RESEARCH ARTICLE

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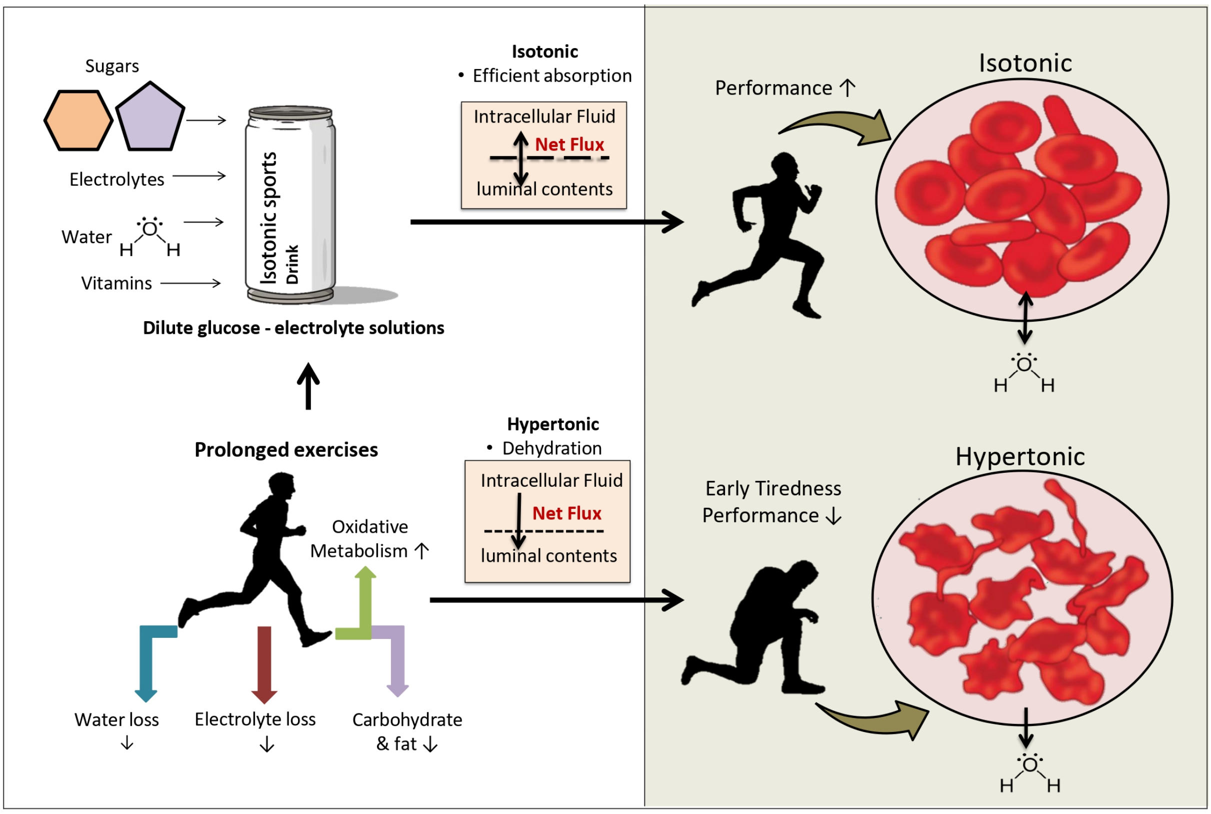

Highlights

- Adhere to European Food Safety Authority regulations for carbohydrate-electrolyte solutions.
- The developed formulas have the osmolarities of 284±1, 317±1, 305±1 (mOsmol/kg).
- Sodium contents of the formulas are 723.7±14.8: 715.5±43.5, 633.3±11.6: 740.0±0 (mg/L).

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RESEARCH ARTICLE

Development of Two Types of Isotonic Beverages with Functional Attributes Using Natural and Synthetic Ingredients

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Abstract: Isotonic drinks maintain a balance of salt and sugar concentrations similar to that of the human body, aiding in the rapid replacement of lost fluids during dehydration. Although the availability of isotonic beverages in the Sri Lankan market is limited, this segment is experiencing rapid growth on the global stage, with numerous brands marketing their products. Moreover, it is especially advantageous to create isotonic beverages tailored to tropical country consumers, given their ability to effectively replenish lost fluids due to dehydration. This study seeks to develop economically viable and commercially feasible formulations of isotonic drinks that cater to the preferences of Sri Lankan consumers, utilizing a combination of synthetic and natural ingredients. In the realm of natural isotonic beverages, coconut water and bee honey serve as excellent sources of electrolytes and sugar, respectively. Following osmolality and sensory evaluations, the chosen samples underwent further analysis to determine their mineral content and physicochemical characteristics. These developed formulations fall within the prescribed ranges for osmolality (270-330 mOsm/kg) and sodium content (460-1150 mg. L^{-1}), aligning with international standards for isotonic beverages. In conclusion, the devised formulas successfully adhere to international specifications for isotonic beverages. The abundant potential of coconut water and bee honey in Sri Lanka with wide availability as valuable sources of minerals and energy for crafting isotonic drinks is evident.

***Keywords*:** Dehydration; Electrolytes; Energy; Osmolality; Sugars

INTRODUCTION

Isotonic beverages, categorized as functional drinks, serve to aid in the recovery of strength for individuals engaged in intense physical activities (Moreno et al., 2013; Orrù et al., 2018; Styburski et al., 2020). Especially in cases of vigorous, prolonged exertion or under tropical conditions, the body's rapid loss of water, electrolytes through sweat, and heightened energy consumption can lead to lowered blood sugar and glycogen levels (Brouns & Kovacs, 1997; Jeukendrup, 2007; Sadowska et al., 2020). To counteract these conditions and prevent performance impairment and injury, swift replacement of lost fluids and essential nutrients, in the form of isotonic beverages, is recommended (Jeukendrup, 2007).

Isotonic beverages possess an osmolality similar to body fluids (270-330 mOsm/kg), facilitating quick absorption, making them ideal for consumption during extended physical activities (Jeukendrup, 2007; Mosler et al., 2020). They typically contain simple ingredients of carbohydrates, minerals, and water (Mosler et al., 2020), and according to European Food Safety Authority regulations, should have specific values for osmolality, sodium content, and carbohydrate proportions (EFSA, 2011; Huang et al., 2020).

In recent years, isotonic beverages have evolved with specialized functions and gained attention in the global and local markets (Girone´s-Vilaplana et al., 2013). However, despite this growth, the local Sri Lankan market still faces an unmet demand for isotonic beverages, possibly due to the tropical climate. To address this gap, the study proposes two isotonic beverage formulations: synthetic and natural. The synthetic formula offers a local alternative, while the natural formula uses readily available ingredients like coconut water and bee honey, aligning with the trend towards natural products.

Both formulations adhere to quality standards and recommendations set by Sri Lanka Standard Institute (SLSI) and Codex Alimentarius (Codex Alimentarius international standards 2016) for additive (preservatives, sweeteners etc.) limits.

MATERIALS AND METHODS

Investigating Fundamental Formulation and Osmolality Adjustment

The research comprised two distinct phases: an initial trial followed by a conclusive assessment, both encompassing synthetic and natural formulas. All the ingredients selected for this study were based on the requirements of the SOZO beverage company Sri Lanka, which funded this research.

Exploring synthetic formulations

In the preliminary stage of the synthetic formula (SF) analysis, diverse sample formulations were meticulously prepared by employing alternative carbohydrate sources (Table 1). These sources included glucose syrup and maltodextrin, each tested at two distinct salt concentration

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levels. Osmolality was initially evaluated using a freeze depression osmometer (Model 3320, Advanced® Micro-Osmometer, USA) for a selected sample. Subsequently, various combinations of carbohydrate sources were explored, striving to align with the upper limit concentrations of salts as recommended by Brouns & Kovacs (1997) at final trial (02). The range of glucose concentrations were extended upon it being a monosaccharide (Table 1). As depicted in Table 2, the optimal ingredient composition that yielded the desired osmolality was then identified and carried forward to the final trial (trial 02) of the SF.

From the pool of identified formulations, three primary samples were chosen based on their osmolality falling within the range outlined by EFSA (2011), signifying their potential to serve as isotonic beverages. These final samples underwent sensory analysis to ascertain their overall sensory attributes and qualities. Through rigorous sensory evaluation, the ultimate SF was refined and established.

Exploring Natural Formulations

The study ventured into two distinct natural formula variants, each presenting its own unique characteristics and potential benefits. These formulations were categorized as the "natural formula with bee honey" and the "natural formula with artificial sweetener."

For the formulation of the "natural formula with bee honey (NBH)", a thoughtful approach was taken, utilizing coconut water as the foundational mineral source (Table 3). This approach was supported by previous research (Khan et al., 2003; Kailaku et al., 2015; Prades et al., 2012; Pratama et al., 2022). Primarily, three combinations of coconut water with deionized water were prepared at consistent levels of flavors, salts, bee honey, preservatives (Potassium Sorbate), and coloring agents (Table 3). Industrially developed natural extracts of watermelon and coconut were utilized as flavoring agents, and hibiscus served as a natural colorant

Table 1: Different carbohydrate sources at various concentrations used for sample preparation in the preliminary trial (01) and final trial (02).

Trial 01				Trial 02					
Carbohydrate source (Glucose syrup and Maltodextrine)				Carbohydrate source (Glucose, Glucose syrup: Glucose in (1:1) and (1:2))					
Glucose syrup		Maltodextrine		Glucose*		Glucose Syrup: Glucose (1:1)		Glucose Syrup: Glucose (1:2)	
Sample	Conc.(g/L)	Sample	Conc.(g/L)	Sample	Conc. (g/L)	Sample	Conc. (g/L)	Sample	Conc. (g/L)
GS60	60	M60	60	G40	40	GS:G40	40	GS:2G40	40
GS70	70	M70	70	G45	45	GS:G50	50	GS:2G50	50
GS80	80	M80	80	G50	50	GS:G60	60	GS:2G60	60
GS90	90	M90	90	G60	60	GS:G70	70	GS:2G70	70
				G70	70	GS:G80	80	GS:2G80	80
				G80	80	GS:G90	90	GS:2G90	90
				G90	90				

* Considering glucose is a monosaccharide, its concentration range has been expanded, conc. = concentration.

Table 2: Basic ingredients for the synthetic formula used in the final trial.

Basic ingredients*	Amount ($mg.L^{-1}$)
Concentration of electrolytes	
Sodium Chloride	900
Calcium Chloride	225
Magnesium Sulphate	100
Potassium Sorbate	200
Ascorbic Acid	200
B vitamin Premix	35
Brilliant Blue FCF Colorant (EFSA, 2010)	40
Citric Acid (EFSA, 2015)	800
Mix Fruit Flavor (Pineapple+ Mango)	200
Sucralose	75

* Ingredients are indicated as in the final formulas for the sensory evaluation, carbohydrate source concentration selected from the trials 01 and 02 not mentioned

derived from the dried flower corolla as described by Singh et al. (2018). Natural coconut flavor was employed to overcome the cooked flavor upon the heating process for preservation of the beverage. As a repercussion to the initial three samples' (Table 3) osmolality test findings, bee honey, renowned for its nutritional qualities, was incorporated alongside a carefully determined proportion of glucose syrup, which served as the primary carbohydrate source in Table 4 (at various concentrations of coconut water). This intricate balance was guided by insights from Bogdanov et al. (1996) & Ali et al. (2021).

Considering the osmolality of derived samples in Table 4, the final formulas of NBH (trial 02) was identified by altering the carbohydrate source concentrations, specifically impartment of glucose syrup at the selected ratio levels of coconut water: deionized water from trial 01 (Table 4). Except for altered carbohydrate source concentrations (bee honey: glucose syrup), coconut water and deionized water concentrations in Table 5, the concentrations of other ingredients are similar to those in Table 3.

Similarly, the "natural formula with artificial sweetener (NAS)" also relied on coconut water as the fundamental mineral source, accompanied by the inclusion of glucose syrup to fulfill the carbohydrate component. As shown in Table 6, combinations of coconut water dilutions with 40 g/L of glucose syrup were prepared. These preliminary formulations underwent osmolality testing, aiding in the selection of specific treatments for subsequent sensory evaluations. Table 7 presents the basic ingredients for the NAS, which were used to prepare samples for the final trial.

Table 3: Ingredients for NBH, using bee honey as the carbohydrate source in the initial trial (01).

Sample	Coconut water (mL)	Deionized Water (mL)	Natural watermelon flavor (g/L)	Natural coconut flavor (mg)	Sodium chloride (1 mmol/L =58.5mg/L)	Bee honey (g/L)	Potassium Sorbate (mg/L)	Dried Hibiscus (mg/L)
BH(50)	50	50	0.05	0.15	700	40	200	0.75
BH(37.5)	37.5	62.5	0.05	0.15	700	40	200	0.75
BH(25)	25	75	0.05	0.15	700	40	200	0.75

Only the carbohydrate source, coconut water, and deionized water concentrations were modified in the subsequent configurations in Tables 4 and 5, BH= Bee honey incorporated sample.

Table 4: Series of coconut water concentrations, after carbohydrate replacement, bee honey: glucose syrup (1:1) in formulation of NBH.

Samples, Bee honey + Glucose Syrup (1 : 1) as the carbohydrate source	Coconut water (%)	Deionized water
BH:GS(50)	50.0	50.0
BH:GS(37.5)	37.5	62.5
BH:GS(30)	30.0	70.0
BH:GS(25)	25.0	75.0
BH:GS(20)	20.0	80.0

BH:GS= Bee honey and glucose syrup incorporated sample.

Table 5: Combinations of coconut water dilutions with varying amounts of glucose syrup in final trial (02) of NBH.

Sample		Bee Honey (g/L) of drink*	Glucose Syrup (g/L) of drink
BH:GS(1)	Coconut water: Deionized water (30: 70)	25	10
BH:GS(2)		25	15
BH:GS(3)		25	20
BH:GS(4)		25	25
BH:GS(5)	Coconut water: Deionized water (25: 75)	25	10
BH:GS(6)		25	15
BH:GS(7)		25	20
BH:GS(8)		25	25

* Bee honey concentration was kept stable, BH:GS= Bee honey and glucose syrup incorporated sample.

Table 6: Combinations of coconut water dilutions in deionized water with artificial sweeteners.

Sample	Coconut water (mL)	Deionized water (mL)
NAS1	50.0	50.0
NAS2	40.0	60.0
NAS3	37.5	62.5
NAS4	25.5	75.0

NAS= Natural formula with artificial sweetener (sucralose).

Table 7: Basic ingredients for the natural formula with artificial sweetener in the final trial.

Basic ingredients	Amount ($mg.L^{-1}$)
Sodium Chloride	700
Glucose Syrup	0.040
Preservative Agent (Potassium Sorbate)	200
Dried Hibiscus Corolla	750
Sucralose	75
Natural Watermelon Flavor	0.05
Natural Coconut Flavor	0.15

Through a meticulous process, three distinct samples were identified for each of the two natural formulas, paving the way for comprehensive sensory analysis. The outcomes of this sensory assessment led to the identification of a single, optimal sample for each natural formula, solidifying their respective formulations.

Preservation Technology

The primary preservation method utilized was pasteurization, involving the treatment of the beverage at 85°C for a duration of 20 minutes. After pasteurizing the beverage, it was filled to pre-sterilized glass container bottles followed by a 30 minute in bottle heating and capping tightly after the process.

Conducting Comprehensive Sensory Assessment

Sensory analysis represented a pivotal aspect of the research, focusing on the ultimate iterations of each formula: the SF, the NBH, and the NAS.

A pre-training was conducted for the panel to investigate the flavor profile of the prepared and served beverages. The beverages were only different among each other in the flavor enhancing agent concentration (Sugar source or coconut water). All other constituents (Color agent (Hibiscus or brilliant blue concentration), flavor additive concentration (artificial sweetener in NAS) etc. were similar in compared formulas. No comparison was made among SF, NBH, and NAS beverages.

Employing a Hedonic scale approach, a form of affective testing involving a 5-point scale as outlined by Lim (2011), the objective was to gauge consumer preference for the selected samples during the conclusive sensory evaluation. This investigation involved the participation of a panel consisting of 30 untrained individuals, all entrusted with the task of sensory evaluation.

To ensure controlled and consistent conditions, the assessment took place within individual booths equipped with regulated illumination and temperature settings. The samples, presented in uniform 50 mL glass cups, maintained a standardized appearance. The evaluation encompassed a range of parameters including color, taste, odor, appearance, and overall acceptability.

Evaluation scores were assigned to the novel beverage products, with each parameter assessed on a scale ranging from 1 to 5. This scale corresponded to different levels of preference: 5 denoted a like very much, 4 indicated like moderately, 3 represented a neutral stance, 2 reflected moderate disapproval, and 1 indicated a significant disliking.

Unveiling Essential Mineral Composition

A pivotal aspect of this investigation entailed an in-depth analysis of mineral content within the conclusive samples, encompassing the SF, the NBH, and the NAS. The elemental constituents under scrutiny included Sodium, Potassium, Calcium, and Magnesium.

This mineral content analysis was facilitated through the utilization of advanced technology, specifically the Atomic Absorption Spectrophotometer (Agilent Technologies/ GTA 120, Graphite Tube Atomizer, Germany). This sophisticated instrument enabled the precise quantification of Sodium, Calcium, and Magnesium levels present within the samples.

Furthermore, the specific measurement of Potassium content was achieved through the employment of the Flame Emission Spectrophotometer (JENWAY PFP7, UK), which added a layer of accuracy and specificity to this analysis.

Exploring Physicochemical Attributes

The final phase of this study encompassed a comprehensive evaluation of the physicochemical properties, serving as quality benchmarks for the ultimate formulations: the SF, the NBH, and the NAS.

To unravel the intricate characteristics of these beverage variants, a multi-faceted approach was undertaken. The pH, Titratable Acidity (TA), Total Soluble Solids (TSS), and color attributes were meticulously assessed, providing essential insights into the overall product quality.

The pH determination, a key indicator of acidity or alkalinity, was meticulously executed using a pH meter (Model; PH100, E*xstik*® pH Meter, USA). This process involved continuous measurement for a phase until a consistent pH value was established, ensuring precision.

Total soluble solids (TSS), a crucial gauge of dissolved substances, were quantified through the employment of a refractometer (Model; N-1E, ATAGO, Japan). Meanwhile, titratable acidity, indicative of the acid content, was determined by titration using 0.1N NaOH, with phenolphthalein employed as an indicator.

Additionally, the evaluation of color attributes was executed using a platinum cobolt colorimeter (Model; LFS- 2002 (SD), LIHERO, China), shedding light on the visual characteristics of the final products.

The quantification of total soluble solids (TSS) was expressed in Brix, offering a measure of sugar content, while titratable acidity (TA) was presented in grams per liter (g/L), accentuating the acidity level.

Statistical Analysis

All the data was analyzed using the SPSS software. An initial Friedman test, designed for nonparametric data (Sensory data), was conducted to determine the significant differences between samples. Tests were performed on the samples using data scores from sensory evaluations and other relevant parameters.

RESULTS AND DISCUSSION

Comparison of the osmolality of formulations created using various carbohydrate sources.

Synthetic formula (SF)

Osmolality refers to the number of moles of dissolved particles (charged or uncharged solutes) per kilogram of solution (Sanders, 2009; Fan & Bai, 2020; Truscott, 2020). Given that beverages with low osmolality enhance water absorption rates (Hunt et al., 1992) and the gut can sense fluid osmolality (Leiper, 2015), studying osmolality plays a crucial role in developing isotonic beverages (Sadowska et al., 2017). Generally, Blood serum osmolality ranges from 280 – 310 mOsm/kg (Thrall et al*.,* 2013), and liquids with osmolarities in that range are rapidly absorbed. Considering the source of the carbohydrates, as the degree of polymerization increases, the osmolality of a fluid decreases due to a reduction in the moles of solutes. Conversely, when the degree of polymerization decreases, the osmolality of the fluid increases due to the presence of a higher number of moles of smaller molecular weight particles (e.g., glucose, which is a monomer) (Truscott, 2020). Regardless of the source of the carbohydrate, the increase in molar concentration allows for the elaborate explanation of the increase in osmolality with concentration.

The osmolality test results obtained for the samples with different carbohydrate sources were compared with the required osmolality range (Table 8). The initial samples obtained from preliminary trials (only with either glucose syrup or maltodextrin) exhibited osmolality levels lower than the required range (270-330 mOsmol/kg) (Tarancon & Lachenmeier, 2015) for an isotonic sports drink. Therefore, attempts were made to correct this by using higher salt concentrations. However, osmolality test results indicated that even with increased salt concentration, the osmolality did not reach the required range in trial 01 (Brouns & Kovacs, 1997; EFSA, 2011; Ruiz, 2022).

Based on the findings for trial 02 depicted in Table 8, samples falling within the specified osmolality range were chosen as the final selections, featuring optimal ingredient combinations. The utilization of glucose as the carbohydrate source enabled the attainment of the required osmolality at concentrations of 40 g/l and 45 g/l. However, adhering to the European Food Safety Authority's regulations for isotonic beverages necessitates a carbohydrate concentration falling within the 40-80 g/l range (EFSA, 2011). While glucose meets this requirement, it does so at the lower limit, prompting the selection of a sample with a carbohydrate concentration closer to the ideal range for isotonic beverages (EFSA, 2011).

Despite sample G:GS60's osmolality aligning with the required range (Tarancon and Lachenmeier, 2015), its proximity to the lower limit led to its exclusion as a final option. In comparison, sample 2G:GS50 displays osmolality within the specified range. Considering this, both 2G:GS50 (50 g/L) and 2G:GS60 (60 g/L) were chosen as the ultimate samples for sensory analysis and were coded T_{SF1} and T_{SF2} respectively. An additional sample (T_{SF3}) was introduced with a middle concentration of 55 g/L carbohydrate content.

Natural formula with bee honey (NBH)

A natural formula incorporating bee honey was developed through preliminary trials to adjust ingredient levels. As previously given the significant impact of bee honey on osmolality (Ali et al., 2017), a strategic approach involved replacing half of the carbohydrate content with an alternate source, thereby reducing the contribution to osmolality. This substitution was accomplished using glucose syrup. Consequently, a range of samples was created, varying coconut water concentrations and featuring half-carbohydrate replacement with glucose syrup (Table 9).

When bee honey + glucose syrup (1:1) substitution was carried out for the initial trial (Trial 01) samples (with only bee honey as the sole carbohydrate source), the osmolality requirement was practically met when coconut water concentrations reached 30% and 25% (Table 9). Consequently, the final trial (Trial 02) involved crafting samples at 25% and 30% coconut water levels, while varying the glucose syrup concentration at 10 g/L, 15 g/L, 20 g/L, and 25 g/L (Table 10). Based on osmolality analysis outcomes, samples containing 15 g/L [BH:GS(2))], 20 g/L [BH:GS(3)], and 25 g/L [BH:GS(4)] of glucose syrup within the 30% coconut water series were selected as treatments for the sensory analysis (Table 10). These three samples were coded T_{NBH1}, T_{NBH2}, T_{NBH3} respectively in further analysis for sensory perception.

Natural formula with artificial sweetener (NAS)

In the case of the natural formula utilizing an artificial sweetener (sucralose), a range of samples was created by adjusting the coconut water concentration to different levels: 50%, 40%, 37.5%, and 25%. As present in Table 11, osmolarity analysis revealed that samples containing coconut water concentrations of 50%, 40%, and 37.5% fell within the isotonic range. As a result, these three samples (NAS1= T_{NAS1}, NAS2= T_{NAS1}, NAS3= T_{NAS1}) were selected to undergo the final phase of sensory evaluation. This beverage is an isotonic beverage that uses natural sources (carbohydrate and electrolytes) as its primary constituents. It is pitched as containing a natural formula, although it is not entirely natural.

Sensory Analysis

Evaluation of Synthetic Formula (SF)

The final sensory evaluation results for the SF are depicted

Table 8: The osmolality values of samples developed using various carbohydrate sources in both trial 01 and trial 02.

Sample code	Carbohydrate source	Concentration (g/L)	Osmolality (mOsmol/kg)
Trial 01			
GS60	Glucose syrup	60	155 ± 1
GS70		70	168 ± 0
GS80		80	189 ± 1
GS90		90	209 ± 1
M60	Maltodextrin	60	97 ± 0
M70		70	102 ± 2
M80		80	104 ± 0
M90		90	114 ± 1
Trial 02			
G40	Glucose	40	281 ± 1
G45		45	305 ± 4
G50		50	339 ± 4
G60		60	390 ± 0
G70		70	430 ± 4
G80		80	490 ± 10
G90		90	523 ± 9
G:GS 40	Glucose: Glucose syrup (1:1)	40	208 ± 3
G:GS 50		50	245 ± 3
G:GS 60		60	274 ± 6
G:GS 70		70	312 ± 3
G:GS 80		80	339 ± 2
G:GS 90		90	380 ± 3
2G:GS 40	Glucose: Glucose syrup (2:1)	40	219 ± 1
2G:GS 50		50	284 ± 1
2G:GS 60		60	321 ± 9
2G:GS 70		70	427 ± 3
2G:GS 80		80	439 ± 1
2G:GS 90		90	471 ± 4

Results were presented in Mean ± SD of three replicates of osmolality values.

Table 9: Osmolality test results for NBH at varied coconut water concentrations in initial trial (trial 01).

Sample code	Carbohydrate source	Coconut water(%)	Deionized water(%)	Osmolality (mOsmol/kg)
BH(50)	Bee honey	50.0	50.0	494 ± 2
BH(37.5)		37.5	62.5	470 ± 0
BH(25)		25.0	75.0	429 ± 3
BH:GS(50)	Bee honey + Glucose Syrup (1: 1)	50.0	50.0	357 ± 1
BH:GS(37.5)		37.5	62.5	349 ± 1
BH:GS(30)		30.0	70.0	310 ± 0
BH:GS(25)		25.0	75.0	280 ± 0
BH:GS(20)		20.0	80.0	212 ± 3

Results were presented in Mean ± SD of three replicates of osmolality values.

Table 10: Osmolality test results for final trial (trial 02) of NBH at diverse glucose syrup concentrations and two coconut water levels.

Sample Code	Coconut water: Deionized water	Glucose syrup concentration (g.L^{-1})	Osmolality (mOsmols/kg)
BH:GS(1)	30 mL: 70mL	10	286 ± 1
BH:GS(2)		15	293 ± 1
BH:GS(3)		20	295 ± 1
BH:GS(4)		25	305 ± 1
BH:GS(5)	25 mL: 75 mL	10	243 ± 3
BH:GS(6)		15	267 ± 2
BH:GS(7)		20	438 ± 1
BH:GS(8)		25	466 ± 1

Results were presented in Mean ± SD of three replicates of osmolality values.

Table 11: Osmolality test results for NAS at varied coconut water concentrations

Sample Code	Coconut water(%)	Deionized water(%)	Osmolality (mOsmol/kg)
NAS1	50.0	50.0	319 ± 4
NAS2	40.0	60.0	317 ± 1
NAS3	37.5	62.5	312 ± 3
NAS4	25.0	75.0	227 ± 2

Results were presented in Mean ± SD of three replicates of osmolality values.

in Figure 1A. Among all three treatments, no statistically significant differences ($P > 0.05$) were observed in terms of flavor. Despite the lack of significant difference, the treatment containing 50 g/L (T_{SF1}) of glucose syrup emerged as the preferred choice and was consequently chosen as the ultimate synthetic sample. This carbohydrate concentration aligns with the European Food Safety Authority (EFSA) guidelines for energy drinks (EFSA, 2011).

Assessment of Natural Formula with Bee Honey (NBH)

Analysis of flavor preferences revealed a significant difference ($P < 0.05$) between T_{NBH1} (50 g/L) and T_{NBH2} (55 g/L), as well as T_{NBH3} (60 g/L), as illustrated in Figure 1B. Conversely, no significant difference ($P > 0.05$) was detected between T_{NBH2} and T_{NBH3}. Based on the industry standards and input from sensory evaluation panelists, T_{NBH3} was designated as the final sample for this natural formula variant.

Evaluation of Natural Formula with Artificial Sweetener (NAS)

The sensory analysis data for the NAS did not exhibit any significant differences ($P > 0.05$) among the various treatments. After considering expert preferences, T_{NAS2} (depicted in Figure 1C) was identified as the optimal choice for the final product. Consequently, the natural formula containing 37.5% coconut water and 62.5% deionized water (T_{NAS2}) was selected as the ultimate iteration of the formula with artificial sweetener.

Flavor profile was considered in the sensory analysis accounting the compared formulas i.e. (T_{SF1}, T_{SF2}, T_{SF3}), ($T_{NBH1,}$ $T_{NBH2,}$ T_{NBH3}) and ($T_{NAS1,}$ $T_{NAS2,}$ T_{NAS3}) were only different in the flavor induced by sugar source or coconut water concentration i.e. (A: 50 g/L, 55 g/L, 60 g/L of glucose: glucose syrup (2:1)), (B: 15 g/L, 20 g/L, 25 g/L of glucose syrup) and (C: 50%, 40%, 37.5% coconut water) among them. No comparison was made between SF, NBH and NAS beverages.

Analysis of minerals

Synthetic formula (SF)

The Atomic Absorption Spectrophotometer revealed concentrations of Ca, Mg, and Na in the developed isotonic beverage as 122.7 ± 0.1 mg/L, 9.2 ± 0.4 mg/L, and 688.2 ± 17.7 mg/L, respectively. K content was assessed at 77.0 ± 8.5 mg/L using the Flame Emission Spectrophotometer. Compliance with the European Food Safety Authority (EFSA) guidelines was achieved, with the sodium content falling within the range of 460 - 1150 mg/L, as required for sports drinks (Table 12).

Natural formula with bee honey (NBH)

Atomic Absorption Spectrophotometry, the isotonic NBH displayed Ca, Mg, and Na concentrations of 189.9 ± 3.8 mg/L, 37.8 ± 6.9 mg/L, and 715.5 ± 43.5 mg/L, respectively. Flame Emission Spectrophotometer measurements indicated a K content of 740.0 ± 0 mg/L (Table 12).

Natural formula with artificial sweetener (NAS)

In the NAS, Atomic Absorption Spectrophotometer analysis showcased Ca, Mg, and Na concentrations of 213.5 ± 8.0 mg/L, 39.0 ± 5.3 mg/L, and 723.7 ± 14.8 mg/L, respectively. K content was estimated at 633.3 ±

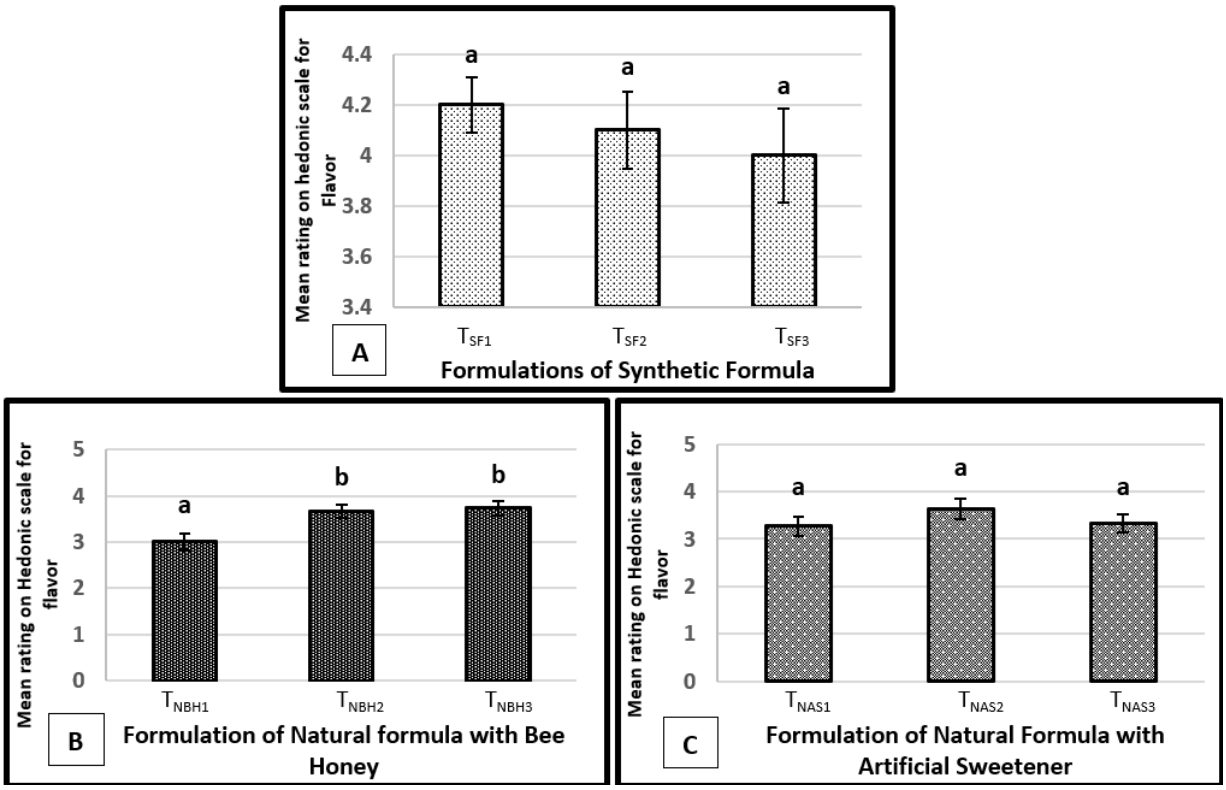

Figure 1: Mean ratings on the hedonic scale for flavor preference of three formulas - A: SF, B: NBH, C: NAS.

Table 12: Analysis Data of Osmolality, Mineral Content, and Physicochemical Characteristics for final selected formulas, synthetic formula (SF), natural formula with bee honey (NBH) and natural formula with artificial sweetener (NAS).

Parameter	SF	NBH	NAS
Osmolality (mOsmol/kg)	284 ± 1	305 ± 1	317 ± 1
Mineral Content (mg/l)			
Sodium	688.2 ± 17.7	715.5 ± 43.5	723.7 ± 14.8
Potassium	77.0 ± 8.5	740.0 ± 0	633.3 ± 11.6
Magnesium	9.2 ± 0.4	37.8 ± 6.9	39.0 ± 5.3
Calcium	122.7 ± 1.0	189.9 ± 3.8	213.5 ± 8.0
Physicochemical Characteristics			
pH	1.6 ± 0.0	2.6 ± 0.0	2.4 ± 0.0
Brix (*B*^{0})	5.1 ± 0.1	5.0 ± 0.1	5.1 ± 0.0
Color (PCU)	130.0 ± 10	356.0 ± 20.8	413.0 ± 15.3
Titratable Acidity (%)	7.9 ± 0.4	6.0 ± 0.0	6.0 ± 0.0

Results were presented in Mean ± SD of three replicates of osmolality, mineral content, and pH, total soluble solids and color measurements.

11.6 mg/L based on Flame Emission Spectrophotometer readings (Table 12).

Analysis of pH, total soluble solids and color

Synthetic formula (SF)

While there exists a limited number of studies conducted on humans, the influence of alterations in acid-base balance on water and solute absorption in the small intestine has been extensively examined in animal studies (Gromova et al., 2021). Consequently, the pH of the final synthetic sample was gauged at approximately 1.6 ± 0.0. Measurement of total soluble solids (°Brix) yielded 5.1 ± 0.1 °Brix, and the color intensity (PCU) was recorded as 130.0 ± 10.0. The titratable acidity was ultimately determined as 7.9 ± 0.4% (Table 12).

Natural formula with bee honey (NBH)

In the case of the NBH, the sample exhibited a color intensity (PCU) of 356.0 ± 20.8, while the pH level was measured at 2.6 ± 0.0. The total soluble solids (°Brix) measured at 5.0 ± 0.1 °Brix. The outcome for titratable acidity culminated in a value of 6.0 ± 0.0% (Table 12).

Natural formula with artificial sweetener (NAS)

For the NAS, the color intensity (PCU) was determined to be 413.0 ± 15.3. The pH level of the sample measured at 2.4 ± 0.0, while the total soluble solids (°Brix) stood at 5.1 ± 0.0 °Brix. The final result for titratable acidity also rested at 6.0 ± 0.0% (Table 12).

CONCLUSION

A successful production of isotonic beverage formulations was achieved, comprising one synthetic variant and two natural options. These formulations, rich in carbohydrates, salts, minerals, and water, underwent comprehensive analytical characterization following osmolality tests and sensory evaluations. All developed formulas were found to adhere to the regulatory standards for isotonic beverages, meeting criteria encompassing osmolality range, sodium content, and carbohydrate levels. Additionally, the developed formula's osmolality lies in the vicinity of 280 - 310 mOsm/kg which is similar to that of the Blood serum osmolality range.

In the synthetic formulation, an optimal combination of glucose and glucose syrup emerged as the most effective carbohydrate source to satisfy regulatory requisites. The natural formulations were grounded in coconut water, supplemented either by bee honey as a natural sugar source or by an artificial sweetener. Glucose syrup was introduced to the natural formulas to ensure compliance with osmolality specifications.

While this achievement is noteworthy, further exploration is warranted. Subsequent investigations should encompass testing the products' shelf life, evaluating their performance in human consumption, and transitioning the laboratory-driven product development into a successful commercial venture.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

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