colour consistency is a hallmark of quality [30].
3. Consumer perception: Colour plays a critical role in consumer perception, and the ΔE differences reflect how subtle changes in the hue and saturation can be perceived. The stronger yellowish hue in Set 3 compared to Set 1 and 2 could affect the visual appeal of a product, especially in industries such as cosmetics or design where colour plays a significant role in consumer choice. Even the small differences between

Set 1 and Set 3 could have implications for industries that require accurate colour matching for aesthetic purposes [26,27].

4. Application in manufacturing: From a manufacturing perspective, the ΔE values indicate a potential need for tight colour control between different product batches. For instance, products that align with Set 1's colour profile (slightly greenish-yellow) may need to maintain this exact appearance throughout production to ensure consistent consumer experience [28]. A shift toward the stronger yellowish tones of Set 3 could affect the final product's appearance, necessitating adjustments in formulation or production processes to maintain the desired colour outcome.
5. Market sensitivity: Some markets or products are more sensitive to colour variation than others. Industries dealing with luxury goods, pharmaceuticals, or premium beverages often require high colour precision, where even small differences can be flagged as a potential quality issues. The moderate to noticeable colour differences ($\Delta E \sim 5-6$) between Set 1 and Set 2, and Set 2 and Set 3, indicate that these variations could be significant in certain sectors [1,23].

Limitation(s)

The present study has several limitations. Firstly, as an in-vitro investigation, it does not replicate the complexity of the oral environment, including factors such as plaque, bacterial biofilms, and saliva, which may influence the staining. Secondly, the cleaning regimen in this study involved rinsing aligners only once every 24 hours, which does not fully reflect patient hygiene practices, such as regular brushing or the use of cleaning agents, potentially affecting colour stability outcomes. Thirdly, the study did not evaluate the effects of thermal fluctuations caused by hot and cold food or beverages, which are common in the oral cavity and may impact aligner materials. Lastly, the findings are based on a single type of aligner material (SmartTrack), limiting the generalisability of the results to other aligner systems with different compositions.

CONCLUSION(S)

In conclusion, the study highlights the detrimental effects of both carbonated beverages and specific mouthwashes on the colour stability of clear aligners. To preserve aligner aesthetics throughout treatment, clinicians should proactively educate patients about these risks and provide customised guidance. Statistical analysis indicates that darker shades are associated with lower L^* values and higher optical density, whereas lighter shades are associated the lower optical density values. Set 2 was observed to be the darkest and Set 3 the lightest, with notable variations in mean L^* values demonstrating the impact of optical density on colour perception. These differences are further quantified by the ΔE calculations, which show noticeable variations that are essential for applications that need exact colour matching. Overall, these results highlight the importance of using optical density measurements in colour analysis and offer useful information to sectors concerned with product development and quality assurance. Future studies could evaluate the long-term effects of dietary practices on orthodontic treatment outcomes and investigate developments in aligner material technology to improve stain resistance.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Jan 22, 2025
- Manual Googling: Sep 27, 2025
- iThenticate Software: Sep 29, 2025 (4%)

ETYMOLOGY: Author Origin

EMENDATIONS: 8

AUTHOR DECLARATION:

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? No
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: Jan 21, 2025

Date of Peer Review: Mar 24, 2025

Date of Acceptance: Oct 01, 2025

Date of Publishing: May 01, 2026