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Article

The Development of a Menthol Solution for Use during Sport and Exercise

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Abstract: Menthol mouth-swilling has been shown to improve performance across differing exercise modalities, yet no work has been conducted to ascertain the preferred concentration of menthol within a swill. Colour has also been shown to influence psychophysiological outcomes, and may influence the efficacy of ergogenic aids. We conducted two experiments: one to ascertain preferred menthol concentration (0.005–0.105% menthol), the second to assess colour preference (Light Blue, Dark Blue, Light Green, Dark Green, Red). Participants rated swills for Smell, Taste, Freshness, Mouth Feel and Irritation (plus Appearance in the second trial) via 15 cm Visual Analogue Scales (VAS), having swilled and expectorated 25 mL of fluid. Both trials employed a crossover design, with tasting order assigned by Latin squares. Differences were assessed for statistical significance ($p < 0.05$) using one-way repeated measures ANOVAs. Standardised mean differences $\pm 90\%$ confidence intervals were calculated to assess the magnitude of any observed differences. No significant differences were found between concentrations for total VAS score, but higher concentrations demonstrated a greater number of small effects. Similarly, no significant differences between colours were found. Small effects were found when Light Green was compared to Dark Green and Red. Effects were trivial when Light Green was compared to Light Blue (0.05 ± 0.20) and Dark Blue (0.19 ± 0.32). We recommend athletes employ a Light Green or Light Blue 0.1% menthol mouth-swill.

Keywords: menthol; exercise; ergogenic aid; mouth swilling

1. Introduction

Mouth-swilling strategies may be useful during exercise to alleviate ‘dry mouth’ brought about by a reduction in salivary flow rate [1]. Other ergogenic effects are likely dependent upon the exercise mode undertaken [2–5] and active ingredients within the swill, e.g., Caffeine [2,6], Carbohydrate [7,8] or Menthol [3]. These ingredients may also be combined with other ergogenic strategies to maximise the influence upon physiological and psychological determinants of fatigue [9–11].

Menthol presents in nature as both a fragrance and flavour molecule, targeting the olfactory and gustatory systems [12,13], typically imparting feelings of coolness and freshness [12–14], hence its seemingly ubiquitous use in consumable products such as confectionary, cosmetics and pharmaceutical applications. A more contemporary application of menthol has been that of an ergogenic aid which can be applied topically [15], used as a mouth swill [16–18] or ingested alongside ice slurry [9,10,19]. This use is fitting, with menthol shown to increase the drive to breathe [20], elevate ventilation [21] and attenuate thirst [13], along with eliciting sensations of coolness and freshness that may alleviate

thermal symptoms during exercise [3]. However, the variability of concentration of menthol within mouth swills and other menthol containing strategies applied to the oral cavity is large. Given such variability, the potential for menthol concentration to affect the efficacy of a treatment, palatability of the menthol solution and any resultant physiological or subjective effects brought about by menthol use is viable. Therefore, an optimal or preferred concentration should be explored.

Similar to concentration, colour has been shown to influence the efficacy of a treatment. For example, studies assessing medical interventions [22], product design [23], solution odour [24] and fictitious sport supplements [25] have reported that the colour of the treatment can significantly influence psychophysiological outcomes. The colour green has been associated with coolness [23,24], tranquilising effects [22] and enhanced endurance performance [25], with blue displaying similar qualities [22–24]. Conversely, red and orange are renowned for stimulatory and warming effects [22,23] but have been shown to decrease motor performance [26]. Such responses are thought to be conditioned through previous experience of colour-associated treatments [22,23], suggesting that previous experience with a coloured product or intervention, may influence participants expectation about the efficacy of that treatment [22,25].

Menthol's novel properties, when coupled with the potential for colour to enhance perceptions of coolness and treatment efficacy, suggest that the development of a menthol solution for experimental application is a process that requires consideration, beyond that of palatability. Therefore, the aims of this study were twofold: (1) to ascertain the preferred concentration of a menthol solution, and (2) to identify preferred colour of a menthol solution. To achieve this, we conducted two separate experiments.

2. Materials and Methods

Two repeated measures, post-only crossover design studies were conducted. In study 1, twenty-one participants (15 male, 6 female, 26.9 ± 5.7 years) were recruited to understand the preferred concentration of menthol. In study 2, thirty-five participants (13 males, 22 females, 22.7 ± 5.7 years) were recruited to identify the preferred colour of a menthol solution. Both studies took place in laboratories at 22 ± 0.3 °C.

Participants in both experiments were excluded if they had any illness that affected their ability to taste or smell, they had anosmia (loss of smell), ageusia (loss of taste), or if they had recently suffered any stomach illnesses such as food poisoning or diarrhoea. Participants were also excluded if they were colour blind. Ethical approval was granted by the School of Social Sciences, Humanities and Law Ethics Committee at Teesside University.

In study 1, menthol crystals ((-)-menthol, Sigma Aldrich, Dorset, UK) were dissolved in ethanol to produce a 5% menthol solution (i.e., 50 g menthol per Litre of ethanol). Ethanol was used as a solvent to ensure thorough dilution of menthol throughout the solution, avoiding a film forming or any clumping of partially dissolved menthol crystals. The ethanol-menthol solution was then diluted to the experimental concentrations, using distilled water. Experimental concentrations ranged from 0.005 to 0.105%, in 0.01% increments. All solutions were colourless/transparent. Participants swilled 25 mL of menthol solution for 10 s [16]. They were then asked to rate the solution for *Smell, Taste, Mouth Feel, Freshness & Irritation*, using 15 cm Visual Analogue Scales (VAS). VAS were marked with polarised descriptors 'Unpleasant' and 'Pleasant' at the left and rightmost extremes of each scale, respectively. This process was repeated for each menthol concentration, with tasting order being assigned via an 11×11 Latin Square, through a custom-made spreadsheet. Water was available ad libitum between tastings. Coffee beans were made available to participants between trials. Inhaling the aroma from the beans provided a contrasting aromatic and olfactory stimulus, with a view to minimising cumulative sensory interference across menthol trials.

In study 2, coloured versions (light blue, dark blue, light green, dark green and red) of the preferred menthol solution identified in study 1 were tasted to assess the effect of colour on participants' perception of solution characteristics. Dark blue and green colours were achieved by adding 2 mL of food colouring (Queen Fine Foods Ltd., Alderley, Queensland, Australia) to solution, whereas light blue and green were produced by adding 0.5 mL of food colouring. The red solution contained 1 mL of food colouring to be independent of green and blue coloured solutions. Participants repeated the VAS as described in study 1, but in addition, were asked to rate the solutions' Appearance. Tasting order was assigned via a 5×5 Latin Square, through a custom-made spreadsheet.

Total VAS score (mean \pm standard deviation) per solution was calculated as the sum of the mean scores for each assessed variable, for each concentration and colour. One-way, repeated measures ANOVAs were used to assess the difference in total solution score, between solution concentrations and colour in study 1 and 2, respectively. Checks for normality and variance of the residuals were performed. All analyses were performed using SPSS (v23, IBM, New York, NY, USA). Effect sizes were calculated as standardised mean differences and 90% Confidence Intervals (C.I.) using a customised spreadsheet [27], with accompanying descriptors [28]. Effect Size thresholds are *Trivial* (>0.20) *Small* (0.2–0.6) *Medium* (0.6–1.2) *Large* (1.2–2.0) *Very Large* (>2.0) as per Hopkins and colleagues [28]. Ninety percent (90%) C.I. are used to differentiate between any observed significant results, and the likely range in which true differences may occur [28,29], rather than as another method of expressing a significant result.

3. Results

3.1. Solution Concentration

Mauchly's test indicated that sphericity had been violated, $\chi^2(54) = 94.11$, $p = 0.001$; therefore, a Greenhouse-Geisser ($\epsilon = 0.470$) correction was applied. There were no significant main differences between menthol mouth swill concentrations, $F_{(4.695, 93.903)} = 0.974$, $p = 0.435$. Standardised mean differences are presented in Table 1. Menthol concentrations of 0.095% and 0.105% demonstrated a greater number of Small effects than other concentrations; specifically, demonstrating Small effects with confidence intervals that did not overlap zero, and values for subjective overall perception of 389 ± 94.73 and 383.14 ± 107.22 , respectively (Figure 1; Panel A). Consequently, a 0.10% solution was used in the colour trial.

3.2. Solution Colour

Mauchly's test indicated that sphericity had been violated, $\chi^2(9) = 24.08$, $p = 0.004$; therefore, a Greenhouse-Geisser ($\epsilon = 0.755$) correction was applied. No significant differences were observed between mouth swill colours, $F_{(3.019, 11211.266)} = 0.835$, $p = 0.479$. Light Green was rated more highly than other solutions (Figure 2), and demonstrated Small differences against Dark Green ($0.28 \pm 90\%$ CI: 0.33) and Red (0.24 ± 0.31) but was only trivially different to Dark Blue (0.19 ± 0.32) and Light Blue (0.05 ± 0.20) solutions. Light Blue displayed a Small difference when compared to Dark Green (0.23 ± 0.38), with all other differences considered Trivial (Dark Blue: 0.14 ± 0.36 ; Red: 0.19 ± 0.32).

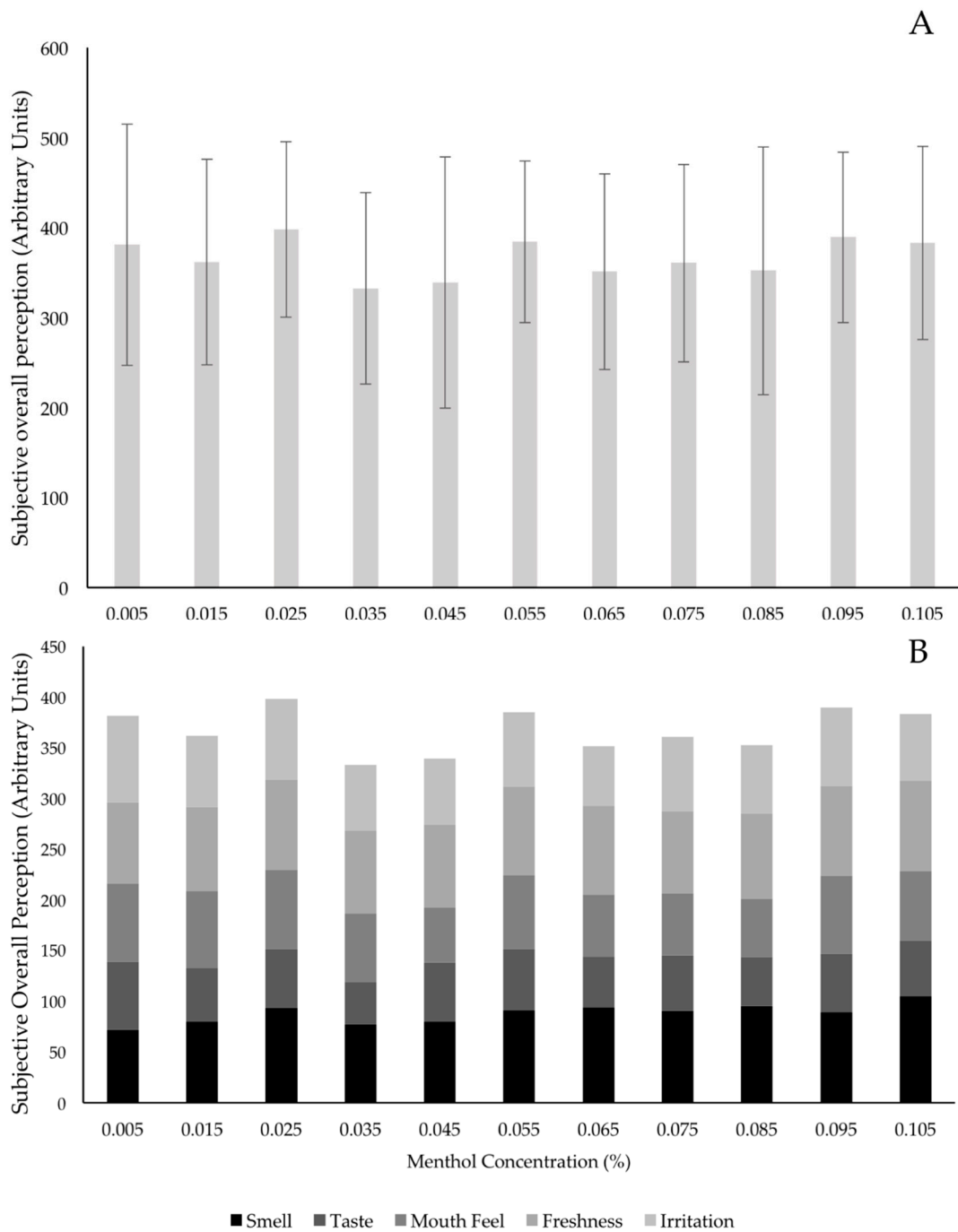


Figure 1. Subjective overall preference for each menthol concentration (%), expressed as Mean VAS rating per solution concentration \pm 1 S.D (**Panel A**) and as a sum of constituent mean VAS for each reported characteristic (**Panel B**).

Table 1. Effect Sizes and accompanying 90% Confidence Intervals for solution concentrations. *Small* effects with confidence intervals not overlapping zero are marked with an asterisk (*).

| | 0.005 | 0.015 | 0.025 | 0.035 | 0.045 | 0.055 | 0.065 | 0.075 | 0.085 | 0.095 | 0.105 | |
|---|-------|-------------|-------------|-------------|-------------|---------------|-------------|-------------|---------------|---------------|---------------|--------------|
| x | | 0.27 ± 0.56 | 0.03 ± 0.59 | 0.52 ± 0.59 | 0.49 ± 0.54 | 0.16 ± 0.48 | 0.44 ± 0.50 | 0.29 ± 0.58 | 0.41 ± 0.35 * | 0.09 ± 0.48 | 0.20 ± 0.55 | 0.005 |
| - | | x | 0.29 ± 0.32 | 0.24 ± 0.24 | 0.19 ± 0.26 | 0.20 ± 0.39 | 0.09 ± 0.37 | 0.01 ± 0.39 | 0.08 ± 0.51 | 0.23 ± 0.48 | 0.17 ± 0.44 | 0.015 |
| - | | - | x | 0.24 ± 0.25 | 0.19 ± 0.27 | 0.19 ± 0.36 | 0.08 ± 0.31 | 0.01 ± 0.35 | 0.08 ± 0.49 | 0.24 ± 0.52 | 0.19 ± 0.47 | 0.025 |
| - | | - | - | x | 0.05 ± 0.37 | 0.44 ± 0.36 * | 0.16 ± 0.35 | 0.25 ± 0.32 | 0.41 ± 0.39 * | 0.50 ± 0.48 * | 0.42 ± 0.35 * | 0.035 |
| - | | - | - | - | x | 0.38 ± 0.41 | 0.10 ± 0.46 | 0.18 ± 0.50 | 0.10 ± 0.45 | 0.39 ± 0.53 | 0.34 ± 0.53 | 0.045 |
| - | | - | - | - | - | x | 0.28 ± 0.39 | 0.20 ± 0.34 | 0.27 ± 0.46 | 0.04 ± 0.39 | 0.02 ± 0.44 | 0.055 |
| - | | - | - | - | - | - | x | 0.08 ± 0.30 | 0.01 ± 0.53 | 0.31 ± 0.43 | 0.26 ± 0.33 | 0.065 |
| - | | - | - | - | - | - | - | x | 0.18 ± 0.51 | 0.25 ± 0.41 | 0.20 ± 0.33 | 0.075 |
| - | | - | - | - | - | - | - | - | x | 0.13 ± 0.41 | 0.08 ± 0.56 | 0.085 |
| - | | - | - | - | - | - | - | - | - | x | 0.06 ± 0.44 | 0.095 |
| - | | - | - | - | - | - | - | - | - | - | x | 0.105 |

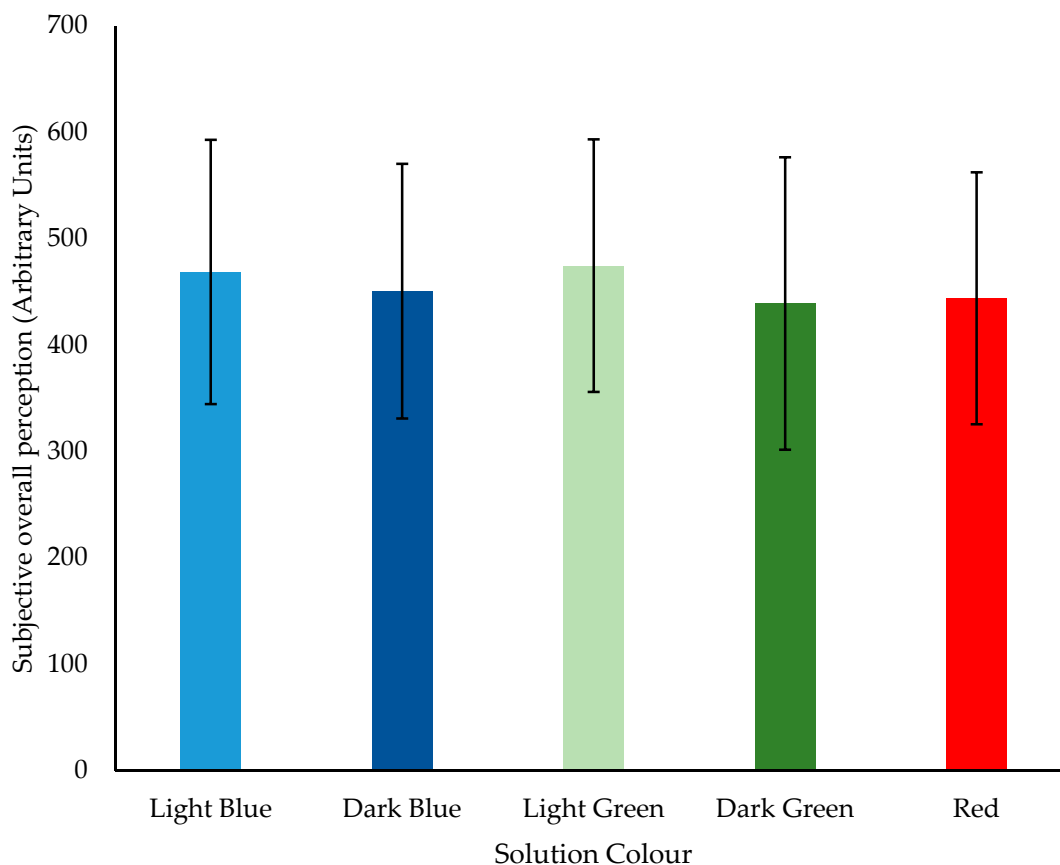


Figure 2. Subjective overall perception (Arbitrary Units) for solution colour, expressed as Mean VAS rating per solution colour \pm 1 S.D.

4. Discussion

Menthol mouth swilling is considered a practical ergogenic strategy before and during exercise in hot environments [11,30] and has been used to improve performance during time trials [17,18] and time to exhaustion [16,31]. Perception of menthol mouth swill concentration may be highly individual in nature, with 200 gene variants of the receptor responsible for menthol detection (TRPM8 [32]). This may in part explain the broad standard deviations and confidence intervals overlapping zero in our results.

Perception may be dependent upon physiological factors such as trigeminal chemosensitivity [33,34] and stratum corneum thickness [35], or environmental influences such as previous or habitual exposure to trigeminal agonists [36,37], e.g., regular use of mentholated products. Habitual menthol use may alter the threshold at which TRPM8 channels and the trigeminal nerve are stimulated [37–40], ultimately habituating thermal sensation [37,38]. Despite no statistically significant differences in the present investigation, menthol concentrations of 0.095–0.105% may lead to small increases in trigeminal stimulation, concomitantly conferring benefits such as an increase in ventilation [3,16], reduced thermal sensation [18] and thirst [14], and improved thermal comfort [9] when exposed to the oral cavity.

The possibility that repeated menthol exposure may confer greater benefits than a single dose of menthol has not been directly explored to date. All menthol-containing studies [9,10,16–19,31] have employed a repeated exposure during the exercise bout—this is quantified via time or distance. Better understanding the time course of menthol mouth swilling responses, and the potential impact of concentrations upon these, would provide insight into possible limitations in application for menthol-containing strategies.

The highest rated solution colour in this investigation (Light Green; 474.40 ± 118.68 Arbitrary Units). The Light Blue solution was also rated highly, with trivial effects reported between Light Green and Light Blue (468.49 ± 124.15 Arbitrary Units; 0.05 ± 0.20 Trivial). Menthol-containing products such as mouth-wash, confectionary and other oral hygiene products are associated with these colours [41,42], and subjective qualities of solution may be enhanced due to this association [23,24,43]. In the absence of significant results between solution colours, researchers may consider using a light green or light blue coloured solution as a starting point for future menthol research.

The perception of colour and concentration may be influenced via the environment in which the solution is administered. The present study was conducted in an ambient temperature laboratory (22 ± 0.3 °C), which may have enhanced the subjective qualities of the menthol solution(s). For example, blue and green are typically associated with cooling [23,24], participants may have therefore perceived these colours as more refreshing than red, which is associated with warming [22,23]. Future research should aim to understand the perception of concentration and colour under differing environmental conditions; especially those conditions that are below 8 °C or exceed 28 °C. These temperatures represent threshold values for the menthol receptor TRMP8 [44], and the human thermoneutral zone (≥ 28 °C [11,45,46]). Investigations at the upper limit of, or exceeding this range are greater than the temperature at which the rate of metabolic heat production exceeds the rate of thermal transfer to the environment (25 °C [47]). This provides an important platform from which to study the perceptual and physiological responses to menthol mouth swilling during exercise. The mode (e.g., running or cycling [48,49]) and nature (continuous or interval; [50]) of exercise are also important experimental concerns, due to differences in heat production, heat storage, and hyperthermia risk [49].

Practically, the effect of colour extends beyond the aesthetic qualities of a solution or treatment; treatment colour may impart emotional modifications that could be tailored to an athlete's psychological profile. Red is typically associated with high arousal states [51,52], anger [53] and danger [54]; red has also been associated with Tae Kwondo match outcome [55]. Conversely, blue and green are perceived as calming [22], only slightly arousing [52], and in congruence with our findings have been shown to be perceived as more pleasant than red [52]. Recently, a green inert drink was used to facilitate an induced beliefs investigation into sprint performance [56]. The colour green was chosen specifically in this investigation due to the potency of belief around green substances' abilities to enhance performance [25,56]. Such expectancy cannot be ignored in our investigation, or the practical application(s) of its findings. Furthermore, perceptions and preferences of colour, vary between individuals, within groups and across cultures [57]. Colours can be interpreted as having opposing meanings in different countries and cultures [58], but in multi-cultural individuals have been reported to be interpreted intermediately [59], careful consideration of cultural perceptions would further enhance the implementation of our findings.

5. Conclusions

Based upon the results of our study, we recommend athletes and practitioners work together to ascertain a menthol concentration for mouth swilling. This concentration would ideally be based upon an individual's perception of the characteristics assessed in this work, and their competitive and training environment(s). Similarly, for practitioners, we advise using a light blue or green solution as a starting point for further investigation, given the synonymy with menthol containing products, but acknowledge that other cultural factors may influence this decision.

Author Contributions: R.B., N.J.A.B. and I.R.S. conceived and designed the experiments; R.B. performed the experiments; R.B. and N.J.A.B. analysed the data; R.B., N.J.A.B., I.R.S. and P.H. wrote the paper.

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