PIONEER AND ACADEMIC STUDIES IN NATURAL SCIENCE AND MATHEMATICS

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Editor
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Operational Developments in Matrix Theory

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ABSTRACT

In this section, operations in matrix theory are discussed. The contribution of the known operations in matrices, addition, subtraction and multiplication as well as division is analyzed. The relation of this operation in other fields and the new results obtained are presented. For example, the relation of matrices to special sequences and the relation of these sequences to each other are shown. Similarity and equivalence issues in matrix theory and the contribution of the operation are studied. The new results obtained are presented.

New results with linear matrix equation systems and division are presented. In addition, the concept of transpose in matrices and the existence of new results between division in detail are introduced.

New results of multiplication in matrices are obtained using division. Some of these results are that a regular matrix has infinite factors and every regular matrix can be written in terms of another regular matrix.

The existence of some known properties in rational numbers was also investigated in matrices. The new features obtained were presented. The property of simplification and expansion in rational numbers is also provided in rational matrices.

Finally, considering the multiplication and division property of matrices, the "POLOID" structure was created. Many properties related to this structure have been investigated.

Keywords – Matrix theory, matrix operations, division, matrix sequences, poloid.

INTRODUCTION

The term matrix is used for the first time in history as the "Magic Square" by the Chinese. Arab mathematicians are aware of the existence of this magic square. Cramer contributed significantly to the subject with the rule he developed in the 18th century with the solution of linear equation systems. The studies of Hamilton and Cayley started to be used significantly in the scientific world in the XIXth century. After a while Cayley defined operations on matrices and established many properties of these operations [1, 2]. Some basic results of the theory were given by Weierstrass, Jordan and Frobenius. Sylvester is introduced the "Matrix" theorem to the literature. [1]. This expression is very important in expressing and explaining systems of linear equations. This theory is studied and many application areas are established. Between 1850 and 1851, Sylvester is established the relationship between matrices and geometry [2].

Many researchers have contributed to the Gaussian elimination method together together with Gauss. The subject is now at its peak with computer technology [3].

Avital made important contributions to mathematics in his time [5]. Two authors Evard, and Gracia are conducted research on the applications of the study of similarity [5]. Many mathematical scientists are made important contributions to mathematical science on the subject in [6, 7, 8, 9, 10, 11, 13, 14]. We are defined poloid [12].

There are many matrix methods for natural structures. Thus, matrices have many application areas focused on solutions for natural structures. Matrices have found applications in science, computer science, physics, chemistry and engineering and have achieved indisputable results.

.

Let $\mathbb{F}$ be a field. Some notations and definitions are given below.

i. The set of matrices over a field $\mathbb{F}$ is

$$\mathbb{K}_n(\mathbb{F}) = \left\{ \left[a_{ij} \right]_n \middle| a_{ij} \in \mathbb{F}, n \in \mathbb{Z}^+ \right\}.$$

ii. The set of regular matrices over a field $\mathbb{F}$ is

$$\mathbb{M}_n(\mathbb{F}) = \left\{ \left[a_{ij} \right]_n \middle| a_{ij} \in \mathbb{F}, n \in \mathbb{Z}^+ \right\}.$$

iii. The transpose of matrix $A \in \mathbb{M}_n(\mathbb{F})$ is A^T.

iv. $A \sim B \Leftrightarrow A = PBP^{-1}, P \in \mathbb{M}_n(\mathbb{F})$.

v. Matrix $A \in \mathbb{M}_n(\mathbb{F})$ is called matrix involutive order k^{th} iff $A^{k+1} = I_n, k \in \mathbb{Z}^+$.

For any a $F \in \mathbb{M}_n(\mathbb{F})$, there are two matrix $A, B \in \mathbb{M}_n(\mathbb{F})$ such that $AB = F$. Also, Matrices A, B are unique.

If $B|F$, then there exists $A \in \mathbb{M}_n(\mathbb{F})$ such that,

$$BA = F \text{ or } F = BA.$$

Except for special cases in matrix multiplication, $AB \neq BA$.

The matrix $\left[d_{ji}^{c} \left({}_{B}^{F} i_j \right) \right]_n$ is the determinant of the matrix obtained by writing the ith column of the F matrix into the jth column of the B matrix in [6].

Definition 1.1.([6]) Let $\mathbb{M}_n(\mathbb{F})$.

i. The following operation "$-$" is defined on

$$-:\mathbb{M}_n(\mathbb{F})\times\mathbb{M}_n(\mathbb{F})\to\mathbb{M}_n(\mathbb{F}).$$

$$(B,F)\to\frac{F}{B}:=\frac{1}{|B|}\left[d_{ji}^{c}\left({}_{B}^{F}i_{j}\right)\right]_n,$$

where $\left[d_{ji}^{c}\left({}_{B}^{F}i_{j}\right)\right]_n$ is the column co-divisor matrix on the B matrix of the matrix F.

$\frac{F}{B}$ is the solution of the linear matrix equation $BX=F$.

$$\mathbb{Q}(\mathbb{M}_n(\mathbb{F}))=\left\{\frac{A}{B}\middle| A,B\in\mathbb{M}_n(\mathbb{F})\right\}$$

ii. The determinant of the new matrix obtained by writing on the jth row of the matrix B the ith row of the matrix A is called the *co-divisor by row* of the matrix A by the row on the matrix B. It is denoted by $d_{ij}^{r}\left(\underset{ij}{AB}\right)$. Their number is n^2. The matrix co-divisor by row is

$$\left[d_{ij}^{r}\left(\underset{ij}{AB}\right)\right], \text{ where } A,B\in\mathbb{M}_n(\mathbb{F}) \text{ [3].}$$

$\frac{1}{B}\left[d_{ij}^{r}\left(\underset{ij}{AB}\right)\right]$ is the solution of the linear matrix equation $XB=A$.

Theorem 1.2. ([12, 18]). If $\frac{A}{B}\in\mathbb{Q}(\mathbb{M}_n(\mathbb{F}))$, then, for least the regular matrix $A_1\in\mathbb{M}_n(\mathbb{F})$ exists such that $A=A_1A_2$ and $B=A_1B_2$ it satisfies equation,

$$\frac{A}{B}=\frac{A_1A_2}{A_1B_2}=\frac{A_2}{B_2}, \text{ where } A_2,B_2\in\mathbb{M}_n(\mathbb{F}).$$

Definition 1.3.([12]) A group is a set $\mathbb{P}$ equipped with a binary operation $*:\mathbb{P}\times\mathbb{P}\to\mathbb{P}$ that associates an element $a*b\in\mathbb{P}$ to every pair of elements $a,b\in\mathbb{P}$, and having the following properties: $*$ is associative, has an identity element $e\in\mathbb{P}$, and every element in $\mathbb{P}$ is invertible More explicitly, this means that the following equations hold for all $a,b,c,d\in\mathbb{P}$:

$P1$. $a*(b*c)=(a*b)*c$, (associativity).

$P2$. $a*e=e*a$, (identity).

$P3$. For every $a\in\mathbb{P}$, there is some $a^{-1}\in\mathbb{P}$ such that $a*a^{-1}=a^{-1}*a=e$ (inverse).

A set $\mathbb{P}$ together with an operation $*:\mathbb{P}\times\mathbb{P}\to\mathbb{P}$ and an element satisfying only conditions (P1), (P2) and (P3) is called a group.
This structure is called *poloid*, when the following property (P4) is added in addition to these properties in [8].

$P4$. For any $a\in\mathbb{P}\setminus\{e\}$, there exists $b,c,d\in\mathbb{P}\setminus\{e\}$ such that $\underbrace{b*\underbrace{c=c}_{=}*d}_{\neq}=a$ with $b\neq d$ (escort).

If it satisfies the following property then a $(\mathbb{P},*)$ monoid is called a *poloid*,
For any $a\in\mathbb{P}$ there some exists $b,c,p\in\mathbb{P}$ such that $a=b*p=p*c$, where $b\neq c$ [12].

Lemma 1.4. Let $(\mathbb{P},*)$ a poloid. If $a\in\mathbb{P}$, then there exists $b,c,d,e,p,q\in\mathbb{P}$ such that $a=b*p=p*c=d*q=q*e$, where $b\neq c,d\neq e$.

Proof. The proof is clear.
Regular matrices are the best example of poloid.

The Narayana sequence is characterized by the third-order recurrence relation as follows

$$N_n=N_{n-1}+N_{n-3}, n\geq 3, \text{ where } N_0=0, N_1=1, N_2=1 \text{ [16]}.$$

Definition 1.4. ([16, 17]).

i. Matrix Narayana sequence (MNS) is defined by

$$\{\mathcal{N}_n\}_{n=0}^{\infty} = \left\{ \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix}, \begin{bmatrix} 1 & 0 & 1 \\ 1 & 0 & 0 \\ 0 & 1 & 0 \end{bmatrix}, \begin{bmatrix} 1 & 1 & 1 \\ 1 & 0 & 1 \\ 1 & 0 & 0 \end{bmatrix}, \begin{bmatrix} 2 & 1 & 1 \\ 1 & 1 & 1 \\ 1 & 0 & 1 \end{bmatrix}, \ldots, \mathcal{N}_n, \ldots \right\},$$

with being the initial matrices

$$\mathcal{N}_0 = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix}, \mathcal{N}_1 = \begin{bmatrix} 1 & 0 & 1 \\ 1 & 0 & 0 \\ 0 & 1 & 0 \end{bmatrix}, \mathcal{N}_2 = \begin{bmatrix} 1 & 1 & 1 \\ 1 & 0 & 1 \\ 1 & 0 & 0 \end{bmatrix}$$

and

$$\mathcal{N}_n = \begin{bmatrix} N_{n+1} & N_{n-1} & N_n \\ N_n & N_{n-2} & N_{n-1} \\ N_{n-1} & N_{n-3} & N_{n-2} \end{bmatrix}.$$

ii. Matrix Lucas sequence (LNS) is defined by

$$\mathcal{L}_n = \begin{bmatrix} L_{n+1} & L_{n-1} & L_n \\ L_n & L_{n-2} & L_{n-1} \\ L_{n-1} & L_{n-3} & L_{n-2} \end{bmatrix}, n \in \mathbb{Z}^+,$$

$$\mathcal{L}_0 = \begin{bmatrix} 0 & 3 & -2 \\ -2 & 2 & 3 \\ 3 & -5 & 2 \end{bmatrix}.$$

MATRICES OF SPECIAL SEQUENCES INVOLUTIVE AND SIMILARITIES

In this section, some of the differences between MNSs and MLSs are analyzed. These investigations include commutativity, similarity and involutive matrices. The relationship between MNS and MLS of multipliers is observed.

Let $\mathcal{N}_1 = \begin{bmatrix} 1 & 0 & 1 \\ 1 & 0 & 0 \\ 0 & 1 & 0 \end{bmatrix}, \mathcal{L}_0 = \begin{bmatrix} 0 & 3 & -2 \\ -2 & 2 & 3 \\ 3 & -5 & 2 \end{bmatrix}$. We write

$\mathcal{N}_1 \mathcal{P} \mathcal{N}_1^{-1} = \mathcal{L}_0$, $P \in \mathbb{M}_3(\mathbb{F})$ [16]. That is

$$\mathcal{L}_0 = \underbrace{\begin{bmatrix} 1 & 0 & 1 \\ 1 & 0 & 0 \\ 0 & 1 & 0 \end{bmatrix}}_{\mathcal{N}_1} \underbrace{\begin{bmatrix} 0 & 3 & -2 \\ -2 & 2 & 3 \\ 3 & -5 & 2 \end{bmatrix}}_{\mathcal{P}} \underbrace{\begin{bmatrix} 0 & 1 & 0 \\ 0 & 0 & 1 \\ 1 & -1 & 0 \end{bmatrix}}_{\mathcal{N}_1^{-1}}.$$

$$\mathcal{L}_0 \sim \mathcal{P}.$$

Property 2.1. For some $\mathcal{P}_1, \mathcal{S}_1 \in \mathbb{M}_3(\mathbb{F})$,

i. $\mathcal{N}_1\mathcal{P}_1 = \mathcal{P}_1\mathcal{N}_1$.

ii. $\mathcal{L}_0\mathcal{S}_1 = \mathcal{S}_1\mathcal{L}_0$.

Proof. Let $\mathcal{N}_1, \mathcal{L}_0$.

i. If $\mathcal{N}_1 | \mathcal{L}_0$, then there exists $\mathcal{P}_1 \in \mathbb{M}_3(\mathbb{F})$ such that

$$\mathcal{N}_1\mathcal{P}_1 = \mathcal{L}_0,$$

If $\mathcal{P}_1 | \mathcal{L}_0$, then $\mathcal{P}_1\mathcal{N}_1 = \mathcal{L}_0$. Thus

$$\mathcal{N}_1\mathcal{P}_1 = \mathcal{P}_1\mathcal{N}_1.$$

ii. If $\mathcal{L}_0 | \mathcal{N}_1$, then some $\mathcal{S}_1 \in \mathbb{M}_3(\mathbb{F})$ such that

$$\mathcal{L}_0\mathcal{S}_1 = \mathcal{N}_1,$$

If $\mathcal{S}_1 | \mathcal{N}_1$, then $\mathcal{S}_1\mathcal{L}_0 = \mathcal{N}_1$. Thus

$$\mathcal{L}_0\mathcal{S}_1 = \mathcal{S}_1\mathcal{L}_0.$$

Corollary 2.2. $\mathcal{P}_1 = \begin{bmatrix} -2 & 2 & 3 \\ 3 & -5 & 2 \\ 2 & 1 & -5 \end{bmatrix}, \mathcal{S}_1 = \frac{1}{31}\begin{bmatrix} 23 & 13 & 19 \\ 19 & 4 & 13 \\ 13 & 6 & 4 \end{bmatrix}$.

Lemma 2.3. For some $\mathcal{P} \in \mathbb{M}_3(\mathbb{F})$, the following holds.

$$\mathcal{N}_n = \mathcal{L}_n\mathcal{P}\mathcal{L}_n^{-1}.$$

Proof. There exists $\mathcal{P}_1 \in \mathbb{M}_3(\mathbb{F})$ such that $\mathcal{N}_n = \mathcal{L}_n\mathcal{P}_1$,

$$\mathcal{P}_1 = \mathcal{P}\mathcal{L}_1^{-1}, \mathcal{P}_1 \in \mathbb{M}_3(\mathbb{F})$$

$$\mathcal{N}_n = \mathcal{L}_n \mathcal{P} \mathcal{L}_n^{-1},$$
$$\mathcal{N}_n \sim \mathcal{P}.$$

Lemma 2.4. For some $\mathcal{S} \in \mathbb{M}_3(\mathbb{F})$, the following holds.

$$\mathcal{L}_n = \mathcal{N}_n \mathcal{S} \mathcal{N}_n^{-1}.$$

Proof. There exists $\mathcal{S}_1 \in \mathbb{M}_3(\mathbb{F})$ such that $\mathcal{L}_n = \mathcal{N}_n \mathcal{S}_1$,

$$\mathcal{S}_1 = \mathcal{S}\mathcal{L}_1^{-1}, \mathcal{S}_1 \in \mathbb{M}_3(\mathbb{F})$$
$$\mathcal{L}_n = \mathcal{N}_n \mathcal{S} \mathcal{N}_n^{-1},$$
$$\mathcal{L}_n \sim \mathcal{S}.$$

Lemma 2.5. For a $\mathcal{P} \in \mathbb{M}_3(\mathbb{F})$, if $\mathcal{N}_n = \mathcal{P}\mathcal{L}_n$, then

$$\left(\frac{(\mathcal{L}_n)^{\mathcal{T}}}{(\mathcal{N}_n)^{\mathcal{T}}} \right)^{\mathcal{T}} = \mathcal{N}_n \mathcal{L}_n^{-1}.$$

Proof. By Definition 1.1.(i) and

$$\mathcal{P} = \left(\frac{(\mathcal{L}_n)^{\mathcal{T}}}{(\mathcal{N}_n)^{\mathcal{T}}} \right)^{\mathcal{T}}, \ \mathcal{P} = \mathcal{N}_n \mathcal{L}_n^{-1},$$

$$\left(\frac{(\mathcal{L}_n)^{\mathcal{T}}}{(\mathcal{N}_n)^{\mathcal{T}}} \right)^{\mathcal{T}} = \mathcal{N}_n \mathcal{L}_n^{-1}.$$

Theorem 2.6. There exist $\mathcal{P}, \mathcal{Q} \in \mathbb{M}_3(\mathbb{F})$ such that

$$\mathcal{L}_n = \mathcal{P}\mathcal{N}_n = \mathcal{N}_n \mathcal{Q}.$$

Proof. By Definition 1.1. and 1.3. it is

$$\mathcal{L}_n = \mathcal{P}\mathcal{N}_n = \mathcal{N}_n \mathcal{Q}.$$

Theorem 2.7. There exist $\mathcal{P}_n \in f(\mathcal{N}_n, \mathcal{L}_n)$ such that

$\mathcal{P}_n = \dfrac{\mathcal{L}_n}{\mathcal{N}_n}$, where $f(\mathcal{N}_n, \mathcal{L}_n)$ function of MNS, MLS.

Proof. By Theorem 1.2.

$$\mathcal{N}_n \mid \mathcal{L}_n \Leftrightarrow \mathcal{L}_n = \mathcal{N}_n \mathcal{P}_n,$$

$$\frac{\mathcal{L}_n}{\mathcal{N}_n} = \frac{\cancel{\mathcal{N}_n} \mathcal{P}_n}{\cancel{\mathcal{N}_n}} = \mathcal{P}_n.$$

Lemma 2.8. Let $A \in \mathbb{M}_n(\mathbb{F})$ be any matrix involutive order k^{th}. If $A^k X = B$, $XA^k = B$ then

$$X = B.$$

Proof. It is clear.

RESULTS AND DISCUSSION

Relationships between sequences of special matrices are obtained. Similarities for MNS, MLS have been transferred. Matrices with common factors are obtained for these sequences. There are escort elements for MNS and MLS. MNS and MLS are calculated to be commutative for quotients derived from some terms. This property can be investigated for general terms.

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General Chemistry Questions and Answers in High Education Level

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ABSTRACT

The academic field consists of questions and answers from the disciplines of General Chemistry in the categories of Physical Chemistry. This chapter contains a total of 13 questions and their features, answers of which are in the field of General Chemistry.

As an academician who has been working in this field for years, this chapter will be useful for the undergraduate and associate degree Chemistry students in our universities, who want to receive master's and doctaral education in the postgraduate field and continue their working life in the academy and for succesful experts who pass practical exams.

Keywords – Selective decoupling, ionic bond, Lewis acid, equilibrium, diamond

INTRODUCTION

Q1. Explain giving a few examples of ionic and covalent bonds?

A1. Ionic bonds: It is formed by adding one or more electrons from the valance shell of one atom to the valance shell of another atom. The atom that loses electrons becomes a positive ion (cation) and the atom that gains electrons becomes a negative ion (cation). Ion bonding occurs as a result of the attraction between these oppositively charges ions such as NaCl, KCl and MgI_2, etc.

Covalent bond: Lewis, chemical bond formation in H_2 is shown as follows.

$$H. + .H \rightarrow H..H \quad (1)$$

This type of electron pairing is an example of covalent bond, which occurs when two electrons one shared by two electrons. Covalent compounds contain only covalent bonds. Structures such as H_2 and F_2 that we use to represent covalent compounds are called Lewis structures. The Lewis structure is a representation of covalent bonding. In this structure, shared electrons between two atoms are shown as dot pairs or lines, and unshared electrons are shown as dot pairs on the atoms. Only valance electrons are shown in the Lewis structure.

Q2. What does absolute alcohol mean? Give examples of polar compounds?

A2. Ethanol is used in many places as a solvent, reaction medium, disinfectant, and fuel oil (as colored spirit). It is 95% ethanol because it is

hygroscopic and cannot be dewatered by distillation because it gives an ezeotropic mixture with water. There are various ways to obtain pure ethanol (absolute or absolute alcohol). One is to boil 95% alcohol with quicklime under reflux and then distill it. Thus,

$H_2O + CaO \rightarrow CaO(OH)_2$ (1)

binds irreversibly. An important method of obtaining alcohols is the hydrolysis of alkyl halides. In reality, the reaction is an equilibrium and yields alcohols or alkyl halides.

$RX + H^+ + OH^- \leftrightarrow ROH + H^+ + X^-$ (2)

Suitable conditions are provided for Eq.2.

This reaction is a nuclephilic substitution and is denoted by SN. They act as X^- or OH^- nucleohiles.

$RCH_2B + OH^- \rightarrow RCH_2OH + Br^-$ (3)

or

$RCH_2OH + Br^- \rightarrow RCH_2Br + OH^-$ (4)

It is named after the organic substrate that gives the reaction, so RX is a substrate; X is a named as a group. Nuclephilic strength depends on the basicity and reducing property of the nucleophile. For example, the bacisity of OH-ion is greater than of H_2O. Therefore, it will react faster with the same substrate. Polarity of the bond, diople moment: Partial positive and negative charges are denoted by δ^+ and δ^-. The H-Cl bond can be represented as $H^{\delta+}$-$Cl^{\delta-}$. This type of bond is defined as a polar covalent bond. H-H and Cl-Cl bonds are non-polar covalent. HCl, HBr, CH_3OH, $S_2O_3^{-2}$. The dipole moment of a bond is calculated by assuming that the bond is completely ionic and that ions carry an electron charge (not actually true). If the bond is completely covalent, the dipole moment is zero.

Q3. What does decoupled mean in NMR?

A3. Carbons form “coupling” with the H. They carry and therefore, “split”. Thus, in the ^{13}C-NMR spectrum; Methyl group ($-CH_3$), quartet Methylene group ($-CH_2$), Triplet text group (-CH), Doublet. Quaternary C (without hydrogen) is observed as a singlet. The ^{13}C atom also “couples” with other ^{13}C atoms directly bonded to it, but the effect of this is very low. In the absence of H, the C resonance appears as a singlet and a “proton decoupled” spectrum is obtained.

Selective irradiation (selective decoupling) method is a method applied to determine which carbon atoms the signals observed in ^{13}C-NMR spectra belong to. In order to apply this method, it is necessary to first determine exatly which protons the peaks observed in the ^{1}H-NMR spectrum of compound belong to.

The resonance frequency of any determined proton is sent onto the sample. However, the intensity of the transmitted field should not be too high as in the off-resonance technique. Because when the field intensity is high, decoupling is observed in protons whose resonances are close. With this method, the signal of the carbon atom whose resonance frequency depends on the irradiated proton is observed as a singlet, while other carbon atoms maintain their spin-spin interactions and give multiple peaks. Thus, the signal of only a single carbon atom in the spectrum is precisely determined.

To precisely identify all carbon atoms, this experiments must be repeated for each nucleus. This is a time-consuming and tiring job. Since the methods developed in recent years, especially heteronuclear 2D-spectrums (HETCOR), show the correlation between all protons and carrbons, spectrum recording with selective irradiation is no longer a method applied in new devices.

Q4. How can aromaticity in IR and NMR be distinquished?

A4. The peak at 1600-1450 cm^{-1} belongs to C=C stretching in aromatic structure. It has the appearance of a crowded, complex bond. This represents aromatiicity. In NMR, it is aromatic between 6.8-8.0 ppm. Aromaticity can be distinqusihed more easily in NMR.

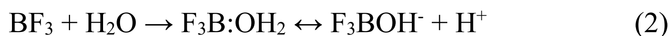
Q5. Why is H_2O used in BF_3?

A5. BF_3 forms “boric acid” and fluoroboric acid as a result of its reaction with water.

$4BF_3 + 3H_2O \rightarrow 3HBF_4 + B(OH)_3$ (1)

Boric acid, $B(OH)_3$, the main product of this reaction, is a substance widely used in industry. For this reason, the aim is to obtain boric acid by using H_2O and BF_3. Lewis acids alone are generally insufficient to initiate cationic

polymerization. They are effective alongside cocatalysts or cocatalyst compounds that have proton donating properties. Compounds such as water and methanol.



$BF_3 + H_2O \rightarrow F_3B{:}OH_2 \leftrightarrow F_3BOH^- + H^+$ (2)

The highest polymerization rate is achieved at an optimum catalyst / cocatalyst ratio that various depending on the type of catalyst and solvent (**Figure 1**).

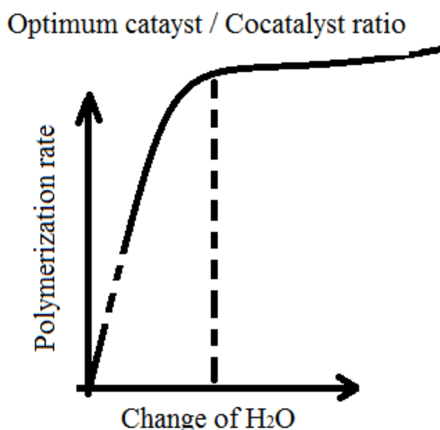


Figure 1. Relationship polymerization rate and H_2O change.

A typical graph showing the effect of cocatalyst/catalyst ratio on the polymerization rate is given in **Figure 1**. The polymerization rate first increases with cocatalyst concentration and then decreases, passing through a maximum. If the curve in the Fig.1. is extrapolated to zero water concentration, the polymerization rate also takes the value of zero. For this reason, it is thought that all cationic initiators exist as ion pairs in the form of H^+A^- in the polymerization environment.

The proton of the ion pair joins the carbons in the monomer's double bond, which has become partially negatively charged due to the side group effect, and forms a stable carbonium ion. The initiation reaction of the catalyst/cocatalyst complex given by boron trifluoride and water with a vinyl monomer is shown below.

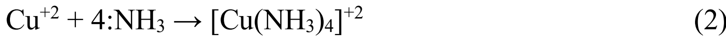
$H^+(BF_3OH)^- + H_2C{=}CR_2 \rightarrow CH_3\text{-}C^+R_2\ (BF_3OH)^-$ (3)

Q6. What does Lewis acid mean?

A6. The substance that accepts electron pairs is called Lewis acid, and the substance that donates electron pairs (donar) is called Lewis base.

$$A + :B \rightarrow A\text{-}B \qquad (1)$$

The electron pair provided by the base is used in bonding pairs of the base are not shown. It is convenient to use the terms acidity and basicity when discussing the equilibrium positions of the reaction. If electron donation or gain is related to a process that determines the rate of the reaction, the terms nucleophilic acid instead of base and electrophile are used instead.



$$Cu^{+2} + 4{:}NH_3 \rightarrow [Cu(NH_3)_4]^{+2} \qquad (2)$$

Lewis acid

$$Ni + 4{:}\ CO \rightarrow Ni(CO)_4 \qquad (3)$$

Lewis acid

$$B(CH_3)_3 + {:}NH_3 \rightarrow H_3NB(CH_3)_3 \qquad (4)$$

Lewis acid (Möricke et al. 2017).

Q7. Draw the shape of the periodic Table? How are atoms arranged in the chart?

A7. Periodic table records are given in **Fig.2.**

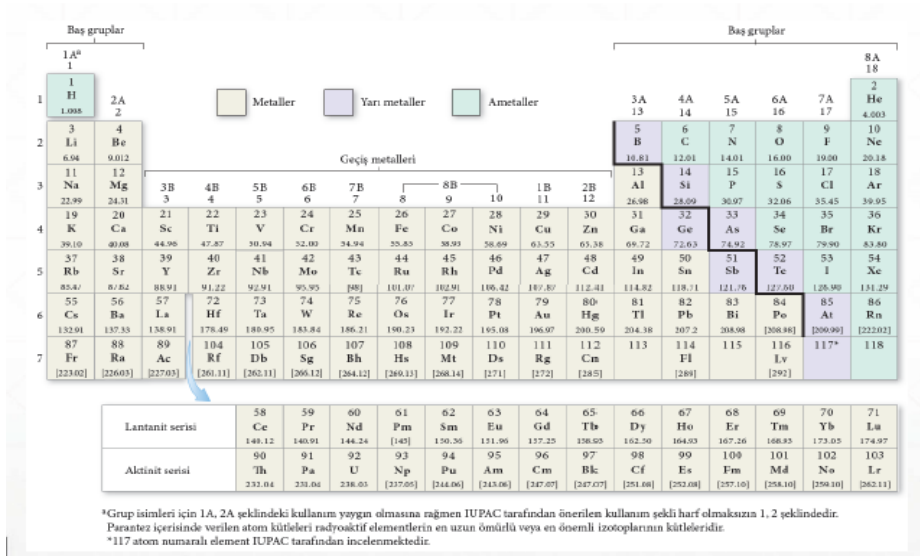


Figure 2. Periodic table.

In the periodic table, elements are arranged in increasing order of their atomic numbers. Atoms in the same group have similar electronic

structures. In general, atomic radii decrease across a period and grow from top to bottom within a group. Mendelev had to exclude some elements from the groups they should be in according to his periodic table. He accepted that these due to errors in atomic masses.

The first two groups are s block elements. The last six groups are p block elements and are the main group elements. They are the d block elements and p block elements and are the main group elements (also called Transition elements). F block elements, also called inner transition elements, expand the table to 32 elements with the main group elements. Since the table is very wide in its current form, f block elements are generally removed from the table and placed at the bottom. The 14 elements following lanthanum (Z=57) are called Lanthanides, and the 14 elements following actinium (Z=89) are called actinides.

Q8. Is the IR spectrum of O_2 taken?

A8. There is no permanent dipole moment, there is a center of symmetry, IR is inactive. Not taken.

Q9. Explain the phenomena of Fluorescence and Phosphorescence?

A9. It is called "Fluorescence" emitted during the transition from an exited singlet system to the ground state singlet system. The expansion emitted during the transition from an exited ternary system to a singlet system in the ground state is called "Phosphorescence". Unlike fluorescence, phosphorescence does not quickly return the properties it absorbs.

Fluorescence is the luminescence phenomenon that occurs when the absorption of a photon in cold nuclei is triggered by the initiator of another photon at a layer wavelength. The difference in energy between the adsorbed and emitted photons appears as conductors or heat. In fluorescence, if the frequency of the light emitted and the frequency of the light stimulating the system are equal, this is called resonance frequence. Resonance fluorescence phenomenon is observed in atoms and molecules in solid state. Since the exited molecules formed in solution get rid of some of their excess energy non-radiatively, the wavelength of the fluorescence observed in them is longer than the wacelengths of the photons that exite the molecule. The wavelengths observed in phosphorescence are longer than the wavelengths observed in fluorescence.

A very short period of time, such as 10-15 s, is required for light absoroption and the lifetime of a molecule in the excited singlet state is 10^{2}-10^{-6} s. The duration of internal transformation and intersystem transition events is approximately 10^{-12} s and 10^{-8} s, respectively. While the fluorescence phenomenon lasts for another 10^{-6}-10^{-10} s when the radiation stimulating rge system disappears, in such a case the phosphorescence continues for another 10^{2}-10^{-6} s. The light emitted dueing the transition from an excited singlet system to the ground state singlet system is called Fluorescence ($h\upsilon_2$).

$^{1}S^{*} + h\upsilon \rightarrow {}^{1}S^{*}$ (arousal) (1)

$^{1}S^{*} \rightarrow {}^{1}S + h\upsilon_2$ (Florescence) (2)

Jablonsky diagram, radiative and non-radiative transitions in a molecule are given in **Figure 3**.

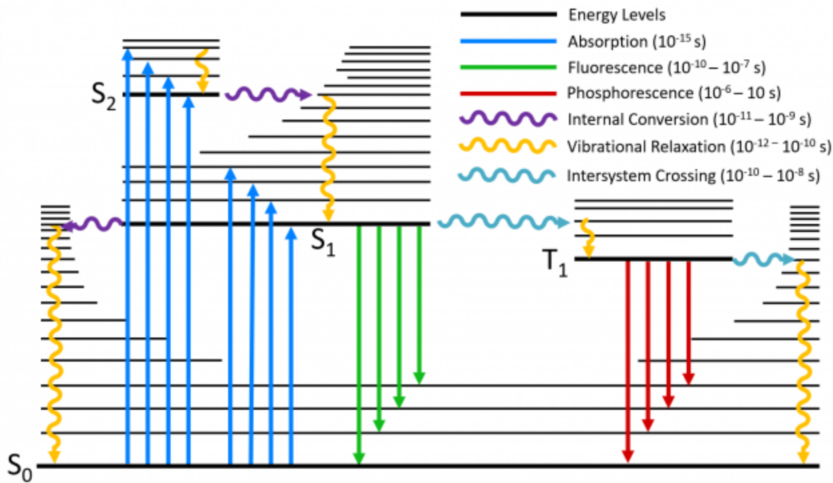


Figure 3. Jablonsky diagram, radiative and non-radiative transitions in a molecule (Khudyakov, 2023).

The light emitted during the transition from an excited triplet system to a singlet system in the ground state is called phosphorescence ($h\upsilon_3$).

$^{1}S^{*} + h\upsilon_1 \rightarrow {}^{1}S^{*}$ (arousal) (1)

$^{1}S^{*} \rightarrow {}^{3}T^{*}$ (Intersystem switching) (2)

$^{3}T^{*} \rightarrow {}^{1}S^{*} + h\upsilon_3$ (Phosphorescence) (3)

Q10. How to write the equilibrium equation? Write the equilibrium between K_p and K_c? In the equation, $\Delta G^o = -R.T.\ln K_p$, is it K_c or K_p?

A10. The equilibrium constant (K_p) shows the ratios of product and reactant concentrations in equilibrium in terms of partial pressure.

$$aA(g) + bB(g) \rightleftharpoons cC(g) + dD(g) \quad (1)$$

K_p expression for the reaction;

$$Kp = \frac{P_C^{\,c}\ P_D^{\,d}}{P_A^{\,a}\ P_B^{\,b}} \quad (2)$$

The molar concentration in terms of K_c is related to the equilibrium constant K_p by formula 3.

$$K_p = Kc\,(RT)^{\Delta\, ngaz} \quad (3)$$

Here Δn_{gas} is the difference of stoichiometric coefficients of gaseous products and reactants (Δn_{gas} = Mole of product gas – mole of reactant gas). When $\Delta n_{gas} = 0$, $K_p = K_c$.

$$\Delta G^o = -RT\ln K_p \quad (4)$$

K in the equation is K_p. K_p becomes $(bar)^{\Delta ng}$, K_c becomes $(mole/L)^{\Delta ngas}$.

Q11. How is Δn_g calculated for an equilibrium reaction? Write the free enthalpy equation for equibrium?

A11. The free emtalpy equation is given as $\Delta G^o = \Delta H^o - T\Delta S$. For a voluntary change;

$\Delta S^o_{evren} = \Delta S^o_{sistem} + \Delta S^o_{çevre} > 0$ (1)

Although this equality is the basic criterion of voluntary change, it is very difficult to implement. Since we cannot always determine all interactions with the environment in order to find the total energy change ($\Delta S^o_{universe}$), a criterion must be determined that can be applied "only to the system and that will not need to be concerned with the changes in its environment. This is the Gibbs free energy.

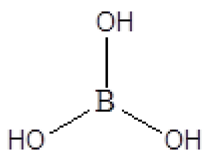
If $\Delta G^o < 0$, the change is voluntary.

$\Delta G^o > 0$, the change is involuntary.

$\Delta G^o = 0$, the system is in equilibrium.

Q12. What is pseudo-asymmetric carbon? What such carbon craking isomer groups?

A12. The term Madabaz asymmetric carbon was given bu Prof.Dr. Cihangir Tanyeli, one of the Professors of METU, Department of Chemistry. Its Emglish name is pseudo (liar). If we consider riboric acid in terms of stereochemistry.

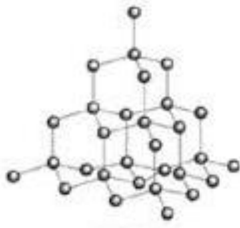


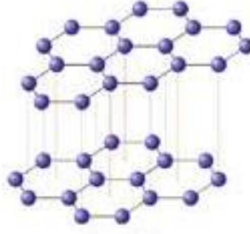
(1)

We see that one is R and the other is S. Therefore, 4 different groups are attached to this carbon and it is asymmetric. Unfortunately, things don't end there, the molecule itself is not chiral (meso) because a plane of symmetry passes through its middle. For same reason, this plane passes over the asymmetric carbon. How does a symmetric plane pass over an asymmetric carbon? Finally, if we swap the two groups on this carbon, we get xylaric acid, a diastereomer of riboric acid. Because of all these, the C3 carbon is stuck between symmetry and asymmetry. C3 carbon is thick ster and thickster.

Q13. What is the structure of diamond and graphite? Types of hybridization? Is diamond a polymer? Why not, Which one has better mechanical properties?

A13. In diamond, each carbon atom is bonded to four other carbon atoms in a regular tetrahedron.





(1)

Strong covalent bonds spread in three dimensions make diamond a crystal. It enables diamond to be a crystal of extraordinary hardness (the hardest known substance) and a high melting point (3550 ºC).

In the crystal structure of graphite, carbon atoms are arranged in hexagonal rings. In the structure, all atoms have sp^2 hybridization and are connected to each other with covalent bonds. The unmetalized 2p orbital is used to make π bonds. Because the electrons in the 2p orbitals move freely, graphite easily conducts electricity along the planes of interconnected carbon atoms. These layers that form graphite are held together by weak Van der Waals forces.

These covalent bonds give rigidity to the graph. However, since these layers can slide on each other, they feel slippery when touched and are also used as a lubricant. Graphite is also used in pencils and the ribbons of computer recorders and typewritters.

Diamond is not a polymer. Because the number of renewed units is limited. The fact that the mechanical properties of diamond are much better than graphite is due to its sp^3 hybridization, regular tetrahedral geometry and strong covalent bonds between C atoms. Another covalent cystal is quartz (SiO_2). The arrangement of silicon atoms in quartz is similar to the arrangement of carbon in diamond, but there is An oxygen atom between each pair of Si atoms. Since the electronegativities of Si and O elements are quite different. The Si-O bond is polar. SiO_2 is very similar to diamond in many properties such as hardness and melting point (1610 ºC) (Ge et al., 2023).

RESULTS AND DISCUSSION

In this chapter, many different question and their answers were presented to understand the general chemistry in University level. These are given in the following questions: ionic bonds, absolute alcohol, polar compounds, decoupled in NMR, dşifferenation aromaticity in IR and NMR, H_2O used in BF_3, lewis acid, periodic table, IR spectrum of O_2, Fluorescence, Phosphoescence, equilibrium, pseudo-asymmetric carbon, diamond and graphite.

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Development of Plant Based Liquid Soap Formulations and Their Stability Sudies

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ABSTRACT

Herbal cosmetics are formulations containing phytochemicals derived from various botanical sources, which influence skin functions and provide bioactive compounds essential for maintaining healthy skin and hair. In this study, natural liquid soaps of plant origin with high cleansing and foaming capacities were produced, and their stability and antibacterial properties were assessed. Accordingly, natural liquid hand soap formulations were developed using both cold and hot processing methods, incorporating plant based fixed oils (such as olive oil, coconut oil, castor oil, daphne oil, avocado oils, palm oil), essential oils (such as lemon oil, lavender, peppermint oil), and necessary auxiliary ingredients. Following the production of natural cosmetic products, stability assessments (including measurements of pH, density, appearance, odor, color, and viscosity) were performed over a 6-month period under both room temperature and hot processing conditions. This research also involved an investigation into the presence of undesirable microorganisms in the manufactured products, both post-production and at the conclusion of their respective shelf lives. Consequently, the products' resistance to potential contamination arising during or after the production process was demonstrated. This study demonstrated that it is possible to develop plant based natural liquid soaps with acceptable microbiological quality and stability. Nonetheless, pre-market stability testing remains essential to validate product safety throughout its shelf life, particularly under variable consumer usage conditions. Continued monitoring and optimization of natural preservative systems are recommended to further enhance product robustness.

Keywords: *Natural liquid soap, hot and cold process, stability testing, microbiological testing, plant oil, essential oil.*

INTRODUCTION

Throughout history, humans have utilized plants for a multitude of purposes, including the treatment of diseases, nutrition, shelter, defense, and warmth, and continue to do so today. In contemporary times, plants play a significant role across various industries such as food, pharmaceuticals, cosmetics, dyes, agriculture, and medicine. Various plant parts including stems, leaves, flowers, seeds, essential oils, fixed oils, bark, and extracts are employed through diverse techniques and for different applications (Joshi ve Pawar, 2015: 1; Kapoor, 2005:306). In the cosmetic industry, plants currently play a significant role both scientifically and economically. Consumers are seeking alternative products to synthetic chemical based artificial cosmetics available on the market. This is because such products often contain harmful

chemicals, including skin irritants like sodium lauryl sulfate and carcinogens or allergens such as triethanolamine that can cause eye and skin allergies. Herbal products offer a natural and healthier alternative for cosmetic formulations and present a broad market opportunity. The compounds most commonly found in plants used in cosmetic products include tannins, fixed oils, essential oils, phenolic acids, carbohydrates, flavonoids, sterols, vitamins, saponins, and minerals. Other compounds such as coumarins, anthocyanins, sesquiterpenes, proteins, alkaloids, resins, and anthraquinones are found less frequently (Kurban, 2018:143).

Soap is one of the oldest therapeutic and cleansing agents in the world. It has been used by humans since ancient times, and its usage continues to increase today. Chemically, soap is defined as a product obtained through the reaction of plant or animal oils with alkaline hydroxides. In short, soap is the alkali salt of fatty acids and is classified as a surfactant. Soaps primarily contain the sodium and potassium salts of various fatty acids, including linoleic acid, stearic acid, oleic acid, palmitic acid, myristic acid, and lauric acid. Therefore, in order to produce high-quality soaps that are safe for human health, it is essential to use fats containing fatty acids with carbon chain lengths between 12 and 18. Fatty acids with fewer than 10 carbon atoms can irritate the skin due to poor cleansing performance, while those with more than 18 carbon atoms are poorly soluble in water (Sert, 2021:1). Today, most of the oils used in soap production are of animal origin. Approximately 75% of bath soaps, 65% of laundry soaps, and 85% of toilet soaps are produced using animal oils, with the remainder derived from plant oils. Soaps manufactured in factories often contain various additives to stabilize texture, adjust color, enhance foaming, and prevent spoilage or cracking. Some of these additives include blankit, cosemin, TiO_2, tinopal, $Al_2(SO_4)_3$, lusidol, HOCl, H_3PO_4, stabilon, tonsil, $NaClO_2$, and sulfur. Again, it is well known that chemicals used in some liquid soaps and antibacterial soaps, particularly triclosan, seriously affect human health (Küçükusta, 2013). According to a study conducted by Halla (2018), antibacterial soaps can weaken the human immune system and destroy beneficial bacteria on the skin, leading researchers to recommend the use of natural soaps instead. The most beneficial soaps for human health are solid soaps made from natural plant oils, free from synthetic chemicals (Göktaş ve Gıdık, 2019:136).

Liquid hand soaps formulated with natural or naturally derived plant-based ingredients can present favorable environments for microbial growth. This is primarily due to their content of minerals, suitable pH levels, moisture, and the presence of various growth factors. Additionally, formulation components such as sugar alcohols, carbohydrates, proteins, amino acids, fatty acids, glycosides, fatty alcohols, peptides, vitamins, steroids, and herbal

materials serve as nutrient sources for microorganisms (Naki Sivri, 2005; Okeke ve Lamikanra, 2001:922).

The identification of microbial spoilage in cosmetics is carried out through microbiological analyses. Standard microbial limit tests are used to assess microbial loads, and the isolated strains are identified using conventional and advanced microbiological techniques (Sutton, 2006:111). The most frequently encountered microbial contaminants in cosmetic products include *Staphylococcus aureus*, *Clostridium tetani*, *Clostridium perfringens*, *Moraxella* spp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter* spp., *Klebsiella pneumoniae*, *Aspergillus* spp., and *Candida albicans* (Durant ve Higdon, 1991; Wilson ve Ahern, 1997:112; Block, 1992; Tenenbaum, 1967:797). Environmental factors during transport and consumer use further contribute to the microbial vulnerability of cosmetic products. Temperature and humidity fluctuations, especially during shipping and storage, as well as consumer habits such as adding water to the product or storing it in humid bathrooms, wet shower stalls, or hot vehicles, create conditions that favor microbial growth and challenge product stability (Fainstein vd., 1988:655). To ensure both product safety and regulatory alignment, stability testing is an essential step prior to product commercialization**.** These studies aim to determine the minimum shelf life during which the cosmetic product remains functional and safe under normal storage conditions. According to industry standards, this period typically ranges from 90 days to six months and involves the evaluation of various physicochemical properties such as pH, density, appearance, viscosity, odor, color, and purity (Wang ve Li, 2014:881; Steinberg, 2006:1; Benderly, 2016:349; Maryam vd., 2020:157; Senny vd., 2018:1267).

In this context, the present study was conducted to develop natural liquid soap formulations using natural or nature-identical plant based raw materials and to assess their microbial load, with particular attention to the product's stability and microbiological safety throughout its expected shelf life.

MATERIAL AND METHODS

Chemicals Used in Liquid Soap Formulations: The liquid soap formulations were prepared using a variety of natural oils, alkaline agents, and functional additives. The raw materials and their suppliers are listed as follows:

Plant Oils: Daphne oil, castor oil, coconut oil, avocado oil, and palm oil were purchased from MSA Kozmetik. Olive oil was obtained from Simya.

Alkaline and Neutralizing Agents: Potassium hydroxide and citric acid were supplied by Simya Grup?, while sodium chloride (NaCl) was sourced from Aromel Kimya.

Functional Additives: PEG-4, glycerin, and lauric acid were obtained from MSA Kozmetik. EDTA was provided by Simya Grup?.

Essential Oils: Lavender oil, peppermint essential oil, and lemon grass essential oil were all purchased from MSA Kozmetik.

All ingredients were of cosmetic or pharmaceutical grade and used without further purification.

Microbiological Analysis Materials: To evaluate the microbiological safety of the formulated liquid soaps, a range of culture media and reagents were used. The specific media and their manufacturers are as follows:

Total Aerobic Microbial Count and Fungal Enumeration: Blood Agar (Biomerioux), Tryptic Soy Agar (TSA; Himedia), Eugon LT Broth (Himedia), and Sabouraud Chloramphenicol Dextrose Agar (SCDA; Himedia) were used.

Selective Media for Pathogenic Bacteria: MacConkey Agar (Himedia) and Levine Eosin Methylene Blue Agar (EMB; Himedia) were employed for Gram-negative bacteria.
Baird Parker Agar (Himedia) was used for the detection of *Staphylococcus aureus*, and Cetrimide Agar (Himedia) for *Pseudomonas aeruginosa*.

Additional Reagents: Tween 80 (Simya) was used as an emulsifier and to aid in the dispersion of hydrophobic substances during sample preparation.

Equipment Used

Distilled Water System (Elga-DV25): Used for supplying water during the formulation process and for the preparation of culture media in microbiological analyses.

Ultrasonic Bath (Wisd, WUC-D06H): Employed during the dissolution phase in the formulation of the products.

Magnetic Stirrer with Heater (Daihan, MSH-20D): Used for simultaneous heating and mixing during the formulation of soaps.

Electronic Balance (Daihan, SC3 model): Used to weigh raw materials during the formulation process.

Blender (Fakir): Used for mixing during the saponification reaction.

pH Meter (Hanna): Utilized to measure and control the pH levels of the formulated products.

Analytical Balance (OHAUS, Pioneer): Used to accurately weigh components for the preparation of microbiological culture media.

Autoclave (Tek-Bal): Employed for the sterilization of culture media, as well as plastic and glassware used in microbiological analyses.

Incubator (Elektro-Mag M5040 BP): Used to incubate inoculated media at appropriate temperatures for bacterial growth.

UV Transilluminator: Used to visualize bacterial colonies growing on Cetrimide agar under UV light.

PRODUCTION OF PLANT BASED NATURAL LIQUID SOAP, STABILITY STUDIES, AND MICROBIOLOGICAL ANALYSIS

Production of Plant Based Natural Liquid Soap

Plant based liquid soap production was carried out using both cold and hot process methods (Saygılı, 2019; Wijana evd., 2019:1; Maryam vd., 2020:157; Senny vd., 2018:1267). The reason for using both methods in soap production was to compare the resulting soaps in terms of appearance and quality control parameters (such as turbidity, viscosity, pH, color, scent, appearance, and foaming properties), in order to determine the most suitable production method. In this context, a total of six different formulations were developed (Figure 1).

Cold process formulations:

Olive oil–daphne oil liquid soap (SS-cold-D1)
Olive oil–avocado oil liquid soap (SS-cold-A2)

Hot process formulations:

Coconut oil–lavender oil liquid soap (SS-hot-HC1)
Coconut oil–lemongrass oil liquid soap (SS-hot-HC2)

Coconut oil–avocado oil–castor oil–peppermint oil liquid soap (SS-hot-A1)
Coconut oil–daphne oil–castor oil–lemongrass oil liquid soap (SS-hot-D1)

Figure 1. Produced natural liquid soaps

Production of Plant Based Natural Liquid Soap Using the Cold Method

This study focuses on the production of natural liquid soap using the cold saponification method. Two distinct formulations were developed for this purpose.

1. formulation: SS-cold-D1**:** Olive oil and daphne oil-based liquid soap
2. formulation: SS-cold-A2**:** Olive oil and avocado oil-based liquid soap

Both formulations were saponified using potassium hydroxide (KOH) solution. The oils and the alkali solution were thoroughly mixed until a homogeneous mixture was obtained. The resulting soap pastes were left to rest overnight. Afterward, they were diluted with hot water to achieve the desired liquid form. To adjust the consistency**,** PEG-4 and sodium chloride were added as emulsifiers. Subsequently, EDTA (ethylenediaminetetraacetic acid-chelating agent**),** Glycerin **(**moisturizing agent) and Lauric acid **(**foaming agent) were incorporated into the formulations. The pH of the mixture was adjusted using a 1% citric acid solution to bring it to the desired level suitable for skin compatibility (Figure 2).

1 2

Figure 2. Natural liquid soaps produced using the cold method (1: SS-cold-D1; 2: SS-cold-A2)

Production of plant Based Natural Liquid Soap Using the Hot Process Method

Within this scope, production was carried out for four formulations under optimized conditions (Figure 3).

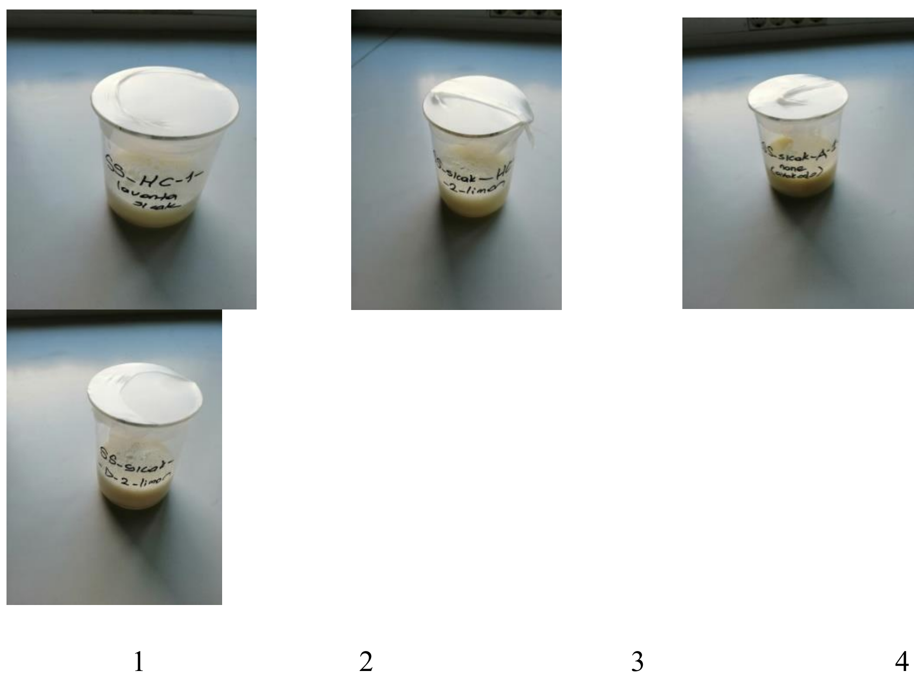
1. formulation: Coconut oil-Lavender oil-based liquid soap (SS-hot-HC1)
2. formulation: Coconut oil-Lemon grass oil-based liquid soap (SS-hot-HC2)
3. formulation: Coconut oil-Avocado oil-Castor oil- Peppermint oil-based liquid soap (SS-hot-A1)
4. formulation: Coconut oil- daphne oil-Castor oil-Lemongrass oil-based liquid soap (SS-hot-D1)

For formulations 1 (SS-hot-HC1) and 2 (SS-hot-HC2), coconut oil was mixed with a potassium hydroxide solution and stirred at 75°C using a magnetic stirrer at 285 RPM for one hour. The mixture was then left to rest overnight. The resulting soap paste was diluted with water and thickened by the addition of PEG-4 (emulsifier) and sodium chloride (emulsifier). EDTA (chelating agent), glycerin (moisturizer), and lauric acid (foaming agent) were subsequently added and mixed thoroughly. The pH was adjusted using a 1% citric acid solution. Finally, a plant based preservative and essential oil (lavender oil and lemongrass oil) were added to complete the process.

In formulation 3 (SS-hot-A1), olive oil, castor oil, palm oil, coconut oil, and avocado oil were mixed with potassium hydroxide solution and stirred at 75°C using a magnetic stirrer at 285 RPM for one hour. The mixture was

then left to rest overnight. The resulting soap paste was diluted with water, and PEG-4 and sodium chloride were added for thickening. EDTA, glycerin, and lauric acid were then incorporated, and the pH was adjusted with a 1% citric acid solution. The process was finalized by adding a plant based preservative and essential oil (peppermint oil).

In formulation 4 (SS-hot-D1), olive oil, castor oil, palm oil, coconut oil, lauric acid, and daphne oil were combined with potassium hydroxide solution and stirred at 75°C using a magnetic stirrer at 350 RPM for one hour. After resting overnight, the soap paste was diluted with water, and PEG-4 and sodium chloride were added to adjust consistency. EDTA, glycerin, and lauric acid were subsequently added, and the pH was regulated using a 1% citric acid solution. Finally, a plant based preservative and essential oil (lemongrass oil) were added to complete the formulation.



1 2 3 4

Figure 3. Natural liquid soaps produced using the hot method (1: SS-hot-HC1; 2: SS-hot-HC2; 3: SS-hot-A1; 4: SS-hot-D1)

Stability Studies of Plant Based Natural Liquid Soap

Following the completion of the saponification reaction, stability studies of the products were initiated. Stability testing (minimum 3 months – typically 6 months) encompasses a set of evaluations aimed at determining the minimum shelf life of a cosmetic product, which is defined as the duration during which the product maintains its original functional properties and remains safe for use under normal storage conditions.

As part of the stability tests of the produced liquid hand soap, parameters such as foaming capacity, density, viscosity, appearance, pH, scent, and color were analyzed to assess the product's shelf life. The application was carried out as follows. Three control samples were taken from the newly formulated product:

Sample 1 was taken immediately at the end of formulation. It was observed for any phase separation or physical deterioration (e.g., turbidity). Its foaming capacity, density, viscosity, appearance, pH, scent, and color were analyzed.

Sample 2 was incubated at 45°C for 1 month**.** During this period, it was observed for phase separation, spoilage, and turbidity. This accelerated aging period is equivalent to a 6-month shelf life under normal conditions**.** The sample's key characteristics, such as foaming capacity, density, viscosity, appearance, pH, odor, and color**,** underwent thorough analysis.

Sample 3 was stored at room temperature (20–25°C) and monitored for 6 months. At the end of this period, full stability tests were conducted on foaming capacity, density, viscosity, appearance, pH, scent, and color (Wang and Li, 2014:881; Steinberg, 2006:1; Benderly, 2016:349; Maryam vd., 2020: 157; Senny vd., 2018:1267).

Foam Determination in Natural Liquid Soaps

To determine the foaming capacity, a 1% aqueous solution of the soap is prepared. This solution is then placed into a measuring cylinder**.** The cylinder is capped and shaken 5 times**.** The foam level is then measured at 0, 3, 5, and 8-minute intervals (Figure 4).

The foam percentage is calculated using the following formula:

$$\% \text{ Foam} = \frac{\text{Foam Lenght (cm)}}{\text{Lenght of Portion Below Foam (cm)}} \times 100$$

Figure 4. Determining the foaming capacity of liquid soaps

pH Determination in Natural Liquid Soaps

To determine the pH of the formulated liquid soap samples, measurements were taken by directly immersing a pH meter (Hanna) into each sample.

Determination of Density in Natural Liquid Soaps

Density is one of the critical physicochemical parameters used to evaluate the quality, consistency, and formulation stability of liquid soap products. In this study, the density of the formulated natural liquid soaps was determined to assess any changes over time that may indicate physical instability or formulation separation (Steinberg, 2006:1; Wang and Li, 2014:881). Density measurements were conducted at room temperature (20–25 °C) using a standard pycnometer method.

First, the empty pycnometer was sealed with its cap and weighed. Then, it was filled with distilled water and weighed again. In the third step, the pycnometer was filled with the liquid soap sample and weighed once more. In each step, the samples were carefully added to the pycnometer, ensuring that no air bubbles were present. The density was then calculated using the formula below (Figure 5).

$$d\left(\frac{g}{cm^3}\right) = \frac{(\text{Sample} + \text{Pycnometer}) - (\text{Pycnometer Tare})}{(\text{Water} + \text{Pycnometer}) - (\text{Pycnometer Tare})}$$

Figure 5. Determination of density of liquid soaps

Determination of Viscosity in Natural Liquid Soaps

In liquid soap formulations, viscosity is a key parameter that directly influences the product’s physical and sensorial properties. Viscosity measurements serve as a critical evaluation tool for assessing product quality, stability, and consumer-perceived performance (Gupta and Gaud, 2015).

Viscosity measurements of the formulated liquid soap samples were carried out through outsourced services provided by Saniter Food and Environmental Laboratory. The analyses were conducted using a B-One Plus Viscometer, equipped with an R-5 spindle at 250 RPM (Analysis method: AOCS 1987) (Figure 6).

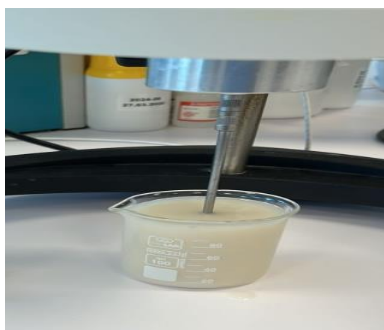


Figure 6. Determination of viscosity of liquid soaps

Microbiological Analysis of Plant Based Natural Liquid Soap

In this study, microbiological analyses were conducted on the initially produced liquid soap samples and on the same samples after being incubated at 45°C for one month. To assess microbial contamination and product safety, various selective and differential culture media were employed as outlined below.

Culture Media and Methods

Eugon LT Broth: Tween 80 (polysorbate) was added to the broth, and it was sterilized in an autoclave at 121°C under 1.5 atm pressure. This medium ensures the neutralization of the samples and supports the proliferation of any bacteria or fungi present, allowing the detection of even minimal amounts of microorganisms. After incubation for 24 hours in this medium, serial dilutions were prepared and inoculated onto different culture media to detect bacteria that should not be present, according to European Commission regulations (Scientific Committee on Consumer Safety, 2023).

Tryptic Soy Agar (TSA)**:** A general-purpose medium used for enumerating aerobic mesophilic bacteria. The medium was sterilized via autoclaving and poured into sterile Petri dishes. Following incubation in Eugon LT Broth, 100 µL of each sample was spread on TSA plates. After incubation at 32.5 ± 2.5°C for 48 hours, colony formation was examined and recorded.

Sabouraud Dextrose Agar with Chloramphenicol (SDA)**:** This medium was used to detect the presence of *Candida albicans*. After sterilization and solidification, 100 µL of each sample previously incubated in Eugon LT Broth was spread onto SDA plates. The plates were incubated at 32.5 ± 2.5°C for 48 hours. The presence of white to beige, convex, creamy colonies was interpreted as indicative of *C. albicans*.

MacConkey Agar (MCA): A selective medium for Gram-negative bacteria due to the inclusion of crystal violet and bile salts. It was employed to detect *Escherichia coli*, which must be absent from cosmetic products. Colonies that appeared large and red in color were presumptively identified as *E. coli*, and further confirmed by sub-culturing on Eosin Methylene Blue (EMB) agar, where *E. coli* forms characteristic metallic green colonies.

Baird Parker Agar (BPA): This selective medium was used to detect *Staphylococcus aureus*. After autoclaving and cooling to 45–50°C, sterile Egg Yolk Tellurite Emulsion was added. Samples (100 µL) incubated in Eugon LT Broth were plated and incubated at 32.5 ± 2.5°C for 48 hours. The appearance of black colonies was considered indicative of *S. aureus*.

Cetrimide Agar (CA): A selective medium for *Pseudomonas aeruginosa*. After sterilization and solidification, 100 µL of each incubated sample was spread on the medium and incubated at 32.5 ± 2.5°C for 48 hours. Blue-green colonies and fluorescence under UV light were taken as indicators of *P. aeruginosa*.

All media were validated using known reference strains to ensure reliability and efficacy prior to application in sample testing (Figure 7).

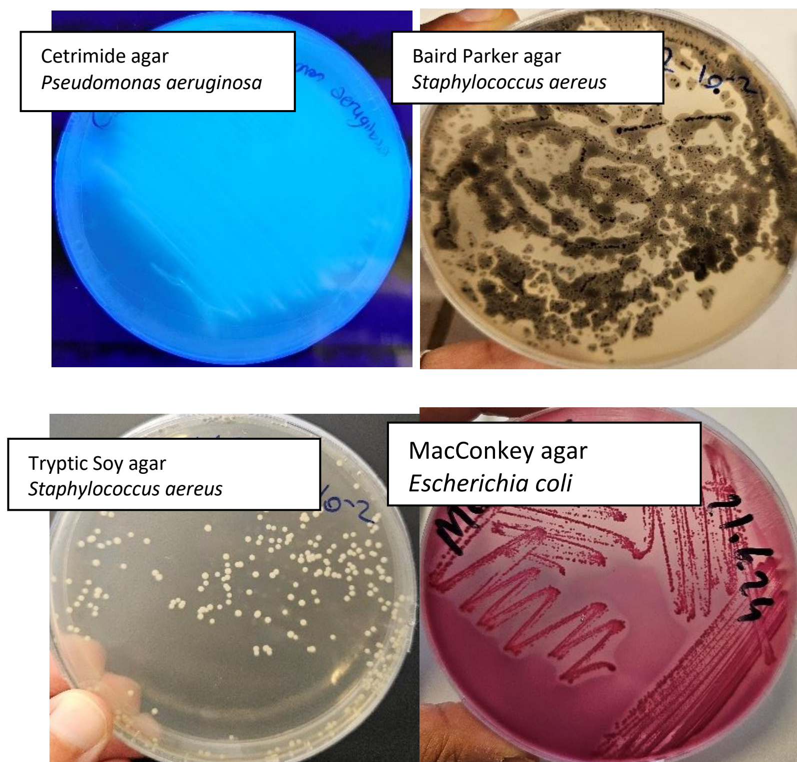


Figure 7. Examples of the culture media utilized

For each liquid soap sample, 1 mL was added to 9 mL of Eugon LT broth supplemented with Tween 80. These mixtures were then incubated at 32.5 ± 2.5 °C for 24 hours for neutralization and enrichment. After this incubation period, serial dilutions (10^{-2} and 10^{-3}) were prepared from the initial 10^{-1} dilution using Eugon LT broth. From each dilution, 100 µL was aseptically inoculated onto the prepared agar media in duplicate using the spread plate method. Finally, the agar plates were examined after being incubated at 32.5 ± 2.5 °C for 48 hours.

RESULTS AND DISCUSSION

Stability Results of Plant Based Natural Liquid Soaps

Stability studies are a series of tests conducted to determine whether the components in soap formulation deteriorate over time or interact with each other to cause undesirable effects. These tests may include physical stability (Color, odor, appearance, consistency (viscosity), sedimentation, phase separation), chemical stability (pH changes, active ingredient degradation, oxidation), microbiological stability (Resistance of the product to microbial contamination, preservative effectiveness) and packaging compatibility (Interaction of the product with the packaging material) (Aral, 1995:1).

In this study, within the scope of stability testing of the produced liquid hand soap, parameters such as foaming capacity, density, viscosity, appearance, pH, odor, and color were analyzed to observe the product's shelf life. The analysis results are presented in Table 1 and Table 2. Based on the stability analysis data obtained at the initial stage, after one month of incubation at 45 °C, and following six months of storage on the shelf, the following conclusions were drawn regarding the stability of the natural liquid soaps produced.

***Appearance*:** No changes were observed in the appearance of the samples at the initial stage, after one month of incubation at 45 °C, and after six months of storage on the shelf.

Color: No color changes were observed in the samples at the initial stage, after one month of incubation at 45 °C (except for SS-Cold-D1), and after six months of shelf storage.

Odor: Throughout all analyses, no changes were detected in the odor of the samples

Density: According to all analysis results, the densities of the samples remained within the acceptable standard range (0.95–1.10 g/cm^3) (Ecer, 2019).

pH: Generally, synthetic liquid soaps based on surfactants are formulated with a pH around 5.5. However, literature suggests that natural liquid soaps typically exhibit a pH between 8 and 11. This is due to the saponification reaction between potassium hydroxide (KOH) and natural solid or liquid oils used in their formulation (Fainstein vd., 1988:655; Maryam vd., 2020:157; Senny vd.,2018:1267). In this context, the pH values of all liquid soap

samples were found to be within the standard range acceptable for natural liquid soaps.

Viscosity: Viscosity determines the fluidity and spreading properties of a product. Consumers generally do not prefer products that are either too runny or excessively thick. Therefore, achieving the target viscosity range in the formulation is of great importance to ensure user satisfaction (Rieger and Rhein, 2007). Moreover, changes in viscosity are also used to assess the product's stability throughout its shelf life. Variations in viscosity over time may indicate undesirable conditions such as emulsion breakdown, microbial growth, or component separation (AOCS, 1987).

The viscosity values of liquid soaps formulated with natural plant oils were generally found to be lower compared to commercial liquid soaps. This is due to the types of ingredients used in the formulation and the production methods applied. In addition, the fatty acid profile of the oils used significantly affects viscosity. Oils with a high oleic acid content, such as olive oil, tend to produce more fluid soaps, whereas oils rich in saturated fatty acids, such as stearic or palmitic acid, result in thicker formulations (Gupta and Gaud, 2015). In commercial products, these fatty acids are usually added as isolated ingredients, while in natural soap production, only the fatty acids derived from raw oils are used.

Every substance contains molecules of varying sizes and properties. Therefore, depending on the amount and nature of the molecules, the force required to induce movement also varies. According to the initial and one-month viscosity results of the natural liquid soap samples, the viscosity of the SS-cold-D1 sample could not be measured as it was below 15 cP, indicating that the sample had very low viscosity and high fluidity. In this sample, a noticeable color change was also observed, suggesting that an error may have occurred during the reaction process. When examining the other samples, the viscosity values of those stored at 45°C for one month and those kept on the shelf for six months were found to be very similar. In the liquid soap formulations, no synthetic surfactants (such as SLES or SLS) were added or reacted. Instead, only natural oils (such as olive oil, coconut oil, and laurel oil) were used. For this reason, the viscosity values of the natural liquid soap samples were found to be lower compared to commercial liquid soaps, which typically have viscosities ranging between 1000 and 1500 cP.

Foaming: With the exception of sample SS-hot-A-1, the foam performance of all other liquid soap samples was observed to be considerably high at the 0, 3, 5, and 8-minutes. The absence of foam in sample SS-hot-A-1, even

initially, is attributed to incomplete saponification, likely caused by weighing errors.

Table 1. Stability analysis results of plat based natural liquid soaps at initial time and after 6 months of shelf storage

Storage Condition	Parameter	SS-cold-D1	SS-cold-A2	SS-hot-HC1	SS-hot-HC2	SS-hot-A1	SS-hot-D2
		Initial / 6 month	***Initial / 6 month***	***Initial / 6 month***	***Initial / 6 month***	***Initial / 6 month***	***Initial / 6 month***
Appearance		Homogeneous paste	Homogeneous paste	Homogeneous paste	H Homogeneous paste	Homogeneous paste	Homogeneous paste
Color		Opaque cream / Milky brown	Opaque white / O.W.	Opaque white / O.W.	Opaque light cream / O.L.C.	Opaque light cream / O.L.C.	Opaque light cream / O.L.C.
Odor		Standard	Standard	Lavender / Lavender	Lemograss / Lemongrass	Peppermint / Peppermint	Lemongrass / Lemongrass
Density (g/cm³)		1.030 / 1.021	1.034 / 1.023	1.021 / 1.020	1.007 / 1.005	1.016 / 1.014	1.025 / 1.017
pH		8.8 / 8.6	8.9 / 8.8	8.8 / 8.5	8.7 / 9.5	8.2 / 8.0	8.6 / 8.4
Viscosity (cP) Room temp.	(R-5 / 250 RPM)	<15 / 80.49	154 / 273.5	139.8 / 155	183 / 205	203 / 184.9	228 / 402
Foam Stability *	(0 / 3 / 5 / 8 min)	29-29-23-16 / 28-28-26-23	25-25-19-18 / 31-29-28-25	41-40-25-20 / 20-19-17-16	27-24-23-23 / 35-34-28-25	No foam / No foam	20-16-14-14 / 19-18-18-17

O.W. = Opaque White
O.L.C. = Opaque Light Cream
* The foam values represent the observed foam height at specific time intervals (e.g., 29–29–23–16 mm per minute)

Table 2. Stability analysis results of plant based atural liquid soaps after one month of incubation at 45 °C

Storage Condition	Parameter	SS-cold-D1	SS-cold-A2	SS-hot-HC1	SS-hot-HC2	SS-hot-A1	SS-hot-D2
Incubated at 45°C (1month)	**Appearance**	Homogeneous paste	Homogeneous paste	Homogeneous paste	Homogeneous paste	Homogeneous paste	Homogeneous paste
	Color	Milky brown	Opaque white	Opaque white	Opaque cream	Opaque light cream	Opaque light cream
	Odor	Standard	Standard	Lavender	Lemongrass	Peppermint	Lemongrass
	Density (g/cm³)	1.016	1.028	1.019	1.050	1.016	1.019
	pH	8.6	8.6	8.5	8.5	8.0	8.4
	Viscosity (cP) R5/250 RPM	<15	280.3	147.3	195.0	215.5	375.0
	Foam Stability* (0 / 3 / 5 / 8 min)	32 / 33 / 23 / 19	51 / 37 / 34 / 25	19 / 18 / 17 / 17	43 / 43 / 27 / 25	No foam	19 / 19 / 19 / 17

* The foam values represent the observed foam height at specific time intervals (e.g., 29–29–23–16 mm per minute)

Results of Microbiological Analysis of Plant Based Natural Liquid Soap

In this study, the microbiological analyses of the plant-based natural liquid soaps were conducted as described in the methodology section. The tests aimed to detect the presence of microorganisms that are not permitted according to European Commission criteria. After a 24-hour neutralization and enrichment step in Eugon LT Broth, the samples were inoculated onto solid media including Tryptic Soy Agar, Sabouraud Dextrose Agar with Chloramphenicol, MacConkey Agar, Baird Parker Agar, and Cetrimide Agar. These selective media were used to assess the presence of bacteria that are prohibited in cosmetic products under European Commission regulations. The results are presented in Table 3.

Table 3. Results of microbiological analysis of plant based natural liquid soap

Storage Condition	Criteria	SS-Cold-D1	SS-Cold-A2	SS-Hot-HC1	SS-Hot-HC2	SS-Hot-A1	SS-Hot-D2
Room Temperature (Initial–6 months)	Total Bacteria (CFU/mL)	$1.45x10^3$	0	0	0	0	0
	Pathogenic Bacteria	N.D	N.D	N.D	N.D	N.D	N.D
Room Temperature (6th month)	Total Bacteria (CFU/mL)	0	0	$1x10^2$	$1x10^2$	0	0
	Pathogenic Bacteria	N.D.	N.D	N.D	N.D	N.D	N.D
Incubator at 45°C(1 month)	Total Bacteria (CFU/mL)	0	0	0	0	0	0
	Pathogenic Bacteria	N.D	N.D	N.D	N.D	N.D	N.D

*Not detected

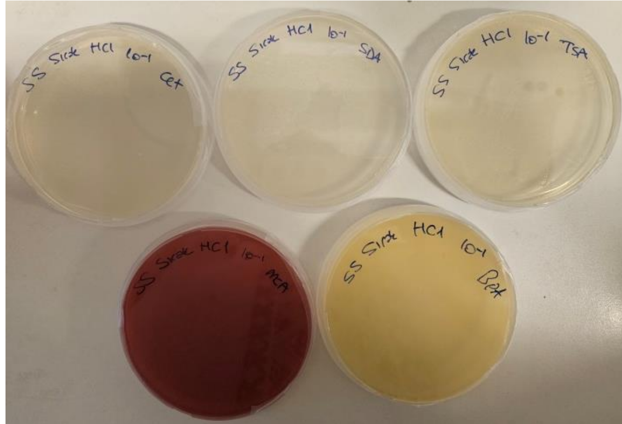


Figure 8. Petri plate images of SS-Hot-HCl samples from the 10−1 dilution of liquid soap samples after incubation on different agar media

Only the initial sample of the SS-cold-D1 liquid soap formulation was found to contain a total aerobic mesophilic bacterial count exceeding the European Commission's permissible limit of 1.0×10^3 CFU/mL. However, in the same sample analyzed after one month of incubation at 45°C and after six months of storage at room temperature, no aerobic mesophilic bacteria were detected. This indicates that the antibacterial effects of the natural oils used in the formulation persisted even after one month. In the other samples, no aerobic mesophilic or pathogenic bacteria were detected either in the initial samples or after one month of incubation at 45°C. Furthermore, after six months of storage, none of the samples showed the presence of microorganisms that are prohibited in Category 1 (rinse-off) cosmetic products according to European Commission regulations. Specifically, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans* were not detected. In the SS-hot-HC1 and SS-hot-HC2 samples, the total aerobic mesophilic bacterial count was 100 CFU/mL, which is well below the allowable limit of 1000 CFU/mL. No pathogenic microorganisms were found in these samples either (Figure 8). These findings demonstrate that the antimicrobial effects of the natural oils persisted throughout the storage period and that the produced liquid soaps are microbiologically safe for use.

CONCLUSION

Herbal cosmetics are preparations that contain phytochemicals derived from various botanical sources, which influence skin functions and provide nourishing substances for healthy hair or skin. In some cases, synthetic cosmetic products can contain harmful chemicals that may cause serious damage to human skin. For this reason, interest in herbal cosmetics has

significantly increased in recent years. In this study, natural liquid soap formulations of herbal origin were developed, and the stability and microbial content of these produced products were analyzed.

Upon examining the stability results (appearance, color, odor, density, pH, foam, viscosity) of the five liquid soap samples formulated, it was found that the stability and microbiological test results of four of the liquid soaps, with the exception of samples SS-cold-D1 and SS-hot-A1, were within standards. All liquid soaps were found to be microbially suitable for use after being stored for both 1 month and 6 months. However, due to the absence of any microbial growth and their suitable stability, the most preferred formulations are SS-hot-D2 and SS-cold-A2**.**

This study has ensured the most effective utilization of promising plant parts (fixed oils, essential oils, extracts, hydrolates, etc.) for use in cosmetic products, by evaluating them from a phytotherapy perspective. Furthermore, by producing non-synthetic, natural herbal cosmetic products that are not harmful to human health, a contribution has been made to the shift towards nature in the personal care industry. Additionally, it is anticipated that this study will benefit the Turkish economy by evaluating plant parts that are effective from a phytotherapy perspective, thus contributing to the cosmetic industry and natural product industry in Turkey.

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Mikroçoğaltılan Bitkilerin Genetik Kararlılığının Moleküler Analiz Yöntemleri ile Belirlenmesi

Burcu ÇETİN

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ÖZET

Bitki doku kültürü yöntemleri bitkilerin kontrollü koşullarda üretilmesini mümkün kılar. Ancak bu işlemler esnasında uygulanan kültür koşulları yada alt kültür sayısı bitkilerde genetik farklılıkların oluşumuna neden olabilmektedir. Bu işlemler sonucunda elde edilen bitkilerin genetik yapılarının belirlenmesi için moleküler tekniklerden yararlanılmaktadır.
Bu çalışmada mikroçoğaltım ile üretilen bitkilerin genetik kararlılığının belirlenmesinde en çok kullanılan RAPD, AFLP, SSR ve ISSR moleküler analizlerinden bahsedilmiştir. Son yıllarda bu yöntemler ile yapılan araştırmalar özetlenmiştir.
Mikroçoğaltım ile üretim yapan firmaların gerek çeşitlerini korumak gerekse yeni çeşitleri saptayabilmeleri için bitki doku kültürü yöntemleri ile üretilen bitkilerin genetik kararlılıklarının belirlenmesi önemlidir.

Anahtar Kelimeler -AFLP, Genetik kararlılık, ISSR, in vitro, mikroçoğaltım, RAPD

GİRİŞ

Hızla artan dünya nüfusunun gerek gıda gerek tıbbi ihtiyaçlarını karşılayan bitkilerin, geleneksel yöntemlerden farklı olarak, biyoteknolojik yöntemlerle üretimi konusunda yapılan çalışmalar son yıllarda önem kazanmıştır. Kum kültürü, su kültürü gibi uygulamalardan sonra, bitkilerin tek bir hücre, doku veya organlarından tam bir bitki elde edilmesini sağlayan bitki doku kültürleri yöntemleri ile kısa zamanda, çok sayıda bitki üretimi yapılabilmektedir (Kocaçalışkan, 2017).

Bitki doku kültürü yöntemlerinden biri olan mikroçoğaltım yöntemi, organize meristemlerden, henüz olgunlaşmamış veya olgunlaşmasını tamamlamış somatik hücrelerden direkt veya indirekt yollar ile hastalıksız, klonal ve istenildiği zaman istenilen miktarda bitki üretimini mümkün kılar (Gürel vd.,2013). Mikroçoğaltımın temeli bitki hücrelerindeki genetik bilginin tam bir bitki oluşturabilme yeteneği olarak açıklanan “totipotansi” özelliğine dayanır. Totipotansi özelliğine sahip bitki hücreleri bitki büyüme düzenleyicileri ile sürgün, kök veya kallusa yönlendirilebilir. Bu sayede tek bir hücreden veya dokudan tam bir bitki elde edilebilir. Mikroçoğaltım uygulamalarında en çok kullanılan bitki büyüme düzenleyicileri oksin ve sitokininlerdir. Her bitki türü veya bir türdeki hücre ve dokulardaki içsel hormon miktarı farklıdır. Bu nedenle en uygun mikroçoğaltım yönteminin belirlenmesi için, bitki doku kültürü ortamına ilave edilecek bitki büyüme düzenleyicilerin oranının her bitki türü için yapılacak uygulamalar ile saptanması gerekir (Babaoğlu vd., 2002).

Bitki büyüme düzenleyicilerine uzun süre maruz kalmanın, *in vitro* çoğaltım sırasında DNA metilasyonu, kromozomal yeniden düzenlemeler, nokta mutasyonları veya ploidi değişiklikleri gibi somaklonal varyasyonları

indüklediği bilinmektedir. Bu varyasyonlar, mikro çoğaltılmış bitkilerin genetik tekdüzeliğini tehlikeye atarak, genetik sadakatin değerlendirilmesini doku kültüründe önemli bir adım haline getirir. Farklı DNA tabanlı belirteçler, çeşitli bitki türlerinde doku kültürüyle yetiştirilen bitkilerde kalıtsal genetik değişiklikleri tespit etmek için yaygın olarak kullanılmıştır (Malhotra vd., 2025).

Somaklonal varyasyonların belirlenmesinde kullanılan başlıca moleküler teknikler, RFLP (Restriction Fragment Length Polymorphism); AFLP (Amplified Fragment Length Polymorphism); RAPD (Random Amplified Polymorphic DNA); SSR (Simple Sequence Repeats); ISSR (Inter Simple Sequence Repeats) ; SCAR (Sequence Characterised Amplified Region) dır.

Literatürler araştırıldığında en çok kullanılan moleküler markörlerin RAPD, AFPL ve ISSR oldukları görülmektedir.

Rastgele Arttırılmış Polimorfik DNA (RAPD), Williams vd., (1990) tarafından özellikle gen haritası çıkarılmamış organizmaların genetik analizi için geliştirilmiş bir yöntemdir. Rastgele nükleotid dizisine sahip tek bir primer, DNA üzerinde zıt ipliklerindeki iki farklı noktaya bağlanır. PCR ile, bu noktalar birbirinden çoğaltılabilir bir mesafede ise farklı DNA ürünleriyle sonuçlanır, Primer bağlanma noktalarından bir veya her ikisindeki dizi farklılıkları, bireyler arasında polimorfizm ile sonuçlanır. Bu polimorfizmler, spesifik bir RAPD bandının varlığı veya yokluğu şeklinde görülür ve bu polimorfizmler baskın genetik belirteçler gibi davranır (Williams vd., 1990; Reshma ve Das, 2021; Yorgancılar vd, 2015)..

Amplifikasyon parça uzunluğu polimorfizmi (AFLP), polimorfizmleri tespit etmek için oldukça hassas bir yöntemdir. Vos vd. tarafından (1995), RAPD-PCR metodunun prensipleri çerçevesinde RAPD tekniğinin dezavantajlarını gidermek amacıyla AFLP metodu geliştirilmiştir. AFLP tekniğinin tekrarlanabilirliği ve polimorfizm düzeyi RAPD-PCR metoduna göre daha yüksektir. Bu teknikte öncelikle DNA enzimler ile kesilir. Her parçanın uçlarına özel olarak tasarlanmış oligonükleotid adaptörleri bağlanır ve bu adaptor dizilerinin tamamlayıcı primerleri PCR ile çoğaltılır. Bu şekilde elde edilen bantlama deseni, popülasyon genetiği, sitematik ve akrabalık analizleri uygulamalarında kullanılır (Marwal ve Gaur, 2020; Yorgancılar vd, 2015).

Basit dizi tekrarları (SSR), mikrosatelitler olarak da adlandırılan SSR'ler, çoğu bitki genomunda yaygın olarak bulunan bir tür tekrarlayan DNA dizisidir. SSR'ler, 1-6 bp uzunluğunda bir motif dizisinin tekrarlarını içerir. Bu yapı nedeniyle SSR'ler sıklıkla mutasyona uğrar, çoğunlukla tekrar biriminin eklenmesi veya çıkarılmasını içeren DNA polimeraz hataları nedeniyle. SSR belirteçleri yüksek polimorfizm, yüksek özgüllük, kullanım kolaylığı, nispeten düşük maliyet, kolay tespit ve genetik eş baskınlık gibi avantajlara sahiptir. Popülasyon farklılaşmasında, genetik çeşitlilik çalışmalarında, akrabalık analizinde, bağlantı analizinde, gen

haritalamasında ve genetik çeşitlilik araştırmalarında yaygın olarak kullanılmış olup çeşitli mahsullerin moleküler ıslahında kolaylık sağlamıştır (Xu vd., 2025

Basit tekrarlı diziler arası polimorfizm (ISSR), komşu, zıt yönlü mikrosatelit bölümleri arasında bulunan, 100 ila 3000 bp arasında değişen boyutlardaki DNA segmentleridir. Mikrosatelitlerin (SSR'ler) ve çoğaltılmış parça uzunluk polimorfizminin (AFLP) avantajlarının çoğunu RAPD'nin evrenselliğiyle birleştiren basit ve hızlı bir yöntemdir. ISSR'ler, primer olarak mikrosatelit çekirdek dizileri ve tekrarlanmayan komşu alanlara (16-18 bp) çapa olarak birkaç seçilmiş nükleotid PCR kullanılarak çoğaltılır. Çeşitli lokuslardan yaklaşık 10-60 parça aynı anda oluşturulur, jel elektroforezi ile ayrılır ve belirli bir boyuttaki parçaların varlığı veya yokluğuna göre puanlanır. ISSR markörlerinin kullanımı hızlı, uygulanması kolay ve primerleri daha uzun olduklarından güvenilirlikleri fazladır. Yeterli bilgi sunan ISSR primerlerini kullanmak düşük bir maliyet, zamandan tasarruf ve genetik analizlerde kolaylık sağlamaktadır (Mir vd., 2023; Yorgancılar vd, 2015).

Son yıllarda, retrotranspozon bazlı moleküler belirteçlerin kullanıldığı çalışmalar artmıştır, çünkü bu mobil genetik elemanlar kendi büyük ve sabit kopyalarını genomun herhangi bir yerine entegre edebilir ve retrotranspozonların eklenme yerleri polimorfizmin bir göstergesi olarak kullanılabilir. iPBS tekniği bunlardan biridir, LTR retrotranspozonunun kendi kopyalarını genomun farklı yerlerine entegre etme olasılığına dayanmaktadtır. iPBS tekniği, PCR sırasında hibridizasyon için PBS (primer bağlanma yeri) dizilerinin korunan kısımlarının kullanılmasına olanak tanır (Kalendar vd., 2014; Fokina vd., 2016)

Moleküler markörler kullanılarak, çok sayıda mikroçoğaltım yapılmış bitkinin genetik kararlılığı araştırılmıştır. *Dendrocalamus asper*'in mikroçoğaltımı üzerinde farklı spektral kalitelerin etkisinin değerlendirildiği çalışmada, moleküler ISSR analizleri için, 24 primerden sadece 10 primer olası polimorfizmlerin tanımlanması için iyi tanımlanmış bantlar sunmuştur. Seçilen tüm primerler monomorfik bantları çoğaltmış ve uzama ve tesadüfi köklenme aşamasından sonra somaklonal varyasyon olmadığı belirlenmiştir (Fernandes vd., 2025). Moleküler analizlerin birden fazla kullanımı, tıbbi açıdan değerli odunsu bir sarmaşık türü olan *Pueraria tuberosa* ile yapılmıştır. Geliştirilen mikroçoğaltım protokolü sonucunda elde edilen bitkilerin genetik sadakati, rejenerasyonun genetik homojenliğini ve *in vitro* çoğaltma yönteminin güvenilirliğini gösteren monomorfik bantlar üreten ISSR ve RAPD belirteçleri kullanılarak değerlendirilmiş ve genetik olarak stabil bitkiler elde edildiği belirlenmiştir (Kanthaliya vd., 2025). *Punica granatum*'un Bhagwa çeşidinin mikroçoğaltımının belirlendiği bir başka çalışmada, mikroçoğaltılmış fidelerin ana bitki ile genetik sadakati, RAPD, ISSR ve SSR'yi içeren moleküler belirteçler kullanılarak değerlendirilmiştir. Bu amaca ulaşmak için, 48 SSR, 20 RAPD ve 12 ISSR'den oluşan bir set

kullanılmıştır. Bunlardan, 14 SSR, 10 RAPD ve 9 ISSR primerleri sırasıyla 35, 94 ve 89 farklı bant üretmiştir. Seçilen tüm belirteçler, %100 homojen bantlar üretmiş ve bu, tüm *in vitro* yetiştirilen Bhagwa fidanlarının yüksek genetik tekdüzeliğini gösterdiğini belirlemiştir. Çalışmanın sonuçlarının Bhagwa nar yetiştiricilerine ve bahçecilik sektörüne kalite güvencesi sağlamada yardımcı olacağı bildirilmiştir (Abhang, vd.,2025). İstenilen özellikleri nedeniyle değer verilen ancak hastalıklara karşı hassas olan 'Oregon Spur' elma çeşidi, etkili çoğaltma yöntemleri ile üretilmiştir. Mikro çoğaltılmış bitkilerin genetik sadakati, 50 RAPD ve 20 SSR belirteçleri kullanılarak değerlendirilmiştir. Sonuçlar, *in vitro* çoğaltılmış bitkilerin genetik olarak ana bitkiyle aynı olduğunu ve hepsinin monomorfik bantlar sergilediğini göstermiştir. Belirlenen protokolün, büyük ölçekli üretim için uygun ve 'Oregon Spur' elma çeşidinin yetiştiriciliği için gerekli olan hastalıksız, tek tip dikim materyali üretmek için güvenilir bir yöntem olduğu bildirilmiştir (Kumari vd., 2025).

Geçici daldırma sistemi rejene bitkilerde somaklonal varyasyon oluşmasına neden olabilmektedir. Genetik veya epigenetik değişikliklerden kaynaklanan fenotipik değişiklikleri kapsayan somaklonal varyasyonlar, şeker kamışı verimliliği için önemli bir faktör olan fidelerin genetik tekdüzeliğini korumada zorluklar sunar. SSR'ların kullanımları ile, geçici daldırma sistemlerinde üretilen şeker kamışı fidelerinin genetik stabilitesini araştırılmıştır. 15 SSR belirteci ana bitkileriyle karşılaştırıldığında SSR desenlerinin Jaccard benzerlik endeksi genetik kararlılık, üç Endonezya şeker kamışı çeşidinde %92 ile %96 arasında değişmiştir. Ayrıca, sahada yapılan morfolojik gözlemler, geçici daldırma sistemi türevi şeker kamışı ile ana bitkileri arasında hiçbir fark olmadığını ortaya koyarak, mikroçoğaltım ürünlerinin türüne sadık olduğunu kanıtlamıştır. (Saptari vd, 2024) Çiçekçilik endüstrisi için önemli olan Karanfil cv. 'Irene' için standardize edilen mikroçoğaltım yöntemi sonucu elde edilen bitkiciklerin klonal sadakatinin belirlenmesi, 12 SSR belirteçlerinin yardımıyla gerçekleştirilmiştir. Araştırmacılar analiz edilecek bitki örnekleri olarak ana bitki ve farklı alttürlerden aldıkları 10 rejenere bitkiyi seçmişlerdir. Mikroçoğaltılan bitkilerden elde edilen tüm bant profilleri monomorfikti ve ana bitkininkilere benzerdi. Sonuçlar, bitkiciklerin doğada türlerine sadık olduğunu, yani ana bitkilere benzediğini açıkça göstermiştir (Maurya vd, 2021).

Melientha suavis Pierre, yüksek besin değerine sahip ve Opiliaceae familyasına ait önemli bir yaprak döken yenilebilir bitki türüdür. Ancak tür, habitat parçalanması ve olumsuz antropojenik etkiler nedeniyle zorluklarla karşı karşıyadır bu nedenlerle in vitro çoğaltımı araştırılmıştır. Genetik doğruluğu sağlamak için, iPBS ve SRAP belirteçleri ile analiz edilmişlerdir. Sonuçlar, mikro çoğaltılan bitkiler ile ana ağaçlar arasında genetik çeşitlilik olmadığını ortaya koymuş ve genetik olarak kararlı yavruların üretimini doğrulanmıştır. Belirlenen prtotokol türün karşılaştığı zorlukların mikro

çoğaltma ve genetik sadakat değerlendirmesi yoluyla ele alınması, tarımsal ve koruma girişimleri için korunmasına ve kullanımına önemli ölçüde katkıda bulunmuştur (Siringam ve Vainijajiva, 2023). Antitümör, antianjiyojenik ve antibakteriyel potansiyeli ile bilinen Rumex hastatus D. Don, aseptik kültürlerinin kurulması için yapılan araştırmada. Bitki köklerinde oluşan kalluslardan rejenere olan bitkilerin genetik sadakati SCoT ve SRAP belirteçleri kullanılarak analiz edilmiştir. Her iki belirteç tarafından elde edilen bantlama profilindeki bu yüksek monomorfizm, mikro çoğaltılmış bitkilerin genetik stabilitesini doğruladı. İlginç bir şekilde, mevcut araştırma kök kaynaklı kallus yoluyla üretilen bitkilerin çok az varyasyona sahip olduğunu ortaya koymuştur (Singh vd., 2025).

Moleküler analiz çalışmalarında AFLP uygulamalarının diğer yöntemlere göre çok daha az olduğu görülmektedir. Ancak yöntemin sonuçlarının daha özgün olması önümüzdeki yıllarda bu yöntem ile yapılacak çalışmaların artacağını düşündürmektedir. Süs değerine ek olarak, bitkinin farklı kısımlarının geleneksel tıpta kullanıldığına dair çeşitli kanıtlar bildirilen Kala zambağının mikroçoğaltım ile üretilmesi sonucu elde edilen bitkiler RAPD, ISSR, AFLP ve SSR yöntemleri ile çeşit tanımlama ve genetik varyasyon değerlendirmesi için kullanılmıştır (Savona vd., 2025). *Magnolia grandiflora* L. için etkili doku kültürü protokolünü incelemek ve genetik belirteç ile rejenerasyon yapılmış bitkilerin genetik stabilitesini tespit etmek amacıyla AFLP primerleri kullanılmıştır. Analizler sonucuda mikroçoğaltılmış bitkiler ana bitkilerle karşılaştırıldığında hiçbir polimorfizm tespit edilmemiş ve bu da genetik stabiliteyi kanıtlamıştır (Mosa vd. 2024).

AFLP belirteç sisteminin çok hassas ve tekrarlanabilir bir yöntem olduğunun bildirildiği çalışma, değerli meyvelerden olan Bektaşi üzümünün (*Ribes grossularia* L.), *in vitro* çoğaltılması, virüssüz dikim materyali üretimi ve pazara yeni çeşitlerin kazandırması amaçları ile yapılmıştır. Sunulan çalışmada dört bektaşi üzümü çeşidinin mikro çoğaltılmış bitkilerinin tarla performansı ve genetik stabilitesi değerlendirilmiştir. Mikroçoğaltılmış bitkilerinin genetik stabilitesini değerlendirmek için iki AFLP ve ISSR kullanılmıştır. AFLP ile değerlendirilen mikro çoğaltılmış bitkilerinin genetik değişkenliği, Hinnonmaki Rot için %0,35'ten Resika için %2,12'ye kadar değişmiştir. Mikroçoğaltım yapılmış bitkilerinin ISSR analizinin sonuçları, Hinnonmaki Rot ve Resika %0'dan Captivator ve Invicta için sırasıyla %4 ve %8,69'a kadar değişkenlik göstermiştir. Yani AFLP belirteçleri tüm bektaşi üzümü genotiplerindeki mikroçoğaltım bitkilerinde genetik değişkenliği tespit ederken, ISSR belirteçleri bunu yalnızca Captivator ve Invicta'da yapmıştır. Araştırma sonucunda ISSR analizinin AFLP'den daha az güvenilir olduğu kanıtlanmıştır. Muhtemelen bunun nedenin her bektaşi üzümü çeşidini analiz etmek için sadece beş primer kullanılması olduğu düşünülmektedir (Wójcik vd., 2020). Sarımsak (*Allium sativum* L.) 'Criollo-9' klonunun mikroçoğaltımı için bir metodoloji

oluşturarak *in vitro* bitkilerin genetik stabilitesinin belirlendiği çalışmada farklı AFLP primer kombinasyonlarını değerlendirmeyi ve *in vitro* elde edilen rejenerantları morfoagronomik olarak değerlendirmesi amaçlamıştır. AFLP sonucunda, analiz edilen her primer kombinasyonu başına ortalama 175,71 bant olmak üzere toplam 130 net bir şekilde ayrılmış bant elde edilmiştir. Belirlenen tüm bantlar monomorfik (%100), bu nedenle filogenetik mesafe 0 olarak belirtilmiştir. Tarla koşullarında 12 t/ha'dan daha yüksek verimler elde edilmiş ve moleküler belirteçlerle elde edilen sonuçlar doğrulanmıştır, bu nedenle mikroçoğaltma tekniğinin bu sarımsak genotipine uygulanması mümkün olduğu belirtilmiştir. Ayrıca, sarımsak (*Allium sativum* L.) 'Criollo-9' klonunun genetik stabilitesini doğrulamak ve bu mikroçoğaltma tekniğinin bu üründe uygulanabilir olduğunu göstermek için AFLP belirteçlerinin ve SSR gibi diğer moleküler belirteçlerin yeni kombinasyonlarının değerlendirilmesi de önerilmiştir (Izquierdo-Oviedo vd., 2017).
Çok değerli bir bitki olan safranın (*Crocus sativus* L.) *in vitro* yetiştirilmesi için bir protokol geliştirilmiştir. Yapılan analizler sonucunda iPBS belirteçleri ile değerlendirilen tarla safranının ve in vitro kültürlerin parmak izi profilleri aynı bulunmuştur, yapılan uygulamalar sonucunda elde edilen bitkiciklerde genetik değişiklik meydana gelmediği anlaşılmıştır (Petrova vd., 2023). Dört farklı moleküler belirteç; ISSR, SCoT, iPBS ve CAAT kutu-türevli polimorfizm (CBDP) yüksek oranda sitokininin kullanılanarak in vitro çoğaltılan Dendrocalamus latiflorus' bitkiciklerinin analizinde kullanılmıştır. Çalışmalar sonucunda *in vitro* klonlar arasında yüksek seviyede monomorfizm tespit edilmiştir; ISSR ve SCoT belirteçleri sırasıyla %98,4 ve %98 monomorfizm, iPBS ve CBDP belirteçleri de %100 monomorfizm belirlemişlerdir. Genotipler arasındaki yüksek monomorfizm (%99,1) ve yakın kümeleme desenleri, ortamdaki yüksek BAP ve KN seviyelerine rağmen yüksek genetik kararlılığın korunduğunu göstermiştir (Tikendra vd., 2022).

Bir çok çalışmada mikroçoğaltım sonucunda genetik olarak kararlı bitkilerin üretildiği bildirilmiştir. Mikroçoğaltım sonucu genetik kararsızlığın elde edildiğinin bildirildiği çalışmalar ise literatürde az sayıdadır. Arjantinde çiftçilerin tarlalarından toplanan 13 ümit verici muz klonundan alınan meristem eksplantları kullanarak mikroçoğaltım çalışmaları yapılmış rejene bitkilerin AFLP analizi sonucu moleküler polimorfizm gösterdikleri belirlenmiştir. Moleküler analiz sonuçları kullanılarak rejene bitkiler ve rejenerasyon yapılmamış bitkilerin tarımsal kantitatif özellikleri arasındaki ilişkiler araştırılmıştır (Ermini vd., 2024). Ananasın *in vitro* çoğaltılmasında ışığın bitki kalitesi üzerinde önemli etkiye sahip olduğunu ve sera koşullarında daha iyi bir agronomik ve anatomik değişim sağladığının görüldüğü çalışmada elde edilen morfolojik, hücresel ve biyokimyasal gözlemlere benzer şekilde ISSR analizi moleküler olarak *in vitro* rejenere edilmiş bitkicikler arasında varyasyonlar ortaya koymuştur ve bu da ananas

bitkilerinde somaklonal varyasyonun varlığına dair daha fazla kanıt sağlamıştır (Kohpaii vd., 2017). *Vanilla planifolia*'nın somaklonal varyasyonu üzerine alt kültür döngülerinin etkisi, ISSR belirteçleri kullanılarak analiz edildiği çalışmada 10 alt kültür döngüsü ile çoğaltılan bitkiler 10 ISSR primeri kullanılarak analiz edilmiştir. Sonuçlar, 8.86 µM BAP'li alt kültür sayısının, *V. planifolia*'nın *in* vitro rejenerasyonunu etkilediği bildirilmiştir (Pastelín Solano vd., 2019).

SONUÇ

Ticari mikroçoğaltım çalışmalarında elde edilen bitkiciklerin genetik olarak stabil ve tek tip olması tercih edilir. Ancak doku kültürü koşullarında çok sayıda yapılan alt kültürleme ya da *in vitro* koşullar bitkilerin genetik yapısında değişikliklere neden olabilir.

Mikroçoğaltım sonucu elde edilen bitkilerdeki fenotipik değişiklikler yapılan gözlemler ile belirlenebilirken, genom seviyesinde meydana gelen değişimler moleküler markör teknikleri ile belirlenebilir. Doku kültürü işlemleri sırasında meydana gelen fizyolojik, embriyolojik, anatomik, biyokimyasal ve moleküler değişimler bize temel bilim çalışma alanlarında da çok değerli bilgiler sunar.

Özellikle son yıllarda doku kültürü teknikleri ile bitki üretimi konusunda ticari birçok firma tarafından klonal olarak üretim yapılmaktadır. Bu firmalar tarafından ticari çeşitlerin üretimleri esnasında herhangi bir genotipik değişimin meydana gelip gelmediğinin belirlenmesi önemlidir. Bunun yanı sıra ıslahçılar tarafından kullanılan bir yöntem olan somaklonal varyasyonlar sonucunda elde edilen çeşitlerin genotipik olarak tescillenmesi gerekir. Sonuç olarak doku kültürü yöntemleri ile üretilen bitkilerin genetik karakterizasyonu için moleküler tekniklerin kullanımı her geçen gün daha da önemli olacaktır.

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