

Production of an Effervescent Powder from *Solanum betaceum* Fruit Having Enhanced Antioxidant Properties

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Abstract Currently, people have an increasing trend towards the consumption of healthy foods having a large content of antioxidants. These substances prevent the oxidative stress caused by several pathologic conditions. Particularly, studies have found a significant amount of phenolic compounds in the fruits of *Solanum betaceum* besides its nutritional properties. This study was focused on the production of an effervescent powder from the *Solanum betaceum* fruit, employing a mixture design in order to get an optimized product with a rapid solubilization time, and good sensory and microbial properties. The phenolic content and antioxidant activity of the optimized effervescent powder were also evaluated. Results showed the product containing lyophilized *Solanum betaceum*, maltodextrin, citric acid, saccharose, sodium carbonate and tricalcium phosphate at 30%, 22.8%, 20.0%, 15.0%, 12.0% and 0.2%, respectively, as optimal. The antioxidant activity of the effervescent powder was higher than that of the lyophilized powder. This was explained by the synergistic effect of the antioxidant power of the fruit and the excipients. The total phenolic content of the effervescent powder and lyophilized product were also measured and was the main responsible for the antioxidant power. On the other hand, the titratable acidity, pH, and total suspended solids of the final drink were 0.20 CAE /100mL, 4.06 and 0.9°, respectively. This product also showed no viable aerobic bacteria, molds or yeasts. This study proved that the *Solanum betaceum* fruit could be processed into an ideal effervescent antioxidant-rich powder.

Keywords: Antioxidant properties, Effervescent powder, Phenolic compounds, Lyophilized powder, *Solanum betaceum*

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1. Introduction

Currently, people are getting conscious about having a healthy lifestyle, consuming fresh and nutritious foods and maintaining a good physical activity. Most exotic fruits such as pomegranate, berries, blueberries, raspberries, strawberries, goji berry, chickpeas, grape, acai berry, hippophaes and many others, are rich in minerals, vitamins, phytochemical and antioxidant compounds meeting the requirements of super foods. Conceptually, superfoods are denoted as foods that are great in nutrition value due to the high concentration of nutrients and the presence of bioactive compounds having a satisfactory bioavailability in the human body [1].

Natural antioxidants, particularly those found in fruits and vegetables have gained attention among consumers and the scientific community due to their remarkable ability to lower the risk of acquiring cancer and cardiovascular diseases [2]. In this scenario, tamarillo shrub (*Solanum betaceum* Cav.) is a small bush (2–4m high) which renders a fruit known for its significant

content of flavonoids and carotenoids with great nutritional and pharmacological properties [3]. Tamarillo seeds also contain lipids, proteins, vitamin E, polyphenols, minerals, and phytosterols. Further, the phenolic compounds of tamarillo are the main contributors of the antioxidant activity of this fruit. Eight hydroxycinnamic acids, two hydroxybenzoic acids, three phenolic glycosides, one flavanol and one flavanone have been identified in the yellow and purple red cultivars at different levels. Table 1 lists the values of main phenolic compounds found in tamarillo [4].

Tamarillo could be ingested either fresh or processed within products preserved in different forms and types. This bush is originated from South America, especially in the Andes region of Colombia, Perú, Chile, Ecuador and Bolivia [5]. Nowadays, Colombia, New Zealand, Australia and USA are the main producers of tamarillo. Recent agricultural efforts have been conducted to improve the fruit quality; however, only few successful tamarillo-based products have been developed [6]. Some variations of the fruit skin color such as yellow, orange, red, and purple are attributed to the presence of chlorophylls, carotenoids and anthocyanins. The

nutritional value of tamarillo fruit is represented by the low content of calories and fat. It is also rich in dietary fibers, minerals, vitamins, proteins, soluble sugars, and organic acids (e.g., malic and citric acids). Further, the fruit juice is a popular form of consumption, but has a high-water content which makes it susceptible to decomposition by microorganisms, chemical and enzymes creating stability concerns [7]. Consequently, the idea of an alternative processed fruit powder could extend the fruit shelf-life reducing the packaging requirements and shipping costs.

Table 1. Phenolic compounds of *Solanum betaceum* Cav fruits

Phenolic compounds	Pulp of <i>Solanum betaceum</i> (g/100g dry weight)
Total phenolics	2.4 – 6.2
Caffeoyl glucoside	1.4 – 29.3
3-O-caffeoylquinic acid	25.0 – 163.6
Dehydrodiferulic acid (I)	0.1 – 21.1
Dehydrodiferulic acid (II)	7.5 – 22.1
5-O-caffeoylquinic acid	0.5 – 2.6
Feruloyl glucoside	0.2 – 3.0
Rosmarinic acid glucoside (isomer I)	3.3 – 14.9
Rosmarinic acid glucoside (isomer II)	2.0 – 14.6
Rosmarinic acid glucoside (isomer III)	3.6 – 19.7
Malonyl derivate of rosmarinic acid glucoside	1.6 – 6.3
Rosmarinic acid glucoside (isomer VI)	0.9 – 5.7
Rosmarinic acid	12.2 – 121.9
Total hydrocinnamoyl acids	60.3 – 421.6
Total anthocyanins (mg/100g dry weight)	0.1 – 0.2
Delphinidin 3-O-rutinoside (Dp-3-rut)	21.8 – 87.4
Cyanidin 3-O-rutinoside (Cy-3-rut)	2.5 – 4.5
Pelargonidin 3-O-rutinoside	77.0 – 78.0

Drying is the conventional way of preserving fruits in order to enhance storage stability, minimizing the packaging requirements and transportation costs. The most common technologies of drying include freezing, and vacuum, osmotic, cabinet, tray, fluidized bed, spouted bed, Ohmic, or microwave drying and their combination thereof. Freeze-drying is one of the best methods of water removal from fruits which results in a high-quality product. Freeze-drying is based on the sublimation of the ice fraction where water passes from solid-to-gaseous state. It employs a very low temperature which hinders deterioration and microbiological activity and provides a better quality to the final product. In fact, freeze-drying is the most reliable dehydration procedure for the preservation of the sensory and nutritional characteristics of foodstuffs, since it does not affect the chemical composition and antioxidant activity of fruits [8].

Effervescent powders are ideal preparations for fruits since the desired flavor and taste could be tune up controlling aspects such as saccharose and acid content. Further, a rapid dissolution and good sensory attributes are the key factors in order to obtain the largest acceptability [9].

The goal of this study was to develop an optimal formulation for an effervescent tamarillo powder having a rapid solubilization upon reconstitution with water busting the antioxidant properties of the phenolic compounds of the fruit. This formulation is expected to have high

sensory properties and a rapid solubilization according to the response optimization analysis.

2. Material and Methods

2.1. Materials

Tamarillo fresh fruits were purchased from a farm located in San Bernardo - Province of Sumapaz, Cundinamarca, Colombia (1600 amsl). The fully ripe red variety was selected. Maltodextrin (lot 1153), sodium carbonate (lot 6852) and citric acid (lot 4512) were purchased by Bell chem international S.A.S. Tricalcium phosphate and saccharose were purchased from Ronas chemicals inc (Sichuan, China).

2.1.1. Liophilization of Tamarillo Powder

Approximately, 2.5 kg of tamarillo fruits were sanitized for 30 min in a bath containing 200 ppm of sodium hypochlorite followed by washing with distilled water. The fruit skin was peeled off and the seeds removed. The pulp (edible fraction) was frozen at -80 °C, lyophilized at 900 mmHg for 72 h and passed through a No.10 mesh to obtain a powder. The powder was then placed in amber plastic bags and stored at -20 °C in a freezer until further testing.

2.1.2. Preformulation Studies

Citric acid and sodium carbonate were prepared at different stoichiometric ratios based on the acid-alkaline effervescence reactions, final pH and sensory attributes. These two materials were selected due to their high aqueous solubility, rapid reaction and low cost. Solubility was defined by the Likert Scale as 1 (very poor), 2 (poor), 3 (average), 4 (good) and 5 (excellent). The cut-off pH criterion was less than 4.5 in order to prevent microbial contamination. Further, critical attributes such as flavor, color, and appearance were measured in order to define the lyophilized tamarillo and saccharose levels employed in a mixture design. The solubilization time and sensory attributes were taken as independent variables [10]. Four components, namely lyophilized tamarillo powder, citric acid, sodium carbonate, and saccharose were taken into account in the experimental design. A total of eight runs of 100g each, having different levels of the compounds were prepared.

2.2. Effervescent Powder Preparation

Lyophilized tamarillo powder (fruit source with antioxidant properties), maltodextrin (stabilizer), citric acid (acidifier), sodium carbonate (CO₂ source), tricalcium phosphate (moisturizer-caking agent) and saccharose (sweetener) were passed through a No.10 mesh. Subsequently, each ingredient was weighed according to the mixture design (Table 2) given by the Design Expert® software vs. 8 (Stat Ease Inc). The powders were then mixed in a V-blender for 10 minutes at 20 rpm, placed in amber plastic bags and stored in a desiccator over silica gel. The moisture content was then measured on an infrared moisture balance (Model 240, Kett U.S.A) in triplicate.

Table 2. Mixture Design for the Effervescent Formulations

Formulation run (%)	1	2	3	4	5	6	7	8
Citric acid	30	30	30	30	20	20	20	20
Sodium carbonate	18	18	12	12	18	18	12	12
Saccharose	15	10	15	10	15	10	15	10
Tricalcium phosphate	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Maltodextrin	6.8	11.8	22.8	27.8	26.8	31.8	22.8	27.8
Lyophilized tamarillo	30	30	20	20	20	20	30	30
Solubilization time (s)	51.0 ± 0.0	48.6 ± 0.0	44.4 ± 0.1	43.2 ± 0.1	40.2 ± 0.0	39.0 ± 0.0	43.8 ± 0.0	42.0 ± 0.0
Sensory attributes (%)	66.3 ± 2.7 ^a	64.0 ± 2.9 ^a	68.3 ± 2.5 ^a	65.7 ± 2.7 ^a	74.7 ± 1.7 ^b	72.9 ± 1.9 ^b	94.0 ± 0.6 ^c	92.1 ± 0.7 ^c

Different superscript letters (a-c) within rows indicate significant differences between samples ($p < 0.05$) according to Fisher's least significant difference (LSD-Fisher) test; values are expressed as mean $\pm$ standard deviation (SD) ($n = 3$).

2.3. Sensory Evaluation

The effervescent tamarillo powders were prepared according to the mixture design shown in Table 2. Approximately, 1g of effervescent tamarillo powder was dissolved in 50mL water. Fifteen panelists in an age group between 20 and 40 years old ranked the samples in a 7-point hedonic scale according to the Meilgaard methodology (2016) [11]. Critical attributes such as aroma, flavor, color, sweetness and bitterness were measured and calculated based on the percentage of acceptability.

2.4. Measurement of Solubilization Time

~1g of powder was placed in a beaker containing 50 mL of purified water at $20 \pm 1^\circ\text{C}$ and stirred with a magnetic stirrer at 200rpm until a clear solution was obtained.

2.5. Physico-Chemical Analysis

The pH of the effervescent solution was determined with ~1g of effervescent tamarillo powder in 50 mL of purified water at $20 \pm 1^\circ\text{C}$ using a pH-meter (Model Tritoline 600, SI Analytics). The total soluble solids were measured employing the AOAC 932.12 methodology using a hand-held refractometer (Model MA871, Milwaukee) [12]. The total acidity was measured according to the method described by the AOAC 942.15 [13]. These experiments were measured immediately once completed the solubilization time in triplicate.

2.6. Total Phenolic Content (TPC)

The TPC of lyophilized tamarillo and effervescent preparations was determined using the method described by AOAC 2017.13 [14]. The absorbance of the solutions was measured at 765 nm using a Spectrofluorophotometer (Synergy H1, Biotek®, USA). Excipients such as maltodextrin, citric acid, saccharose, sodium carbonate, and tricalcium phosphate at 22.8%, 20.0%, 15.0%, 12.0% and 0.2%, respectively were used as negative controls. The total phenolic content in each formulation was determined using a standard curve prepared with gallic acid and expressed as milligrams of gallic acid equivalents (GAE) per 100 g of powder.

2.7. Antioxidant Activity

Several complementary assays such as 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS);

2,2-diphenyl-1-picrylhydrazyl (DPPH); ferric reducing antioxidant power (FRAP); and the oxygen radical absorption capacity (ORAC) were employed to estimate the antioxidant activity [15].

2.7.1. Oxygen Radical Absorbance Capacity (ORAC)

It was conducted following the method described by the AOAC 2012.23 [16]. The fluorescence readings were measured with an excitation at 485 ± 20 nm and emission at 530 ± 25 nm using a Spectrofluorophotometer (Synergy H1, Biotek®, USA), using the excipients (maltodextrin, citric acid, saccharose, sodium carbonate, and tricalcium phosphate at 22.8%, 20.0%, 15.0%, 12.0% and 0.2%, respectively) as blanks. The ORAC value in each formulation was determined using a standard curve prepared for trolox and expressed as μmol of Trolox equivalents per 100 g of powder.

2.7.2. Trolox Equivalent Antioxidant Capacity (TEAC)

The DPPH (mg eq. Trolox/ 100g) and ABTS (mg eq. Trolox/100mL) assays were used to determine the antioxidant capacity according to a previously established procedure by Hatano, and Plank and collaborators [17,18]. The absorbance of the resulting solutions was measured at 517 nm and 732 nm using a spectrophotometer (UV-2602, Labomed, inc, USA). The excipients dissolved in methanol were used as controls. The TEAC was expressed as milligrams of gallic acid equivalents (GAE) per 100 g of powder.

2.7.3. Ferric Reducing Antioxidant Power (FRAP)

It was used to measure the antioxidant potential in samples by reducing the ferric iron in the solutions. The FRAP assay was conducted according to the methodology described by Benzie and collaborators [19]. Absorbance measurements of the colored product (ferrous tripyridyltriazine complex) were taken at 593 nm with a spectrophotometer (UV-2602, Labomed, inc, USA). Data were express in mg of ascorbic acid equivalents per 100 g of powder.

2.8. Viable Microbial Count

The total aerobic viable bacterial count, and the viable yeast and mold counts were determined according to the methodology described by the AOAC 2015.13, AOAC 991.14 and AOAC 997.02, respectively [20,21,22].

3. Results and Discussion

3.1. Preformulation Studies

Formulations having citric acid and sodium carbonate at the 3:2 stoichiometric ratio were selected as ideal in terms of solubilization time, pH and the preliminary sensory evaluation. Likewise, the lyophilized tamarillo having the same acid-alkali (3:2) ratio had the best preliminary sensory characteristics. Further, eight formulations with different levels of the compounds are listed in Table 2.

The solubilization time and sensory attributes were taken as dependent variables. The lyophilized tamarillo at the 20-30% level contributed to the good palatability which was in agreement with the requirements of the regulatory entities for beverages (fruit content larger than 10 %) [23]. All runs containing lyophilized tamarillo, citric acid, sodium carbonate and saccharose had a rapid solubilization (<51 s) and good acceptability (>64%)

(Table 2). Further, high levels of citric acid and lyophilized tamarillo along with low levels of maltodextrin resulted in larger solubilization times. The moisture content remained under 2% in all formulations, which is essential for an effervescent product. Table 1 and Figure 1 depict the results of the sensory properties taken in triplicate per panelist. There were no significant differences between the average score for aroma, color and appearance showing a high score. However, attributes such as flavor, sweetness, bitterness (sourness) were significantly different ($p < 0.05$). Although all the attributes had a great influence on the overall average acceptability of the product, the high scores were related to high levels of maltodextrin. Thus, Figure 2 depicts the optimization analysis based on the average sensory properties. It also shows that high levels of sugar and tamarillo along with low levels of sodium carbonate and citric acid resulted in the best sensory properties. These conditions matched those found in formulation 7 and this product was selected for further testing.

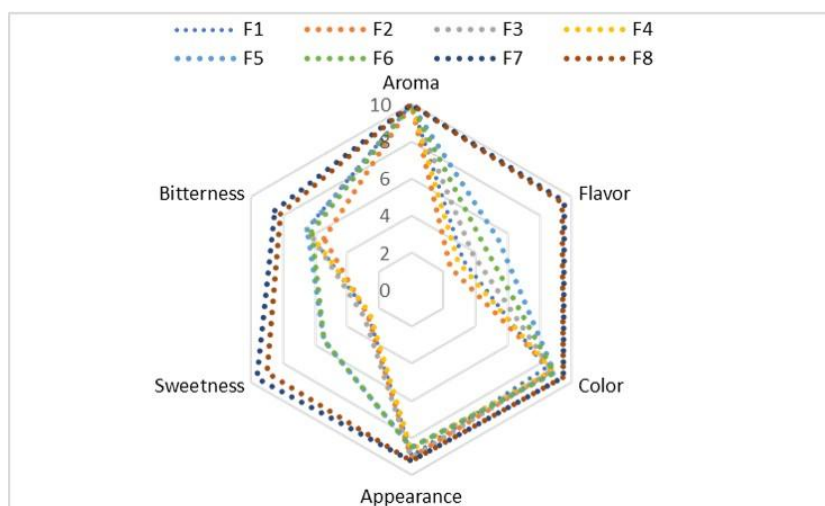


Figure 1. Sensory evaluation of each formulation. Values are expressed as mean $\pm$ standard deviation (SD) of 15 panelists

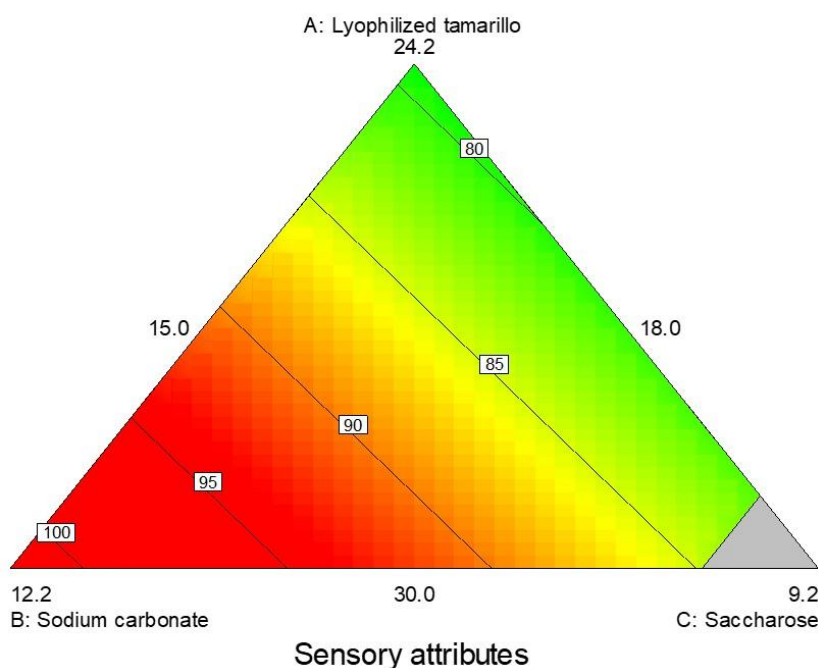


Figure 2. Sensory evaluation of each formulation. Values are expressed as mean $\pm$ standard deviation (SD) ($n = 15$) of 15 panelists

3.2. Physico-chemical and Antioxidant Studies

The physico-chemical properties of the lyophilized tamarillo and the optimal effervescent powder are listed in Table 3. It can be observed that pH, acidity and total suspended solids of the final reconstituted formulation met the requirements established by the food regulatory agencies. Thus, an instant beverage should contain TSS <0.3% acidity and pH below 4.5 [23]. A low pH value is required to hinder further microbial growth. The Brix value was similar to those values given by commercial drinks [24]. Likewise, the titrable acidity was comparable to that recommended to preserve the natural flavor of the fruit as well as to inhibit microbial growth. This could be attributed to the contribution of the inherent acidity present in the tamarillo fruit. The results of the antioxidant activity conducted by the ORAC, TEAC and FRAP assays from each formulation are listed in Table 3.

Table 3. Physico- chemical and Antioxidant Properties of lyophilized tamarillo and the optimal effervescent formulation

Property	Lyophilized tamarillo (1g/ 50mL)	Effervescent Tamarillo powder (1g/ 50mL)
pH	3.5 ± 0.2	4.1 ± 0.1
Titrable acidity g/100mL (Citric acid equivalents)	0.3 ± 0.0	0.20 ± 0.0
Total suspended solids (TSS)	0.7 ° ± 0.1*	0.9 ° ± 0.1*
Moisture (g/100g)	1.9 ± 0.2*	1.9 ± 0.2*
ORAC value (μmol Trolox/100g)	34620.0 ± 143.3	37220.9 ± 162.7
TEAC value DPPH (mg eq. Trolox/ 100g)	5022.0 ± 20.2	7646.2 ± 67.3
TEAC value ABTS (mg eq. Trolox/100mL)	3941.6 ± 96.2	7377.7 ± 73.2
FRAP value (mg Eq. Ascorbic acid/100 g)	6010.9 ± 82.7	6291.9 ± 87.0
Total phenolic compounds (mg eq. GAE/100g)	3773.1 ± 7.7	4463.7 ± 12.9

Asterisk within rows indicate no significant differences between samples ($p < 0.05$) according to Fisher's least significant difference (LSD-Fisher) test; values are expressed as mean ± standard deviation (SD) (n= 3).

Further, the antioxidant activity resulted from the ORAC, TEAC and FRAP tests was higher in the best effervescent product as compared to the lyophilized tamarillo. Therefore, the antioxidant activity of citric acid *per se* enhanced the total antioxidant activity of the optimal formulation. The FRAP technique showed the highest correlation ($r = -0.924$) with the content of ascorbic acid and phenolic compounds. Likewise, the total phenolic content of the optimal product was higher than that of the lyophilized tamarillo. This is explained by the synergistic antioxidant activity of citric acid incorporated as additive (ORAC value 608.45 ± 101.17).

3.3. Microbial Analysis

No viable bacteria, yeasts or molds were found in these tests. This phenomenon is explained by the hindering effect of microbial growth in an acidic environment. These results are in compliance with the regulatory specifications. The total bacteria, yeast and mold counts

should not exceed 50.0 cfu/mL and 2.0 cfu/mL, respectively. Further, enterobacters such as *E. coli* should be absent in 100mL of sample [23].

Other effervescent fruit formulations have been previously reported [25]. For instance, the development of water-soluble effervescent products containing probiotics, facilitates daily ingestion of probiotic organisms, improving absorption due to rapid dissolution and stabilization of the gastric mucosa caused by carbon dioxide [26]. Other studies have shown that ascorbic acid in *G. tenax* fruit acts as an absorption factor for dietary iron [27]. Compression of fruit powders has also gained popularity due to the ease of storage, transportation, and administration. Further, effervescent products could be a good alternative to accomplish fast product dissolution [28]. These studies are in agreement with our results confirming that fruit-based effervescent products are a good strategy to produce fast-dissolving fruit powders with biological properties.

4. Conclusions

An effervescent product based on tamarillo powder was produced by employing a mixture experimental design with eight runs. Citric acid, sodium carbonate and saccharose were employed as excipients at different levels. The best formulation had tamarillo powder, maltodextrin, citric acid, saccharose, sodium carbonate, and tricalcium phosphate at 30%, 22.8%, 20.0%, 15.0%, 12.0% and 0.2%, respectively. This formulation had a rapid solubilization time and high acceptability among the consumer panelists. The ORAC, TEAC and FRAP assays proved that this formulation had a higher antioxidant activity than the lyophilized product. The antioxidant activity was correlated with the total phenolic compounds and ascorbic acid present in the final product.

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Competing Interests

The authors have no competing interests.

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