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CDK inhibitors as anti-cancer drugs; Present and Future

Omer Kacar^{1*}

Editorial

The cell reproduces itself via replication. The cyclical replication processes from the resting state (G1 phase) to cell division (M Phase) called cell cycle. Cell cycle requires complicated steps are composed of G1, S, G2, and M. These step transitions from one to another are strictly controlled. Control of eukaryotic cell growth and division occurred at the three critical points: late G1, G2/M, and metaphase-to-anaphase transition. These critical steps are also known as "checkpoints" which ensure correct timing of cellular events. The checkpoints trigger the passaging through the phases of the cycle which requires organized set of proteins checks proper cell growth and DNA integrity. The most well-known monitoring proteins for late G1 and G2/M checkpoints are cyclic dependent kinases (CDKs) which are activated by cyclin proteins, however, unlike the other two checkpoints, metaphase-to-anaphase transition checkpoint is controlled by anaphase promoting complex (APC/C). Many CDKs have specificity for cell cycle checkpoints, for instances CDK4 and CDK6 for late G1, CDK2 for S and CDK1 for G2/M (1).

Central player of cell cycle progress is CDKs and associated proteins cyclins. CDKs are activated or inhibited by CDK cyclins or inhibitors, respectively. CDKs are highly affected kinase enzymes that are blocked by inhibitors which stop the cell cycle progression and induce cell growth arrest. Cancer is characterized by loss of cell cycle control as a result of accumulation of mutations or overlooked DNA damages by the check point enzymes. These mutations also result in increased mutagenic signals and defective anti-mitogen signalling pathways. The expression and inhibition of CDKS are deregulated in cancer, hence the successful inhibition of CDKs is focused for cancer therapy. During the past 20 years, many drugs for CDKs inhibition were investigated to fight against cancer (2).

To drive the cells from G0 or G1 phase into S phase through CDK4 and CDK6, the D-type cyclins (that is, cyclin D1, cyclin D2 and cyclin D3) are needed to control activity of the CDKs. The different D-type cyclins seem to show tissue specific expression in normal cells.

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Completed his Ph.D. in Istanbul University Faculty of Science, Dept. of Molecular Biology, Istanbul, Turkey. Dr. Kacar is focused to Differentiation, Regeneration, cell cycle and new cancer Therapeutics during his carrier. Dr. Kacar already is working at TUBITAK Marmara Research Center, Genetic Engineering and Biotech. Inst. Gebze Kocaeli



The transcriptional regulation of CDK4 and CDK6 is the one of way to control late G1 checkpoint, whereas interaction of cyclin D with CDK4 are tightly regulated. Inhibitor proteins of CDK4 (INK4 (INhibitors of CDK4)) are composed of p16INK4a, p15INK4b, p18INK4c, p19INK4d which interact with CDK4 and CDK6 inhibits the activity of these CDKs. The INK proteins can bind both CDK4, CDK6 and D type cyclins. CDK4 and CDK6 with the contribution D-type cyclins phosphorylate the tumor suppressor retinoblastoma proteins (RB) which drive the cell cycle arrest at late G1 phase. The CDK4/6-RB pathway is critical to continue cell duplication; therefore, deregulation of this pathway is expected in most cancers. CDK4 and CDK6 are also hyper activated or overexpressed in a wide variety of tumors (3).

CDK2 and cyclin E association is essential to drive the G1 to S transition. INK4 inhibitor proteins does not regulate the CDK2, however which is regulated by CDK, interacting protein/kinase inhibitory protein (CIP/KIP) class of CDK inhibitors. The CIP/KIP proteins, p21CIP1, p27KIP1, inhibit CDKs activity by binding CDK2-cyclin complexes(4).

CDK1 associated with cyclins cyclin A2 and Cyclin B1 is essential for initiating M phase in eukaryotes. The cell in the M phase with the mis-replicated DNA leads to the cell death. The onset of M phase is under strictly controlled by checkpoint signalling kinases, CHK1 and WEE1, to overcome contributing the death of cell with mis-replicated DNA (5).

In addition to the mentioned CDKs above, there are cell cycle-independent CDKs (i.e. CDK7, CDK8, CDK9) which contribute to the basal transcriptional regulation of the cell cycle. In order to importance of

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1 TUBITAK Marmara Research Center, Genetic Engineering and Biotech. Inst. Gebze Kocaeli *Corresponding Author: Omer Kacar. E-mail: kacaromer@yahoo.com transcription-regulating CDKs which are taken attention as anti-cancer drugs.

The 21 human CDKs are encoded by the genome, however only 7 CDKs have functional role in the cell cycle progression. Hyper activation of CDKs may lead to the tumor progression by unregulated proliferation. Also INK4 inhibitors are blocked by some mutations in some type of cancers. (6). The CDK inhibitors as therapeutic targets have been investigated for 20 years. The CDKs inhibitors are divided three groups which are ATP-competitive, ATP-noncompetitive (including some small mimetic peptides for p21, p27 and p57), Allosteric inhibitors.

CDK4 and CDK6 are valuable targets to fight against cancer due to their essential role in the starting of cell cycle. Although the importance of the CDK4 and CDK6 for proliferation, the first investigated CDK inhibitors are "pan CDKs" (i.e. flavopiridol (alvocidib; developed by Sanofi-Avantis), olomucine (not commercial), roscovitine (seliciclib; developed by Cyclacel)), which have relatively low affinity for CDK4 and CDK6 (7).

The first CDK inhibitor approved by US FDA is palbociclib (Ibrance), a CDK4/6 inhibitor, in Feb 2015. In currently, there are 11 inhibitors -Palbociclib, Pfizer (Onyx Pharmaceuticals); Abemaciclib, Eli Lilly; Ribociclib, Novartis/Astex; Alvocidib, Sanofi (Tolero); Milciclib, Nerviano; MM-D37K, MetaMax; G1T28-1, G-1 Therapeutics; TG-02, Tragara Pharmaceuticals; Seliciclib, Cyclacel; AT-7519, Astex; Roniciclib, Bayer- under clinical evaluation (8).

All types of CDK inhibitors have great interest as therapeutic cancer agents for researchers, but still more specified CDKs inhibitors are needed to take control of cell division in different check points.

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Original Article

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Slant helices in dual Lorentzian Space D_1^3

Derya Saglam^{1*}, Serhat Ozkan¹, Duygu Ozdamar¹

Abstract

In this paper, we consider a unit speed dual Lorentzian curve $\tilde{\alpha}$ in dual Lorentzian space D_1^3 and denote by $\{\tilde{T}, \tilde{N}, \tilde{B}\}$ the dual Frenet frame of $\tilde{\alpha}$. We say that α is a slant helix if there exists a non-zero dual constant vector field \tilde{U} in D_1^3 such that the dual function $\langle \tilde{N}, \tilde{U} \rangle$ is a dual constant. Moreover, we give some characterizations of slant helices in terms of their dual curvatures. Finally, we show that dual tangent indicatrices and dual binormal indicatrices of slant helices are dual helices.

Keywords: Dual Lorentzian space; Dual Frenet equations; Dual slant helices; Dual curves

Introduction

Dual numbers were introduced by W. K. Clifford (1849-1879) as a tool for his geometrical investigations. After him E. Study used dual members and dual vectors in his research on the geometry of lines and kinematics. He devoted special attention to the representation of directed lines by dual unit vectors and defined the mapping that is known by his name. There exists one-to-one correspondence between the points of dual unit sphere S^2 and directed lines in R^3 [2,8].

If we take the Minkowski 3-space R_1^3 instead of R^3 of the E. Study mapping can be stated as follows: The dual timelike and spacelike unit vectors of dual hyperbolic and Lorentzian unit spheres H_0^2 and S_1^2 in the dual Lorentzian space D_1^3 are in one-to-one correspondence with the directed timelike and spacelike lines in R_1^3 , respectively. Then a differentiable curve on H_0^2 corresponds to a timelike ruled surface in R_1^3 . Similarly, the timelike (resp. spacelike) curve on S_1^2 , corresponds to any spacelike(resp. timelike) ruled surface in R_1^3 [11, 8]. We will survey briefly the fundamental concepts and properties in the Lorentzian space. We refer mainly to O'Neill [5, 8].

Let R_1^3 be the 3-dimensional Lorentzian space with Lorentzian metric $\langle , \rangle = dx_1^2 + x_2^2 - x_3^2$. It is known that in R_1^3 there are three categories of curves and vectors, namely, spacelike, timelike and null, depending on their causal characters. Let \vec{x} be tangent vector of Lorentzian space. Then \vec{x} is said to be spacelike $\langle \vec{x}, \vec{x} \rangle 0$ or $\vec{x} = \vec{0}$, timelike if $\langle \vec{x}, \vec{x} \rangle \langle 0$, null (lightlike) if $\langle \vec{x}, \vec{x} \rangle = 0$ and $\vec{x} \neq \vec{0}$. Let $\alpha: I \subset R \to R_1^3$ be a regular curve in R_1^3 . Then, the curve α is spacelike if all velocity vectors are spacelike. Similarly, it is called timelike and null curve if all its velocity vectors are timelike and null vectors, respectively [8].

A dual number \overline{x} has the form $x + \varepsilon x^*$ with properties

$$\varepsilon \neq 0, 0\varepsilon = \varepsilon 0 = 0, 1\varepsilon = \varepsilon 1 = \varepsilon, \varepsilon^2 = 0$$

where x and x^* are real numbers and ε is the dual unit (for the properties of dual vectors, see [7,12]). An ordered triple of dual numbers ($\overline{x}_1, \overline{x}_2, \overline{x}_3$) is called a dual vector and the set of dual vectors is denoted by

$$D^{3} = D \times D \times D = \{ \tilde{x} | \tilde{x} = (\overline{x}_{1} + \varepsilon \overline{x}_{1}^{*}, \overline{x}_{2} + \varepsilon \overline{x}_{2}^{*}, \overline{x}_{3} + \varepsilon \overline{x}_{3}^{*})$$
$$= (\overline{x}_{1}, \overline{x}_{2}, \overline{x}_{3}) + \varepsilon (x_{1}^{*}, x_{2}^{*}, x_{3}^{*})$$
$$= \vec{x} + \varepsilon \vec{x}^{*}, \quad \vec{x}, \vec{x}^{*} \in \mathbb{R}^{3} \}.$$

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 D^3 is a module on the ring D. For any $\tilde{x} = \vec{x} + \varepsilon \vec{x}^*$, $\tilde{y} = \vec{y} + \varepsilon \vec{y}^* \in D^3$, if the Lorentzian inner product of dual vectors \tilde{x} and \tilde{y} defined by

$$\langle \tilde{x}, \tilde{y} \rangle = \langle \vec{x}, \vec{y} \rangle + \varepsilon \left(\langle \vec{x}, \vec{y}^* \rangle + \langle \vec{x}^*, \vec{y} \rangle \right)$$

then the dual space D^3 together with this Lorentzian inner product is called dual Lorentzian space and it is shown by D_1^3 [8]. A dual vector \tilde{x} in D_1^3 is said to be spacelike, timelike and lightlike (null) if the vector \vec{x} is spacelike, timelike and lightlike (null), respectively. Dual Lorentzian cross product of dual vectors $\tilde{x} = (\bar{x}_1, \bar{x}_2, \bar{x}_3)$ and $\tilde{y} = (\bar{y}_1, \bar{y}_2, \bar{y}_3)$ in D_1^3 is defined by

$$\begin{split} \tilde{x} \times \tilde{y} &= \begin{vmatrix} -\vec{e}_1 & \vec{e}_2 & \vec{e}_3 \\ \overline{x}_1 & \overline{x}_2 & \overline{x}_3 \\ \overline{y}_1 & \overline{y}_2 & \overline{y}_3 \end{vmatrix} \\ &= (\overline{x}_3 \overline{y}_2 - \overline{x}_2 \overline{y}_3, \overline{x}_3 \overline{y}_1 - \overline{x}_1 \overline{y}_3, \overline{x}_1 \overline{y}_2 - \overline{x}_2 \overline{y}_1) \end{split}$$

[8]. If $\vec{x} \neq 0$, the norm $\|\vec{x}\|$ of $\vec{x} = \vec{x} + \varepsilon \vec{x}^*$ is defined by

$$\|\tilde{x}\| = \sqrt{\left|\left\langle \tilde{x}, \tilde{x} \right\rangle\right|}$$
.

A dual vector \tilde{x} with norm 1 is called a dual unit vector. Let $\tilde{x} = \vec{x} + \varepsilon \vec{x}^* \in D_1^3$. Then,

i) The set

 $S_1^2 = \{\tilde{x} = \vec{x} + \varepsilon \vec{x}^* | \|\tilde{x}\| = (1,0); \ \vec{x}, \vec{x}^* \in R^3 \text{ and the vector } \vec{x} \text{ is spacelike} \}$ is called the pseudo dual sphere in D_1^3 .

ii) The set

 $H_0^2 = \{\tilde{x} = \vec{x} + \varepsilon \vec{x}^* | \|\tilde{x}\| = (1,0); \ \vec{x}, \vec{x}^* \in R^3 \text{ and the vector } \vec{x} \text{ is timelike} \}$ is called the pseudo dual hyperbolic space in D_1^3 [7, 11, 8].

If every real valued functions $\alpha_i(t)$ and $\alpha_i^*(t)$ $1 \le i \le 3$, are differentiable, dual Lorentzian curve

$$\begin{split} \tilde{\alpha} : I \subset R \to D_1^3 \\ t \to D_1^3 &= (\alpha_1(t) + \varepsilon \alpha_1^*(t), \alpha_2(t) + \varepsilon \alpha_2^*(t), \alpha_3(t) + \varepsilon \alpha_3^*(t) \\ &= \vec{\alpha} \ (t) + \varepsilon \vec{\alpha}^*(t) \end{split}$$

is differentiable in D_1^3 . The real part $\vec{\alpha}$ (*t*) of the dual Lorentzian curve $\tilde{\alpha} = \tilde{\alpha}(t)$ is called indicatrix. The dual arc length of the curve $\tilde{\alpha}(t)$ from t_1 to *t* is defined as

(1.1)
$$\overline{s} = \int_{t_1}^t \|\widetilde{\alpha}'(t)\| dt = \int_{t_1}^t \|\widetilde{\alpha}'(t)\| dt + \varepsilon \int_{t_1}^t \langle \overrightarrow{T}, \overrightarrow{\alpha}^{*'}(t) \rangle dt = s + \varepsilon s^*,$$

where \vec{T} is a unit tangent vector of the indicatrix $\vec{\alpha}$ (t). From now on we will take the arc length s of $\vec{\alpha}$ (t) as the parameter instead of t [7, 8].

Now we can give dual Frenet equations for unit speed dual timelike and spacelike curves in D_1^3 similar to that in R_1^3 [1, 3, 10, 7].

Timelike Case ($\langle \tilde{T}, \tilde{T} \rangle = -1$):

Let $\tilde{\alpha}$ be a unit speed dual timelike curve in D_1^3 . The dual Frenet frame { $\tilde{T}, \tilde{N}, \tilde{B}$ } of $\tilde{\alpha}$ is given by

$$\widetilde{T} = \widetilde{\alpha}', \ \widetilde{N} = \frac{\widetilde{T}'}{\overline{\kappa}} = \frac{\widetilde{\alpha}''}{\|\widetilde{\alpha}''\|}, \ \widetilde{B} = \widetilde{T} \times \widetilde{N}$$

where \times is dual Lorentzian cross product. The dual Frenet equations are

(1.2)
$$\frac{d}{d\overline{s}} \begin{pmatrix} T\\ \widetilde{N}\\ \widetilde{B} \end{pmatrix} = \begin{pmatrix} 0 & \overline{\kappa} & 0\\ \overline{\kappa} & 0 & \overline{\tau}\\ 0 & -\overline{\tau} & 0 \end{pmatrix} \begin{pmatrix} T\\ \widetilde{N}\\ \widetilde{B} \end{pmatrix}$$

where $\overline{\kappa} = \kappa + \varepsilon \kappa^*$ is nowhere pure dual curvature and $\overline{\tau} = \tau + \varepsilon \tau^*$ is nowhere pure dual torsion.

Spacelike Case ($\langle \tilde{T}, \tilde{T} \rangle = 1$):

We separate three cases depends on causal character of \tilde{T} '.

i) Let \widetilde{T}' be a dual spacelike vector field. Since $\overline{\kappa} = \|\widetilde{T}'\| = \sqrt{\langle \widetilde{T}', \widetilde{T}' \rangle}$, $\overline{\tau} = -\langle \widetilde{N}', \widetilde{B} \rangle$, $\widetilde{N} = \widetilde{T}'/\overline{\kappa}$ and

 $\widetilde{B} = \widetilde{T} \times \widetilde{N}$ then the dual Frenet equations:

(1.3)
$$\frac{d}{d\overline{s}} \begin{pmatrix} T\\ \widetilde{N}\\ \widetilde{B} \end{pmatrix} = \begin{pmatrix} 0 & \overline{\kappa} & 0\\ -\overline{\kappa} & 0 & \overline{\tau}\\ 0 & \overline{\tau} & 0 \end{pmatrix} \begin{pmatrix} T\\ \widetilde{N}\\ \widetilde{B} \end{pmatrix}$$

where $\overline{\kappa} = \kappa + \varepsilon \kappa^*$ is nowhere pure dual curvature and $\overline{\tau} = \tau + \varepsilon \tau^*$ is nowhere pure dual torsion.

ii) Let \tilde{T} be a dual timelike vector field. Since $\bar{\kappa} = \|\tilde{T}\| = \sqrt{-\langle \tilde{T}, \tilde{T} \rangle}$, $\bar{\tau} = \langle \tilde{N}, \tilde{B} \rangle$ then the dual Frenet equations:

(1.4)
$$\frac{d}{d\overline{s}} \begin{pmatrix} \widetilde{T} \\ \widetilde{N} \\ \widetilde{B} \end{pmatrix} = \begin{pmatrix} 0 & \overline{\kappa} & 0 \\ \overline{\kappa} & 0 & \overline{\tau} \\ 0 & \overline{\tau} & 0 \end{pmatrix} \begin{pmatrix} \widetilde{T} \\ \widetilde{N} \\ \widetilde{B} \end{pmatrix}$$

where $\overline{\kappa} = \kappa + \varepsilon \kappa^*$ is nowhere pure dual curvature and $\overline{\tau} = \tau + \varepsilon \tau^*$ is nowhere pure dual torsion.

iii) Let \tilde{T}' be a dual lightlike vector field. The dual Frenet frame is $\tilde{T} = \tilde{\alpha}'$, $\tilde{N} = \tilde{T}'$ and \tilde{B} is the unique dual lightlike vector field orthogonal to \widetilde{T} such that $\langle \widetilde{N}, \widetilde{B} \rangle = 1$. Then the dual Frenet equations:

(1.5)
$$\frac{d}{d\overline{s}} \begin{pmatrix} \widetilde{T} \\ \widetilde{N} \\ \widetilde{B} \end{pmatrix} = \begin{pmatrix} 0 & 1 & 0 \\ 0 & \overline{\tau} & 0 \\ -1 & 0 & -\overline{\tau} \end{pmatrix} \begin{pmatrix} \widetilde{T} \\ \widetilde{N} \\ \widetilde{B} \end{pmatrix}$$

where $\overline{\tau} = \tau + \varepsilon \tau^*$ is nowhere pure dual torsion.

Slant helices ever been studied by many researchers [1, 4, 6, 9, 13, 14]. Recently, slant helices have been studied in Minkowski space E_1^3 and the authors have given characterizations of the curves [1]. In this paper we study dual slant helix in dual Lorentzian space and investigate dual version of some well-known results for dual slant helices in dual Lorentzian space. Also we take a dual Lorentzian curve $\tilde{\alpha} = \tilde{\alpha}(\bar{s})$ in dual Lorentzian space D_1^3 and denote by $\{\widetilde{T}, \widetilde{N}, \widetilde{B}\}$ the dual Frenet frame of $\widetilde{\alpha}$. We say that $\widetilde{\alpha}$ is a dual slant helix if there exists a fixed direction \widetilde{U} of D_1^3 such that the function $\langle \widetilde{N}, \widetilde{U} \rangle$ is dual constant [13, 14]. In this work we give characterizations of dual slant helices in terms of the dual curvature and dual torsion of $\tilde{\alpha}$. Finally, we discuss the dual tangent and dual binormal indicatrices of dual slant curves, proving that they are dual helices in D_1^3 .

Some Characterizations of Slant Helices in D_1^3

Theorem 2.1. Let $\tilde{\alpha}$ be a unit speed dual timelike curve with not pure dual curvature $\bar{\kappa}$ and not pure dual torsion $\overline{\tau}$ in D_1^3 . Then $\tilde{\alpha}$ is a dual slant helix if and only if one the next two dual functions:

(2.1)
$$\frac{\overline{\kappa}^2}{\left(\overline{\tau}^2 - \overline{\kappa}^2\right)^{3/2}} \left(\frac{\overline{\tau}}{\overline{\kappa}}\right)^{\prime} \text{ or } \frac{\overline{\kappa}^2}{\left(\overline{\kappa}^2 - \overline{\tau}^2\right)^{3/2}} \left(\frac{\overline{\tau}}{\overline{\kappa}}\right)^{\prime}$$

is dual constant where $\overline{\tau}^2 - \overline{\kappa}^2$ is not pure dual.

Proof. Let $\tilde{\alpha}$ be a unit speed dual timelike curve in D_1^3 . In order to prove the theorem, we first assume that $\tilde{\alpha}$ is a dual slant helix. Let \tilde{U} be the fixed dual vector field such that the function $\langle \tilde{N}, \tilde{U} \rangle = \bar{c}$ is dual constant. There exist smooth dual functions \bar{a}_1 and \bar{a}_3 such that

(2.2)
$$\widetilde{U} = \overline{a}_1 \widetilde{T} + \overline{c} \widetilde{N} + \overline{a}_3 \widetilde{B}$$

Since \widetilde{U} is dual constant, a differentiation in (2.2) with using (1.2) gives

(2.3)
$$\begin{cases} \overline{a}_1 + \overline{c}\,\overline{\kappa} = 0\\ \overline{a}_1\overline{\kappa} - \overline{a}_3\overline{\tau} = 0\\ \overline{c}\,\overline{\tau} + \overline{a}_3 = 0 \end{cases}$$

From the second equation of (2.3), we obtain

(2.4)
$$\overline{a}_1 = \overline{a}_3 \left(\frac{\overline{\tau}}{\overline{\kappa}}\right)$$

On the other hand

(2.5)
$$\left\langle \widetilde{U}, \widetilde{U} \right\rangle = -\overline{a_1}^2 + \overline{c}^2 + \overline{a_3}^2 = \text{dual constant}$$

Considering (2.4) and (2.5) together, let \overline{b} be the not pure dual constant given by

$$\delta \overline{b}^2 := \overline{a}_3^2 \left(\left(\frac{\overline{\tau}}{\overline{\kappa}} \right)^2 - 1 \right), \quad \delta \in \{-1, 0, 1\}$$

If $\delta=0$ then $\overline{a}_3 = 0$ and from (2.3) $\overline{a}_3 = 0$, $\overline{a}_1 = \overline{c} = 0$. Then $\widetilde{U} = 0$ and it is contradiction. Thus $\delta=1$ or $\delta=-1$ and since $\overline{\tau}^2 - \overline{\kappa}^2$ is not pure dual, then

$$\overline{a}_3 = \pm \frac{\overline{b}}{\sqrt{\left(\frac{\overline{\tau}}{\overline{\kappa}}\right)^2 - 1}} \quad \text{or} \quad \overline{a}_3 = \pm \frac{\overline{b}}{\sqrt{1 - \left(\frac{\overline{\tau}}{\overline{\kappa}}\right)^2}}.$$

From the third equation of (2.3), we obtain

$$\frac{d}{d\overline{s}} \left(\pm \frac{\overline{b}}{\sqrt{\left(\frac{\overline{\tau}}{\overline{\kappa}}\right)^2 - 1}} \right) = \overline{c}\,\overline{\tau} \quad \text{or} \quad \frac{d}{d\overline{s}} \left(\pm \frac{\overline{b}}{\sqrt{1 - \left(\frac{\overline{\tau}}{\overline{\kappa}}\right)^2}} \right) = -\overline{c}\,\overline{\tau} \;.$$

According to this

$$\frac{\overline{\kappa}^2}{\left(\overline{\tau}^2 - \overline{\kappa}^2\right)^{\frac{3}{2}}} \left(\frac{\overline{\tau}}{\overline{\kappa}}\right) = \pm \frac{\overline{c}}{\overline{b}} \text{ or } \frac{\overline{\kappa}^2}{\left(\overline{\kappa}^2 - \overline{\tau}^2\right)^{\frac{3}{2}}} \left(\frac{\overline{\tau}}{\overline{\kappa}}\right) = \pm \frac{\overline{c}}{\overline{b}}.$$

Thus we obtain (2.1). Conversely, we suppose that the condition (2.1) is satisfied. For the sake of simplicity, we assume that the first dual function in (2.1) is a dual constant, namely \overline{c} (the other case is analogous). We define

(2.6)
$$\widetilde{U} = \frac{\overline{\tau}}{\sqrt{\overline{\tau}^2 - \overline{\kappa}^2}} \widetilde{T} + \overline{c} \, \widetilde{N} + \frac{\overline{\kappa}}{\sqrt{\overline{\tau}^2 - \overline{\kappa}^2}} \widetilde{B}$$

A differentiation of (2.6) together the dual Frenet equations gives $\widetilde{U}' = 0$, that is, \widetilde{U} is a dual constant vector. Moreover $\langle \widetilde{N}, \widetilde{U} \rangle = \overline{c}$ and so $\widetilde{\alpha}$ is a dual slant helix. Hence we prove this theorem.

Theorem 2.2. Let $\tilde{\alpha}$ be a unit speed dual spacelike curve in D_1^3 .

(a) If the dual principle normal vector of the dual curve $\tilde{\alpha}$ whose dual curvature $\bar{\kappa}$ and dual torsion $\bar{\tau}$ are not pure dual, is spacelike, then $\tilde{\alpha}$ is a dual slant helix if and only if one the next two dual functions

(2.7)
$$\frac{\overline{\kappa}^2}{\left(\overline{\tau}^2 - \overline{\kappa}^2\right)^{\frac{3}{2}}} \left(\frac{\overline{\tau}}{\overline{\kappa}}\right) \quad \text{or} \quad \frac{\overline{\kappa}^2}{\left(\overline{\kappa}^2 - \overline{\tau}^2\right)^{\frac{3}{2}}} \left(\frac{\overline{\tau}}{\overline{\kappa}}\right)$$

is dual constant where $\overline{\tau}^2 - \overline{\kappa}^2$ is not pure dual.

(b) If the dual principle normal vector of the dual curve $\tilde{\alpha}$ whose dual curvature $\bar{\kappa}$ and dual torsion $\bar{\tau}$ are not pure dual, is timelike, then $\tilde{\alpha}$ is a dual slant helix if and only if the dual function

$$\frac{\overline{\kappa}^2}{\left(\overline{\tau}^2 + \overline{\kappa}^2\right)^{\frac{3}{2}}} \left(\frac{\overline{\tau}}{\overline{\kappa}}\right)^{\frac{1}{2}}$$

is dual constant where $\overline{\tau}^2 + \overline{\kappa}^2$ is not pure dual.

(c) Any dual spacelike curve, whose dual curvature $\overline{\kappa}$ and dual torsion $\overline{\tau}$ are not pure dual, with dual lightlike principle normal vector is a dual slant helix.

Proof. Let $\tilde{\alpha}$ be a unit speed dual spacelike curve in D_1^3 . In the case that the dual principle normal vector \tilde{N} of $\tilde{\alpha}$ is spacelike or timelike, the proof of Theorem 2.2 is similar to the given for Theorem 2.1.

Now we consider that the dual principle normal vector \tilde{N} of the dual curve is a lightlike vector. We show that any such dual curve is a dual slant helix. Let \bar{a}_2 be any non-trivial solution of the O.D.E.

$$\overline{y}' + \overline{\tau} \overline{y} = \overline{0}$$

and define $\widetilde{U} = \overline{a}_2 \widetilde{N}$. By using (1.5) and $\overline{a}_2 + \overline{a}_2 \overline{\tau} = \overline{0}$ so $\widetilde{U} = 0$, that is, \widetilde{U} is a non-zero dual constant vector field of D_1^3 and then $\langle \widetilde{N}, \widetilde{U} \rangle = \overline{0}$. It proves that $\widetilde{\alpha}$ is a dual slant helix.

Indicatries and Involutes of a Dual Slant Helices

In this section we study the dual tangent indicatrix, dual binormal indicatrix of a dual slant helix and its involutes. We restrict to non-null dual curves whose dual principle normal vector \tilde{N} is spacelike or timelike. Thus we are considering dual timelike curves or dual spacelike curves with dual spacelike or dual timelike principle normal vector. Given a unit speed dual curve $\tilde{\alpha}: I \to D_1^3$, the dual tangent indicatrix (resp. dual binormal indicatrix) is the dual curve $\tilde{\mathbf{T}}: I \to D_1^3, \tilde{\mathbf{T}}(t) = \tilde{T}(t)$, (resp. the dual curve $\tilde{\mathbf{B}}: I \to D_1^3, \tilde{\mathbf{B}}(t) = \tilde{B}(t)$), where \tilde{T} (resp. \tilde{B}) is the dual tangent vector (resp. dual binormal vector) to $\tilde{\alpha}$. If the image of a dual curve lies in H_0^2 or S_1^2 we say that the curve is dual spherical. In particular, the dual tangent indicatrix and the dual binormal indicatrix are dual spherical.

We say that $\tilde{\alpha}$ is a dual helix in dual Lorentzian space D_1^3 if there exits a fixed direction \tilde{U} of D_1^3 such that the function $\langle \tilde{T}, \tilde{U} \rangle$ is a dual constant. Dual helices are characterized by the fact that the ratio $\frac{\overline{\tau}}{\overline{\kappa}}$ is a dual constant along the dual curve, where $\overline{\tau}$ and $\overline{\kappa}$ is not pure dual torsion and dual curvature of $\tilde{\alpha}$ respectively.

Theorem 3.1. Let $\tilde{\alpha}$ be a unit speed dual timelike curve or a spacelike (with dual spacelike or timelike principle normal vector) curve. Let be dual curvature $\overline{\kappa}$, dual torsion $\overline{\tau}$, the dual functions $\overline{\tau}^2 - \overline{\kappa}^2$ and $\overline{\tau}^2 + \overline{\kappa}^2$ are not pure dual. If $\tilde{\alpha}$ is a dual slant helix in D_1^3 , then the dual tangent indicatrix $\tilde{\mathbf{T}}$ of $\tilde{\alpha}$ is a dual (spherical) helix.

Proof. Denote the dual curvature and the dual torsion of $\tilde{\mathbf{T}}$ by $\bar{\kappa}_{\tilde{\mathbf{T}}}$ and $\bar{\tau}_{\tilde{\mathbf{T}}}$ respectively. We will prove that the ratio $\bar{\tau}_{\tilde{\mathbf{T}}}/\bar{\kappa}_{\tilde{\mathbf{T}}}$ is dual constant. Let the dual tangent indicatrix $\tilde{\mathbf{T}}$ be not dual arclength parametrized. In general and if $\tilde{\beta}(t)$ is non-parametrized by the dual arclength dual curve, the corresponding formulae of the dual curvature and the dual torsion are:

$$\overline{\kappa}_{\tilde{\beta}}^{2}(t) = \varepsilon \frac{\left\|\tilde{\beta}'(t)\right\|^{2} \left\|\tilde{\beta}''(t)\right\|^{2} - \left\langle\tilde{\beta}'(t), \tilde{\beta}''(t)\right\rangle^{2}}{\left\|\tilde{\beta}'(t)\right\|^{6}}, \ \overline{\tau}_{\tilde{\beta}}(t) = -\varepsilon \frac{\det(\tilde{\beta}'(t), \tilde{\beta}''(t), \tilde{\beta}''(t))}{\overline{\kappa}_{\tilde{\beta}}^{2}(t) \left\|\tilde{\beta}'(t)\right\|^{6}}$$

where ε is 1 or -1 depending on $\tilde{\beta}''(t)$ is a dual spacelike or dual timelike vector, respectively.

Consider that $\tilde{\alpha}$ is a dual spacelike curve with dual principle normal vector \tilde{N} spacelike or timelike. Denote $\varepsilon = 1$ or -1 depending on \tilde{N} is spacelike or timelike, respectively. Then the dual tangent indicatrix $\tilde{\mathbf{T}}$ is a dual spacelike curve or a dual timelike curve. For both cases,

(-)

$$\overline{\kappa}_{\widetilde{\mathbf{T}}}^2 = \frac{1}{\overline{\kappa}^2} \left(\overline{\kappa}^2 - \varepsilon \overline{\tau}^2 \right), \quad \det(\widetilde{\mathbf{T}}', \widetilde{\mathbf{T}}'', \widetilde{\mathbf{T}}''') = \varepsilon \overline{\kappa}^5 \left(\frac{\overline{\tau}}{\overline{\kappa}} \right), \quad \overline{\tau}_{\widetilde{\mathbf{T}}} = \varepsilon \frac{\overline{\kappa} \left(\frac{\overline{\tau}}{\overline{\kappa}} \right)}{\overline{\kappa}^2 - \overline{\tau}^2}$$

In the case that $\tilde{\alpha}$ is a dual timelike curve, then $\tilde{\mathbf{T}}$ is a dual spacelike curve and

$$\overline{\kappa}_{\widetilde{\mathbf{T}}}^{2} = -\frac{1}{\overline{\kappa}^{2}} \left(\overline{\kappa}^{2} - \overline{\tau}^{2} \right), \quad \det(\widetilde{\mathbf{T}}', \widetilde{\mathbf{T}}'', \widetilde{\mathbf{T}}''') = -\overline{\kappa}^{5} \left(\frac{\overline{\tau}}{\overline{\kappa}} \right), \quad \overline{\tau}_{\widetilde{\mathbf{T}}} = \frac{\overline{\kappa} \left(\frac{\overline{\tau}}{\overline{\kappa}} \right)}{\overline{\kappa}^{2} - \overline{\tau}^{2}}$$

Thus we obtain

$$\frac{\overline{\tau}_{\tilde{\mathbf{T}}}}{\overline{\kappa}_{\tilde{\mathbf{T}}}} = \varepsilon \frac{\overline{\kappa}^2 \left(\frac{\overline{\tau}}{\overline{\kappa}}\right)}{\left(\overline{\kappa}^2 - \varepsilon \overline{\tau}^2\right)^{\frac{3}{2}}} \quad \text{or} \quad \frac{\overline{\tau}_{\tilde{\mathbf{T}}}}{\overline{\kappa}_{\tilde{\mathbf{T}}}} = \frac{\overline{\kappa}^2 \left(\frac{\overline{\tau}}{\overline{\kappa}}\right)}{\left(\overline{\tau}^2 - \overline{\kappa}^2\right)^{\frac{3}{2}}}.$$

By considering Theorems 2.1 and 2.2, the ratio $\overline{\tau}_{\tilde{T}} / \overline{\kappa}_{\tilde{T}}$ is dual constant. Hence \tilde{T} is a dual helix.

Theorem 3.2. Let $\tilde{\alpha}$ be a unit speed dual timelike curve or a dual spacelike (with spacelike or timelike dual principle normal vector) curve. Let be dual curvature $\bar{\kappa}$, dual torsion $\bar{\tau}$, the dual functions $\bar{\tau}^2 - \bar{\kappa}^2$ and $\bar{\tau}^2 + \bar{\kappa}^2$ are not pure dual. If $\tilde{\alpha}$ is a dual slant helix in D_1^3 , then the dual binormal indicatrix $\tilde{\mathbf{B}}$ of $\tilde{\alpha}$ is a dual (spherical) helix.

Proof. Denote $\overline{\kappa}_{\tilde{B}}$ and $\overline{\tau}_{\tilde{B}}$ the dual curvature and dual torsion of the dual curve \tilde{B} respectively. Consider $\tilde{\alpha}$ a dual spacelike curve. Then the dual binormal indicatrix \tilde{B} is a dual timelike or a dual spacelike curve, depending on \tilde{N} is timelike or spacelike, respectively.

$$\overline{\kappa}_{\tilde{B}}^{2} = \frac{\overline{\kappa}^{2} - \varepsilon \overline{\tau}^{2}}{\overline{\tau}^{2}}, \quad \det(\widetilde{\mathbf{B}}', \widetilde{\mathbf{B}}'', \widetilde{\mathbf{B}}''') = \varepsilon \overline{\kappa}^{2} \overline{\tau}^{3} \left(\frac{\overline{\tau}}{\overline{\kappa}}\right)', \quad \overline{\tau}_{\tilde{\mathbf{B}}} = \frac{\overline{\kappa}^{2}}{\overline{\tau} \left(\overline{\kappa}^{2} - \varepsilon \overline{\tau}^{2}\right)} \left(\frac{\overline{\tau}}{\overline{\kappa}}\right)'.$$

where ε is 1 or -1 depending on \widetilde{N} is a spacelike or timelike, respectively.

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If $\tilde{\alpha}$ is timelike, then \tilde{B} is a dual spacelike curve. We have

$$\overline{\kappa}_{\widetilde{\mathbf{B}}}^{2} = \frac{\overline{\tau}^{2} - \overline{\kappa}^{2}}{\overline{\tau}^{2}}, \quad \det(\widetilde{\mathbf{B}}', \widetilde{\mathbf{B}}'', \widetilde{\mathbf{B}}''') = \overline{\kappa}^{2} \overline{\tau}^{3} \left(\frac{\overline{\tau}}{\overline{\kappa}}\right), \quad \overline{\tau}_{\widetilde{\mathbf{B}}} = \frac{\overline{\kappa}^{2}}{\overline{\tau} \left(\overline{\tau}^{2} - \overline{\kappa}^{2}\right)} \left(\frac{\overline{\tau}}{\overline{\kappa}}\right)^{2}.$$

Thus we obtain

$$\frac{\overline{\tau}_{\tilde{B}}}{\overline{\kappa}_{\tilde{B}}} = \delta \frac{\overline{\kappa}^2 \left(\frac{\overline{\tau}}{\overline{\kappa}}\right)}{\left(\overline{\kappa}^2 - \varepsilon \overline{\tau}^2\right)^{\frac{3}{2}}} \quad \text{or} \quad \frac{\overline{\tau}_{\tilde{B}}}{\overline{\kappa}_{\tilde{B}}} = \delta \frac{\overline{\kappa}^2 \left(\frac{\overline{\tau}}{\overline{\kappa}}\right)}{\left(\overline{\kappa}^2 - \overline{\tau}^2\right)^{\frac{3}{2}}},$$

where δ is 1 or -1 depending on $\overline{\tau}$ is positive or negative, respectively. By using (2.1), (2.7) and (2.8) in the above equations are dual constant, which proves that the dual binormal indicatrix $\tilde{\mathbf{B}}$ is a dual helix in D_1^3 .

Now we give a characterization of a dual slant helix in terms of its involutes. Firstly, we define an involute of a dual curve. $\tilde{\alpha}: I \subset R \to D_1^3$ is a dual curve, an involute of $\tilde{\alpha}$ is a dual curve $\tilde{\beta}: I \to D_1^3$ such that for each $s \in I$ the point $\tilde{\beta}(s)$ lies on the dual tangent line to $\tilde{\alpha}$ at *s* and $\langle \tilde{\alpha}'(s), \tilde{\beta}'(s) \rangle = 0$. If $\tilde{\alpha}$ is a non-null dual curve, the equation of an involute is $\tilde{\beta}(s) = \tilde{\alpha}(s) + (\overline{c} - s)\tilde{T}(s)$, where \overline{c} is dual constant and \tilde{T} is the unit dual tangent vector of $\tilde{\alpha}$.

Theorem 3.3. Let $\tilde{\alpha}$ be a unit speed dual timelike curve or a spacelike (with spacelike or timelike dual principle normal vector) curve. Let be dual curvature $\bar{\kappa}$, dual torsion $\bar{\tau}$, the dual functions $\bar{\tau}^2 - \bar{\kappa}^2$ and $\bar{\tau}^2 + \bar{\kappa}^2$ are not pure dual. Let $\tilde{\beta}$ be an involute of $\tilde{\alpha}$. Then $\tilde{\alpha}$ is a dual slant helix if and only if $\tilde{\beta}$ is a dual helix.

Proof. We denote by $\overline{\kappa}_{\tilde{B}}$ and $\overline{\tau}_{\tilde{B}}$ the dual curvature and the dual torsion of $\tilde{\beta}$, respectively. If $\tilde{\alpha}$ is a dual timelike curve, then

(3.1)
$$\overline{\kappa}_{\widetilde{B}} = \frac{\overline{\tau}^2 - \overline{\kappa}^2}{\overline{\kappa}^2 \left(\overline{c} - \overline{s}\right)^2}, \ \overline{\tau}_{\widetilde{B}} = -\frac{\overline{\kappa}}{\left(\overline{c} - \overline{s}\right)\left(\overline{\tau}^2 - \overline{\kappa}^2\right)} \left(\frac{\overline{\tau}}{\overline{\kappa}}\right)^2$$

Thus we obtain

$$\frac{\overline{\tau}_{\widetilde{B}}}{\overline{\kappa}_{\widetilde{B}}} = -\frac{\overline{\kappa}^2}{\left(\overline{\tau}^2 - \overline{\kappa}^2\right)^{\frac{3}{2}}} \left(\frac{\overline{\tau}}{\overline{\kappa}}\right)^{\frac{1}{2}}.$$

If $\tilde{\alpha}$ is a dual spacelike curve

(3.2)
$$\overline{\kappa}_{\tilde{B}} = \frac{\overline{\kappa}^2 - \varepsilon \overline{\tau}^2}{\overline{\kappa}^2 \left(\overline{c} - \overline{s}\right)^2}, \ \overline{\tau}_{\tilde{B}} = -\frac{\overline{\kappa}}{\left(\overline{c} - \overline{s}\right)\left(\overline{\kappa}^2 - \varepsilon \overline{\tau}^2\right)} \left(\frac{\overline{\tau}}{\overline{\kappa}}\right)^2$$

and

$$\frac{\overline{\tau}_{\tilde{B}}}{\overline{\kappa}_{\tilde{B}}} = \frac{\overline{\kappa}^2}{\left(\overline{\kappa}^2 - \varepsilon\overline{\tau}^2\right)^{\frac{3}{2}}} \left(\frac{\overline{\tau}}{\overline{\kappa}}\right)^{\frac{1}{2}}.$$

Here ε is 1 or -1 depending if \widetilde{N} is a spacelike or a timelike dual vector, respectively. The proof finishes using the equations (3.1), (3.2) and Theorem 2.1 and Theorem 2.2.

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Detection of gamma irradiated spices with OSL method and its reliability

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Abstract

Objective: Irradiation has been accepted as an effective food safety method for various foods over 50 years. Gamma rays from radioactive isotopes of Cobalt 60 or Cesium 137 are used for food irradiation applications. Main concern about food irradiation is the detection of these irradiated foods and also loss of detection of irradiation through storage period. Photo Stimulated Luminescence (PSL) is one of the physical techniques that can be used for irradiation detection. The aim of this study is to analyse the behaviour of PSL signals of irradiated spices with respect to storage period, storage temperature, origin and type of samples.

Material and Methods: Red pepper, thyme and cumin were used as samples and 4°C and 25°C were selected as storage temperature. Storage period was set as six months after irradiation application. During six months storage period, PSL signal was not detectable for most of the origin and sample type. At the end of sixth month, an spin resonance spectroscopy (ESR) analysis was performed to detect the accuracy of the PSL technique.

Results: With respect to the results of these experiments, it was seen that, most of the samples was observed as false un-irradiated by PSL technique, however ESR analyse the samples as irradiated at the end of sixth month.

Conclusion: According to the statistical analysis, origin and type of sample were determined as the dependent parameters of PSL detection.

Keywords: Spices; Gamma Irradiation; Photo Stimulated Luminescence (PSL)

Introduction

Food irradiation is applied as controlled amount of ionizing radiation (with sufficient energy to create positive and negative charges) which includes gamma rays from radioactive isotopes Cobalt-60 and Cesium-137 (1).

Gamma rays are the specific energies that normally come from the spontaneous decay of radionuclide which is not naturally occured and unstable. The radionuclide that is used for the irradiation of food materials is mainly Cobalt-60. The aim of food irradiation applications are the preventing germination of some foods, killing of insects in grains or dried foods, retardation of ripening period, increasing shelf life and killing microorganisms in herbs and spices (2).

Mainly; gamma irradiation in food industry is used for the prevention of microbial growth and health problems as well as decreasing the economic losses because of microbial deteriorations of food products, unexpected recalls because of outbreaks, loss of consumers' confidence and loyalty. Due to such reasons, microbial inactivation mechanism of the technique becomes important for its application process (3).

The parameters that effect the microbial reduction due to irradiation process can be listed as: size of the microorganisms, age of the microorganisms, radiation absorbed dose, type of microorganisms, absence or presence of oxygen and time of exposure.

1.1 Irradiation Detection and Regulations

The level of irradiation in different food systems is regulated by different authorities in every country; in general this value is in the range of 1-10 kGy for pasteurization processes and above 10 kGy for sterilization purposes (4). Foods such as wheat, wheat flour, white potatoes, pork, fruits, vegetables, herbs, spices, poultry, meat and animal feeds and also enzymes are approved for irradiation application process by most of the irradiation authorities (5). The main concern of irradiation process for the market is the consumer acceptance and loss of detection of irradiation through storage time. At this step, detection of irradiation after processing becomes an important point for both consumer acceptance and reliability of irradiation.

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1.1.1 Detection Methods

There are several valid and accurate methods used all over the world for food irradiation detection. The irradiation process, when applied at usual doses; namely equal to or less than 10 kGy, involves few chemical changes on food rather than other treatments such as heating or freezing (6).

The most important irradiation detection methods are electron spin resonance spectroscopy (ESR), thermoluminescence (TL), Photo Stimulated Luminescence (PSL) and DNA Comet Assay. Among them; the luminescence techniques are some of the common used techniques for irradiation detection. Luminescence can arise from the thermal or optical stimulation of minerals that have been previously exposed to ionizing radiation. Irradiation creates free charges in the solid which may be captured by lattice defects acting as traps. If the matter has a crystalline structure, the excited charge carriers can remain trapped in the crystalline lattice defects. When heat or light is applied to the sample, the stored charges are released and recombined resulting in a light emission (7). If the system is stimulated by heat, mechanism is called as thermoluminescence (TL), and if the system is stimulated by light, it is called as photo stimulated luminescence (PSL). The recorded luminescence intensity is proportional to the absorbed radiation dose.

Photo stimulated luminescence (PSL) is one of the accurate methods used all over the world for determination of irradiated foods. Luminescence can arise from the thermal or optical stimulation of minerals that have been previously exposed to ionizing radiation. Irradiation creates free charges in the solid which may be captured by lattice defects acting as traps. If the sample has materials in crystalline structure, the excited charge carriers can remain trapped in the crystalline lattice defects (8).

Food is contaminated by very small quantities of silicate minerals such as quartz and feldspar for most of the time and presence of these minerals on the food materials can be important for a reliable signal measurement. For both TL and PSL techniques, signal sources are the minerals in the sample. In TL measurements, due to the high temperature, organic materials in samples are burned and can cause failure of detection and also the system. Therefore, it is reasonable to separate inorganic minerals and organic compounds and analyze only inorganic compounds. However in PSL, there is no need for separation of inorganic minerals and organic compounds during measurement since no heating is required, therefore samples are not denatured (9).

PSL is specified as a method for the detection of irradiated foods by European Standards and it is based on optical stimulation of mineral debris, typically silicates, bioinorganic materials such as calcite, feldspar. Irradiation of food causes such minerals to store energy in their charge carriers. When stimulated with optical energy, the trapped energy is released in the charge carriers as luminescence (10). In this technique, trapped electrons are stimulated with appropriate wavelength and intensity of light, and luminescence is monitored as a function of stimulation time. Observed luminescence is due to recombination of electrons at whole traps which act as recombination centers (Figure 1).



Figure 1: Simplified band model for describing luminescence mechanism (http://rses.anu.edu.tr)

If the food is irradiated, the signal is strong; obviously a weak signal would indicate a non- irradiated food. In occurrence of an intermediate signal, the sample can be estimated as a mixture of irradiated and non- irradiated foods or the sample may have a low sensitivity (7). Due to this analysis, PSL sensitivity of the product becomes important. Sensitivity depends on quantity and type of minerals in the sample.

In PSL detection, if irradiated samples are treated once more, just a small increase is observed in PSL signals since only left electrons are transferred to the upper level; however unirradiated samples cause a substantial increase in PSL after the first irradiation (11).

Materials and Method

Radiation Source

Cobalt-60 is not naturally occurred and is produced by neutron bombardment in a nuclear reactor of the metal Nickel-59. It is then doubly encapsulated in stainless steel "pencils" to prevent any leakage during its usage in a radiation plant. When not used, the gamma "source" is stored in a pool of water (4). In the case of foods or some another products irradiation process, the source is pulled out of the water into a chamber with massive concrete walls. Foods or medical products to be irradiated are brought into the chamber, and are exposed to the rays for a defined period of time. After the process is finished, the source is returned to the water tank. The irradiation treatment is done within an irradiation room in a typical plant. The radiation source is fixed on the elevator system and when it is not used the source is localized in a water tank.

Irradiation Treatment

In TAEA (Turkish Atomic Energy Authority; the boxes were placed in 45x45x90 cm size irradiation boxes and loaded on horizontal conveyor and transported to the irradiation room. The irradiation treatment takes place in this room and samples are passing across the gamma source (Cobalt-60) and absorbed the radiation.

The process is continued till reaching the wanted absorbed dose (10 kGy) for samples. After process ends, gamma source is placed back in the water.

Samples

Spice samples (red pepper, thyme and cumin) were obtained from local spice markets located in Adana, İzmir, Maraş and Ankara without subjected to gamma irradiation. Gamma irradiated and unirradiated samples were stored both at refrigeration temperature (4°C) and room temperature (25°C). The spice samples were kept in small bags and covered with aluminum foil and placed in opaque boxes which are not transparent to light.

Irradiation Detection

For irradiation detection, the samples were loaded on 10 mm aluminum disks in a red lightened room. Disks were initially covered with silicon oil in order to paste spices. A thin layer of spice was put on aluminum disk. After samples were prepared, they were loaded into PSL equipment. Each sample was monitored for 200 seconds in order to obtain reliable signals from the samples. During the analysis, spice samples were placed in a light sealed system which did not expose any interfering light except the light coming from the system source.

2.1 PSL Detection System

PSL detection system is composed of a stimulation light source, a sensitive photo detector and sample holder (Figure 2). Depending on the type of mineral in the sample, Infra-red (IR) (\sim 880 nm) and blue light (\sim 470 nm) can be used as light sources.



Figure 2: System of PSL detector (15)

Luminescence was detected in photon counting mode using a photomultiplier tube with a bi alkali photocathode (Electron Tubes, 9532 Q) with a UV band pass filter (Hoya U-340) transmitting wavelengths between 280-380 nm. Stimulation was done with a blue light source employing a cluster of 24 blue light emitting diodes. Power density on the sample was 30mW/cm^2 . Intense blue light was used in order to measure lower doses in spice samples.

For the stimulation, light emitting diodes (LED) were used due to their long life and easier availability. In this technique, photo multiplier tubes (PMT) were used as photo detectors. The signals obtained from PMT were in the form of pulses and pulses were counted by a computer connected to the equipment with USB cable.

2.1.1 Irradiation Detection

For PSL measurement, data was taken (200 seconds with one second intervals) until a stabilized signal value was reached. PSL measurements were made in triplicates for each samples studied. In order to confirm the irradiation process, background PSL signal values were monitored for all unirradiated samples. Background value is defined as the lowest value of PSL signal monitored before irradiation. The experiment parameters were defined as time of storage, storage temperature, sample type and origin. Measurements were carried for 6 months based on the counter stay of samples on markets.

2.2 Statistical Analysis

The analysis of regression was carried out to investigate the effect of experiment parameters on the final treated product irradiation detection by using MiniTAB (Version 16). Multi way ANOVA (analysis of variance) were used for comparison of means. Significance was accepted at 0.05 level of probability (p<0.05). Mean separation was performed by LSD (least significant difference) for multiple comparisons of means. All measurements were performed in triplicate.

Results and Discussion

During experiments, three different samples (red pepper, thyme, cumin) from four different origins (Adana, Izmir, Maras, Ankara) were used to obtain reliable data and minimize the error coming from the origin of the samples such as amount of inorganic materials or dust and also type of inorganic materials. According to the results of PSL measurements; background PSL signal value is mostly affected by origin and type of samples. Samples from different origins were observed to have different background PSL signal intensity values (between 450 and 1230 cps) (Table 1).

Tuble 1: Duckground 1 51 signal values of Red 1 opper, editin and Thylie Sumples for an origins			
Origins	Red Pepper	Thyme	Cumin
Adana	462±5.4%	991±5.8%	1214±4.6%
İzmir	1013±10.2%	969±4.2%	787±2.7%
Maras	402±5.4%	864±12.4%	1256±4.2%
Ankara	755±8.5%	783±21%	1014±4.6%

Table 1: Background PSL signal values of Red Pepper, Cumin and Thyme Samples for all origins

3.1 PSL Signal Values After Irradiation

After irradiation (10 kGy), the samples were analyzed for their PSL signal values (Table 2). In order to prevent the optical fading for long term storage, meaning losing PSL signals due to light, samples were stored in dark (12).

Table 2: Average PSL Signal Values for Irradiated Samples from All Origins

<u> </u>		1 0	
Origins	Red pepper	Thyme	Cumin
Adana	20778±6.2%	1425±7.1%	3515±2.9%
İzmir	1223±3.1%	1054±10.3%	756±2.8%
Maras	1024±2.9%	1631±10.5%	994±4.9%
Ankara	663±9.2%	1421±17.4%	969±7.4%

The reason of measuring high PSL signal value of Adana samples after irradiation can be due to the amount of dust in the sample and also the amount of traps in the mineral debris. This situation might have caused high amount of electron transfer resulted high luminescence formation. However, PSL signal of other three red pepper samples were lower than the expected. The reason for such observation may be due to the presence of shallow traps (lack of deep traps) and the lower amount of dust when compared to Adana red pepper. The captured electrons in such traps can be lost at room temperature with little optical effect (8).

The increase of PSL signal value levels for all samples of irradiated thymes were approximately in the same range. However, difference in PSL signal values in Maras and Ankara originated thyme samples seem more detectable than Adana and İzmir originated thyme samples.

Tuble of Tereont Change of Toll Signal Intense Values Thter Intalation Treatment				
Origins	Red pepper	Thyme	Cumin	
Adana	97.8%	30.5%	65.5%	
İzmir	17.2%	-	-	
Maras	60.7%	46.9%	-	
Ankara	-	44.9%	-	

 Table 3: Percent Change of PSL Signal Intense Values After Irradiation Treatment

Cumin from Adana is the only sample that resulted in observable increase of PSL signal value after irradiation. PSL signal value levels of other cumin samples were only background PSL signals.

3.2 PSL Signal Value Change with respect to Time and Temperature

Spice type and origin show different responses to the irradiation process, so similar increase or decrease behaviors in PSL signal values were not observed after irradiation of the samples. This shows that spice type and origin are the key parameters for irradiation detection using PSL method. In order to determine whether time was a parameter for PSL detection, monthly analyses were done for each origin and sample type. In addition to time dependency, the samples were stored at 4°C as refrigeration temperature and 25°C as room temperature to analyze the effect of storage temperature on PSL measurement. With this approach, the presence of shallow traps were also studied (if there are shallow traps in samples, electrons in these traps are lost at room temperature with time) (13).

First group consist of red pepper, thyme, cumin from Adana, red pepper and thyme from Maras and thyme from Ankara. Those samples had detectable PSL signals during the storage time, at two different temperatures (Table 4-6-7).





Table 6: PSL Value Analyses of Maraş Red Pepper/Thyme Between Background and Six Month Storage (-1 refers to unirradiated samples, 0 as just irradiated samples and 1, 2, 3, 4 and 6 refer to the analysed months)







Second group consisted of red pepper and thyme of İzmir and had detectable signals, though in low levels, in the early mounths of storage , however, the observed levels dropped down to the ground level after then. The most probable reason of this is the presence of shallow traps in the studied samples (Table 5).

Cumin samples from İzmir, Ankara, Maras and red pepper from Ankara were in the third group and nearly no increase of PSL signal value was observed after their irradiation.

3.3 ESR Results of Samples

ESR detection was done for only red pepper samples of Adana, İzmir, Maras and Ankara at the sixth month in order to compare the obtained OSL results with ESR results at the end of the experimental duration. Also the reason of making ESR for only sixth month is that, no detection observation for İzmir, Maras and Ankara at the end of sixth month. By this way, significant difference between Adana samples and the others can be explained clearly.

 Table 5: PSL Value Analyses of İzmir Red Pepper/Thyme Between Background and Six Month Storage (-1 refers to unirradiated samples, 0 as just irradiated samples and 1, 2, 3, 4 and 6 refer to the analysed months)



In ESR analyses, all the samples show a similar cellulose peak (Figure 3). This means that, they were irradiated homogeneously with the same amount of gamma ray. The cellulose peak is clearly observed and this means that, detection with ESR is more appropriate after six month. At this step, it can be easily understood that, there is enormous effect of light, so optical fading, on irradiation detection by using PSL system.

In addition to these, the significant difference on PSL signals of Adana samples from others can be explained as the difference of amount of dust and also the structural difference of dust.



Figure 3: Results of ESR measurements at the end of six month for red peppers from all origins (Arrows represent radiation induced cellulose peaks and magnetic field difference between peeaks is nearly 60 Gauss)

3.4 Statistical Analysis

PSL signal was used as the response parameter in order to analyze the detection loss. The parameters having effect on PSL were chosen as time, temperature, origin of spices and type of spices.

In order to determine the effect of time, monthly PSL signal value measurements were performed for 6 month (average time of the spices to be on the market shelf was given as 4 months).

For the effect of temperature on PSL signals, storage temperatures were set as 4°C and 25°C.

4 way ANOVA (General Linear Model) was used in order to analyze the parameters' effects on PSL signal value detection. Data were obtained as PSL versus spice types, origin, time and temperature.

Spice types and origin were considered as suitable to be taken as parameters of PSL detection. However, the selected time interval and selected temperatures could not be seen as parameters of PSL detection according to the resulted p- values. The confidence interval was selected as 95%, so p values lower than 0.05 were considered as significantly different and were selected as significant determiners of the system (Table 8).

According to the results of the Tukeys Test (95%), red pepper was significantly different than cumin and thyme with respect to PSL response. This can be due to the structure or dust content/amount of red pepper. It may have more free electrons than other samples which might cause higher PSL signals after irradiation applications.

With respect to origin Adana samples were significantly different from İzmir Spices, Maras and Ankara samples. This may be most probably due to the amount and type of dust on the samples.

Conclusion

The results of the studies show that PSL can be used as an efficient technique in the laboratories or customs for determination of the irradiation application of spices because it is rapid, cost effective and samples are not affected by the system. Main drawback experienced in these studies is the inconvenience of the system to be applicable to all type of food.

PSL signal value deviations in red pepper samples were more than the ones in other spice samples. This shows that type of sample and origin have a significant effect on background PSL values of the samples.

The reasons for such kind of a deviation may be explained as, non-homogenous nature of spices, different humidity values of different samples and type and amount of dust in the nature of spices.

In statistical analysis, origin and spice types were determined as significant parameters of PSL detection of irradiated samples. However storage time and storage temperature were not significant on PSL signal detection during storage.

The advantages of PSL system can be summarized such; it is rapid, cost effective. However, the disadvantages may be explained as follows; there may be risk of inaccuracy and there are decays of signals with storage time (if they are not stored at dark) and on repeated measurements (14).

Conflict of interest: The authors declare they have no potential conflicts of interest with respect to the research, authorship, and/or publication of this article, and declare study has ethical permissions if required..

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Original Article

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Seroprevalence of Toxoplasmosis in Free Range chickens in Tabriz area of Iran by using ELISA test

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Abstract

Objective: As consumption of chicken meat may be as one of the sources of human infection, this study was undertaken to determine the prevalence of *T. Gondii* among the free Range Chicken, by using ELISA in Tabriz, Northwest of Iran.

Materials and Methods: One hundred blood samples were collected from free Range chickens by a cluster random sampling method and tested for toxoplasmosis by Enzyme Linked Immunosorbent Assay (ELISA). The results were analysed by SPSS software using Chi-Square test and a P value <0.05 was considered statically significant.

Results: Results revealed that 18% of Free-Ranging chickens positive by ELISA respectively. Although the difference observed in the percentage of *T. Gondii* antibodies among different areas of Tabriz city, Iran, there were no significant differences P < 0.05 detected by the ELISA method.

Conclusion: Based on cultural and food habits in our area, the meat and viscera of chicken may be important sources of infection in human when consuming semi-cooked meats. Considering the high prevalence of toxoplasmosis in chickens, standards in chicken breeding, education of environmental health personnel and standardization for preparation and handling techniques are required by Health and Veterinary organizations.

Key words: Toxoplasmosis, Free-Ranging Chickens, ELISA, Tabriz, Iran.

Introduction

Toxoplasma gondii is an obligate intracellular protozoan that infects humans and a wide range of mammalian and bird (1). Its high infection rates and its benign co-existence with the host, T.gondii is regarded as one of the most successful parasites on earth. is a global parasites with no known geographical boundaries (2).Serological surveys done in various parts of the world show that in some countries more than a third of the human population have antibodies against T.gondii . This high prevalence of infection in human proves the importance of toxoplasmosis as azoonotic disease(3,4).T.gondii infection in free-range chickens (FR) is considered important as FR chickens are one of the best indicators for soil contamination with T.gondii oocysts because they feed from the ground, and tissues of infected chickens are considered a good source of infection for cats. Additionally, ingestion of infected chicken meat can be a source of infection for T.gondii infection in humans and other animals. Rarely, toxoplasmosis can cause clinical disease in chickens (5).Soil contaminated with oocysts can be taken up by pastoral animals, such as sheep and goats, during grazing. Poultry having outdoor access will also take up considerable amounts of soil and can thus become infected with Toxoplasma.

Therefore, free-ranging chickens are now used as sentinel animals to isolate and characterise *Toxoplasma* strains throughout the world (6). The aim of this study was to investigate the utility of enzyme linked immunosorbent assay (ELISA) for detection infections with *T. gondii* in chickens.

Material and Methods

Study area

East-Azerbaijan Province is one of the 31 provinces of Iran. It is located in Iranian Azerbaijan, bordering with Armenia, Republic of Azerbaijan, Ardabil Province, West Azerbaijan Province, and Zanjan Province. The capital of East Azerbaijan is Tabriz. East Azerbaijan Province is in Regions 3 of Iran, with its secretariat located in its capital city, Tabriz.

The province covers an area of approximately 47,830 km², it has a population of around four million people. The historical city of Tabriz is the most important city of this province, culturally, politically, and commercially (Fig. 1). Generally speaking, East Azerbaijan enjoys a cool, dry climate, being in the main a mountainous region. But the gentle breezes off the Caspian Sea have some influence on the climate of the low-lying areas.

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Temperatures run up to 8.9 °C in Tabriz, and 20 °C in Maraqeh, in the winter dropping to -10-15 °C at least (depending on how cold the overall year is). The ideal seasons to visit this province are in the spring and summer months.



Figure 1: The study area of Tabriz City, East Azerbaijan Province, North-west Iran

Samples Collection

Free range chicken samples

In 2015, a total 100 blood samples were collected from free range chickens by a cluster random sampling method in sub-urban regions of Tabriz city in East-Azerbaijan Province. The chickens were 1-4 years old females used as a source of meat and egg in the region.

Blood samples

Five millilitre (ml) of blood was drawn from each bird Wing by disposable syringe. Blood were collected in sterile plain tubes and left 30 minutes at room temperature to clot, then centrifuged at 3000 rpm for 5 minutes for serum collection which was aspirated by using micropipette and dispensed into another sterile tubes, each serum was divided into 2 tubes, and kept in deep freeze at -20°C.

The Diagnostic Methods

Enzyme Linked Immunosorbent Assay (ELISA)

This is kit product of the cusabio company, Chicken toxoplasma circulating antigen (TCA) ELISA, and was performed according to the manufacturer's instructions, this kit used the whole parasite protein of cell membrane as antigen and determined the antibodies in the sera of animals, and stored at $\pm 4^{\circ}$ C until used. The ELISA test has the advantage that it can be automated and is convenient for large-scale surveys. In chickens fed oocysts, Biancifiori et al. (1986) studied the kinetics of IgG-ELISA using the soluble fraction of tachyzoites. They reported IgG titres of 1 : 800 or higher starting at day 12 p.i. and

titres peaked to 1 : 12 800 at day 41 when the experiment was terminated.

Statistical Analysis

The results were analysed by SPSS software using Chi-Square test and a P value <0.05 was considered statically significant.

Results

This present study showed the considerable percentage of antibody titer (18%) against *T. gondii* positive by ELISA among FR chicken (Table-1). This result indicates to high distribution of toxoplasmosis among chicken in the studied area. Our finding demonstrated that anti *T. gondii* antibodies were high in FR chickens in the studied area.

Table 1: The rate of chicken	Toxoplasmosis	in
different parts of Tabriz city,	Iran	

Part	Number	Percent
Northern	27	17.6
Southern	23	18.4
Western	24	15.7
Eastern	26	20.3
Total	100	18

Discussion

Our finding demonstrated that anti T. gondii antibodies were high in FR chickens in the studied area. It seems that become infected mostly during feeding on the ground contaminated with oocysts. (10). The prevalence rate of anti-Toxoplasma antibody in FR chicken (18%) in present study was lower than that of EL massry et al survey (47.2%) from Giza province in Egypt (11). The role of these FR chicken as intermediate host and disseminator of oocysts (12). Since industrial chicken that reared in saloons rose for meat production in short duration and less exposed to cat feces, these chicken had the lowest prevalence compared to FR chicken. T. gondii antibodies were reported to be present in 53.3% of chickens in Egypt by sabin-feldman dye test ,indirect hemagglutination assay (IHA), or the complement fixation test (15,16). However, IHA and the dye test were found to be insensitive for detecting T. gondii antibodies in experimentally infected chicken The prevalence of T. gondii in chickens ,as determined by the modified agglutination test (MAT)(17) varies within countries, ranging from 10% to 47% (11,14,18,19,20). The many factors such as management and hygienic standards in breeding, density of cat and environmental condition are effect on the acquisition of T. gondii oocyst by animal.

ELISA is of a great sensitivity, objective, quantitative and may be automatically adopted, although it needs a refinement in the procedures.(24).A significant difference $P \le 0.05$ was detected in the percentage of T. gondii antibodies among FR Chicken by ELISA, Bird

and rodents are two of the most important intermediate hosts. They become infected easily through ingestion of oocysts (25, 32). Dubey et al. (1993) studied the serologic response of four-weekold chickens to T. gondii following oral oocyst inoculation. Other workers have used an ELISA test to demonstrate that chickens and pigeons inoculated with T. gondii oocysts seroconvert within 2 and 3 weeks, respectively (26, 33). Soil is the most important source of infection for intermediate hosts and, owing to the feeding behavior of terrestrial species, e. g. chickens and partridges, the prevalence of T. gondii in these hosts is a good indicator of environmental contamination with parasite oocysts (14). which indicates that FR Chicken are more likely to get infection than those which are Industrial chicken. The prevalence rate of toxoplasmosis in FR chicken by ELISA was nearly similar to those of Chinese infected FR Chicken (34.7% (27-33).

Conclusion

Based on cultural and food habits in our area, the meat and viscera of chicken may be important sources of infection in human when consuming semi-cooked meats. Considering the high prevalence of toxoplasmosis in chickens, standards in chicken breeding, education of environmental health personnel and standardization for preparation and handling techniques are required by Health and Veterinary organizations.

Conflict of Interest: The authors declare that there are no conflicts of interest.

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Original Article

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The latest innovative study in neurotechnology: A fully implantable external rechargeable and controlled neuroimplant system

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Abstract

Objective: One of the main problems for the bio-implants is the charge life of implant. International Neurotechnology Center is working to develop nerve stimulators and charge units for bio-implants.

Material and Methods: A general object of the present invention is to provide an improved method for transmission of stimulating signals to an electrode implanted in the body. The new neuro implant presented here is a fully implantable, externally rechargeable and controlled system.

Results: To overcome the problems encountered with the existing fully implantable neuro implants such as component failure, limited battery life, programming difficulties and high cost, a new system, that is based on fully implanted externally charged and controlled principles, has been developed.

Conclusion: The newly designed neuro implant system, which is patented and having full quality assurance certificates (CE, ISO9001 and ISO13485), appears to be competitive to the presently known neuro implants in terms of quality, safety, reliability, reduction in size and cost.

Key words: Vagal Nerve Stimulators; Fully implantable; External rechargeable; External controlled; neuro implant.

Introduction

Neuro implantation is a well-established surgical technic to apply neuro stimulation under the skin following operation. Neuro stimulation is a process, by which nerves partially losing their function as a result of disease or trauma, are stimulated using artificial electrical pulses for regeneration. Electrical signals used for this purpose must be consistent with the nature of human neurophysiology (1).

The application, started with epidural spinal cord stimulation to control chronic pain in 1967, nowadays has clinically been approved for other cases, e.g. peripheral nerve stimulation to control pain, vagus nerve stimulation for the management of epilepsy, depression and Alzheimer's disease, phrenic nerve stimulation for diaphragm pacing in breathing disorders, hypoglossal nerve stimulation to treat sleep apnea and snoring, deep brain stimulation for the management of Parkinson's disease (2-9).

Historically, implant technologies used were radiofrequency (RF) transmission, fully implantation, semiimplantation (the Tulgar neuro-implant system), system respectively (10).

Material and Methods

A general object of the present invention is to provide an improved method for transmission of stimulating signals to an electrode implanted in the body. The new neuro implant presented here is a fully implantable, externally rechargeable and controlled system. The system includes both implanted and external parts.

The implanted parts are stimulator and electrode. Stimulator, that is housed in a medical grade titanium case Ti64 (grade 5, permanently implantable in human body) as shown in Figure 1, consists of a 3-fold sandwich, from bottom to up LFP rechargeable battery of 3.2V 120mAh, gold plated PCB mounted with the necessary electronic components and internal charging coil on the top. Implanted bipolar electrode is completely novel with a brand new designed easy-fit connector and cuff electrode that is not wrapped along the nerve thus enhancing the surgery (Figure 2a and b). External parts compose a specially designed Communicator with an external charging coil to awake the embedded circuit and to charge the implanted battery. The external Communicator also has a LCD display to show the status of battery, and to measure the tissue impedance during the operation. The software (treatment program) was optimized to allow the implanted battery lasts for at least 3 months. This user friendly systems allows to start or stop stimulation when needed, e.g. it is possible to start soon before feeling the aura in epileptic patients in case of VNS, vagus nerve stimulation; or to start just

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before going to sleep and to stop just after awaking, sleep apne patients in case of hypoglossal nerve stimulation; or to start anytime when pain is felt in case of peripheral nerve stimulation. In addition, the treatment program is consistent with the body's own language as scientifically approved by our previous study (11).



Figure 1: Titanium housing.



Figure 2A: Implanted bipolar electrode. user friendly cuff contacts (not wrapped around the nerve)



Figure 2B: Implanted bipolar electrode. easy-fit connector

Results and Discussion

Our latest innovative study, presented here, reports a fully implanted externally recharged and controlled neuro-implant model which is now ready to apply.

The newly designed neuro implant system, which is patented and having full quality assurance certificates (CE, ISO9001 and ISO13485), appears to be competitive to the presently known neuro implants in terms of quality, safety, reliability, reduction in size and cost.

Conflict of Interest: The authors declare that there are no conflicts of interest.

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