

Valorizing Underutilized Crops for Dairy Innovation: Creating a Market-Ready, Nutraceutical-based, Preservative-Free Yoghurt with Added Value from Zobo and Turmeric

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Abstract: This study investigates the effects of Zobo (*Hibiscus sabdariffa*) and turmeric (*Curcuma longa*) extracts on the organoleptic and microbial quality of laboratory-prepared yoghurt. With growing interest in natural additives due to their potential health benefits, the use of Zobo and turmeric extracts was explored as natural preservatives and flavor enhancers. Fresh yoghurt samples were prepared and divided into three batches: one with Zobo extract, another with turmeric extract, and a control without any additives. Sensory evaluation was conducted to assess organoleptic properties such as taste, color, texture, and overall acceptability, while microbial analysis was performed to evaluate the yogurt's safety and shelf life over a 7-day storage period. The results indicated that both Zobo and turmeric extracts significantly improved the color and flavor profiles, with Zobo enhancing a pleasant red hue and tart flavor, while turmeric imparted a subtle earthy taste. Microbial analysis revealed a reduction in bacterial growth, particularly in the turmeric-enhanced yoghurt, demonstrating its antimicrobial properties. Overall, the study highlights the potential of Zobo and turmeric extracts as natural preservatives that not only enhance the sensory qualities of yoghurt but also improve its microbial safety, making them promising additives for the dairy industry.

Keywords: Dairy, Yoghurt, Zobo, Shelf-life and Turmeric.

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INTRODUCTION

Yoghurt is a widely consumed fermented milk product valued for its unique flavor, texture, and various health-promoting properties (Awari et al., 2023). It is commonly produced by the activity of lactic acid-producing bacteria, particularly *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Tamime & Robinson, 2018; Awah et al., 2016b). Fermentation not only improves the keeping quality of milk but also enhances its nutritional profile by increasing nutrient availability and introducing beneficial probiotic microorganisms (Ezenwelu et al., 2024; Obianom et al., 2023). In recent years, interest in functional foods has encouraged the use of natural supplements that can improve both the nutritional and sensory qualities of yoghurt (Agu et al., 2014; Ubaoji et al., 2020). Among such additives, Zobo (*Hibiscus sabdariffa*) and turmeric (*Curcuma longa*) have been studied for their potential contributions to sensory appeal and microbiological safety (Irkin & Esmer, 2015; Dhakal et al., 2019).

Zobo, which originates from the dried calyces of *Hibiscus sabdariffa*, is rich in anthocyanins responsible for its characteristic deep red coloration and strong antioxidant capacity (Da-Costa-Rocha et al., 2014; McKay et al., 2015). Anthocyanins are recognized for their ability to neutralize free radicals, thereby lowering oxidative stress and possibly reducing the incidence of chronic illnesses (Kumar et al., 2021; Anazodo et al., 2024). Incorporating Zobo extract into yoghurt may provide improved coloration as well as added health benefits. Previous research indicates that plant-based additives can enhance the antioxidant properties of yoghurt, potentially improving both product stability and consumer wellness (Mbachu et al., 2014; Agu et al., 2013a, Onuorah et al., 2019; Donkor et al., 2025).

Turmeric, obtained from the rhizomes of *Curcuma longa*, contains curcumin, a bioactive compound known for its anti-inflammatory, antimicrobial, and antioxidant activities (Hewlings & Kalman, 2017; Gupta et al., 2013). When used in yoghurt production, turmeric may help reduce microbial spoilage and extend shelf life while contributing distinctive sensory attributes (Adindu et al., 2016; Awah et al., 2017). Scientific investigations have explored turmeric's role in promoting health, particularly in relation to metabolic disorders and prolonged inflammatory conditions (Agu et al., 2013b).

Sensory attributes including taste, aroma, texture, and colour, play a major role in consumer preference for yoghurt products (Granato et al., 2018). Using natural ingredients such as Zobo and turmeric to improve these organoleptic characteristics aligns with increasing consumer interest in foods that offer additional health advantages (Umeoduagu et al., 2023b). Conducting sensory assessments is therefore crucial to determine how these extracts influence overall acceptability (Awah et al., 2011; Agu et al., 2014; Anaukwu et al., 2015).

The microbiological quality of yoghurt is equally important, as it affects food safety, product quality, and shelf longevity (Awah et al., 2016a; Ezenwelu et al., 2024). Natural antimicrobial constituents, like those found in turmeric, may suppress spoilage microorganisms and pathogenic contaminants, thereby enhancing the microbiological stability of the product (Dhakal et al., 2019; Umeoduagu et al., 2023a). Research shows that plant-derived

antimicrobials can be effective in improving the microbial profile of dairy-based foods (Irkin & Esmer, 2015; Agu et al., 2015).

Therefore, this study aims to investigate how Zobo and turmeric extracts influence the organoleptic attributes and microbial quality of laboratory-prepared yoghurt. It focuses on evaluating sensory properties (taste, aroma, texture, and color), determining changes in microbial load and shelf life, and assessing the antimicrobial actions of these extracts against yoghurt spoilage organisms.

MATERIALS AND METHODS

3.1 Study Area

The study was carried out in Nnamdi Azikiwe University in the Department of Applied Microbiology and Brewing Laboratory, Awka, Anambra State. Anambra State is located in South-East geopolitical zone of Nigeria. The latitude of Awka is 6.210528 while the longitude is 7.072277. Its GPS coordinates are 6° 12'37.9008"N and 7° 4'20.1972"E.

3.2 Sample Collection

Instant powdered milk (449 g), dried zobo (*Hibiscus*) leaves, turmeric powder, and a commercial yoghurt starter culture were obtained from a retail shop at Ifite market, Anambra State, Nigeria. The materials were packaged in sterile Ziploc bags and immediately transported to the microbiology laboratory for further processing.

3.3 Sample Preparation

The zobo leaves were boiled at 100°C and the resulting liquid was filtered to obtain the extract. Turmeric extract was similarly produced by pressing the mixture through a clean white cloth. Five experimental samples were prepared: 10% zobo extract, 10% turmeric extract, 20% zobo extract, 20% turmeric extract, and a control (distilled water). Each preparation received 71 g of powdered milk.

3.4 Yoghurt Production

All mixtures were heated and supplemented with 71 g of powdered milk before thorough mixing. Pasteurization was achieved by maintaining the temperature at 80°C for 25 minutes, after which the samples were cooled to 45°C. Each sample was inoculated with 0.71 g of starter culture and incubated under refrigeration for 14–18 hours to allow fermentation.

3.5 pH and Temperature Determination

The pH and temperature of each yoghurt sample were recorded 24 hours after fermentation. Subsequent readings were taken at three-day intervals, for four sessions, until spoilage was evident.

3.6 Microbial Analysis

Microbial load was evaluated 24 hours after fermentation and again after spoilage. Total bacterial count, *Lactobacillus* count, enteric bacteria count, *Salmonella* count, and *Shigella* count were determined. Lactic acid bacteria were quantified on MRS agar using the pour-plate method and incubated anaerobically for 24 hours. Enteric organisms (e.g., *E. coli*, *Enterobacter* spp.) were enumerated on EMB agar, while *Salmonella* and *Shigella* were assessed on SSA medium. One millilitre of each sample was plated

on sterile Petri dishes and incubated for 24 hours to observe growth. Some analyses were repeated once rancidity developed.

3.7 Characterization and Identification of Bacteria

Microbial isolates were characterized based on colony morphology, Gram reaction, and standard biochemical assays (Chessbrough, 2006). Tests performed included Gram staining, catalase, citrate utilization, sugar fermentation, coagulase, motility, oxidase, urease, indole, methyl red, and Voges-Proskauer, as outlined in Bergey's Manual of Determinative Bacteriology (9th ed., 1994).

3.7.1 Gram Staining

A thin smear of each isolate was prepared on clean slides, air-dried, heat-fixed, and flooded with crystal violet for one minute. After rinsing, Gram's iodine was applied for 60 seconds, followed by decolorization with acetone-alcohol. Safranin was then applied as a counterstain. Slides were viewed under oil immersion ($\times 100$). Purple cells indicated Gram-positive bacteria; pink or red cells indicated Gram-negative bacteria.

3.7.2 Catalase Test

Three millilitres of 3% hydrogen peroxide were dispensed into a sterile test tube, and three loopfuls of 24-hour culture were added. Immediate effervescence signified a positive catalase reaction; absence of bubbles signified a negative reaction.

3.7.3 Indole Test

An 18–24-hour culture was inoculated into 3 mL of sterile tryptone water and incubated at 35–37°C for up to 48 hours. After incubation, 0.5 mL of Kovac's reagent was added. Formation of a red ring at the surface within 10 minutes indicated a positive result; no colour change was recorded as negative.

3.7.4 Citrate Utilization Test

Sterile Simmons citrate agar slants were inoculated with a 24-hour culture and incubated for 24 hours. A positive reaction was indicated by a colour shift from green to blue on the agar surface, while no colour change indicated negative utilization.

3.7.5 Sugar Fermentation Test

To assess carbohydrate utilization, 1 g of the chosen sugar and 1 g of peptone were dissolved in 100 mL of water. Five millilitres of this mixture were dispensed into test tubes containing inverted Durham tubes and bromothymol blue as indicator. After sterilization and cooling, cultures were inoculated and incubated for three days. Acid production was shown by a shift from green to yellow; gas production was detected by bubbles in the Durham tube.

3.7.6 Urease Test (Christesen's Urea Agar)

This test detects organisms capable of hydrolyzing urea to ammonia and carbon dioxide, particularly differentiating urease-positive Proteae from other Enterobacteriaceae. A heavy inoculum from an 18–24-hour culture was streaked across the agar slant without stabbing the butt. Tubes were incubated at 35°C with loose caps and observed periodically up to six days. Development of a bright pink colour indicated urease activity. A control medium lacking urea was included to avoid false positives due to protein degradation. Strong positives (e.g., *Proteus* spp., *Morganella morganii*, *Providencia stuartii*) occurred within 1–6 hours, while weak, delayed reactions were observed in organisms such as *Klebsiella* and *Enterobacter*. Negative results remained yellowish.

4.0 RESULTS

Table 1: Shows the result of the total bacterial count, *Lactobacillus* count, *Salmonella* and shigella count, enteric organism count after yoghurt production ($\times 10^2$ CFU/ml)

Table 2: Shows the result of total bacterial count, *Lactobacillus* count, *Salmonella* and shigella count, enteric organism count, 10 days after yoghurt production, after spoilage ($\times 10^{-3}$ CFU/ml)

Table 3: Shows the result of temperature and pH values of yoghurt samples after production

Table 4: Morphological and Biochemical Identification of the Various Bacterial Isolates

Table 1: Total bacterial count, *Lactobacillus* count, *Salmonella* and shigella count, enteric organism count after yoghurt production

	No of bacterial total colonies on plate	No of Lactobacillus Total colonies on plate	No of Salmonella and Shigella Total colonies on plate	No of Enteric organism Total colonies on plate
G1	TNTC	4.6×10^4	6.6×10^4	1.05×10^5
G2	TNTC	6.5×10^4	TFTC	6.5×10^4
G3	TNTC	3.1×10^4	TFTC	1.03×10^5
G4	TNTC	8.8×10^4	1.20×10^5	1.35×10^5
G5	7.2×10^4	3.5×10^4	NG	NG

Table 2: Total bacterial count, *Lactobacillus* count, *Salmonella* and *Shigella* count, enteric organism count, 10 days after yoghurt production, after spoilage

	No of bacterial total colonies on plate	No of <i>Lactobacillus</i> Total colonies on plate	No of <i>Salmonella</i> and <i>Shigella</i> Total colonies on plate	No of Enteric organism Total colonies on plate
G1	TNTC	TNTC	TFTC	TFTC
G2	TNTC	TNTC	TFTC	TFTC
G3	TNTC	TNTC	TFTC	9.8×10^5
G4	TNTC	TNTC	5.4×10^5	7.6×10^5
G5	9.8×10^5	TNTC	TFTC	7.9×10^5

NG: NO GROWTH

TFTC: Too few to count(Colonies that are less than 30 on a plate)

TNTC: Too numerous to count(Colonies that are more than 300 on a plate)

Table 3: Temperature and pH values of yoghurt samples after production

	DAY 2	DAY 4	DAY 6	DAY 8
	pH and Temp	pH and Temp	pH and Temp	pH and Temp
G1(Control)	6.51 30.3°C	6.50 30.3°C	5.7 30.4°C	5.1 29.8°C
G2	6.57 30.2°C	6.51 30.3°C	5.5 30.6°C	5.3 27.6°C
G3	6.54 30.2°C	6.53 30.3°C	5.6 30.2°C	5.4 27.5°C
G4	6.53 30.2°C	6.50 30.3°C	5.5 30.7°C	5.4 28.5°C
G5	6.54 30.2°C	6.51 30.3°C	5.4 30.6°C	5.3 28.6°C

Table 4: Morphological and Biochemical Identification of the Various Bacterial Isolates

Isolate	Form	Surface	Colour	Margin	Elevation	Opacity	Gram	Cat	Ind	Cit	Lac	Glu	Suc	Fru	Mal	Ure	Identity
1	Circular	Glistening	Cream	Entire	Raised	Transparent	- Rod	+	-	+	+	+	+	-	+	+	<i>Proteus mirabilis</i>
2	Irregular	Glistening	Cream	Entire	Raised	Opaque	-Rod	+	-	+	+	+	+	(-)	+	+	<i>Klebsiella pneumoniae</i>
3	Circular	Shiny	White	Entire	Convex	Moist	-Rod	+	-	+	+	+	+	+	+	-	<i>Enterobacter aerogenes</i>
4	Circular	Smooth	Whitish	Entire	Convex	Translucent	-Rod	+	+	-	+	+	Var	-	-	-	<i>Escherichia coli</i>
5	Circular	Smooth	Cream	Entire	Convex	Opaque	-Rod	-	+	var	var	+	+	var	+	-	<i>Vibrio cholerae</i>

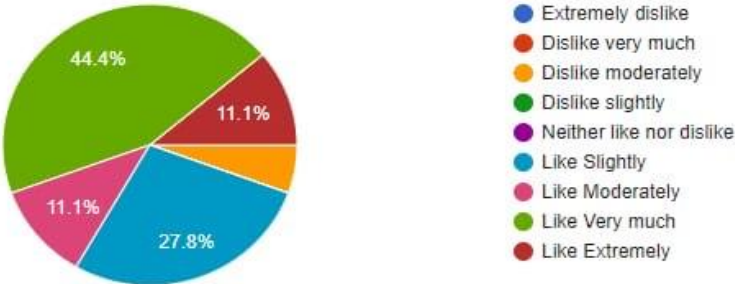
Pie Chart data collection from respondents on the questionnaire on effects of zobo and turmeric extract on the organoleptic qualities of yoghurt:

SAMPLE 1 (CONTROL)

Colour

18 responses

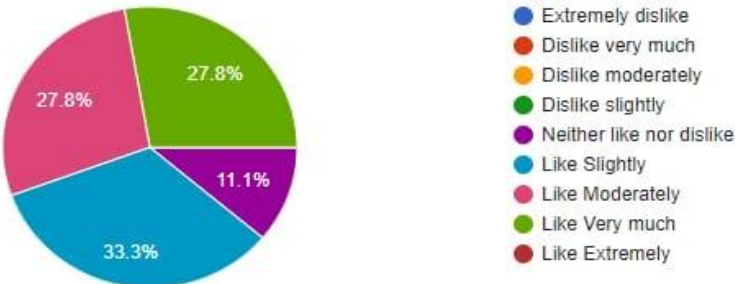
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Consistency

18 responses

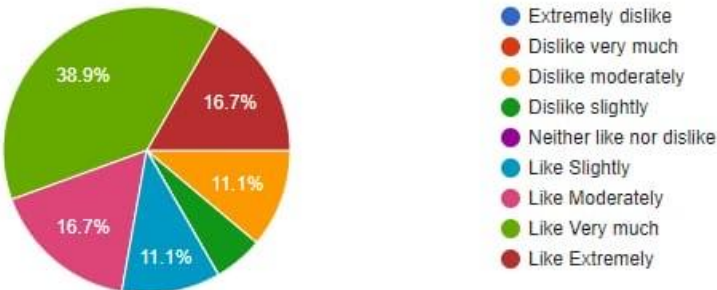
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Aroma

18 responses

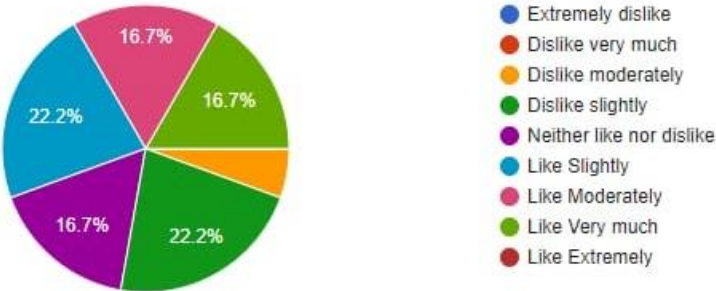
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Sourness

18 responses

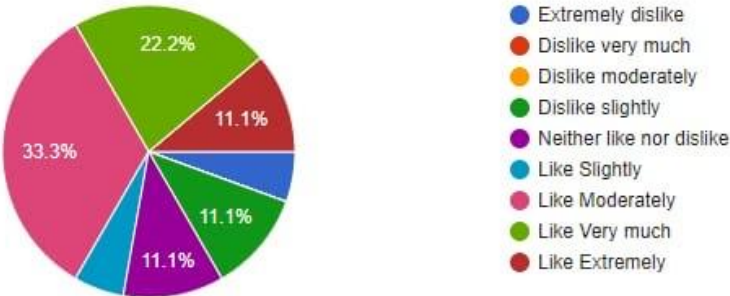
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Taste

18 responses

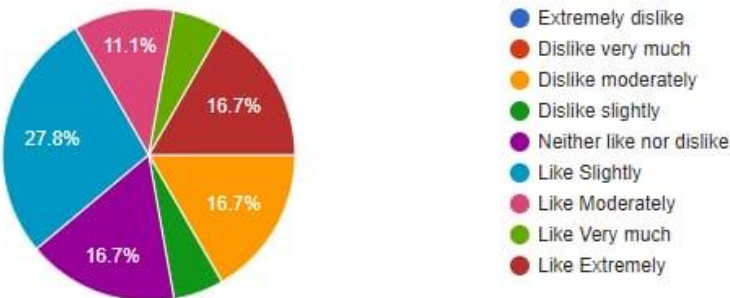
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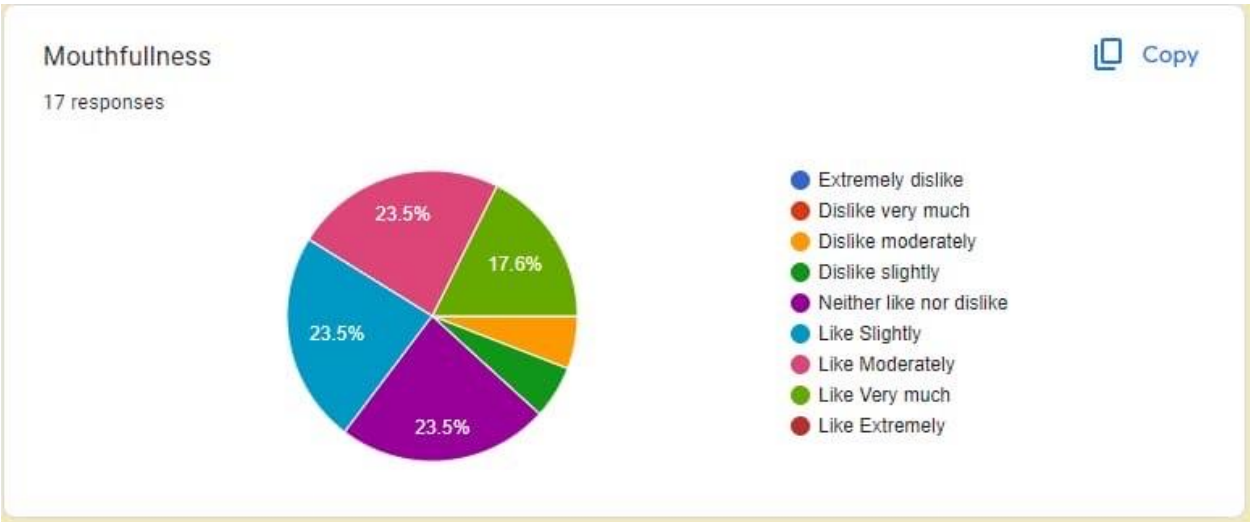


After Taste

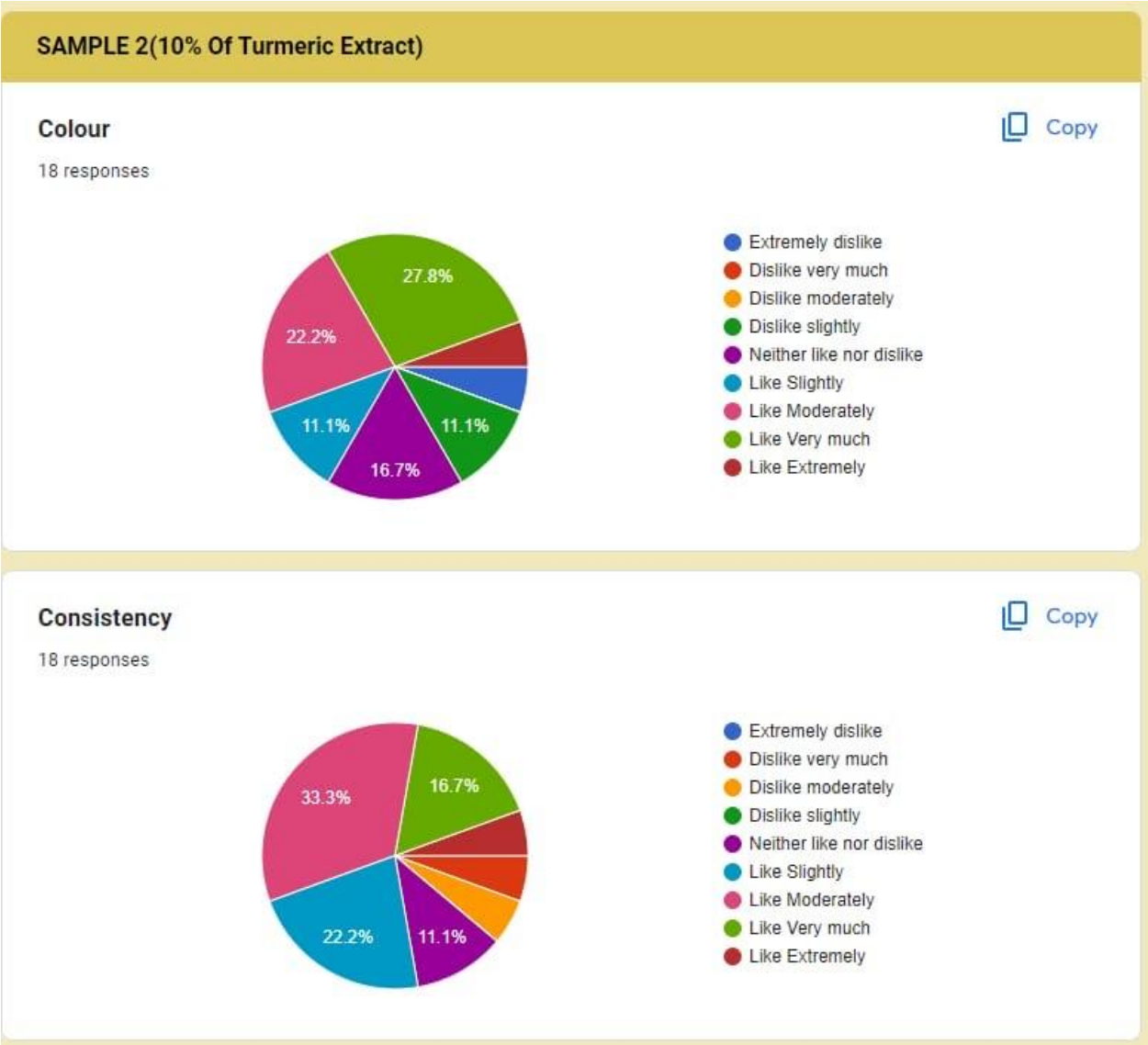
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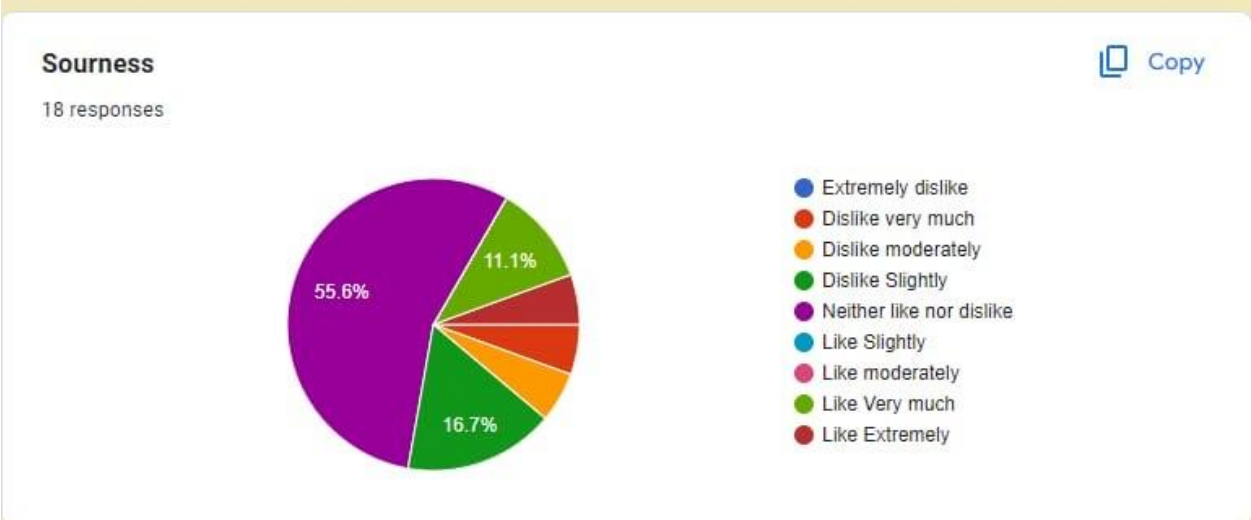
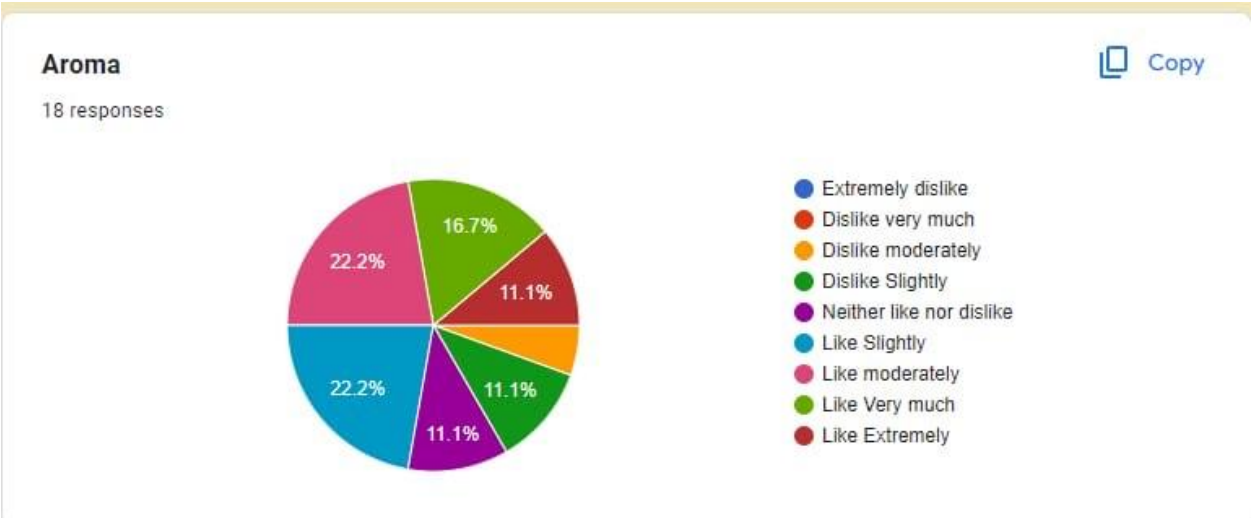
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Sample 2

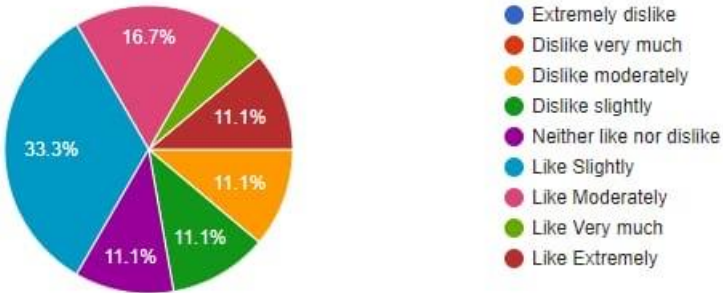




Taste

18 responses

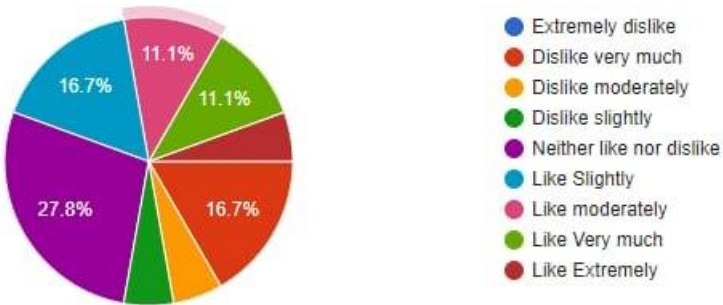
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After Taste

18 responses

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SAMPLE 3(20% Of Turmeric Extract)

Colour

18 responses

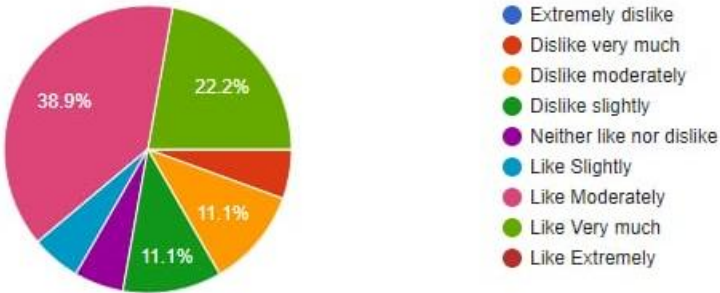
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Consistency

18 responses

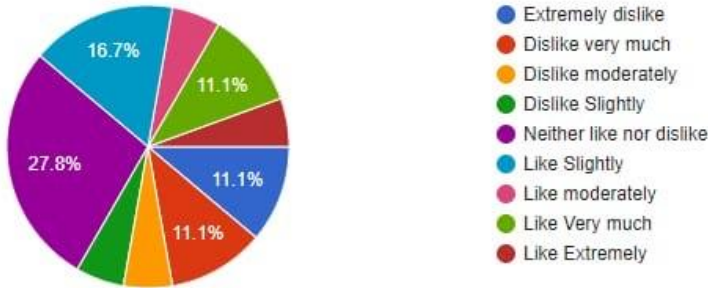
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Mouthfullness

18 responses

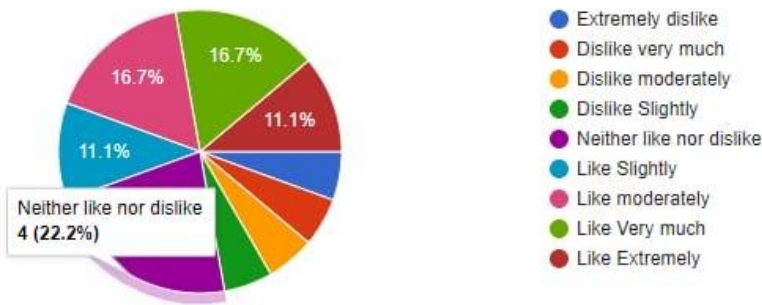
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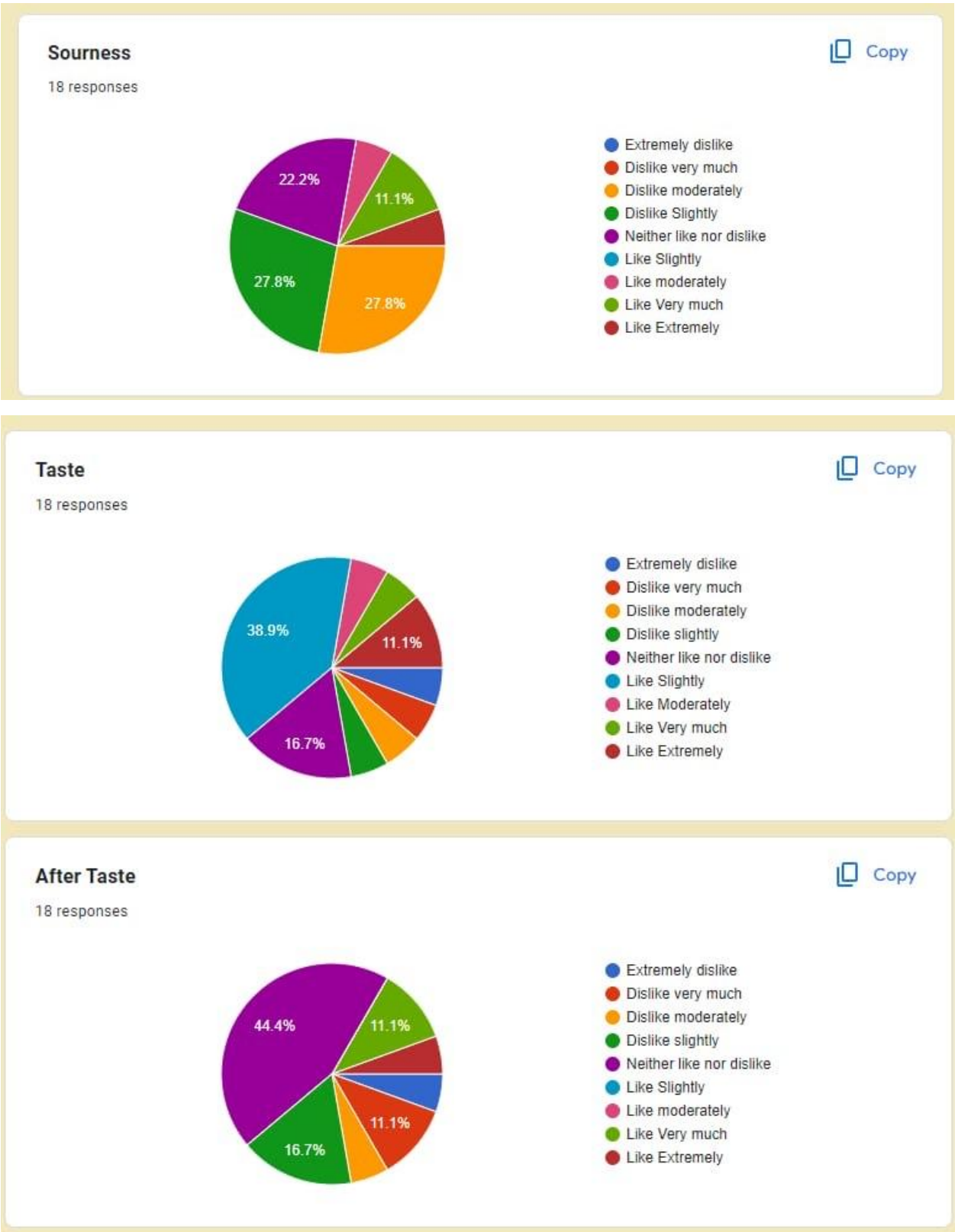


Aroma

18 responses

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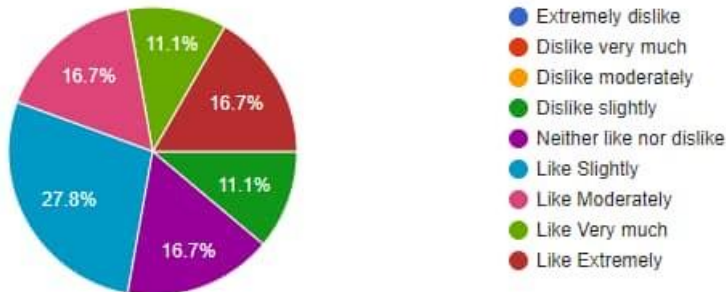


SAMPLE 4(10% Of Zobo Extract)

Colour

18 responses

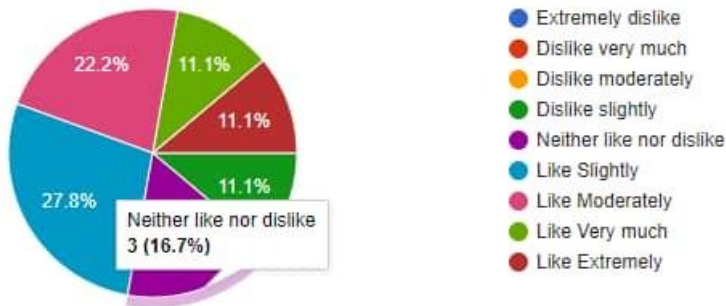
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Consistency

18 responses

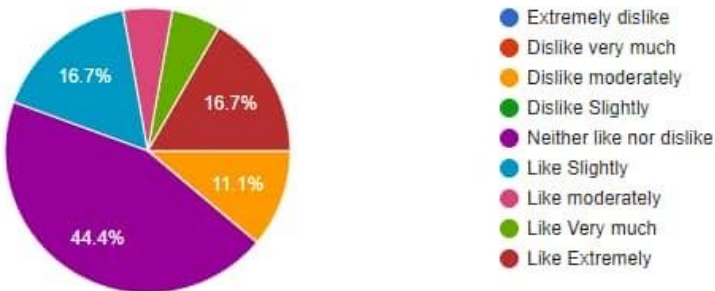
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Mouthfullness

18 responses

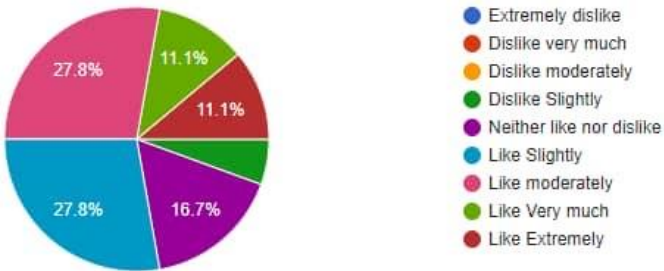
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Aroma

18 responses

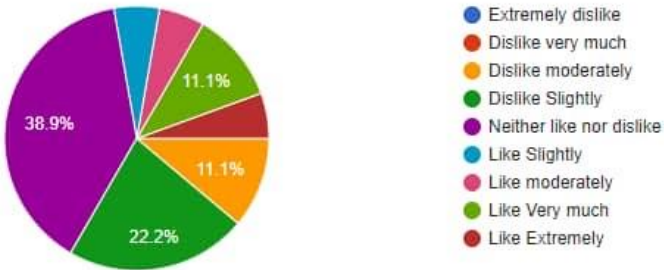
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Sourness

18 responses

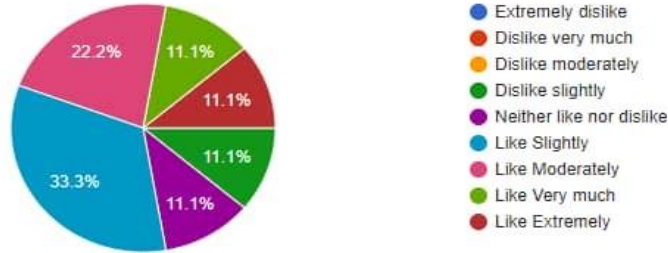
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Taste

18 responses

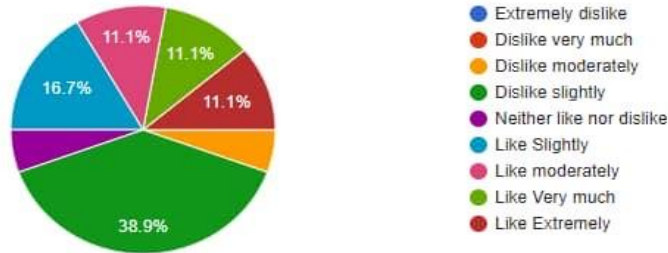
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After Taste

18 responses

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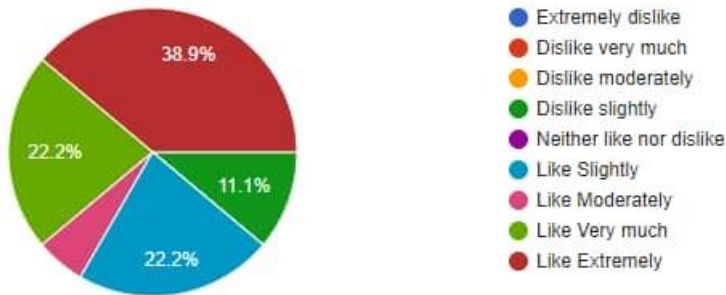


SAMPLE 5(20% Of Zobo Extract)

Colour

18 responses

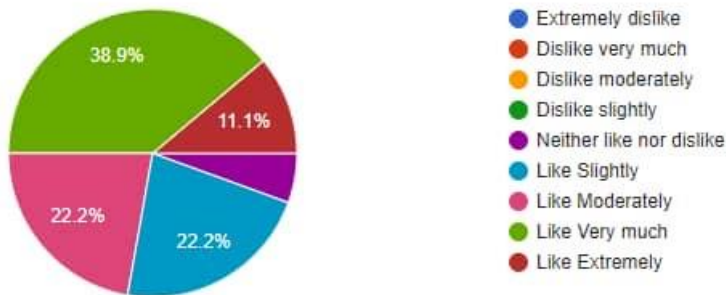
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Consistency

18 responses

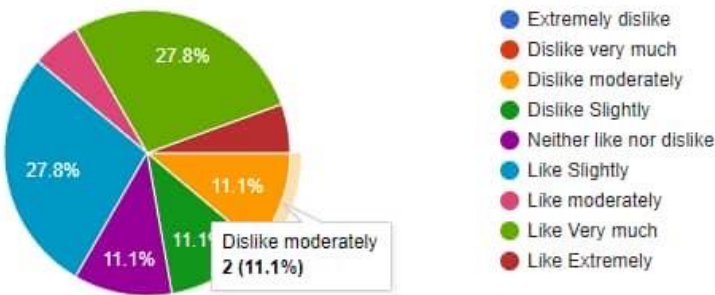
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Mouthfullness

18 responses

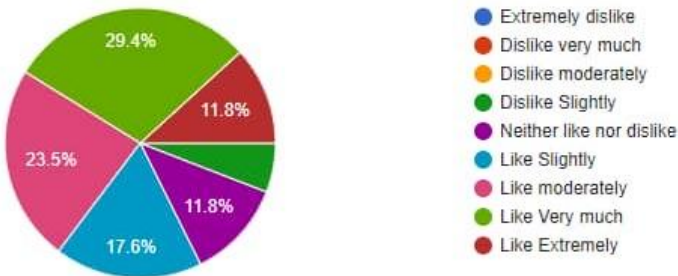
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Aroma

17 responses

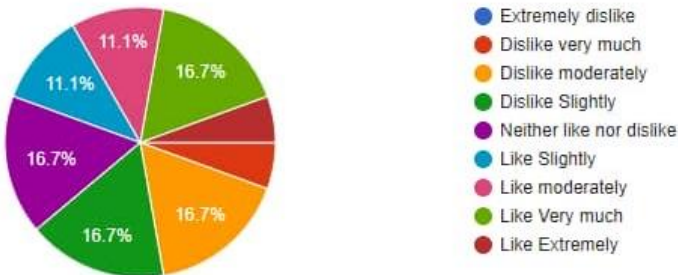
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Sourness

18 responses

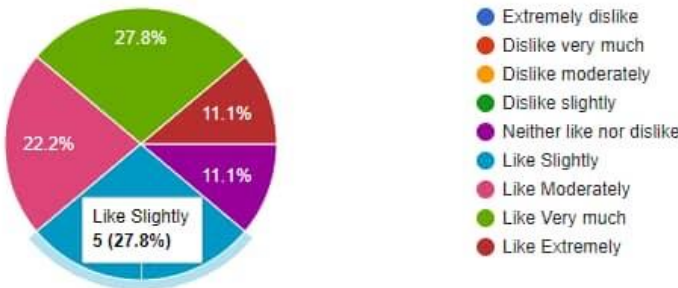
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Taste

18 responses

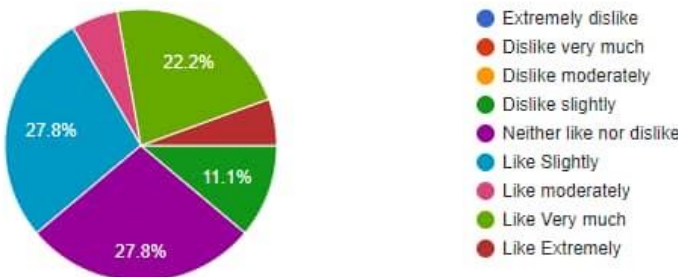
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After Taste

18 responses

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5.1 DISCUSSION

In this study, the effects of Zobo (*Hibiscus sabdariffa*) and turmeric (*Curcuma longa*) extracts on the organoleptic and microbial quality of laboratory-prepared yoghurt were evaluated. The inclusion of these natural additives was motivated by their known antimicrobial and antioxidant properties, aiming to enhance both the sensory attributes and microbial stability of yoghurt. The findings contribute to the growing body of research on the use of plant-based extracts in food preservation and safety, addressing consumer demand for healthier, more natural food products.

The organoleptic properties of yoghurt, including taste, aroma, texture, and color, were assessed to determine the impact of Zobo and turmeric extracts. Zobo, with its vibrant red color, imparted a noticeable hue to the yoghurt, which may have influenced consumer preferences. The study's results are consistent with prior research by Adebayo-Tayo et al. (2017), which demonstrated that *Hibiscus sabdariffa* can significantly enhance the visual appeal of food products, although its acidic taste may require balance to prevent it from overpowering the flavor profile. In contrast, turmeric's yellow pigmentation provided a more subtle change in appearance, while its distinct flavor and potential bitterness had to be moderated, aligning with the findings of Prasad et al. (2015) regarding turmeric's impact on food sensory characteristics.

Regarding microbial quality, the introduction of Zobo and turmeric extracts was hypothesized to reduce the microbial load in yoghurt due to the known antimicrobial properties of both plants. Turmeric, in particular, contains curcumin, which has been widely studied for its antibacterial activity. The study's findings corroborate previous research by Bamidele et al. (2018), who reported that curcumin effectively inhibited the growth of several pathogenic microorganisms in dairy products. Similarly, *Hibiscus sabdariffa* has been shown to possess significant antimicrobial properties, particularly against Gram-positive bacteria such as *Staphylococcus aureus*, as highlighted by Olayinka et al. (2020).

However, despite the antimicrobial benefits, the inclusion of these extracts did not entirely eliminate microbial growth, especially in samples stored at ambient temperatures. This finding underscores the complexity of microbial control in food products, where environmental factors such as storage conditions and initial contamination levels play a significant role. It also reflects the need for additional preservatives or refrigeration to ensure complete microbial safety, as discussed by Okpala et al. (2021) in their work on natural preservatives in dairy products.

The microbial analysis of the yoghurt samples indicated a reduction in total viable counts in the formulations containing Zobo and turmeric extracts compared to the control. This aligns with the work of Abdulkadir et al. (2016), who found that the antimicrobial compounds in *Hibiscus sabdariffa* can disrupt bacterial cell walls, leading to reduced microbial growth in food products. The reduction in microbial load in this study was particularly notable in samples stored for extended periods, suggesting that these extracts may contribute to prolonged shelf life. The study by Onyenekwe et al. (2017) similarly highlighted the shelf-life extension potential of natural extracts in fermented dairy products.

In terms of sensory attributes, the study found that while both Zobo and turmeric positively influenced the color and texture of yoghurt,

their impact on taste was more divisive. While some participants appreciated the unique flavors imparted by these extracts, others found them less favorable, particularly at higher concentrations. This variation in sensory perception is supported by the findings of Adeoye et al. (2019), who noted that consumer acceptance of herbal extracts in food products often depends on individual taste preferences and cultural familiarity with the ingredients.

In conclusion, this study demonstrates that Zobo and turmeric extracts can positively influence the microbial quality and sensory attributes of yoghurt, though their effects on taste may require careful calibration to suit consumer preferences. The antimicrobial properties of these extracts provide a natural alternative to synthetic preservatives, contributing to food safety and shelf life extension. Future studies could explore the optimal concentrations of these extracts to maximize their benefits without compromising the sensory appeal of the final product. This research highlights the potential of plant-based additives in improving the quality and safety of dairy products, supporting the trend towards more natural and health-conscious food production methods (Adebayo-Tayo et al., 2017; Abdulkadir et al., 2016; Bamidele et al., 2018; Olayinka et al., 2020).

CONCLUSION/ RECOMMENDATIONS

The findings from this study demonstrate the potential of Zobo (*Hibiscus sabdariffa*) and turmeric (*Curcuma longa*) extracts to enhance both the organoleptic and microbial quality of laboratory-prepared yoghurt. The incorporation of these extracts contributed to improved color and texture, while also exhibiting antimicrobial properties that reduced the microbial load, thereby promoting shelf life extension. However, variations in sensory perception, particularly in taste, indicate the need for careful calibration of these extracts to achieve optimal consumer acceptance.

Based on these results, it is recommended that further research should focus on determining the ideal concentrations of Zobo and turmeric extracts to balance their antimicrobial efficacy with sensory appeal. Additionally, combining these natural extracts with other preservation methods, such as refrigeration or pasteurization, could offer a comprehensive approach to improving both the safety and quality of dairy products. The development of plant-based preservatives aligns with the growing demand for natural food additives, supporting the creation of healthier, more sustainable food products for the market.

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