

Supporting Information

Development of a Novel Low-Calorie Lime Juice-Based Prebiotic Beverage Using a Combined Design Optimization Methodology

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Material and methods

Preparation of iodine solution

To prepare of the iodine solution, 5 g potassium iodide (KI) and 0.268 g potassium iodate (KIO₃) were dissolved in 200 ml of distilled water. Then, 30 ml of 3 M sulfuric acid (H₂SO₄) was added and diluted with distilled water to the 500 ml solution.

Titrateable acidity (TA)

The TA was determined by titrating a 10 mL sample with NaOH (0.1 N) using phenolphthalein till the sample turned light pink (pH 8.1) [1] and was calculated as [2]:

$$\%TA = \frac{V \times 0.1N \text{ NaOH} \times 0.064 \times 100}{V_s}$$

Where V is the titer volume of NaOH (ml) and V_s is the volume of the sample (ml).

Results were expressed as % citric acid in TA and °Brix in TSS.

Table S1. Optimization criteria and dependent variables optimization

Variables/Responses	Goal	Lower limits	Upper limits	Weights	Importance
LEO solution (0.1 v/v) (%)	In range	0	100	1	3
ME (%)	In range	0	100	1	3
Lutein (mg/100 ml)	In range	1	3	1	3
Prebiotics	In range	Level 1	Level 2	1	3
Taste	Maximize	5.25	7.62	1	5
Flavor	Maximize	5.85	7.92	1	5
Texture	Maximize	5.90	8.05	1	4
Color	Maximize	4.30	7.12	1	5
Overall acceptance	Maximize	5.28	8.25	1	5

Table S2. Physicochemical properties of the optimized formulations

Optimized sample	TSS (°Brix)	pH	Ascorbic acid ^a	Titrateable acidity ^b
Opt 1	1.0 ± 0.0	2.62 ± 0.07 ^a	23.28 ± 1.54 ^a	0.56 ± 0.01 ^c
Opt 2	1.0 ± 0.0	2.59 ± 0.03 ^a	22.33 ± 1.27 ^a	0.56 ± 0.04 ^c
Opt 3	3.5 ± 0.0	2.48 ± 0.02 ^b	25.16 ± 0.64 ^a	0.60 ± 0.02 ^{b, c}
Opt 4	3.5 ± 0.0	2.46 ± 0.04 ^b	23.46 ± 1.65 ^a	0.64 ± 0.04 ^{a, b}
Opt 5	3.5 ± 0.0	2.41 ± 0.04 ^b	25.11 ± 1.92 ^a	0.65 ± 0.03 ^a
Opt 6	3.5 ± 0.0	2.33 ± 0.03 ^c	24.43 ± 1.52 ^a	0.68 ± 0.01 ^a

Data are presented as mean ± SD, n = 3.

^a mg/100 g

^b g of citric acid/100 ml

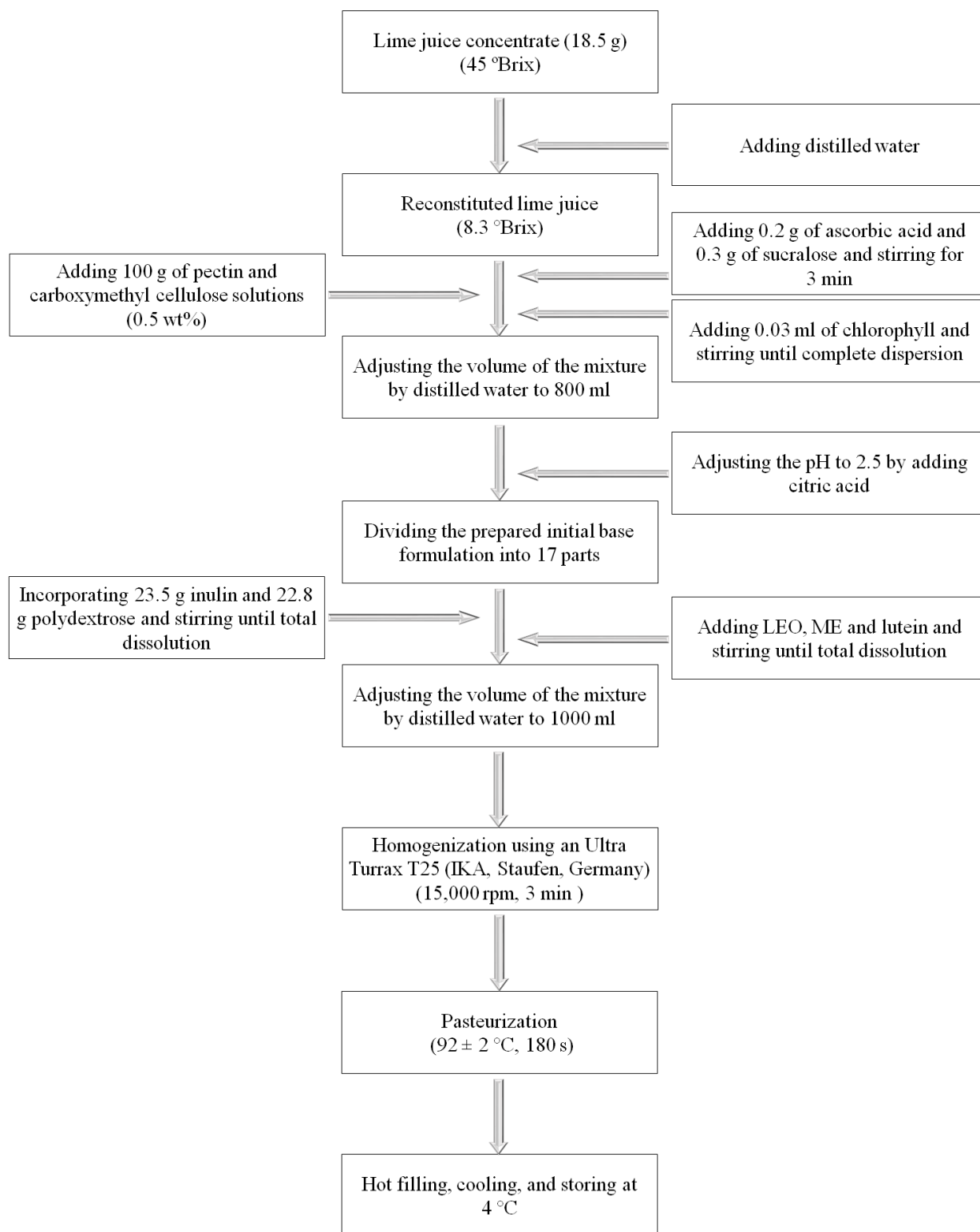


Figure S1. Protocol for the preparation of 1 L functional beverages in a laboratory scale. (black and white)

References

1. AOAC. AOAC official method 942.15: Acidity (titratable) of fruit products. *Official Methods of Analysis of AOAC International* **1996**.
2. Tyl, C.; Sadler, G.D. pH and titratable acidity. In *Food analysis*, Springer: 2017; pp. 389-406.