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Effect of Deuterium Oxide (D_2O) on Foodborne Amino and Fatty Acid Stability Under Thermal and Irradiation Exposures

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Abstract

Deuterium oxide (D_2O) has recently gained interest in pharmaceutical, biological and spectroscopic applications. However, its implications have not yet been investigated in foods and macronutrients. This study aimed to examine the effect of D_2O on amino- and fatty acids of pasteurized liquid egg white (EW) and egg yolk (EY) products under thermal (T) and irradiation (γ and UV) exposures. First, two test groups (TG_{EW} and TG_{EY}) were prepared with EW and EY, each having three vials for each exposure and the control. TG_{EW} contained 40 mL of EW, while TG_{EY} had 40 mL of EY. Next, 6 mL of D_2O (~16% of egg product by weight) was added to all TGs except the controls, the vials were sealed and homogenized using a plate shaker at 20 rpm/250 s. After that, TGs were subjected to thermal (61.5 °C/24 h), γ (5 to 15 kGy/180 min), and UV (280 to 400 nm/120 min) irradiation. Finally, the amino and fatty acid contents were determined using sensitive LC-MS/MS and GC-MS techniques. The results showed that D_2O significantly reduced amino acid degradation to T (86.2%), γ (93.0%) and UV (90.8%) exposures, as well as unsaturated fatty acid degradation to γ (71.3%) and UV (91.6%), and saturated fatty acid degradation to T (93.8%) and γ (90.0%), ($P<0.05$). Overall, our data highlights that D_2O, constitutes an interesting next challenge for the preservation, stability, and quality control of foods and macronutrients, taking care of safety and toxicity considerations.

Keywords
Amino acid
Deuterium oxide
Fatty acid
Irradiation
Temperature

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Introduction

Deuterium oxide (D_2O), also known as heavy water (CAS no. 7789-20-0), includes two deuterium (D) atoms instead of two ordinary hydrogen (H) atoms, and the isotope deuterium (2 H, D) has one additional neutron (Linse & Hub, 2021). D is about 0.015% of H atoms in regular water (H_2O), providing unique physicochemical properties for D_2O (Kselíková *et al.*, 2019). It is not radioactive and toxic if not taken in large amounts (i.e., 50 mg/kg body weight for a 70-kg person), (Makhatadze *et al.*, 1995; Sen *et al.*, 2009).

The influence of D_2O on the structure of H_2O and biological macromolecules can be through (a) solvent isotope effect or (b) D isotope effect. Hence, it has gained interest in pharmaceutical (i.e., therapeutic agent against cancer, deuterated drugs, antibiotics), biological (i.e., cell development, metabolism, tissue homeostasis, drug resistance, and aging), and spectroscopic (i.e., mass-spectrometry, nuclear magnetic resonance spectroscopy, Fourier transform infrared spectroscopy) applications (Chauhan, 2020; da S. Mariano *et al.*, 2017; Efimova *et al.*, 2007; Kleemann *et al.*, 2020; Kopf *et al.*, 2022; Pica & Graziano, 2018; Sen *et al.*, 2009; Wang *et al.*, 2022). One of its unique properties relates to enhancing the thermal stability of lysozyme and bovine serum albumin and isotopic labelling (Linse & Hub, 2021; Smith, 2021).

Chemical process optimization relies on an equilibrium assumption for chemical reactions (i.e., temperature, stoichiometry, reaction time, etc.), (Taylor *et al.*, 2023). However, food processing is a much more complicated issue, and foods do not generally follow this assumption due to

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various structural length scales (Burbidge & Le Révérend, 2016). From the pure physics perspective, foods are considered '*dirty*' systems with their strongly metastable thermodynamics, referring to complicated, applied, and advanced in engineering, processing and applied science (Vilgis, 2015; Yousefi & Abbasi, 2022). Adapting food constituents to physical conditions requires complicated changes in intermolecular interactions, determining food stability and quality (Zhang *et al.*, 2021). The behaviour of specific amino- and fatty acids has been investigated in H_2O/D_2O mixture (Makhatadze *et al.*, 1995), such as peptization of Phe, His, Pro, Cys, and Met and thermal stability of lysozyme (Efimova *et al.*, 2007; Fulczyk *et al.*, 2019; Kresheck *et al.*, 1965), and oxidative stability in corn oil and linoleic acid-D_2O models (Kim *et al.*, 2014; Lee *et al.*, 2018; Oh *et al.*, 2016; Oh *et al.*, 2017). However, to our knowledge, a reliable rationalization of D_2O's stabilizing effect has not yet been explored for foods and nutrients against thermal and irradiation stresses. For instance, hen's egg is a unique biological entity rich in protein (12.6%), fat (9.5%) and H_2O (76.1%), which possess functional properties such as emulsifying, foaming and gelation capacities, making them eligible and most widespread ingredients in food industry (Miguel *et al.*, 2020; Sarantidi *et al.*, 2023; Usturoi *et al.*, 2025). On the other hand, its higher H_2O content makes whole egg and egg-derived products highly perishable commodities, such as unsaturated fatty acids' susceptibility to oxidation, leading to reduced shelf life with bad taste and unpleasant odour (Kocetkovs *et al.*, 2022; Liu *et al.*, 2022; Romero *et al.*, 2022) because H_2O is a fundamental determinant of biomacromolecules' structure and thermodynamic character (Marques *et al.*, 2020). Primarily when interactions exist between the solvent and the solute. This diversity, therefore, results in compositional variations of biomacromolecules, such as amino- and fatty acids in food with varied reactivity during decomposition (Wang *et al.*, 2024), also known as an isotopic effect, that is more significant in D_2O/H_2O due to H-bonds involving D atoms. For instance, the C-D bond is 10 times energetically stronger than the C-H bond and is more resistant to chemical or enzymatic cleavage (Goldenzweig & Fleishman, 2018; Pica & Graziano, 2018; Sen *et al.*, 2009).

Most studies have focused on the potential use of D_2O in slowing down the thermal degradation of biological entities (Giubertoni *et al.*, 2023; Reslan & Kayser, 2018). However, to our knowledge, its effects on foods and macronutrients under harsh physical conditions have not been investigated. With this growing understanding of D_2O's moderating role on the foods and macronutrients, this study aims to examine its effects on the stability of the hen egg's amino- and fatty acids to T, γ, and UV exposures.

Materials and methods

Materials

D_2O (99.9 atom %D) was purchased from Sigma Aldrich (Catalogue no: 151882-250G, Darmstadt, Germany). Pasteurized liquid EW and EY products were delivered in 1 kg carton boxes by İPAY Inc., (İzmir, Türkiye). The samples were stored at 4 °C till further analyses.

Sample preparation

Two TGs were prepared from EW and EY at room temperature (25 °C), regulated by an air conditioning system. TG_{EW} (EW_T, EW_γ, and EW_{UV}) had 40 mL of EW product in a vial for each exposure, including the control (Isolab Boro 3.3, Germany). 6 mL of D_2O (~16% of egg product by weight) were added to all TGs except the controls according to the median percentage (%) of D_2O's protein denaturation preventive concentration, 7-25% D_2O (Rowe *et al.*, 2019). The same procedure was repeated for TG_{EY} (EY_T, EY_γ, and EY_{UV}). Finally, the vials were sealed and homogenized using a plate shaker at 20 rpm for 250 s (Biosan PSU-20i, Istanbul, Türkiye).

Temperature, gamma, and UV exposures

The thermal exposure was conducted to EW_T and EY_T at 61.5 ± 0.1 °C during 24 h in an etuve (Nüve EN 055, Ankara, Türkiye). The applied T was selected as the median pasteurization temperature of EW and EY products, which are 57 °C for EW and 66 °C for EY.

The gamma (γ) treatment was performed in Gammapak Sterilization Ind. & Trd. Inc., (Çerkezköy, Türkiye). EW_γ and EY_γ were irradiated with 5 to 15 kGy (median ~10 kGy) for 180 min using the JS 9600 Gamma Irradiation Device with Co-60 radioactive source (registered with serial number IR-185), as suggested (10 to 30 kGy) for microbial safety by Perchonok *et al.* (2012).

The ultraviolet (UV) stress was applied to EW_{UV} and EY_{UV} using the Vital 300W E UV source (Overall band: 280-400 nm; UVA: 315-400 nm and UVB: 280-315 nm), (Osram 4008321543929, Augsburg, Germany) during 120 min, to mimic UV-induced oxidative stress of active oxygen species, which react with lipids and proteins (Nasibi & M-Kalantari, 2005).

Chromatography techniques

Amino acid content was obtained quantitatively (g/1000 g) using liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS), and fatty acid content was determined semiquantitatively (%) by gas chromatography-mass spectrometry (GC-MS). Both techniques were selected as highly sensitive approaches over other methods. All chemicals and solvents were of analytical and chromatography grade purity. Chromatographic tests were performed in the SEM Laboratory Systems and Solutions Inc. (İstanbul, Türkiye).

LC-MS/MS and GC-MS analyses

16 amino acids (Val, Ala, Gly, Phe, Trp, Pro, Met, Cys, Tyr, Glu, Asx, His, Lys, Arg, Thr, and Ser) were quantified in TG_{EW} with LC-MS/MS spectra of aminoacids. 0.500 g of EW was hydrolyzed with 4 mL of aminoacid reagent (JASEM JSMCL-508, Istanbul, Türkiye) at 110 °C for 24 h. It was cooled at room conditions and centrifuged at 4000 × g for 5 min. After that, 100 µL of supernatant was diluted to 1 mL by deionized water to obtain 800-fold diluted hydrolysates. Jasem Quantitative Amino acids Kit's protocol was followed to prepare aminoacid hydrolyzates due to its advantages of fast and simple preparation, analysis without derivatization, and short analysis time (7.5 min). Then, 50 µL of hydrolysate was pipetted into a vial, and 50 µL of internal standard solution

and 700-µL of Jasem acidic hydrolysis reagent were added. The mixture was thoroughly vortexed for 5 s to speed the hydrolysis rate and productivity. The supernatant was decanted into an HPLC vial, and 3-µL of supernatant was injected into the aminoacid column (JSM-CL-575). Agilent 1290 Infinity-Agilent 6470 Triple Quad System (Santa Clara, USA) quantitatively analyzed aminoacid content in µmol/L, then converted to g/1000 g equivalent (Table 1), (Sonmezdag *et al.*, 2018).

Table 1. LC-MS/MS analytical parameters for aminoacid quantification

Instrument	Agilent 1290 infinity-agilent 6470 triple quad
Column	Jasem Aminoacid Kit Column
HPLC Parameters	
Initial Pressure	110 bar
Column Temperature	30 °C
Auto-sampler	Set a 2-sec needle wash using a flush-port
MS/MS parameters	
Ion Source	ESI (Agilent jet stream)
Polarity	Positive
Gas Temperature	150 °C
Gas Flow	10 L/min
Nebulizer Pressure	40 psi
Sheath Gas Temperature	400 °C
Sheath Gas Flow	10 L/min
Capillary Voltage	2000 V (positive)

Table 2. GC-MS analytical parameters for fatty acid semiquantification

Instrument	Agilent 8890-5977 B GC/MSD system
Column	Agilent-HP5MS Ultra inert
Oven initial temperature	40 °C (5 min holding)
Oven ramping rate	10 °C per min for a total of 5 min
Oven maximum	250 °C 5 min holding
Inlet temperature	220 °C
Electron impact ionization	70 eV
Mode	Scan
Wash solution	A-B Chloroform
MS Source	230 °C
MS Quad	150 °C
Aux Heaters (MSD Transfer Line)	280 °C

8 fatty acids were semi-quantified in TG_{EY} using GC-MS, as conducted by Kuang *et al.* (2018). Of them, 5 were unsaturated fatty acids, including gamma-linolenic acid (GLA), linoleic acid (ALA), oleic acid (OA), arachidonic acid (AA), and docosahexaenoic acid (DHA). In contrast, saturated fatty acids were myristic acid (MA), palmitic acid (PA), and stearic acid (SA). In GC-MS analysis, a solvent-assisted extraction method was used to extract fatty acids efficiently. First, 2 g of EY were poured into a headspace vial, and 10 mL of dichloromethane (DCM) solvent (Merck 106050, Darmstadt, Germany) was added to all vials, including the control. DCM was selected to be the most suitable solvent for extraction with a low boiling point (39.6 °C) and immiscibility with H_2O (Asuzu *et al.*, 2023; Zhao *et al.*, 2023). Next, the vial was agitated at 400 rpm for 2 h. After that, 2 mL of extract was filtered through a 0.45 µm syringe filter (Phenex, CA, USA) and transferred to a 2 mL vial. Finally, 1 µL was injected into the Agilent-HP5MS column (30 m length, 0.25 mm inside diameter, 0.25 µm film thickness) of the Agilent 8890-5977 B GC/MSD system (Table 2). Fatty acids were identified based on their mass spectra against the National Institute of Standards and Technology (NIST) Mass Spectral Library. After identification, corresponding area counts were collected for semi-quantification (%), (Sonmezdag *et al.*, 2018).

Statistical evaluation

The effect of D_2O on amino and fatty acid degradation between TG_{EW} and TG_{EY} was statistically evaluated with the Pearson Correlation Coefficient test using the SPSS program version 20.0 (IBM Corporation, New York, USA), ($P < 0.05$).

Results and discussion

LC-MS/MS results

TG_{EW} was subjected to thermal stress at 61.5 °C for 24 h, which is the median pasteurization T of EW and EY products, or close to the pasteurization T of whole egg (62 to 64 °C up to 10 min) to preserve whole egg functionalities (Lechevalier *et al.*, 2017). The LC-MS/MS analysis showed that the median content of non-degraded amino acids was 86.2 ± 11.3%, indicating D_2O significantly reduced amino acid degradation to T, γ and UV stresses ($P = 0.00001 < 0.05$), (Table 3).

The food industry has recently extensively preferred liquid whole egg and egg-derived products (Uysal *et al.*, 2017). Pasteurization T is limited to 60-64 °C in the egg industry for 3.5 min (Lechevalier *et al.*, 2017). If pasteurization is longer than 3.5 min, the whole egg viscosity significantly increases and good functional properties such as foaming, gelation and emulsification are adversely affected at ~60 °C (Fan *et al.*, 2024; Jia *et al.*, 2021). Similarly, EW is pasteurized at 55.0-57.2 °C for 1-8 min, to avoid thermal denaturation and unfolding (Weiss *et al.*, 2018). Briefly, heating promotes oxidative modification, alters conformation, lowers solubility, and makes particle size smaller, especially in egg protein, leading to commercially undesirable products (Bhat *et al.*, 2021; Li *et al.*, 2023; Rahaman *et al.*, 2016; Wen *et al.*, 2023). Therefore, adopting egg and egg-derived products to thermal extremities and higher water activity improves their stability during food design, processing, and storage (Tekiner *et al.*, 2024). The current study studied pasteurized liquid EW product rich in amino acids, which is initially processed at 57 °C for 4.1 min by the supplier, to clarify the knowledge gap about the moderating effect of D_2O addition on amino acids' thermal stability. Our findings showed that D_2O significantly reduced amino acid degradation to thermal stress ($P = 0.00001 < 0.05$), making EW protein more compact or less flexible, relative to H_2O (Clark *et al.*, 2019). Protein compactness is an important factor determining the mechanism of protein folding (Galzitskaya *et al.*, 2008). It correlates to the protein's kinetic (i.e., the unfolding rate) and thermodynamic stability (Anderson *et al.*, 2023). Notably, increased kinetic stability is associated with enhanced resistance to proteolytic degradation (Colón *et al.*, 2017). In the literature, some studies report that the presence of 95% D_2O is equivalent to a 4-5 °C reduction in storage temperature (Rowe *et al.*, 2019), higher temperature raises D_2O's heat capacity and melting point (T_m: 3.81 °C at 101.325 kPa) (Linse & Hub, 2021), and protein is more stable in D_2O (Rowe *et al.*, 2019). Based on our robust experimental data, D_2O, relative to H_2O, increases EW proteins' kinetic stability against degradation as a barrier. However, its

mechanism of action on controlling the degree of protein degradation needs further research for its practical application in the food industry.

Lys, Arg, Tyr, and Trp have been suggested to be critical amino acids in the thermostabilization of food proteins (Bellmaine *et al.*, 2020). In this study, Trp had the poorest thermal stability (50%) even with D_2O, while 97.2% of Lys, 90.5% of Arg, and 90.9% of Tyr remained non-degraded. Trp, an aromatic amino acid, is susceptible to oxidation with reactive oxygen species (ROS), carbonyl-containing compounds, and cleavage of its reactive indole ring (Hellwig, 2020; Mallamace *et al.*, 2021; Wen *et al.*, 2023), leading to undesired quality issues such as color changes, off-flavours and off-odours (Zhang *et al.*, 2013). According to Zhu *et al.* (2024), carbonyl-containing compounds in the EW pattern and oxidative modifications might yield this result for Trp. Moreover, D_2O might not stabilize Trp's native state (Pica & Graziano, 2018) because the applied 61.5 °C to Trp aligned with the hydrophobicity range, thereby influencing thermal stability (Zhang *et al.*, 2021). In summary, Trp level can be used as a useful quality indicator with or without the presence of D_2O during thermal processing and storage of foods, unless catalysts or potent oxidizing agents are present (Ren *et al.*, 2023).

In this study, the median content of non-degraded polar hydrophobic amino acids (Cys and Tyr) was determined to be 92.1 ± 1.7%, whereas it was 80.1 ± 14.6% for non-polar hydrophobic group (Val, Ala, Gly, Phe, Trp, Pro, and Met), indicating a ~15% improvement with the impact of polarity and hydrophobicity. Polar amino acids are generally found at the surface of proteins needed for interactions with the aqueous environment, making them soluble in aqueous solutions and protein assembly (Qiao *et al.*, 2019). Non-polar amino acids play a critical role in maintaining the stability and integrity of protein structures, tend to avoid contact with H_2O due to the presence of aliphatic or aromatic groups, and and aggregate in the core of the protein itself (Dongmo Foumthuim & Giacometti, 2023; Yau *et al.*, 2021). Hydrophobic amino acids shield hydrophobic side chains from H_2O through the hydrophobic effect, which is a T-dependent driving factor in protein folding in the range of hydrophobic force peaks (30–80 °C), (Luo *et al.*, 2022; van Dijk *et al.*, 2015). In the current work, the non-polar hydrophobic group (Phe, Trp, Val, Met, Trp, Cys, and Ala) exposed to 61.5 °C/24 h has relatively high hydrophobicity, aligning with hydrophobic force peaks. At this point, D_2O needs to be considered with its smaller molecular polarizability than H_2O (Luo *et al.*, 2022). Besides, several studies report that Tyr and Phe exposed to 80 °C/24 h, as well as Ala of the whole egg liquid pasteurized at 68 °C/6 min, remained non-degraded by 96% (Tabak *et al.*, 2021), which aligns with our findings. Overall, our work suggests that D_2O might weaken van der Waals attractions between amino acids and H_2O molecules, protecting H bonds and electrostatic interactions responsible for the stable structure of proteins.

The recent study subjected EW to γ-irradiation of 5 to 15 kGy (median ~10 kGy) for 180 min. The median percentages of non-degradation were 96.9 ± 2.5% for Glu and Asx, 94.7 ± 4.9% for His, Lys and Arg, 92.5 ± 9.0% for Cys and Tyr, 90.4 ± 2.9% for Thr and Ser, and 90.0 ± 6.9% for Val, Ala, Gly, Phe, Trp, Pro and Met, respectively. The γ-irradiation is a non-thermal processing technology and has drawn special attention due to its dual effects for sterilization of certain perishable food products (i.e., meat, fruits, herbs, spices) (Wang *et al.*, 2018). Its dose below 10 kGy does not affect the nutritional properties of most foods, and the shelf life of food is notably extended (Yao *et al.*, 2022). The γ-irradiation technology can modify protein structure (i.e., unfolding and denaturation) and improve protein's functionality (i.e., solubility, water and oil retention, emulsification, degree of hydrolysis) by exposing hydrophobic groups (Wang *et al.*, 2017; Wang *et al.*, 2020). In the literature, some studies report that γ-irradiation of 3.99 kGy could nearly degrade Tyr, Phe, and Trp (Tabak *et al.*, 2021), and 1 kGy almost degraded 30.5% of Tyr and 22.7% of Cys (Blanco *et al.*, 2018). In contrast, a 3-kGy dose had no significant effects (Badr, 2006). Exceptionally, in this study, 6 mL of D_2O (~16% of EW by weight) was added to the γ-irradiated vial because 7-25% D_2O prevent protein degradation in vaccines, as previously suggested by Rowe *et al.* (2019). Our data shows that the median percentages of non-degraded Phe, Tyr and Cys, irradiated by 10 kGy for 180 min, were found to be 94.7, 98.8 and 86.1%, respectively, with significant improvement (~69.5 to 100%), compared to Tabak *et al.* (2021) and Blanco *et al.* (2018). These high non-degradation rates might be associated with higher heat capacity and coolant ability of D_2O, relative to H_2O (Bila *et al.*, 2017). Overall, in the case of non-thermal processing, D_2O may be used as a reference solvent for estimating radiation damage at food quality inspections.

The current study applied UV irradiation (Overall: 280-400 nm, UVA: 315-400 nm, and UVB: 280-315) for 120 min to EW. The median non-degradation percentage was 90.8 ± 7.5%, ranging between 84.3 ± 4.1 and 93.6 ± 7.2% for amino acid categories. Proteins are susceptible to UV photodestruction and primarily absorb UV radiation because of the presence of non-polar hydrophobic Trp and Phe and polar hydrophobic Tyr residues at 280 (UVB band), (Biter *et al.*, 2019), making the quantification of protein concentrations possible (Gooran & Kopra, 2024). Besides, UV induces browning and formation of large protein aggregates by disulfide exchange and protein backbone cleavage (Manzocco *et al.*, 2012). The photoreduction is proposed to result from electron donation from excited Tyr or Trp residues (Gammelgaard *et al.*, 2020). UV-irradiation leads to disruption of their native structure. Protein does not have a "molten form". As the irradiation dose increases, it transforms into stable small aggregates with a partially preserved secondary structure (Korolenko *et al.*, 2025). Tyr, Phe, and Trp are the most reactive aromatic amino acids (Sun *et al.*, 2023). According to Tabak *et al.* (2021), in the case of UVA and B irradiations after 24 h, Phe Tyr did not significantly undergo degradation (~0 to 10%). However, Schubert *et al.* (2024) reported a stronger Cys, His, Trp, and Tyr decay, treated with a UVC dose of 100.0 J/mL for 3 min. Our data demonstrate that Trp shows the highest degradation under heat (50%), whereas it remains undegraded under UV exposure (100%). T has a significant effect on Trp's UV degradation, and it is speculated that T promotes the direct degradation of Trp. On the other hand, in the case of non-thermal stress UV, this feature may be associated with a larger mass of the heavy water molecule D_2O (Shuaibov *et al.*, 2009). Based on our findings, D_2O can contribute to understanding the fate of aromatic amino acids and their quantification as an off-odour quality marker for the foods under UV irradiation.

Table 3. The content of non-degraded amino acids after T, γ, and UV exposures (g/1000 g)

No	Category	Amino acid	Content (g/1000 g)						
			$m_{Control}$	$T_{exposure}$		$\gamma_{exposure}$		$UV_{exposure}$	
				$m_{non\text{-}degraded}$	% $_{non\text{-}degraded}$	$m_{non\text{-}degraded}$	% $_{non\text{-}degraded}$	$m_{non\text{-}degraded}$	% $_{non\text{-}degraded}$
1	Non-polar hydrophobic aminoacids	Val	18.40	14.38	78.15	16.38	89.02	18.25	99.18
2		Ala	41.25	38.59	93.55	38.79	94.04	39.16	94.93
3		Gly	15.33	11.78	76.84	14.09	91.91	12.03	78.47
4		Phe	24.12	20.54	85.16	22.85	94.73	23.11	95.81
5		Trp	0.04	0.02	50.00	0.03	75.00	0.04	100.00
6		Pro	23.87	22.10	92.58	22.16	92.84	22.03	92.29
7		Met	15.17	12.31	81.15	14.05	92.62	14.36	94.66
		Median ± Sd	19.74 ± 11.52	17.10 ± 11.01	79.63 ± 13.54	18.34 ± 10.88	90.02 ± 6.36	18.43 ± 11.98	93.62 ± 6.66
8	Polar hydrophobic aminoacids	Cys	8.50	7.93	93.29	7.32	86.12	6.92	81.41
9		Tyr	15.34	13.94	90.87	15.16	98.83	13.38	87.22
		Median ± Sd	11.92 ± 4.84	10.94 ± 4.25	92.08 ± 1.71	11.24 ± 5.54	92.47 ± 8.99	10.15 ± 4.56	84.32 ± 4.11
10	Polar acidic negatively charged amino acids	Glu	41.48	37.44	90.26	39.45	95.11	39.77	95.88
11		Asx	32.59	30.83	94.60	32.16	98.68	25.50	78.24
		Median ± Sd	37.04 ± 6.29	34.14 ± 4.67	92.43 ± 3.07	35.81 ± 5.15	96.89 ± 2.53	32.64 ± 10.09	87.06 ± 12.47
12	Polar basic positively charged amino acids	His	11.50	9.25	80.43	10.26	89.22	11.19	97.30
13		Lys	49.04	47.69	97.25	48.24	98.37	43.70	89.11
14		Arg	21.34	19.31	90.49	20.62	96.63	20.09	94.14
		Median ± Sd	27.29 ± 19.47	25.42 ± 19.93	89.39 ± 8.46	26.37 ± 19.63	94.74 ± 4.86	24.99 ± 16.80	93.52 ± 4.13
15	Polar amino acid with uncharged R group	Thr	18.09	15.97	88.28	17.26	95.41	14.36	79.38
16		Ser	29.46	27.24	92.46	29.27	99.36	27.87	94.60
		Median ± Sd	23.78 ± 8.05	21.61 ± 7.97	90.37 ± 2.96	23.27 ± 8.49	97.38 ± 2.79	21.12 ± 9.55	86.99 ± 10.76
Overall		**Median ± Sd**	**22.85 ± 13.13**	**20.58 ± 12.80**	**85.96 ± 11.43**	**21.76 ± 12.90**	**92.99 ± 6.14**	**20.74 ± 12.22**	**90.79 ± 7.54**

*Quantitatively, g/1000 g. Mean ± standard deviation.

Table 4. The content of non-degraded fatty acids after T, γ, and UV exposures (%)

No	Category	Abbreviation	Content (%)						
			$\%_{initial}$	$T_{exposure}$		$\gamma_{exposure}$		$UV_{exposure}$	
				$\%_{final}$	$\%_{non\text{-}degraded}$	$\%_{final}$	$\%_{non\text{-}degraded}$	$\%_{final}$	$\%_{non\text{-}degraded}$
1	Unsaturated fatty acid	GLA	0.06	0.06	100.00	0.05	83.33	0.05	83.33
2		ALA	15.48	15.38	99.35	14.84	95.87	14.66	94.70
3		OA	36.51	36.11	98.90	36.43	99.78	34.84	95.43
4		AA	2.61	2.29	87.74	1.86	71.26	2.39	91.57
5		DHA	0.68	0.58	85.29	0.00	0.00	0.62	91.18
		Median ± Sd	11.07 ± 15.55	10.88 ± 15.54	94.26 ± 7.13	10.64 ± 15.69	70.05 ± 40.73	10.51 ± 14.85	91.24 ± 4.80
6	Saturated fatty acid	MA	0.27	0.27	98.9	0.26	96.30	0.23	85.19
7		PA	23.51	22.02	93.6	20.08	85.41	22.55	95.92
8		SA	12.21	10.84	88.8	11.04	90.42	11.32	92.71
		Median ± Sd	12.00 ± 11.62	11.04 ± 10.88	94.15 ± 5.63	10.46 ± 9.92	90.71 ± 5.45	11.36 ± 11.16	91.27 ± 5.51
Overall		**Median ± Sd**	**11.42 ± 13.30**	**10.94 ± 13.04**	**94.22 ± 6.17**	**10.57 ± 12.99**	**77.80 ± 32.77**	**10.83 ± 12.72**	**91.25 ± 4.67**

Semiquantitatively, %. Mean ± standard deviation.

GC-MS results

8 fatty acids (5 unsaturated and 3 saturated) were semiquantitatively screened in the TG_{EY} using GC-MS. The obtained data showed that the median percentages of the non-degraded fatty acids after thermal, γ and UV stresses were 93.7 ± 5.6, 78.3 ± 32.8 and 91.4 ± 4.2%, respectively, and D_2O significantly reduced unsaturated fatty acid degradation to γ (71.3 ± 41.4%) and UV (91.6 ± 3.7%) irradiations, and saturated fatty acid degradation to thermal (93.8 ± 5.1%) and γ-irradiation (90.0 ± 4.4%) ($P = 0.04 < 0.05$), (Table 4). The heat accelerated lipid oxidation, also known as thermal oxidation, increases the degrees of oxidation of lipids by raising T, leading to sensory degradation, nutritional loss, and production of some toxic substances (e.g., furan formation) at ~118 °C (Fan, 2015; Liu *et al.*, 2019). The thermal oxidation can also induce the formation and aggregation of polar compounds, such as Triacylglycerol (TAG) oligomers, TAG dimers, oxidized TAG monomers, DGs, monoglycerides, and free fatty acids (Chen *et al.*, 2021; Han *et al.*, 2020). Therefore, slowing down thermal oxidation, thereby increasing the stability of lipids for consumers' health, has attracted significant attention in the food industry (Wang *et al.*, 2023). The major strategies to improve the lipids' oxidation stability are focusing on food packaging and antioxidants (Atta *et al.*, 2022). In this study, our data demonstrate that D_2O can slow down lipids' thermal oxidation as a strategic approach, relative to packaging and antioxidant alternatives.

Based on the fatty acid category, 93.6 ± 6.5% of the unsaturated fatty acids did not degrade, and ALA had the highest degree (99.4%), followed by OA (98.9%), GLA (96.7%), AA (87.5%), and DHA (85.7%). On the other hand, the median percentage of non-degraded saturated fatty acids

was 93.8 ± 5.1%, and MA exhibited the highest rate (98.9%), followed by PA (93.6%) and SA (88.8%), respectively. During thermal treatment, unsaturated fatty acids undergo oxidation and may produce some radicals (Ma *et al.*, 2019). Hence, controlling thermal oxidation of lipids in food matrix has considerable practical relevance and theoretical interest (Zhuang *et al.*, 2022). In the pasteurized liquid egg products industry, EY is generally processed at 58-63 °C for 2.5-4.0 min due to its heat sensitivity, which restricts its application in food products (Li *et al.*, 2021; Zhu *et al.*, 2021). Zhuang *et al.* (2022) observed a reduction in the content of unsaturated fatty acids at 100 °C by 11.7%. Similarly, heating at 143 °C for 4 s reduced fatty acids' concentration down to 12% (Kilic-Akyilmaz *et al.*, 2022). For instance, DHA content decreased from 15.5 to 10.1 mg per 50 g egg at 180 °C (Javed *et al.*, 2018), while it was 14.3% in this study. Hence, there was 14.3% degradation in our study versus 28% in Javed *et al.* (2018), indicating a 49% improvement. Besides, OA and ALA were fully degraded at 90 °C for 30 min (Charuwat *et al.*, 2018). In our study, the degrees of degraded OA and ALA in D_2O/EY mixture were 1.1 and 0.6%, respectively, indicating a ~99% improvement. Overall, D_2O might protect the thermal degradation of EY's fatty acids against prolonged heat treatment. However, the number of studies examining the effect of D_2O on lipids remains insufficient and is limited to several studies, such as volatile formation and oxidative stability in edible bulk oils (Kim *et al.*, 2014; Lee *et al.*, 2018), and linoleic acid content (Oh *et al.*, 2016). Thus, our data significantly contributes to this knowledge gap for the food industry by considering D_2O as an alternative antioxidizing agent for lipids other than packaging and chemical antioxidant approaches.

This study subjected TGEY to γ-irradiation with a median dose of 10 kGy for 180 min. The median content of unsaturated fatty acids GLA, ALA and OA was reduced by ~4.7% (95.3 ± 4.9%), followed by AA (29.1%). However, DHA was degraded entirely (100%) in the presence of D_2O. In EY, DHA concentration ranges between 0.4 and 3.7% of total fatty acid (Kaeoket & Chanapiwat, 2013). Amongst saturated fatty acids, the median degree of non-degradation of MA, PA, and SA was 90.0 ± 4.4%. In the literature, some studies show that OA was not significantly affected by γ-exposure of max. 60 kGy (Hong *et al.*, 2010), and 3-kGy γ-treatment had no significant effect on the liquid EY's fatty acid content (Blanco *et al.*, 2018). Debbarma *et al.* (2025) observed a substantial decrease in polyunsaturated fatty acids content at 4 and 6 kGy doses. Another study by Olotu *et al.* (2014) reported that a 10 kGy γ-irradiation entirely degraded DHA. The γ-irradiation might damage DHA, a long chain polyunsaturated fatty acid, by rapturing the covalent bonds as a direct result of a photon depositing energy into the molecule, or by producing free radicals and non-radical reactive oxygen species, acting with H_2O molecule indirectly, that are in turn responsible for the protein damage (Richard & Monk, 2024; Zbikowska *et al.*, 2006). Our data aligns with the previously reported findings. Overall, the effect of γ-irradiation on foods is of utmost importance, and an insight into the quality aspects of unsaturated fatty acids and D_2O may help food scientists prevent γ-irradiation-associated lipid oxidation and thereby improve the nutritional quality of foods.

In recent work, the median content of unsaturated fatty acids, irradiated with UV (Overall band: 280-400 nm, UVA: 315-400 nm, and UVB: 280-315) during 120 min, decreased by ~4.5%. Of them, OA had the highest non-degraded content (95.4%), followed by ALA (94.7%), AA (91.3%), DHA (90.4%), and GLA (86.1%). Regarding the saturated fatty acids, the median content was 91.1 ± 5.7%. Amongst them, 95.9% of PA remained non-degraded, followed by SA (92.6%) and MA (84.8%). Foods are generally processed by thermal and non-thermal technologies. Of non-thermal ones, UV irradiation has also gained attraction in recent years. In literature, many studies have typically focused on UV irradiation and food microbial safety. However, a work by Zhu *et al.* (2021) reports the furan formation from ALA and GLA, irradiated with UVC at 22 °C for 25 min. Considering D_2O, to our knowledge, the effect of D_2O on foods and fatty acids has not been explored yet. As is well known, D_2O has found extensive application as a moderator in nuclear reactors Larm *et al.* (2025). Nevertheless, our data suggest that D_2O may be considered a tool in quality control-related analyses and spectral characterizations of proteins and lipids, as well as for food preservation and stability challenges, despite its being overlooked with critical awareness by the food industry.

Conclusion

Studying the stability and physico-chemical response of amino and fatty acids to physical stress conditions during food processing and storage, as well as with preservation and quality monitoring, is of great interest among food researchers. This study, the first to our knowledge, investigated the fate of amino- and fatty acids of EW and EY origin to thermal and irradiation (γ and UV) exposures, to clarify the knowledge gap about the moderating effect of D_2O as an alternative stabilizing agent other than food packaging and antioxidant options despite being overlooked with a critical awareness by the food industry. Our data ultimately highlights, which probably are just the tip of the iceberg, that D_2O, with different physical properties from ordinary H_2O, can be considered as a moderating substance in food preservation-related stability challenges, quality-related spectral characterization and quality monitoring at processing and storage stages in the food industry. Thus, we believe that D_2O constitutes an interesting next challenge for the food researchers studying protein and lipid assemblies to decode its mode of action, taking care of safety and toxicity considerations.

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Author contributions

Nesibe Nur Yalçın: Data collection, Writing the draft of the manuscript, Data analysis; **Leila Mehdizadehtapeh**: Data collection, Writing the draft of the manuscript, Data analysis; **Kübra Ekmekçi**: Data collection, Writing the draft of the

manuscript, Data analysis; **Büşra Çalık**: Data collection, Writing the draft of the manuscript, Data analysis; **Ahmad Nimatullah Al-Baarri**: Revising and editing the manuscript; **Marwa Tainsa**: Revising and editing the manuscript; **İsmail Hakkı Tekiner**: Presentation of research idea and study design, Data analysis and interpretation, Supervising the study, Approval of the final version

Conflicts of interest

The authors declare that there is no conflict of interest

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