ISSN 2007-9737

Optimization of Essential Oil Extraction from Agastache Mexicana Subsp. Xolocotziana through Surfactant-assisted Hydrodistillation: A Response Surface Approach and Pareto Front Analysis for Enhancing Antioxidant Activity and Yield

Anette Gisela Botello-Ojeda[1], María Beatríz Bernábe-Loranca[2,*], Lilia Alejandra Conde-Hernández[3], David Pinto-Avendaño[4]

[1] Tecnológico de Monterrey, Puebla, Departamento de Biotecnología, Puebla, Mexico

[2] Benemérita Universidad Autónoma de Puebla, Facultad de Ciencias de la Computación, Puebla, Mexico

[3] Benemérita Universidad Autónoma de Puebla, Facultad de Ingeniería Química, Puebla, Mexico

[4] Benemérita Universidad Autónoma de Puebla, Director General de Innovación y Transferencia del Conocimiento, Puebla, Mexico

{maria.bernabe, lilia.conde, david.pinto}@correo.buap.mx

Abstract Agastache mexicana subsp. mexicana and Agastache mexicana subsp. xolocotziana are endemic plants of Mexico known as red and white toronjil, respectively. These plants are popular in Mexico due to their ornamental and medicinal value. With the aim of obtaining the essential oil of Agastache mexicana subsp. xolocotziana with the highest quality, the extraction method known as surfactant-assisted hydrodistillation was employed. Subsequently, an evaluation of the extraction yield and antioxidant activity of the oil was conducted. Given that various variables influence the quality of the essential oil, it was necessary to employ the response surface methodology. Parameters such as the concentration of Tween 20, liquid-solid ratio, and extraction time were varied. The response variables included the extraction yield and antioxidant activity expressed through the IC50 value, determined using the DPPH method (IC50 DPPH). In this context, the IC50 value represents the concentration of essential oil required to neutralize 50% of free radicals in a solution, serving as an inverse indicator of antioxidant activity: the lower the IC50, the higher the antioxidant activity. As a result, the criterion for optimizing the extraction process, i.e., finding the overall optimal solution, is based on maximizing the extraction yield and minimizing the IC50 DPPH value (equivalent to maximizing antioxidant activity). The task of performing this optimization is challenging, and a method supported by the theory of order was employed to identify non-dominated solutions. This led to the identification of a set of non-dominated and non-comparable solution pairs, forming the non-dominated set used to construct the Pareto front. The obtained results reveal that the extracted essential oils exhibit the best antioxidant activity (represented by the lower IC_{50} DPPH value) and the highest extraction yield (%w/w).

Keywords. Essential oil extraction, multiobjetive, optimization, response surface.

ISSN 2007-9737

1 Introduction

Agastache mexicana subsp*. xolocotziana*, commonly known as "toronjil blanco" (white hyssop), is an endemic plant of Mexico with a minty aroma and flavor, lanceolate olive-green leaves with a crenate margin, and a white corolla with trichomes on its lower lip. It has a robust rhizome and is only found in a cultivated state in central Mexico. Hybridization between *Agastache mexicana* subsp*. mexicana* and *Agastache palmeri* is suggested as the origin of this taxon, given the absence of wild populations with white flowers, pollen, and sterile fruits, and vegetative reproduction through rhizomes [1].

In traditional medicine, a combination of *Agastache mexicana* subsp*. xolocotziana* and *Agastache mexicana* subsp*. mexicana* is recommended for its tranquilizing, sleep-inducing, nervous relaxing, anti-hypertensive, and rheumatism treatment properties, as well as for treating stomach pain and menstrual cramps. *Agastache mexicana* subsp*. xolocotziana* is specifically used to treat heart diseases and is internationally commercialized due to its therapeutic properties.

Its essential oil has been found to contain more than 38 compounds, with estragole and methyl eugenol being the most abundant. Despite its great economic value and scientific interest, there are no reports on the antioxidant activity of its essential oil [2-6].

Surfactant-assisted hydrodistillation is a novel technique for extracting essential oils that involves adding surfactants to the water used in hydrodistillation to create micelles, which reduce the surface tension between the aqueous phase and the plant tissues. This technique aims to eliminate the physical barrier for water diffusion inside the cell, help moisten the plant material, and prevent oil deposits on the surface [7,8].

Tween 20 has been used as a surfactant in the extraction of essential oils from *Rosa damascena* Mill [9] and *Lavandula hybrida* L. [10], resulting in increased essential oil extraction yield. However, the use of amphiphilic compounds in essential oil extraction did not result in changes in the composition of the essential oil obtained, according to Solanki *et al*. [11].

The structure of this paper is presented as follows: In Section 1, an introduction is provided that addresses the characteristics of the plant from which the essential oil was extracted, as well as the extraction method employed. Section 2 details the materials and methods used in the essential oil extraction process, as well as in determining its extraction yield and antioxidant activity.

Section 3 addresses the multi-objective process, and to conclude, an table is included that provides details on the terminology used. Finally, the work concludes with the conclusions drawn from this research and the relevant bibliographic references.

2 Materials and Methods

2.1 Plant Material

Fresh aerial parts of *Agastache mexicana* subsp*. xolocotziana* were acquired from the "Central de Abastos de Puebla," a local market in Puebla, Mexico, in March and April of 2021. The plants were gathered at coordinates 19°14'50.9"N 98°28'00.1"W and verified by botanist Dr. Cristobal Sánchez Sánchez from the Ethnobotanical Garden Francisco Peláez Roldán in San Andrés Cholula, Puebla, Mexico.

A voucher (155907) was preserved for each specimen to ensure accurate identification. The plant material was then air-dried for ten days in a sheltered area at room temperature to eliminate moisture. After that, the dried plant material, which had a humidity level of 6.93±0.12 (%w/w), was tightly packed in plastic bags to prevent water contamination.

2.2 Surfactant-assisted Extraction

For the essential oil extraction, a Clevenger type distillation apparatus was used, comprising a heating source, a 2 L pear-shaped flask for generating steam by boiling water, a straight glass condenser, and a glass collector to separate essential oil from water. The experiments were conducted by introducing 30 g of dried aerial parts of *Agastache mexicana* subsp. *xolocotziana* into the 2 L pear-shaped distillation flask along with the necessary amount of distilled water and Tween 20.

The oils were extracted over the period required for each experiment (refer to Table 1). The extraction yield of the essential oil was calculated as a percentage (%) using the following formula:

$$\%\, extraction\, yield = \frac{Weight\ of\ essential\ oil}{Weight\ of\ sample} \times 100. \quad (1)$$

2.3 Antioxidant Activity

2.3.1 DPPH Radical Scavenging Activity

The antioxidant activity of *Agastache mexicana* subsp. *xolocotziana* essential oil (AMXEO) was determined by measuring its ability to scavenge radicals using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), following the method described by Rashidi *et al*. [12] with slight modifications. A 100 µL methanolic oil mixture of appropriate concentration was combined with 3.9 mL of freshly prepared 0.06 mM methanolic DPPH solution.

The mixture was shaken vigorously and allowed to stand at room temperature for 30 minutes to permit any reaction to occur. The absorbance values of the resulting solutions were measured at 517 nm using a ThermoFisher Scientific UV-Vis Spectrophotometer, with methanol as a blank. The antioxidant activity was expressed as the percentage inhibition of the DPPH radical (% inhibition), calculated using the following formula:

$$\%inhibition = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100, \quad (2)$$

where A_{blank} represents the absorbance values of the control reaction and A_{sample} is the absorbance value following the reaction with the essential oil. The antioxidant activity was expressed as the IC_{50} DPPH, which is the concentration of the oil that causes 50% inhibition. The IC_{50} was calculated from the percentage of inhibition versus the EO concentration plot. A lower IC_{50} value corresponds to better antioxidant activity.

The experimental data were fitted to an empirical model obtained by the Response Surface Methodology [13].

The following equation 3 was obtained by fitting the estimated constants and coefficients:

$$Y = 1.3587 - 0.1737\,A + 0.2600\,B - 0.0150\,C - 0.1272\,AB - 0.1113\,AC - 0.1862\,BC + 0.2971\,A^2 + 0.1664\,B^2 + 0.1462\,C^2, \quad (3)$$

where Y represents the extraction yield of *Agastache mexicana* subsp. *xolocotziana* EO (%), A Tween 20 concentration (g/mL), B solid-liquid ratio (g/L), and C extraction time (h).

The experimental data for the antioxidant activity (IC_{50} DPPH) was subjected to a regression analysis. The test variables were fitted to a second-order polynomial equation using coded values, as shown below:

$$IC_{50}DPPH = 71.47 + 18.33\,A - 12.48\,B - 17.14\,C - 0.24\,A^2 + 16.59\,B^2 + 17.30\,C^2 - 11.85\,AB - 7.35\,AC - 7.37\,BC\ , \quad (4)$$

where IC_{50} DPPH refers to the AMXEO antioxidant activity determined by the DPPH assay (mg/mL), A represents Tween 20 concentration (g/mL), B represents the solid-liquid ratio (g/L), and C represents the extraction time (h).

The previous two equations were used to generate theoretical data, which were then filtered and used to construct a Pareto's front.

3 Multi-objective Solutions for the Antioxidant Activity and Extraction Yield Issue in the Essential Oils

In this section, a set of solutions that fall within the Pareto's front is presented. These solutions have been filtered from a large set of solutions obtained through a design of experiments with two objectives: 1) antioxidant activity (IC_{50}) and 2) yield. The design of experiments yielded 500 solutions, and the method for obtaining a non-dominated subset generated 90 solutions.

Therefore, we have obtained an elite set of solutions for decision-makers to choose the most suitable ones based on specific criteria (lower IC_{50} and higher extraction yield, or vice versa).

The method for solving this problem consists of determining the extraction conditions that allow for maximum extraction yield and minimum IC_{50} DPPH (the best antioxidant activity). This involved finding a global optimal solution that maximizes extraction yield and minimizes IC_{50} DPPH. This type of optimization is not an easy task, so a method based on the theory of ordering was used to identify non-dominated solutions.

This allowed us to find a set of non-dominated and incomparable solution pairs that formed the non-dominated set used to construct the Pareto's front (Fig1) [14]. Efficiency (Pareto optimality) is crucial because any inefficient solution does not represent a preferred alternative for decision- making.

The data used to construct the Pareto's front were obtained through regression analyses performed using Minitab Version 21.3 software, as previously presented (equations 2 and 3). The application called NODOM was used to find the set of non-dominated and incomparable solution pairs, which form the non-dominated set used to construct the Pareto´s front (Fig1).

NODOM works as follows: it accepts a set of vectors as input (a txt file). NODOM also requires the number of objectives as input. The output is a file containing the set of non-dominated and incomparable solutions [14].

According to the literature, and in broad terms, the multi-objective problem at hand can be informally expressed as follows:

Let A = Min/Max $\{(f_1, f_2)|\ f_1$ is the c antioxidant activity value and f_2 is the yield value}. The set A is subject to the constraints of antioxidant activity and extraction yield, which are detailed in the past sections. Then, if (A, non-comparable Pareto's order) is a partially ordered set, then (f_1, f_2) = set of minimals of A. The non-comparable Pareto order is the negation of Pareto Dominance (DP) [15].

Pareto Dominance (PD). It is said that a vector $\vec{u} = (u_1, \dots, u_k)$ dominates $\vec{v} = (v_1, \dots, v_k)$ (denoted by $\vec{u} \preccurlyeq \vec{v}$) if and only if it is partially smaller than $\vec{v}$. In other words, if $u_i \leq v_i \forall\ i \in \{1, \dots, k\}$ and $\exists\ i \in \{1, \dots, k\}: u_i < v_i$

3.1 Multi-objective Problems: Basic Theory

In many disciplines, solving a problem or making a decision means finding the best common solution to a set of relationships.

Solving a multi-objective problem can be challenging, but it can be approached more effectively by identifying the relationships between the problem's characteristics, constraints, and main objectives that are sought to be improved together. In this context, it is sensible to express these problems using mathematical functions, which facilitates their understanding and treatment. When we talk about improving together, we refer to the simultaneous optimization of all the associated functions. In this way, a problem of the type described below is defined.

Definition 1. A multi-objective problem (MOP) can be defined in the case of minimization (and analogously for the case of maximization) as:

The simultaneous optimization of more than one objective means optimizing a function of the form $f: S \to T$, where $S \subseteq R^n$ and $T \subseteq R^k$.

It is clear that there is no element in S that produces an optimum simultaneously for each of the k objectives that compose f.

The multi-objective optimization problem can be formulated as follows:

Find a vector $x^* = [X_1^*, X_2^*, \dots, X_n^*]^n$ x that satisfies the m constraints:

$$g_i \geq 0 \text{ where } i = 1,2, \dots, m, \tag{5}$$

and the p constraints :

$$h_i(x) = 0 \text{ where } i = 1,2, \dots, p, \tag{6}$$

and optimize the vectorial function:

$$f(x) = [\mathrm{f}_1(x), \mathrm{f}_2(x), \dots, \mathrm{f}_\mathrm{k}(x)]^n, \tag{7}$$

where $x = [x_1, x_2, \dots, x_n]^T$ is the vector of decision variables.

In other words, we want to determine the particular solution, $X_1^*, X_2^*, \dots, X_n^*$ from the set S formed by all the values that satisfy (1) and (2), which lead to the optimal values for all the objective functions [16]. Sometimes, it is necessary to adjust or change the sign of the objective functions of the problem to present it in the manner described above.

Originally, multi-objective problems are presented in three forms: one in which all the functions are maximized, one in which all are minimized, and one in which some are maximized while others are minimized.

Starting from definition 1, common sense leads us to conceive multi-objective optimization as the search for a vector that represents the set of decision variables and optimizes (either maximizing or minimizing) the objective functions simultaneously. However, in this case, it is important to note that these functions may conflict with each other [16].

It is clear that multi-objective problems generate multiple solutions, and for the problem of obtaining essential oils, the method based on the concept of Pareto efficiency has been employed, which involves reaching a set of non-dominated vectors.

This leads to the concept of partially ordered set, which is studied in mathematics within the areas of algebra and combinatorics [17].

A more extensive explanation of the ordering relationships for multi-objective problems can be found in various literature, however, for the problem addressed in this work, a summary of the theory of order can be seen in [15].

The following definitions cannot be excluded in order to understand the concept of non-dominated solutions that make up the Pareto front.

Definition 2. Given a set A and $(\preccurlyeq)$ a partial order relation on it, we call the pair $(A, \preccurlyeq)$ a **partially ordered set**, also referred to as a **Poset**.

Definition 3. Given $(A, \preccurlyeq)$ a Poset, the subset $X \subseteq A$ is said to be a **total order** or chain with respect to $(\preccurlyeq)$ if and only if it satisfies $x \prec y$ or $y \prec x$ for all $x, y \in X$. In this case, $(X, \preccurlyeq)$ is called a totally ordered set.

If $x \npreceq y$ holds for all x, y ∈ X, then X is said to be an antichain. Chains can be finite or infinite and can also be stationary at one of their ends, such as:

$$x_0 \prec x_1 \prec x_2 \prec \dots \prec x_{i-1} \prec x_i \prec x_{i+1} \prec \dots$$

0

$$\dots \prec x_{i+1} \prec x_i \prec x_{i-1} \prec \dots \prec x_2 \prec x_1 \prec x_0$$

The following statement is crucial for generating non-dominant solutions:

Starting from a partial order, the dominance relation $(\prec)$ can be defined as follows:

$$x \prec y \Leftrightarrow x \preccurlyeq y \wedge x \neq y.$$

When it happens that $x \npreceq y \wedge y \npreceq x$, they are said to be incomparable, denoted by $x \parallel y$.

On the other hand, it is also important to mention Zorn's lemma, also known as Kuratowski-Zorn lemma [18]. It is a proposition in set theory that states the following:

Every non-empty partially ordered set in which every chain (totally ordered subset) has an upper bound, contains at least one maximal element.

A **maximal element** of a partially ordered set P is an element of P that is not smaller than any other element. The term minimal element is defined in a dual way.

Definition 4. Let $(P, \leq)$ be a partially ordered set; $m \in P$ is a **maximal** element of P if the only $x \in P$ such that $m \leq x$ is $x = m$.

The definition of minimal element is obtained by replacing ≤ with ≥.

At first glance, it might seem that m should be a maximum element, which is not always true: the definition of a maximal element is slightly weaker. In fact, there can be maximal elements without a maximum.

The reason is that, in general, ≤ is only a partial order in P; if m is maximal and $p \in P$, it is possible that neither $p \leq m$ nor $m \leq p$, so m would not be a maximum. This also allows for the possibility of having more than one maximal element in a set.

However, if $m \in P$ is maximal and P has a maximum, it will hold that $max(P) \leq m$; by the definition of a maximum, we must have $m \leq max(P)$ and therefore $m = max(P)$; in other words, a maximum, if it exists, is also the unique maximal element.

It is not hard to see that if ≤ is a total order in P, the notions of maximum and maximal coincide: let $m \in P$ be a maximal element and $p \in P$ be arbitrary; by the total order condition, either $p \leq m$ or $m \leq p$; in the second case, we would have $p = m$ and so, $m = max(P)$.

Not always do maximal elements exist, even in the case where P is totally ordered [17].

Non-dominated solutions are minimal in a Hasse diagram, which is a simplified graphical representation of a finite partially ordered set. This is achieved by eliminating redundant information. To do this, an upward edge is drawn between two elements only if one follows the other without any intermediate elements.

The Hasse diagram of S is defined as the set of all ordered pairs (x, y) such that y follows x, in other words, the Hasse diagram can be identified with the relation of "*follow*".

3.2 Dominance Scheme

In the field of multi-objective optimization, a certain scheme must be decided upon for improving one solution over another, that is, which solutions will be chosen to be more suitable. This relationship of improvement of one individual over another is what we call a *Dominance Scheme*.

The definition of a Dominance Scheme is mainly based on the fact that a multi-objective problem does not have a unique solution, and therefore the decision maker must choose from a range of possible solutions that cannot be improved upon each other, meaning that they are non-dominated, and select the one that best suits their needs.

At this point, the expert who solved the multi-objective problem of antioxidant activity of essential oils, when they have obtained the Pareto Front, will have a set of solutions to choose from depending on their interests.

Within the field of real numbers, the ordering is naturally defined. For R^n, we can extend the concept using the following definition:

Definition 5. Given x, y vectors in R^n, $x \leq y$ if and only if $x_k \leq y_k$ for every $k \in \{1 \dots n\} \wedge x < y$ if and only if $x \leq y$ with $x \neq y$.

3.3 Optimum and Pareto Front

A common option used as a dominance relation is the well-known Pareto dominance defined as follows:

Definition 6. Given the multi-objective problem

Minimize: $f(x)$

where $f: F \subseteq R^n \rightarrow R^q \; q \geq 2$.

With $A \subseteq F$ as the feasible region. We say that a vector $x^* \in A$ is non-dominated or a Pareto optimum if there does not exist a vector $x \in A$ such that $f(x) < f(x^*)$.

Thus, the answer to the problem of finding the best solutions (the non-dominated solutions, however dominance is defined within the technique) in a multi-objective problem is what we call the *solution set* of the problem.

The set of objective function values restricted to the vectors in the solution set (i.e., the non-dominated vectors) is known as the Pareto's front.

In general, and specially for real-life problems, it is not easy to find the Pareto front analytically (and in most cases, it is impossible).

A concept closely related to the Pareto front is the Pareto optimum. Both the Pareto optimum and the Pareto front are the framework for working within the multicriteria decision-making.

Definition 7. The set $E(A; f)$ of efficient Pareto solutions (also known as the **set of Pareto optima**) is defined as follows $E(A; f) \coloneqq \{a \in A : \nexists b \in A$ that meets $f(b) < f(a)\}$

In other words, it is the set of all non-dominated vectors under the Pareto scheme.

In summary, the set of Pareto optima is the solution space of the problem, and the Pareto front is its image with respect to the function:

$$f: F \subseteq R^n \rightarrow R^q q > 2$$

to be optimized.

The Pareto optimum for a given multi-objective problem is a partially ordered set, formally speaking. In multi-objective problems, we seek the minimal elements of the solution space R^n viewed as a Poset under the naturally defined $\leq$ relation.

3.4 Method for Finding the Maximum: Non Dominated Solutions

When considering two objectives simultaneously (maximizing yield, minimizing IC50) and conducting the simultaneous measurement of both responses, it becomes evident that these responses are conflicting and require resolution through multi-objective techniques.

If we were to address each objective separately, we might opt to solely maximize yield or minimize antioxidant activity. However, by combining both goals, we achieve a higher-quality and more profitable oil. Essentially, independently evaluating these aspects does not allow for the effective design of a process to obtain high-quality essential oil, as this quality, although intrinsically related to the oil's chemical components and reflected in its antioxidant activity, is effectively measured by determining its antioxidant activity, thus ensuring the quality of the essential oil along with its yield.

Maximizing yield, in this context, implies the ability to obtain the maximum possible amount of essential oil from the plant, optimizing the process to reduce costs. By integrating both aspects, we can obtain a quality oil at an efficient cost, representing an ideal combination of cost and quality.

Ndominated or nodom is an application derived from the article "On finding the maxima of a set of vectors" [19].

Nodom is the implementation of the algorithm "Algorithms to identify nonodom solutions in a multi-dimensional set". The program is implemented in C code and identifies non-dominated solutions from a data set. The code and details for its use are available at http://www.cs.cinvestav.mx/~emoobook/nodom/nonodom.html.

Two versions of this algorithm have been implemented, the first by Luis Vicente Santana Quintero and Antonio López Jaimes. This algorithm has a complexity of O(n2) and is currently the most widely used.

The second version was developed by Kung, Luccio, and Preparata [19] and finds a set of non-dominated solution vectors (also known as maxima elements). This algorithm requires $O(n \log n)$ comparisons for $k = 2$, and $O(n(\log k - 2n))$ $O(n(\log k - 2n))$ comparisons for $k >= 3$, where n is the size of the solution set in the input and k is the dimension of the vector.

Algorithm for Finding the Set of Non-Dominated Solutions: The Kung, Luccio, and Preparata algorithm (1975) provides an efficient solution for finding the set of non-dominated solutions in a multi-dimensional solution space. It utilizes a divide-and-conquer approach along with intelligent comparisons to minimize the number of required operations. This technique is widely used in multi-objective optimization problems and data analysis where identifying optimal or non-dominated solutions is necessary.

a) Coordinate Sorting: The first step of the algorithm involves sorting the solution set V by coordinates in each dimension. This means that solutions are sorted based on their values in the first dimension, then based on their values in the second dimension, and so on.

b) Division: After sorting the solutions, a "divide and conquer" approach is applied to find the non-dominated solutions. The sorted set of solutions is divided into two parts. Then, non-dominated solutions are recursively found in each of the two parts. Finally, the non-dominated solutions from both parts are combined to obtain the global set of non-dominated solutions.

c) Merging Non-Dominated Solutions: During the merging process, solutions from both parts are compared, and those not dominated by any other solution are selected. This is efficiently done using a comparison where each solution is compared with the last selected element from the other part. If a solution is dominated by the last selected element, it is discarded; otherwise, it is added to the set of non-dominated solutions.

The "Merge of Non-Dominated Solutions" is the step where non-dominated solutions from two subsets are combined after dividing the original set of solutions. The merging of non-dominated solutions is done by comparing each solution in one subset with the last selected element of the other subset and adding the solutions that are not dominated by this last selected solution. This efficient approach helps to find the global set of non-dominated solutions optimally.

Algorithmic Strategy:

i. Comparison with the Last Selected Element: During merging, each solution from the current subset is compared with the last selected element from the other subset.

Suppose we are merging two subsets A and B, where A contains the non-dominated solutions selected so far, and B contains the non-dominated solutions from the other subset. Each solution in subset A is compared with the last selected solution from subset B, and vice versa.

ii. Discard or Addition of Solutions: If a solution in A is dominated by the last selected solution from B, it is discarded as it cannot be non-dominated in the global set.

If a solution in A is not dominated by the last selected solution from B, it is added to the set of non-dominated solutions. The same applies to the solutions in B: if a solution in B is dominated by the last selected solution from A, it is discarded; otherwise, it is added to the set of non-dominated solutions.

iii. Efficiency: This strategy is efficient as it avoids comparing each solution in one subset with all solutions in the other subset. Instead, only one comparison is made with the last selected

ISSN 2007-9737

Table 1. Data of Pareto's front

Yield (%)	IC50 DPPH (mg/mL)	Yield (%)	IC50 DPPH (mg/mL)	Yield (%)	IC50 DPPH (mg/mL)	Yield (%)	IC50 DPPH (mg/mL)	Yield (%)	IC50 DPPH (mg/mL)	Yield (%)	IC50 DPPH (mg/mL)
1.240	127.61	1.311	74.356	1.303	75.557	1.322	72.595	1.363	68.527	1.637	58.167
1.265	100.54	1.312	74.276	1.283	78.734	1.322	72.610	1.361	68.755	1.670	57.158
1.364	68.327	1.310	74.436	1.303	75.618	1.322	72.639	1.398	66.420	1.672	57.120
1.344	71.339	1.313	74.171	1.325	72.063	1.286	78.186	1.399	66.364	1.674	57.064
1.426	64.507	1.311	74.329	1.321	72.981	1.321	72.713	1.354	70.601	1.686	56.709
1.282	78.962	1.306	75.109	1.303	75.742	1.321	72.728	1.361	68.956	1.690	56.596
1.488	63.617	1.281	79.246	1.324	72.096	1.321	72.743	1.361	68.849	1.704	56.184
1.277	79.957	1.317	73.717	1.324	72.108	1.321	72.759	1.354	70.517	1.761	54.628
1.278	79.717	1.323	72.375	1.324	72.130	1.321	72.789	1.425	64.555	1.356	69.746
1.362	68.643	1.322	72.538	1.302	75.898	1.290	77.536	1.772	54.347	1.355	70.148
1.268	82.535	1.322	72.624	1.324	72.153	1.321	72.821	1.436	63.989	1.355	70.058
1.271	81.770	1.282	79.039	1.324	72.165	1.288	77.943	1.440	63.785	1.355	70.022
1.267	93.079	1.320	73.454	1.324	72.177	1.287	78.113	1.377	67.369	1.355	70.004
1.272	81.226	1.316	73.815	1.324	72.200	1.321	73.098	1.367	67.933	1.355	69.986
1.270	82.003	1.314	74.016	1.324	72.212	1.302	75.921	1.367	67.919	1.356	69.934
1.274	80.805	1.315	73.940	1.323	72.236	1.321	73.133	1.368	67.794	1.356	69.882
1.267	93.361	1.313	74.145	1.323	72.249	1.286	78.309	1.490	61.793	1.356	69.830
1.388	67.226	1.313	74.093	1.323	72.286	1.321	73.237	1.376	67.399	1.356	69.763
1.277	80.011	1.327	71.815	1.323	72.298	1.321	73.255	1.376	67.392	1.356	69.697
1.278	79.904	1.307	74.965	1.323	72.324	1.321	73.309	1.519	60.984	1.356	69.680
1.600	59.361	1.327	71.833	1.323	72.336	1.370	67.648	1.486	63.658	1.356	69.632
1.307	74.879	1.327	71.842	1.323	72.349	1.361	68.817	1.379	67.301	1.356	69.552
1.308	74.823	1.327	71.852	1.323	72.362	1.789	53.915	1.487	63.637	1.356	69.536
1.308	74.794	1.326	71.861	1.323	72.402	1.781	54.122	1.383	67.248	1.357	69.474
1.308	74.766	1.326	71.880	1.322	72.415	1.356	69.813	1.383	67.242	1.357	69.443
1.309	74.682	1.305	75.197	1.322	72.428	1.776	54.234	1.555	60.204	1.357	69.428
1.309	74.655	1.305	75.256	1.322	72.455	1.353	70.828	1.586	59.698	1.357	69.413
1.309	74.627	1.326	71.928	1.284	78.583	1.559	60.142	1.598	59.417	1.357	69.383
1.310	74.545	1.304	75.375	1.322	72.496	1.340	71.767	1.618	58.783	1.357	69.368
1.310	74.490	1.325	71.968	1.322	72.524	1.423	66.078	1.608	59.081	1.357	69.353
1.310	74.463	1.325	71.978	1.322	72.552	1.359	69.072	1.607	59.118	1.358	69.251
1.308	74.738	1.325	71.989	1.322	72.567	1.390	67.184	1.583	59.939	1.358	69.237
1.311	74.382	1.325	71.999	1.322	72.581	1.322	72.483	1.626	58.521	1.830	52.900

element from the other subset, significantly reducing the number of required comparisons.

iv. Iteration: This process of comparison and addition or discarding is repeated until all solutions in both subsets have been processed.

To understand how non-dominated solutions are recursively found in each of the two parts during the divide and conquer, it is important to consider how solution sets are divided and how algorithm steps are applied in each subdivision.

Division of Solution Sets:

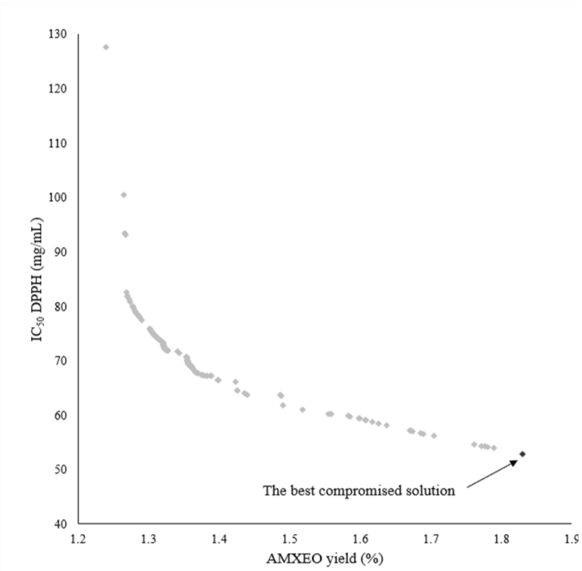

Fig. 1. Pareto's front

I. The algorithm begins with a solution set V. This set is divided into two subsets V1 and V2 using some splitting criterion such as half of the set or some other approach.

II. Recursion in Each Subset: Once subsets V1 and V2 are formed, the algorithm recursively applies the same process on each subset to find the non-dominated solutions in them.

That is, the same algorithm is called recursively, but this time with V1 as input in one call and V2 as input in the other call.

III. Merging Non-Dominated Solutions: After non-dominated solutions have been found in each of the subsets, merging of non-dominated solutions is performed. This involves comparing solutions from both subsets and selecting those not dominated by any other solution.

IV. Global Set of Non-Dominated Solutions: Finally, the global set of non-dominated solutions is obtained by combining the non-dominated solutions from both subsets after merging.

Considering the above, the following repository shows all the solutions generated with the design of experiments for antioxidant activity (IC_{50} DPPH) and extraction yield understood as f1 and f2 respectively.

Table 1 shows the Minima set, which is the set of minimals that form the Pareto's front. This set contains the non-dominated and incomparable solutions.

The above table produces the following figure (Fig1) representing the solution set of the problem and the values of the objective functions, restricted to the non-dominated vectors, that is, the Pareto frontier. Microsoft 365 Excel software was used to construct Fig1. The objective functions correspond to the maximization of AMXEO extraction yield (objective 1) and the minimization of IC_{50} DPPH (objective 2).

The optimal levels of the operating parameters are observed as 1.83% of AMXEO extraction yield and an IC_{50} DPPH of 52.9 mg/mL, corresponding to the following process conditions: 0.001 g/mL concentration of Tween 20, 30.8 g/L solid-liquid ratio, and 105 min extraction time. The optimization was performed by assigning equal weights to each function.

In the Fig1, the points corresponding to the following criteria can be distinguished:

Max.min This represents the desired point since, in this particular process, the goal is to maximize the yield and minimize the IC_{50} value (indicative of better antioxidant activity). At this point, an AMXEO extraction yield of 1.83% and a DPPH IC_{50} of 52.9 mg/mL are obtained, under the following process conditions: a concentration of 0.001 g/mL of Tween 20, a solid-liquid ratio of 30.8 g/L, and an extraction time of 105 minutes.

Min.max This point represents a scenario of minimum yield and maximum IC_{50} value (indicative of poorer antioxidant activity). This result would be precisely the opposite of what we aim to achieve, being the least favorable option and possibly the one we want to avoid at all costs. It would entail a costly process due to the low yield and the production of essential oil of poor quality, with low antioxidant activity.

This scenario translates to a yield of 1.27% and an IC_{50} of 100.5 mg/mL (almost double the value of the best predicted antioxidant activity), under the following process conditions: a concentration of 0.001 g/mL of Tween 20, a solid-liquid ratio of 15.8 g/L, and an extraction time of 30 minutes.

Min.min The inflection point, marked by a minimum yield and a minimum value of antioxidant activity, would imply obtaining an essential oil of

ISSN 2007-9737

Table 2. List of the used terms

IC_{50} DPPH	Concentration of antioxidant required to reduce the initial absorbance of DPPH• by 50%.
Essential oil	Natural compounds with a strong aroma produced as secondary metabolites in various aromatic plants.
Surfactant-assisted hydrodistillation	Method for extracting essential oils in which polysorbates are added as surfactants and emulsifiers to the water used in EO extraction with the aim of forming micelles that simultaneously reduce the surface tension between the aqueous phase and the hydrophobic surface of plant tissues and, on the other hand, between the aqueous and oily phases. This way, the physical barrier to water diffusion within the cell is eliminated, helping to moisten the plant material and preventing oil deposition on the
Antioxidant activity	The ability of a substance to inhibit oxidative degradation, slowing it down or even delaying it.
Extraction yield	Amount or proportion of essential oil obtained in relation to the amount of dry raw material used in the process. It is expressed as a percentage and is calculated by dividing the amount of extracted essential oil by the initial amount of plant material or substance from which it is extracted. A higher yield indicates a more efficient and effective extraction of the desired essential oil. This parameter is essential for assessing the efficacy of extraction methods and for optimizing processes in the essential oil industry.

the highest quality, reflected in the best possible antioxidant activity. However, this would come at the expense of a sacrifice in yield, resulting in a costly process due to reduced production. In this work, we focus on the situation of max.min.

The points assigned by the Pareto front provide a clear indication of the solution to our problem. However, at the inflection point, there are four points that it is important to evaluate to determine their relevance to our problem.

At the moment, these points are beyond our scope, but it is proposed in future work to utilize multicriteria analysis as a tool to identify and define this subset, as well as to determine the substantial or relative importance of this set of inflection points on the Pareto front for our problem.

Given this situation, we can affirm that the previously presented solutions (min.max, max.min) are convenient and depend on specific circumstances. For example, if our goal is to obtain the maximum possible amount of essential oil regardless of its antioxidant activity because the practical application of the oil serves another purpose (such as aroma, for example), then the min.max approach would be the best option.

However, if the application of the essential oil requires its best antioxidant activity and we cannot afford to sacrifice economic resources, our objective would be to maximize antioxidant activity while minimizing costs, that is, max.min.

4 Conclusions and Future Work

The analysis of the Pareto´s front highlighted the importance of considering multiple objectives in the optimization process. By simultaneously optimizing both performance and antioxidant activity, we were able to achieve a more holistic approach in the extraction process, ensuring both economic efficiency and high-quality products. By comparing the solutions along the Pareto’s front, we were able to identify the parameter value ranges that lead to the best compromised solutions of extraction yield and antioxidant activity.

In general, this research emphasizes the importance of considering multiple objectives in the optimization of essential oil extraction processes. The analysis of the Pareto’s front provides a powerful tool for decision-making, allowing stakeholders to make informed decisions based on their preferences and priorities.

Finally, the Pareto front indicates that higher antioxidant activity corresponds to higher extraction yield; however, this may not necessarily be applied. When the cost of enhancing antioxidant activity becomes prohibitive, a solution with low extraction yield and moderate antioxidant activity (such as solution 23 from the set of solutions) can be chosen from within the Pareto front.

As a future work, it is proposed to use multicriteria analysis to narrow down the subset related to the inflection point and determine the substantial or relative importance of that set of inflection points on the Pareto front for our problem.

Acknowledgment

Author Anette Gisela Botello Ojeda expresses gratitude to the Consejo Nacional de Ciencia y Tecnología (CONACyT) in Mexico and the Benemérita Universidad Autónoma de Puebla (BUAP) for the financial support that facilitated the completion of her doctoral studies.

References

1. **Carrillo-Galván, G., Bye, R., Eguiarte, L. E., Cristians, S., Pérez-López, P., Vergara-Silva, F., Luna-Cavazos, M. (2020).** Domestication of aromatic medicinal plants in Mexico: Agastache (Lamiaceae)—an ethnobotanical, morpho-physiological, and phytochemical analysis. Journal of Ethnobiology and Ethnomedicine, Vol. 16, pp. 1–16. DOI: 10.1186/s13002-020-00368-2.
2. **Estrada-Reyes, R., López-Rubalcava, C., Ferreyra-Cruz, O. A., Dorantes-Barrón, A. M., Heinze, G., Aguilar, J. M., Martínez-Vázquez, M. (2014).** Central nervous system effects and chemical composition of two subspecies of Agastache mexicana; an ethnomedicine of Mexico. Journal of Ethnopharmacology, Vol. 153, No. 1, pp. 98–110. DOI: 10.1016/j.jep.2013.12.057.
3. **Juárez, Z. N., Bach, H., Sánchez-Arreola, E., Bach, H., Hernández, L. R. (2016)**. Protective antifungal activity of essential oils extracted from Buddleja perfoliata and Pelargonium graveolens against fungi isolated from stored grains. Journal of Applied Microbiology, Vol. 120, No. 5, pp. 1264–1270. DOI: 10.1111/jam.13092.
4. **Najar, B., Marchioni, I., Ruffoni, B., Copetta, A., Pistelli, L., Pistelli, L. (2019).** Volatilomic analysis of four edible flowers from Agastache genus. Molecules, Vol. 24, No. 24, p. 4480. DOI: 10.3390/ molecules24244480.
5. **Rodríguez-Acosta, M., Jiménez-Merino, A., Coombes, A. J. (2010).** Plantas de importancia económica en el estado de Puebla. Puebla: Herbario y Jardín Botánico, Benemérita Universidad Autónoma de Puebla.
6. **Ventura-Martínez, R., Rodríguez, R., González-Trujano, M. E., Ángeles-López, G. E., Déciga-Campos, M., Gómez, C. (2017).** Spasmogenic and spasmolytic activities of Agastache mexicana ssp. mexicana and A. mexicana ssp. xolocotziana methanolic extracts on the guinea pig ileum. Journal of ethnopharmacology, Vol. 196, pp. 58–65. DOI: 10.1016/j.jep.2016.12.023.
7. **Charchari, S., Abdelli, M. (2014).** Enhanced extraction by hydrodistillation of sage (Salvia officinalis L.) essential oil using water solutions of non-ionic surfactants. Journal of Essential Oil Bearing Plants, Vol. 17, No. 6, pp. 1094-1099. DOI: 10.1080/0972060X.2014.935041.
8. **Tongnuanchan, P., Benjakul, S. (2014).** Essential oils: extraction, bioactivities, and their uses for food preservation. Journal of food science, Vol. 79, No. 7, pp. R1231-R1249. DOI: 10.1111/1750-3841.12492.
9. **Baydar, H., Baydar, N. G. (2005).** The effects of harvest date, fermentation duration and Tween 20 treatment on essential oil content and composition of industrial oil rose (Rosa damascena Mill.). Industrial crops and products, Vol. 21, No. 2, pp. 251-255. DOI: 10.1016/j.indcrop.2004.04.004.
10. **Filly, A., Fabiano-Tixier, A. S., Louis, C., Fernandez, X., Chemat, F. (2016).** Water as a green solvent combined with different techniques for extraction of essential oil from lavender flowers. Comptes Rendus Chimie, Vol. 19, No. 6, pp. 707–717. DOI: 10.1016/j.crci.2016.01.018.
11. **Solanki, K. P., Desai, M. A., Parikh, J. K. (2020).** Improved hydrodistillation process using amphiphilic compounds for extraction of essential oil from java citronella grass. Chemical Papers, Vol. 74, No. 1, pp. 145–156. DOI: 10.1007/s11696-019-00861-3.
12. **Rashidi, S., Eikani, M. H., Ardjmand, M. (2018).** Extraction of Hyssopus officinalis L. essential oil using instant controlled pressure drop process. Journal of Chromatography A, Vol. 1579, pp. 9–19. DOI: 10.1016/j.chroma.2018.10.020.
13. **Myers, R. H., Montgomery, D. C., Anderson-Cook, C. M. (2016).** Response surface methodology: process and product optimization using designed experiments. John Wiley & Sons.

14. **Bernábe-Loranca, B., Coello-Coello, C. A., Osorio-Lama, M. (2012).** A multiobjective approach for the heuristic optimization of compactness and homogeneity in the optimal zoning. Journal of Applied Research and Technology, Vol. 10, No. 3, pp. 447-457.
15. **Bernábe, B. (2006).** Desarrollo de un Modelo para la Determinación de Zonificación Óptima. Proyecto de tesis doctoral en desarrollo.
16. **Ehrgott, M. (2005).** Multicriteria optimization. Vol. 491. Springer Science & Business Media.
17. **Gierz, G., Hofmann, K. H., Keimel, K., Lawson, J. D., Mislove, M., Scott, D. S. (2003).** Encyclopedia of mathematics and its applications. Continuous lattices and domains, Vol. 93.
18. **Campbell, P. J. (1978).** The origin of Zorn's lemma. Historia Mathematica, Vol. 5, No. 1, pp. 77–89. DOI:.1016/0315-0860(78)90136-2.
19. **Kung, H. T., Luccio, F., Preparata, F. P. (1975).** On finding the maxima of a set of vectors. Journal of the ACM (JACM), Vol. 22, No. 4, pp. 469–476. DOI: 10.1145/321906.32191.

Article received on 16/04/2024; accepted on 21/07/2024.
**Corresponding author is María Beatríz Bernábe-Loranca.*