ANTICANCER AND ANTIOXIDANT ACTIVITIES OF PAPAIN-HYDROLYZED A-LACTALBUMIN

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Abstract. In the present study, α-lactalbumin (α-La) was hydrolyzed with papain for different periods (30, 60, 90, and 120 min). The α-La hydrolysate, which was obtained by subjecting papain to a hydrolysis process for 120 min, had the most significant antioxidant activity. The anticancer activity of these hydrolysates was evaluated on the Mcf-7, Caco-2, and A-549 human cancer cell lines using MTT assay, recording IC_{50} at 45.24, 79.9, and 49.17 μg/mL, against Mcf-7, Caco2, and A-549, respectively. The expression levels of caspase-9 in Mcf-7, A-549, and Caco-2 cells treated with the IC50% (45.24, 49.17, and 79.9 μg/mL α-La hydrolysates) for 2 h increased by about 3.591, 2.609, and 3.195-fold, respectively, as compared to control. α-La hydrolysates inhibited VEGFR-2 in Mcf-7, A-549, and Caco2 cells by about 23, 42, and 47%, respectively). The findings demonstrated that α-La, hydrolyzed with papain, exhibited significant anticancer properties against human cancer cells.
Keywords: *bioactive peptides, proliferation, apoptosis, DPPH, ESI- MS*

Introduction

Disruptions in prooxidant/antioxidant homeostasis cause chronic and degenerative diseases, in addition to genetic and environmental factors. Endogenous and exogenous antioxidants destroy reactive oxygen species (ROS) to preserve homeostasis. The escalation of free radical activities triggers robust and enduring oxidative stress, resulting in lasting alterations in the structures of DNA, proteins, and lipids. These activities result in harm to cellular structures and genes, leading to metabolic problems and the development of cancerous growths (Abdel-Rahim and El-Beltagi, 2010; George and Abrahamse, 2020). The human body has innated ways to mitigate these dangers. The body is safeguarded against the harmful and mutation-inducing effects of ROS, RNS, and xenobiotics by enzymatic and non-enzymatic defense mechanisms (Afify and El-Beltagi, 2011; Pizzino et al., 2017; El-Beltagi et al., 2024a, b, c). Incorporating

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physiologically active peptides, which contain both hydrophilic and lipophilic antioxidants, into the diet can boost human activity.

Cancer is a terrible disease that is on the rise due to changes in lifestyle, nutrition, and global warming (Sharma and Majumdar, 2009; Shallan et al., 2010). The medicine used for cancer treatment is not always effective, and some drugs can cause side effects. Consequently, scientists are investigating organic substances obtained from therapeutic plants as a possible remedy for cancer. According to the WHO, over 80% of the global population, particularly individuals residing in underdeveloped nations, depend on herbal remedies for their healthcare requirements (Bodai et al., 2018; Ramadan et al., 2022). Researchers frequently use enzymatic hydrolysis to produce bioactive peptides (Halim et al., 2018). Enzymatic hydrolysis of milk proteins with papain enhances biological activities (Abdel-Hamid et al., 2016, 2017, 2020). Many harmful by-products such as whey are released into the environment by the dairy industry. Whey proteins consist of β-lactoglobulin (β-Lg) in 40-50%, α-lactalbumin (α-lac) in 12-15%, and bovine serum albumin (BSA) in 5% (Yadav et al., 2015).

α-lactoglobulin is particularly suitable for deriving anticancer peptides. It is rich in sequences that can, upon enzymatic hydrolysis, release bioactive peptides with various biological activities, including anticancer. It also exhibits strong antioxidant activities that counter cancer development (Soloshenko et al., 2020; Landim et al., 2021).

The enzymatic breakdown of food byproducts to produce physiologically active peptides is appealing due to its cost-effectiveness, precision, and less harmful effects compared to mechanical and chemical methods (Karami et al., 2019). Consequently, the present study sets out to determine the active peptide sequences produced by papain digestion and to assess the antioxidant and anticancer properties of α-la hydrolysate.

Materials and methods

Protein hydrolysates preparation and characterization

α-Lactalbumin from bovine milk (CAS No: 9051-29-0) obtained from Merck was hydrolyzed with papain (E/S ratio 1:200) for different periods (30, 60, 90, and 120 min) as described by Abdel-Hamid et al. (2017). To find hydrolysis degree (DH), trichloroacetic acid (TCA) method was used as described by Hoyle and Merrltt (1994) and calculated from the following formula:

$$DH\ (\%) = [\text{Soluble nitrogen in TCA 10\% / Total nitrogen in the sample}] \times 100 \quad \text{(Eq.1)}$$

Free amino acids were estimated using the ninhydrin method after 30, 60, 90, and 120 min-hydrolysis (Lie, 1973). Absorbance was recorded at 570 nm. Calibration equation for leucine was

$$y = 0.0011x + 0.1499\ (R2 = 0.9455) \quad \text{(Eq.2)}$$

where y and x are leucine absorbance and concentration, respectively. SDS–PAGE of α-la hydrolysates with papain was run according to Laemmli (1970) using 3% stacking acrylamide gel and 17% resolving acrylamide gel. The structural conformation and the functional groups of α-lactalbumin hydrolysate were analysed by FTIR (Rosli and Sarbon, 2015). For the most antioxidant active protein hydrolysate, which was collected after 120 min, positive ions electrospray ionization mass spectrometry (ESI-MS) was

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utilized (Al-Mohammadi et al., 2020). An aliquot (10 µL) of the peptide solution was injected into a XEVO TQD triple quadruple instrument, Water Corporation, Milford, MA01757, U.S.A. The samples were run through the column, ACQUITY UPLC-BEH C18 1.7 -m2.1 × 50 mm at 0.2 mL/min flow rate, using a solvent system consisting of (A) 0.1% formic acid aqueous solution and (B) 0.1% formic acid in acetonitrile The masses of the resulting peptides were matched to the original amino acid sequence of α-Lactalbumin registered in https://www.uniprot.org and using also the potential enzymatic cleavage sites ending with the basic amino acids (arginine, lysine and histidine) to deduce their sequences.

Antioxidant activity (AA) evaluation

The AA of α-lactalbumin hydrolysate with papain (1000 µg/mL) obtained at different times was estimated. The α-La 120 min hydrolysates were selected for this analysis.

DPPH radical scavenging activity (DPPH-RSA) assay

DPPH-RSA was estimated (Ramadan et al., 2008). one mL of each sample (50–1000 µg/mL) was added to 4 mL of 0.15 mM DPPH (in 95% ethanol) and shaken vigorously. Absorbance was recorded at 517 nm after incubation for 30 min. TBHQ was used as a standard sample. The RSA was calculated as follow:

$$\text{RSA (\%)} = [(\text{A control} - \text{A sample}) / \text{A control}] \times 100 \quad \text{(Eq.3)}$$

with A = absorbance at 517 nm.

Ferric reducing antioxidant power (FRAP)

Reducing power of α-lactalbumin hydrolysates and tertiary butylhydroquinone (TBHQ) was estimated by recording the absorption of Perl's Prussian blue complex resulting from reduction of Fe^{3+} to Fe^{2+} at 700 nm as described by Göçer and Gülçin (2011).

Anticancer activity evaluation

Cell viability in vitro (MTT-assay)

The impact of α-lactalbumin (α-La) 120 min hydrolysate with papain (concentration range 31.25-1000 µg/mL) on MCF7, A549, and Caco-2 viability was estimated in vitro using MTT-assay. Cell viability was estimated after incubation for 48, measuring absorbance at 550 nm (Hansen et al., 1989). The following formulas calculated the cell viability (%) and cytotoxic activity (%):

$$\text{Cell viability (\%)} = (\text{Absorbance sample} / \text{Absorbance control}) \times 100 \quad \text{(Eq.4)}$$

$$\text{Cytotoxic activity (\%)} = 100\% - \text{cell viability (\%)} \quad \text{(Eq.5)}$$

Hydrolysate concentration which gives 50% growth inhibition is referred to as IC_{50}.

Analysis of the results by GraphPad Prism software provides the IC_{50} (inhibition concentration; 50%) values and their statistical errors based on several repetitions of the measurement.

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Quantification of mRNA levels of caspase-9

Step One Plus Real-time PCR (Applied Biosystems, Foster City, CA, USA) was employed to quantitatively analyze caspase-9 in Mcf-7, Caco-2, and A-549, before and after treatments, using gene-specific primers or SYBR Green master mix. Cells were treated with IC_{50} of the tested hydrolysates for each cell line for 24 h based on MTT-assay. The relative expression level of caspase-9 in mRNA levels was estimated by quantitative real-time PCR (Asadi et al., 2018).

In vitro VEGFR-2 kinase assay

The α-lactalbumin 120 min hydrolysate with papain produced resulting at 45.24, 79.9, and 49.17 μg/mL for Mcf-7, Caco-2, and A-549, respectively, were selected to evaluate their inhibitory activities against VEGFR-2 following manufacturer's instructions (Abou-Seri et al., 2016) as described in El-Helby et al. (2019). Comparing the treated compounds to the control incubations yielded the inhibition %.

$$\text{Inhibition (\%)} = [(\text{control} - \text{treatment}) / \text{control} \times 100] \quad \text{(Eq.6)}$$

Statistical analysis

To examine the data, we used one-way ANOVA and then Tukey's post hoc test to see if there were any differences. We used SPSS version 16.0 (SPSS Inc., Chicago, Release 16.0.0, 2007) for all of our statistical calculations. $P < 0.05$ was utilized to determine statistical significance.

Results and discussion

Alpha-lactalbumin hydrolysates characterization

SDS-PAGE of α-La hydrolyzed with papain is presented in *Figure 1A*. The purified α-La showed two bands at zero time (1 and 2). With increasing the hydrolysis time, the intermediate band (band 1) disappeared while the low-sized one (band 2) remained but faded gradually with time. So, it can be understood that in the first 30 min, the band fissure targeted the bonds in the middle of the molecule, while with time, the degradation targeted the distal parts of the peptide's fragments leading to the disappearance of the staining color. It is expected then that small-sized peptides will be predominant with increasing hydrolysis time. Also, band No 1, appearing in the native αLa resisted hydrolysis and was only partially degraded. Similar results were shown previously (Garcia-Mora et al., 2015, 2016).

The DH (*Fig. 1B*) and the free amino acids concentration (*Fig. 1C*) were estimated in α-La hydrolysates at different intervals, 30-120 min. The DH gradually increased from 7.012 after 30 min to 23.11% after 120 min. Typically, changes in DH are caused by changes in enzyme reaction time, which affects breaking of peptide bonds. DH is an essential parameter since it can affect the molecular peptide size and structural specification, which greatly influence their biological interactions and activities (Xie et al., 2019).

Changes in the chemical structure of α-La before and after hydrolysis with papain were estimated using FTIR spectroscopy and data are presented in *Figure 1D*.

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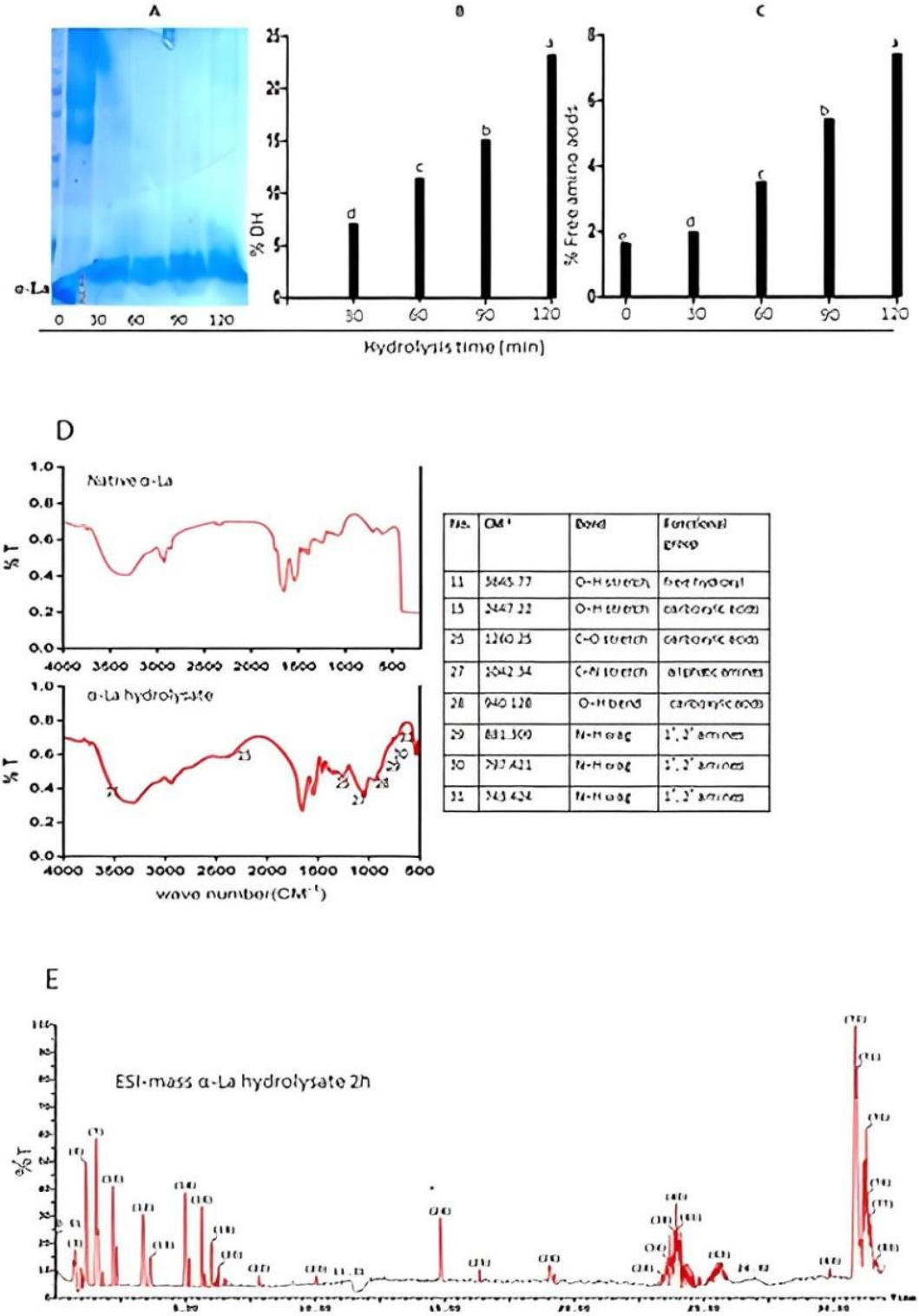

Figure 1. *(A) SDS-PAGE of α-lactalbumin, (B) the degree of hydrolysis (DH %) of α-lactalbumin with papain (E/S = 1:200) during 120 min at 37°C and pH 6, and (C) the extent (%) of free amino acids. Values indicated by different small letters are significantly different according to Tukey's HSD test ($p \leq 0.05$). (D) IR spectra of native and 2-h α-lactalbumin hydrolysate by papain (E/S = 1:200) at 37°C and pH 6. (E) Mass spectrometric chromatogram of peptides formation from α-lactalbumin hydrolysate with papain (E/S = 1:200) for 2 h at 37°C and pH 6 by electro-spray-ionization-MS (ESI-MS)*

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Peaks for intact α-La were found at 3305, 2924, 1742, 1651, 1540, 1456, 1339, 1241, 1076, 702, and 610 Cm^{-1}. The FTIR spectra of α-La 120-min hydrolysate with papain showed eight new peaks which were absent in that of the intact protein, i.e., at 3645, 2447, 1260, 1042, 940, 881, 797, and 743 Cm^{-1}. Hydrolysis likely releases free carboxylic and amine groups, confirming the process.

ESI- MS estimated the peptide components of the α-la hydrolysate with papain at the positive ion mode. The primary peaks caused by papain (*Fig. 1D; Tables 1*, *2* and *3*) included 81 peptides of molecular masses ranging from 101.79 to 841.74 Da. These comprised 46 dipeptides, 9 tripeptides, 5 tetrapeptides, 5 pentapeptides, 2 hexapeptides and 1 heptapeptides. Quantitatively, the dipeptides represented 47.38% of the total peptides, and16.36% amino acids. The rest of the peptides represented about 36.26% of the total and consisted of tripeptides (27.84%), tetrapeptides (6.3%), pentapeptides (1.11%), hexapeptides (0.67%) and heptapeptides (0.33%). Thus, tripeptides quantitatively represented the major components, followed by tetrapeptides and pentapeptides. Nevertheless, the dipeptides group represented the biggest number of peptides (46 peptides and was classifiable into basic (40.65%), hydrophobic (51.01%), neutral (1.33%and acidic dipeptides (7.01%) of the total dipeptides, i.e., the collective nature of dipeptides is rather hydrophobic and basic. basic group included two Arginine containing peptides (RC), and (RN). The dipeptide NR (Asparagine-Arginine) was the most frequent one. There were also two Lysine-containing dipeptide (KS) and (KF). The peptides with more than two amin acids, i.e., a tri-to heptapeptide with medium molecular weights in the 2-h α-La hydrolysate (ca 36.26% of the total). These peptides were classified according to their alkalinity into basic, hydrophobic, neutral, and acidic (*Table 4*). The total basic peptides represented ca. 63.97% of the total peptides, while 79.9% hydrophobic, 20.2% neutral and 1.8% acidic peptides.

Table 1. *Possible di-peptide compositions of 2-h α-lactalbumin hydrolysate with papain (E/S = 1:200), produced at 37°C and pH 6*

Peak ID	Area % total	Molecular weight (Da)	Composition
2:3	2.19	174.8211	CA
4	0.68	172.9981	AT
6	5.73	216.9909	KS
7	6.5	261.0088	FL
8	1.89	274.9846	KF
10	4.78	305.0522	MC
11	1.58	319.0379	RC
12	4.16	349.0814	WC
13	1.47	363.0526	WC
24	3.05	219.0592	CD
25	0.27	244.1514	EN, DK
26	0.65	301.0468	NW
32:45 and 47	9.92	271.1661	RN
48:49	0.53	337.1517	CY
50	0.09	152.0149	PG
54	0.1	115.8119	GG
56	0.13	271.1396	RN
57:67	3.23	297.1873	MM
68:69	0.41	263.1262	FL

No. of peptides: 81; 46 di peptides, 9 tri-peptedes, 5 tetra- peptides, 5 penta-peptides, 2 hexa-peptides, 1 hepta-peptides, 13 amino acids

A: alanine, T: theronine, G: glycine, M: methionine, R: arginine, Q: glutamine, W: tryptophane, S: serine, L: leucine, Y: tyrosine, D: aspartic acid, H: histidine, T: theronine, P: proline, C: cysteine, F: phenylalanine, K: lysine, E: glutamic acid, and N: Asparagine

Amino acids names followed the one-letter abbreviations: https://www.genscript.com/Amino_Acid_Code.html

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Table 2. *Classification of the di-peptides according to their contents of basic, acidic, and hydrophobic amino acid residues*

Basic	Hydrophobic	Neutral	Acidic
KS	CA	CY	CD
KF	AT	GG	EN, DK
RC	FL		
RN	MC		
	WC		
	PG		
	MM		
	FL		
40.65%	**51.01%**	**1.33%**	**7.01%**

Basic: red, Hydrophobic: green, Acidic: orange, Neutral: black. The percentage was calculated relative to the total di-peptides

Table 3. *Possible tri, tetra, Penta, hexa, and hepta peptide compositions of 2-h α-lactoalbumin hydrolysate with papain (E/S = 1:200), produced at 37 °C and pH 6*

Peak ID	Molecular weight (Da)	Peptide sequence	Fragment sequence*	Area % total	Relative proportion (%)
Triapeptides					**27.84%**
5	377.845	TEF	20:22	0.3	
14	393.0827	DKF	97:99	4.1	
15	407.0594	Undefined		1.02	
19	402.1424	Undefined		0.22	
27	383.0841	Undefined		0.37	
70:71	413.2046	WCK	79:81	21.82	
Tetrapeptides					**6.3%**
9	430.1654	CAKK	110:113	0.42	
16	437.1293	SCDK	95:98	2.9	
17	451.0933	SSEF	82:85	0.36	
18	481.1746	YGLF	69:72	1.55	
20	525.2275	Undefined		0.65	
52:53	430.0251	CAKK	110:113	0.43	
Pentapeptides					**1.11%**
21	569.2643	TCVLF	46:50	0.17	
28:30	678.6484	Undefined		0.67	
31	669.3976	Undefined		0.27	
Hexapeptides					**0.67%**
23	777.2665	RFVPLF	3:8	0.36	
51	707.5917	TEYGLF	67:72	0.31	
Heptapeptides					**0.33%**
22	841.7412	Undefined		0.33	

*Fragment sequence calculated based on α-La sequence of https://www.uniprot.org/uniprotkb/P02754/entry

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Table 4. *Classification of the tri-heptapeptides according to their contents of basic, acidic, and hydrophobic amino acids residues*

Basic	Hydrophobic	Neutral	Acidic
WCK	TEF	DKF	TEF
CAKK	DKF	SCDK	SSEF
CAKK	WCK	TEYGLF	
TCVLF	YGLF		
RFVPLF	SSEF		
	TCVLF		
	RFVPLF		
	TEYGLF		
63.97%	**79.9%**	**20.2%**	**1.8%**

Basic: red, Hydrophobic: green, Acidic: orange, Neutral: black. The percentage was calculated relative to the total di-peptides

Antioxidant activity

DPPH-RSA of α-La hydrolysates produced using papain at different times are depicted in *Figure 2A*. Increasing the degree of hydrolysis of α- α-La from 7.012% to 23.11%, significantly ($P < 0.05$) and highly increased the DPPH-RSA from 17.75% to 87.82%, i.e., after 120 min hydrolysis. This antioxidant capacity is very close to that produced by TBHQ i.e., 89.17%. Antioxidant activity can be found in unhydrolyzed α-La (18.99%). The DPPH-RSA of α-La hydrolysates produced using papain for 120 min (the highest antioxidant activity) at different concentrations is depicted in *Figure 2B*. When the concentration was increased from 50 to 1000 µg/mL, the DPPH-RSA increased significantly ($P < 0.05$) from 78.68% to 87.82%. Nonsignificant increases were recorded between 250, 500, and 1000 mg/mL. Antioxidants are known to interact with free radicals and stop oxidation. DPPH is commonly used to evaluate reducing substances (You et al., 2009). Based on the findings, α-La hydrolysates are effective electron donors and can halt radical chain reaction by reacting with free radicals. The amount of histidine and other hydrophobic amino acids in α-Lactalbumin and their concentrations are both related to its antioxidant activity (Grażyna et al., 2017).

The α-La hydrolysates FRAP produced using papain at different times is shown in *Figure 2C*. The protein that was not hydrolyzed had a weaker reducing effect than the hydrolysates ($P < 0.05$). After 120 min, hydrolyzed α-La had a higher FRAP value (absorbance = 1.528) and DH = 23.11% compared to 1.476 for TBHQ. The FRAP of α-La hydrolysates produced using papain for 120 min (the highest antioxidant activity) at different concentrations is shown in *Figure 2D*. When the concentration of α-La hydrolysates was increased from 50 to 1000 µg/mL, the FRAP value increased significantly ($P < 0.05$) from 0.545 to 1.528.

Anticancer activity

The concentration of α-La hydrolysates was directly proportional to the cell viability percentage (*Fig. 3A, B*). Based on the results of the MTT experiment, the α-La hydrolysates showed a concentration-dependent inhibition of the proliferation of Mcf-7, A-549, and Caco-2. Nonsignificant increases were recorded between 250, 500, and

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1000 mg/mL α-La hydrolysates exhibited the lowest IC_{50} against Mcf-7 at 45.24 µg/mL, while the highest value 79.9 µg/mL was realized against Caco-2. It can be concluded that α-La 120-min hydrolysate by papain is generally effective against the three studied carcinogenic cell lines, where IC_{50} was in the range 42.8 – 76.92 µg/mL, particularly against Mcf-7 cell-line. To study the impact of α-La hydrolysates on cell morphology, we utilized the Mcf-7, Caco-2, and A-549 cell lines, and the results are documented in *Figure 3B*. α-La hydrolysates inhibited the growth of Mcf-7, Caco-2, and A-549 cell lines in a a dose-dependent manner, leading to noticeable changes in cell morphology.

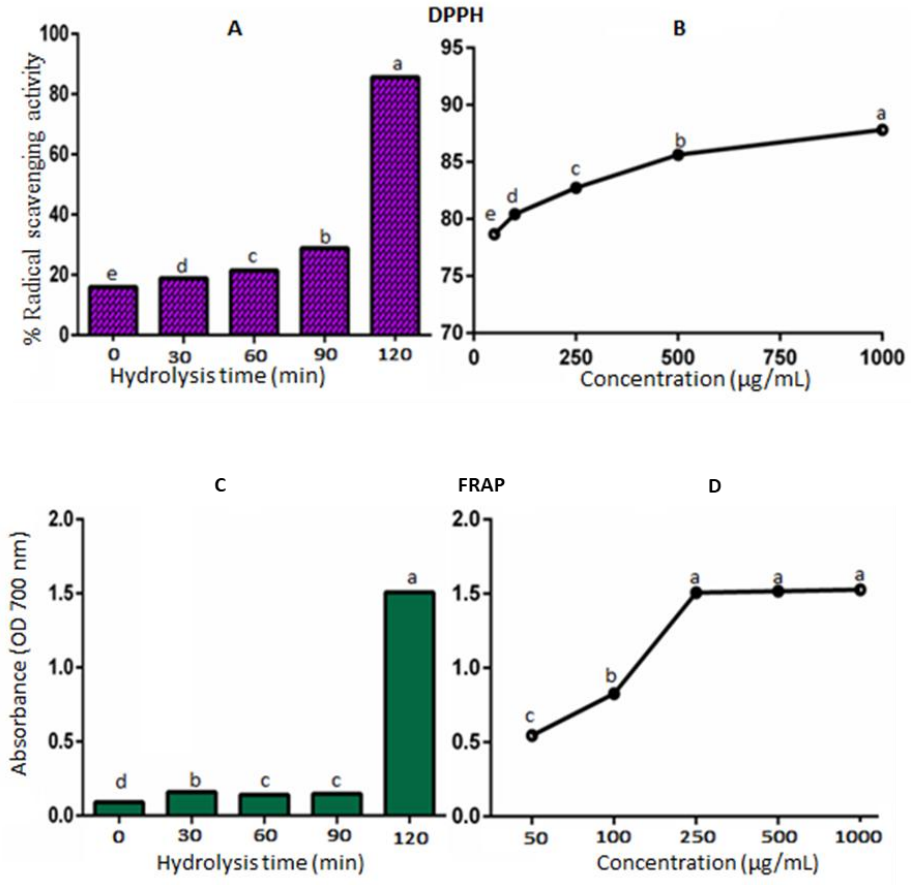

Figure 2. *(A) DPPH free radical scavenging activity of α-lactalbumin (α-La) hydrolysates(100 µg/ml) produced by papain (E/S = 1:200) at 37°C and pH 6 at different time intervals, and (B) DPPH radical scavenging activity of 2-h α-La hydrolysates with papain (the highest antioxidant activity) at different concentrations. (C) Ferric reducing antioxidant power (FRAP) of α-lactalbumin (α-La) hydrolysates (250 µg/ml) produced by papain (E/S = 1:200) at 37°C and pH 6 at different time intervals and (D) FRAP of α-La hydrolysates with the highest antioxidant activity (produced after 120 min hydrolysate with papain) at different concentrations. Different small letters indicate significant differences following Tukey's HSD test ($p \leq 0.05$)*

Gene expression of Caspase-9 transcripts seems highly multiplied by the application of α-La hydrolysates (*Fig. 4A*). The expression of caspase-9 in Mcf-7, A-549, and Caco-2 cells treated with 45.24, 49.17, and 79.9 µg/mL α-La hydrolysates for 24 h was multiplied by 3.591, 2.609, and 3.195-fold, respectively, as compared to control. The

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ability of many anticancer drugs to induce apoptosis may construct their mechanism of action (Sen and D'Incalci, 1992; Motomura et al., 2008). However, many of the newly developed anticancer medications suffer from undesirable side effects and are resistant to treatment (Khan and Mlungwana, 1999). As a result, there is growing interest in development of safe and more effective cancer treatment agents based on natural compounds (Panchal, 1998). Proteins have been scientifically proven to be promoters in prevention of certain diseases, including cancer, and are thought to be an essential source of therapeutic peptides (Ramkisson et al., 2020). Several established pathways are believed to regulate anticancer effects of protein hydrolysates and peptides derived from food. These pathways include nucleic acid impairment, immune system regulation, protease inhibition, apoptosis induction, cell cycle arrest, and inhibition of intracellular signaling systems (RCK Rajendran et al., 2017). Apoptotic cell death, in which the body maintains a steady population of cells by regulating their division and death, is one of the most efficient mechanisms the body uses to govern cell proliferation and death (Indran et al., 2011). To achieve their anticancer effects, bioactive peptides and protein hydrolysates can orchestrate cellular DNA damage. One example of this is the common bean-derived peptide GLTSK, which was found to impair DNA function in HCT116 cells through the overexpression of the histone γH2AX (Luna-Vital et al., 2016).

The inhibitory activities against VEGFR-2 were tested utilizing anti-phospho-tyrosine antibody with Alpha Screen system (PerkinElmer). The α-La 120-min hydrolysates by papain that exhibited the most antiproliferative action against Mcf-7, A-549, and Caco-2, at 45.24, 49.17, and 79.9 μg/mL, respectively, were chosen for this study.

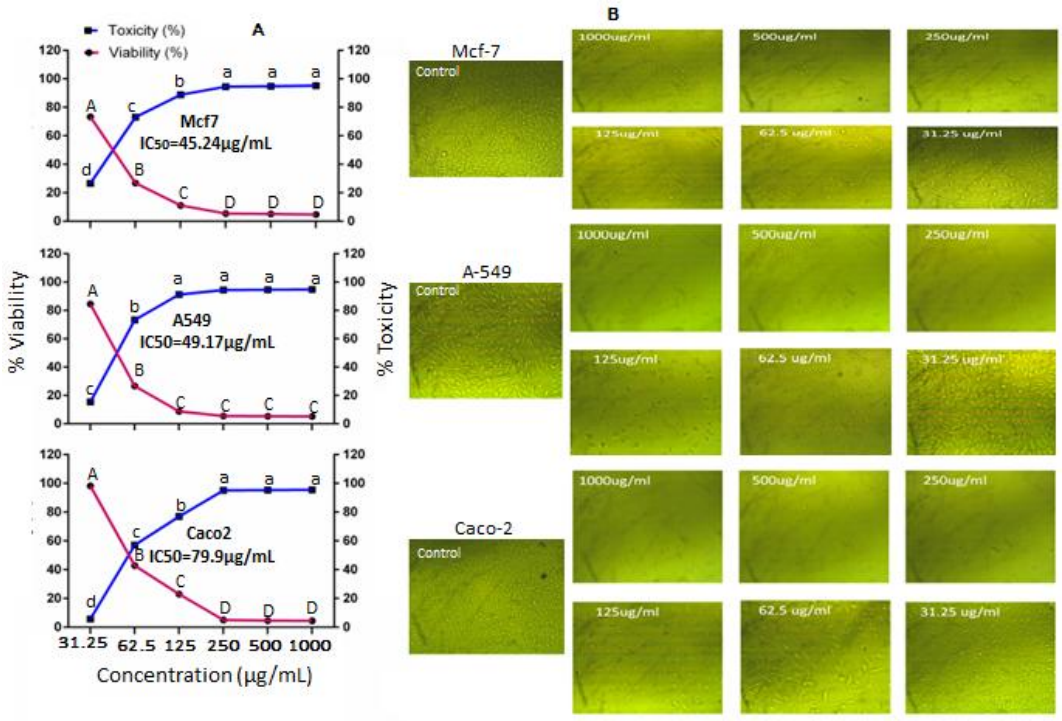

Figure 3. *(A) Relationship between toxicity (%) and cell viability (%) of Mcf-7, Caco-2, and A-549 cell lines treated with α-La hydrolysates produced using papain (E/S = 1:200) for 120 min at 37°C and pH 6 (the highest antioxidant activity) at different concentrations. Different letter indicates significant differences among the cell viability (capital letters) and toxicity (small letters) according to Tukey's HSD test ($p \leq 0.05$). (B) Light microscopic images of cells lines after treatment with α-La hydrolysates using Reichert-Jung microscope*

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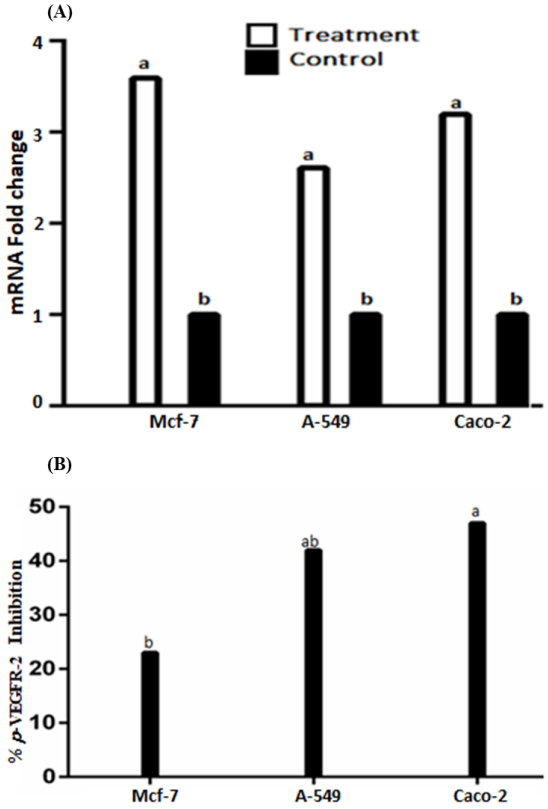

Figure 4. *(A) Effect of 120-min α-La hydrolysate with papain (E/S = 1:200) at 37°C and pH 6 (the highest antioxidant activity) on caspase 9 gene expression of three human cancer cell lines (Mcf-7, A-549, and Caco-2). Cells were treated with the concentration causing the IC_{50} for each cell line for 24 h and their mRNA levels were evaluated by quantitative real-time PCR. (B) Inhibition (%) of VEGFR-2 in three human cancer cell lines (Mcf-7, A-549, and Caco-2) after treatment with 120-min α-La hydrolysates by papain (E/S = 1:200) at 37°C and pH 6 (IC_{50} for each cell line). Different small letters refer to significant differences to Tukey's HSD test ($p \leq 0.05$)*

The results are shown in *Figure 4B*. It is observed that α-La hydrolysates strongly inhibited VEGFR-2 in Mcf-7, A-549, and Caco2 cells by about 23%, 42%, and 47%, respectively. This is an important result since VEGFR-2 is the most significant transducer of VEGF-dependent angiogenesis (Holmes et al., 2007) and thus the studied factor seems an efficient anticancer factor. The VEGF signaling pathway plays a fundamental role in regulating tumor angiogenesis. The therapeutic potential of VEGF has been demonstrated in a variety of human cancers (Niu and Chen, 2010). VEGF-receptor 2 (VEGFR-2) is regarded as the most significant transducer of VEGF-dependent angiogenesis because it is major target of angiogenesis-related kinases (Holmes et al., 2007). As a result, it is thought that inhibiting VEGF/VEGFR signaling pathway is a promising therapeutic target for preventing tumor angiogenesis or subsequent tumor growth (Vayner and Ball, 2000; Tugues et al., 2011; Eldehna et al., 2015; Abou-Seri et al., 2016).

Conclusion

The results of this investigation demonstrated direct anticancer action of α-La hydrolysates on human cancer cells. The 120-min α-La hydrolysate, containing

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predominantly medium-sized peptides (tri, tetra, pepta, hexa, and heptapeptides) with molecular weights ranging from 101.79 to 841.74 Da, exhibited the highest effectiveness, which aligns with its strong antioxidant properties. The biological activities of the α-La hydrolysate with papain are determined by its chemical composition and characteristics. The presence of significant amounts of basic and hydrophobic peptides may be responsible for anti-carcinogenic activity. The α-La hydrolysates exhibit significant efficacy against three carcinogenic cell lines (Mcf-7, A-549, and Caco-2), with IC50 values of 45.24, 49.17, and 79.9 μg/mL, respectively. Additionally, they demonstrate inhibitory effects on VEGFR-2, with inhibition percentages of around 23%, 42%, and 47% for the corresponding cell lines. Future research may aim to optimize the anticarcinogenic potency of the peptides by isolating and assessing the most prominent individual peptide, as well as by varying the treatment circumstances.

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