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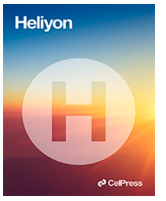

Research article

Investigation of fatty acid profile of eyes recovered from slaughterhouse waste

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ABSTRACT

Polyunsaturated fatty acids (PUFAs), principally Docosahexaenoic acid (DHA, 22:6n-3), the foremost omega-3 PUFAs in the brain and eyes, have been implicated in maintaining the structural and functional properties of the retina and cornea. Another PUFA, Arachidonic Acid (AA, 20:4n-6), primary omega-6 PUFA in the cell membrane of phospholipids, is a central inflammatory mediator involved in many molecular and cellular functions under physiological and pathological conditions, including dry eye disease (DED) and age-related macular degeneration (AMD). This study investigated the fatty acids (FA) composition of the vitreous humor, retina, cornea, and whole eye in two mammals, the Arabian sheep (*Ovis aries*) and Arabian camel (*Camelus dromedarius*), with the aim of exploring new paths for beneficial PUFA production. In *Ovis aries*, the retina exhibited the highest content in DHA and AA with 4.30 ± 0.63 % and 13.48 ± 1.33 % of the total fatty acid content, respectively. In *Camelus dromedarius,* the DHA content was greater in the retina compared to all samples, and AA was detected in the vitreous humor, cornea, retina, and whole eye, with the highest content in the retina (15.38 ± 0.71 %). Comparing both mammals, the DHA fraction was higher in camel's retina than in sheep's retina, whereas no differences were noticed for AA accumulation. In conclusion, ocular tissues collected from agri-food waste in slaughterhouses could serve as a sustainable source for FA production and provide an innovative and emerging prospect in the nutrition, pharmaceutical, and healthcare sectors.

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1. Introduction

Upcycling food waste has become an urgent matter due to the ever-growing global demand for animal-derived protein-based food products, which is projected to rise by 70 % by 2050 [1]. This surge in production and consumption of animal proteins is closely linked to a substantial increase in food-related waste [2]. Remarkably, only 30–40 % of the products from meat processing post-slaughter are used for human consumption [3]. Disposing of these by-products not only results in lost economic opportunity but also contributes to harmful environmental consequences [4]. Moreover, stricter regulations, limited disposal options, and growing public safety concerns have driven up recycling and disposal costs [5].

The eyes, which have a complex yet surprisingly homogeneous fatty acid (FA) profile across vertebrates [6,7], are among the discarded materials from slaughterhouses. These eyes have exhibited substantial potential for repurposing as biomaterial substrates in sustainable tissue engineering [8–13].

Indeed, lipids, including FAs, play key roles in the central nervous system (CNS), from synaptic stabilization/signaling to DNA regulation/neuroprotection [14]. FAs are essential in modulating inflammatory responses, maintaining cellular membrane structure, and ensuring the homeostatic balance of ions and signaling molecules [14]. Furthermore, alterations in the FA composition of the eyes have been associated with numerous retinal diseases, such as age-related macular degeneration (AMD), retinitis pigmentosa (RP), and diabetic retinopathy [15].

Polyunsaturated fatty acids (PUFA), including omega-3 Docosahexaenoic Acid (DHA, 22:6n-3) and omega-6 Arachidonic Acid (AA, 20:4n-6), are essential for the proper functioning of mammalian eyes,. Inague et al. (2023) showed that rats subjected to oxygen-induced retinopathy, which leads to pathological angiogenesis, experience “intense lipid remodelling that favours pathways for neutral lipid synthesis, cholesterol import/export, and lipid droplet formation” [16]. The study emphasized “profound changes in pathways for long-chain fatty acid production, vital for retina homeostasis” [16]. Furthermore, Dasyani et al. (2020) revealed that the inactivation of genes responsible for PUFA synthesis in Zebrafish (*Danio rerio*) embryos resulted in alteratiosn in lipid composition and visual behaviour, confirming the significant functions of PUFAs in healthy vision [17].

Nevertheless, aside from studies on humans, research on the FA profile of mammalian eyes in remains limited, with most specifically focusing on the retina, given its crucial role within the eye. For instance, it has been reported that the retina of the oxen contains a lower proportion of Oleic Acid (C18:1n-9), comprising 17.3 % of total FA, a higher content of AA and DHA [18]. Additionally, studies on bovine and rabbit retinas were generally composed of Stearic Acid (C18:0), Palmitic Acid (C16:0), Tetracosanoic Acid (C24:0), and DHA [19].

Research has shown that retinal tissue is particularly responsive to dietary changes. For instance, a 4.5-fold increase in Eicosapentaenoic Acid (EPA, C20:5 n-3) levels was observed in the retina of lambs fed with freeze-dried-feed from the microalgae *Nannochloropsis oceanica*, compared to control lambs [20]. As for other structures in mammalian eyes, studies have revealed that phospholipids from the endothelium in rabbit corneas had the highest proportion of AA, while the epithelium contained the lowest degree of unsaturation [21].

To reduce slaughterhouse waste, including mammalian eyes, and capitalize on their biomolecules, recovering PUFA from these tissues, particularly from the retina, holds great promise for upcycling and extracting. Currently, no studies have been conducted, either nationally or internationally, on the FA profile of the eyes of common mammalian reared in the United Arab Emirates (UAE), such as the Arabian sheep, *Ovis aries* (*O. aries*) and Arabian camel, *Camelus dromedarius (C. dromedarius)*. These two mammals were chosen for this study due to their significant agricultural and economic importance in the UAE, where they are well-adapted to arid environments with limited access to dietary polyunsaturated fatty acids (PUFAs). This unique adaptation makes their ocular fatty acid profiles particularly intriguing and presents a promising opportunity for addressing environmental and nutritional challenges. The UAE faces rising food insecurity and health issues such as obesity and micronutrient deficiencies. Repurposing animal tissues, including those from local livestock, into valuable products like PUFAs and other biomolecules, aligns with the nation’s focus on biowaste sustainability. This cost-effective approach offers potential solutions for transforming agricultural waste into supplements and therapeutic products, contributing to a more sustainable, healthier, and secure future.

In this study, for the first time, the FA profile of the whole eyes and individual compartments, vitreous humor, retina, and cornea, of *O. aries and C. dromedarius* were analyzed. Discarded slaughterhouse waste from both species was collected from the Automated Slaughterhouse in the Municipality of Abu Dhabi, UAE, and different eye compartments were isolated. Total lipids extraction was performed on all samples, and fatty acid methyl esters (FAME) were produced. The various FAs in each compartment were qualitatively identified using Gas Chromatography coupled with a Flame Ionization Detector (GC-FID), with FAME standards for comparison. Finally, the relative amount of each FA was quantified as a percentage of the total FA extracted.

2. Materials and methods

2.1. Sample collection and dissection of ocular compartments

The project involved animal-based studies which have been approved by the Khalifa University Research Ethics Committee and the University Animal Research Oversight Committee (protocol #H22-036).

Ten eyes from Arabian sheep *(O. Aries)* and ten eyes from Arabian camels *(C. Dromedarius)* were collected at the Abu Dhabi Automated Slaughterhouse, Municipality of the City of Abu Dhabi. The entire ocular globes were collected post-slaughter and transported on ice in zip-locked bags filled with saline containing antibiotics to our laboratory.

Upon arrival, the eyes were removed from the saline, placed in sealed containers, and stored at −80 °C to preserve the integrity of their biological and biochemical components, prevent degradation and enzymatic activity, minimize oxidation of sensitive molecules like polyunsaturated fatty acids, and maintain tissue structure prior to lipid extraction.

For lipid extraction, the frozen containers were placed in a 37 °C water bath for approximately 20 min. After thawing, the globes were thoroughly rinsed with saline containing antibiotics. Five eyes from each species were then dissected, and various ocular compartments, including the vitreous humor, retina, and cornea (Supplementary Figs. 1a–d), were isolated. Lateral incisions were made in the sclera to facilitate the extraction and isolation of the various ocular components. All isolated compartments were rinsed in the saline solution and weighted (Supplementary Table 1). The remaining intact globes were reserved to examine lipid content that can be extracted from the whole eye.

2.2. Lipids extraction

Each vitreous humor, cornea, retina, and whole eye sample was placed separately in a 30 ml Pyrex glass vial with a phenoplast screw cap fitted with a composite PTFE/ethylene propylene seal purchased from Sigma-Aldrich, St. Louis, MO, USA. The samples were cut into small pieces using scissors and then crushed with a Wheaton® Safe-Grind® Potter-Elvehjem Tissue Grinder, purchased from Duran Wheaton Kimble Life Sciences, USA.

Total lipids were extracted from different ocular compartments and whole eyes following the Bligh and Dyer protocol [22]. In brief, to each gram of tissue, 1 volume of ethanol, 2 vol of chloroform, and 0.03 volume of anti-oxidant butylated hydroxytoluene (BHT) 5 μM were added. 1-heptadecanoyl-2-hydroxy-sn-glycero-3-phosphoethanolamine (17:0 LysoPE) was added as an internal standard. All chemicals and reagents used were of analytical grade and high purity. Organic solvents, including chloroform and ethanol, were acquired from Carlo Erba, Val de Reuil Cedex, France. BHT was purchased from Supelco (Sigma-Aldrich, St. Louis, USA), and 17:0 LysoPE was obtained from Avanti polar lipids.

The mixture was homogenized and further ground for 2–3 min with a IKA Ultra-Turrax T8 Homogenizer Disperser obtained from IKA Labortechnik Staufen, Germany, then and centrifuged at 450 g, 4 °C, 10 min. The lower organic phase containing the lipids was collected. The tissue homogenate was re-extracted by adding 1 volume of ethanol and 2 vol of chloroform per g of tissue and re-centrifuged again. After the second centrifugation, the lower organic phase was collected and combined with the organic phase obtained with the first extraction. The solvent was evaporated under nitrogen, and the lipids were weighed and stored at −20 °C for further analysis.

2.3. FAME production and quantification

In order to produce Fatty Acid Methyl Esters (FAME), FA esterification was performed overnight using 300 μl toluene with 600 μl of 1 % sulfuric acid (H_2SO_4). The mixture was kept at 50 °C for 12 h following Breteler et al. (1999) and modifications by Bermúdez et al. (2016). Afterward, any non-FA was removed by splitting phases using a 600 μl 5 % sodium chloride (NaCl) solution. FAMEs were separated by adding 100 μl n-Hexane 3 times and transferred into a new test tube, evaporated, and 100 μl (final volume) was added. Finally, the 100 μl were transferred into a GC vial purchased from Sigma-Aldrich, St. Louis, MO, USA, flushed with nitrogen N_2 and stored at −20 °C until analysis. H_2SO_4, NaCl, toluene, and n-hexane were acquired from Supelco.

For FAME quantification, GC-FID was conducted using an Agilent 7890B GC-FID System with a Gas Chromatography column SP®-2560 Capillary GC Column (L × I.D. 100 m × 0.25 mm, df 0.20 μm) purchased from Merck KGaA, Darmstadt, Germany. A standard of 44 FAME mix was procured from GLC-569, Nu-Chek Prep, Inc. Elysian, Mn. The carrier gas was helium at a constant flow of 2 ml/min. The FID was set to 280 °C, with a gas flow of 350, 35, and 30 ml/min of synthetic air, hydrogen, and helium, respectively. The injected sample volume was 1 μl.

A series of blanks (Hexane, HPLC grade) and 44 FAME standard mix were prepared in GC vials and measured along the samples in order to qualitatively identify the FA and quantify each FA in the total fatty acids extracted per sample based on GC-FID analysis (Supplementary Fig. 2, Table 2).

Initially, the column oven was set to 80 °C and held for 10 min. The temperature was then increased at the rate of 7 °C/min until reaching 170 °C, where it was held for 10 min. A second temperature increase was applied at 12 °C/min to 205 °C, followed by a 20-min hold. The third temperature increase was at 20 °C/min until reaching 220 °C, with a hold for 15 min. Finally, the temperature was raised at 15 °C/min to 230 °C and maintained for 20 min.

2.4. Statistical analysis

In order to determine significant differences and similarities in terms of FA content between eye compartments, a one-way Analysis of Variance (ANOVA) followed by a Bonferroni post-hoc test was applied. A two-way ANOVA was performed to compare the relative FA content between camel and sheep samples. All results were expressed as percentages of FAs and presented as the mean ± standard error of the mean (SEM) from five replicates. To determine similarities between the eye compartments in terms of relative FA content, Principal Component Analysis (PCA) was conducted. Data analysis, plots, and figures were generated using GraphPrism or R Software [23].

3. Results

3.1. Fatty acid prolife of Arabian sheep (O. aries) eyes

This preliminary study investigated the FA profile in the entire eye and several of its individual compartments. After total lipid extraction and FAME production, GC-FID analysis was used to calculate the percentage by mass of each FA in the samples based on the area method. whereby the area of each FA was divided by the sum of areas for all peaks. This value represented the percentage of FA in the sample.

In the eyes of the Arabian sheep *(O. aries)*, different ocular compartments revealed distinct FA profiles. For example, in the vitreous humor, Palmitic Acid (C16:0) was the most abundant FA, accounting for 44.93 ± 4.72 % of the total FA content, followed by Stearic Acid (C18:0) with 36.95 ± 1.03 % of total FA (Fig. 1a). In contrast, in the cornea, Oleic Acid (C18:1) was the predominant FA with 38.69 ± 1.27 % of total FA content, followed by C16:0 at 28.87 ± 1.77 %, and DHA with 1.06 % (Fig. 1b).

Notably, DHA was more prominent in the retina, where it accounted for 4.31 ± 0.63 % of the total FA content. The predominant FA in the retina was C18:0 at 17.84 ± 0.72 %, followed by C16:0 and Arachidonic Acid (C20:4), at 14.43 ± 0.25 % and 13.48 ± 1.33 %, respectively (Fig. 1c). In the whole eye of the *O. aries*, C18:1 was the dominant FA, followed by C16:0 and C18:0, at 34.2 ± 7.99 %, 26.17 ± 2.53 %, and 20.45 ± 2.48 %, respectively (Fig. 1d).

The main unsaturated FA, including DHA (Fig. 2a), Oleic Acid, OA (Fig. 2b), AA (Fig. 2c), and Linolenic Acid LA (Fig. 2d) were investigated in vitreous humor, cornea, retina and whole eye of Arabian sheep (*O. aries*).

DHA was detected in the cornea, retina, and whole eye, but not in the vitreous humor. The retina had the highest DHA content, which accounted for 4.3 ± 0.63 % of total FA content (Fig. 2a). No significant differences in DHA content were observed between the vitreous humor and cornea or between the vitreous humor and whole eye. However, significant differences were detected between the retina and vitreous humor (**: $p < 0,01$), as well ass between the retina and cornea with (*$p < 0,05$). These findings align with the literature, which indicates that DHA is predominantly found in the retina.

Additionally, the monounsaturated FA, OA, was detected in all analyzed samples. The highest content of OA was observed in the cornea (38.69 ± 1.27 %) and whole eye (34.21 ± 7.9 %), while the lowest was found in the vitreous humor (14.93 ± 4.1 %) and retina (12.58 ± 0.57 %), with no significant differences (Fig. 2b). Significant differences were observed between the cornea and retina (**: $p < 0,01$), cornea and vitreous humor (*: $p < 0,05$), and whole eye and retina (*: $p < 0,05$).

The omega-6 PUFA, AA, was detected in all analyzed samples, with the highest content in retina (13.48 ± 1.33 %) (Fig. 2c). Significant differences were observed between the retina and whole eye ($p < 0,01$; ***), retina and cornea (**: $p < 0,01$), and retina and vitreous humor (****: $p < 0,0001$).

Another omega-6 PUFA, LA, which is considered an essential FA, was detected in all samples except the vitreous humor. No significant differences were noted among the cornea, retina, and whole eye (Fig. 2d).

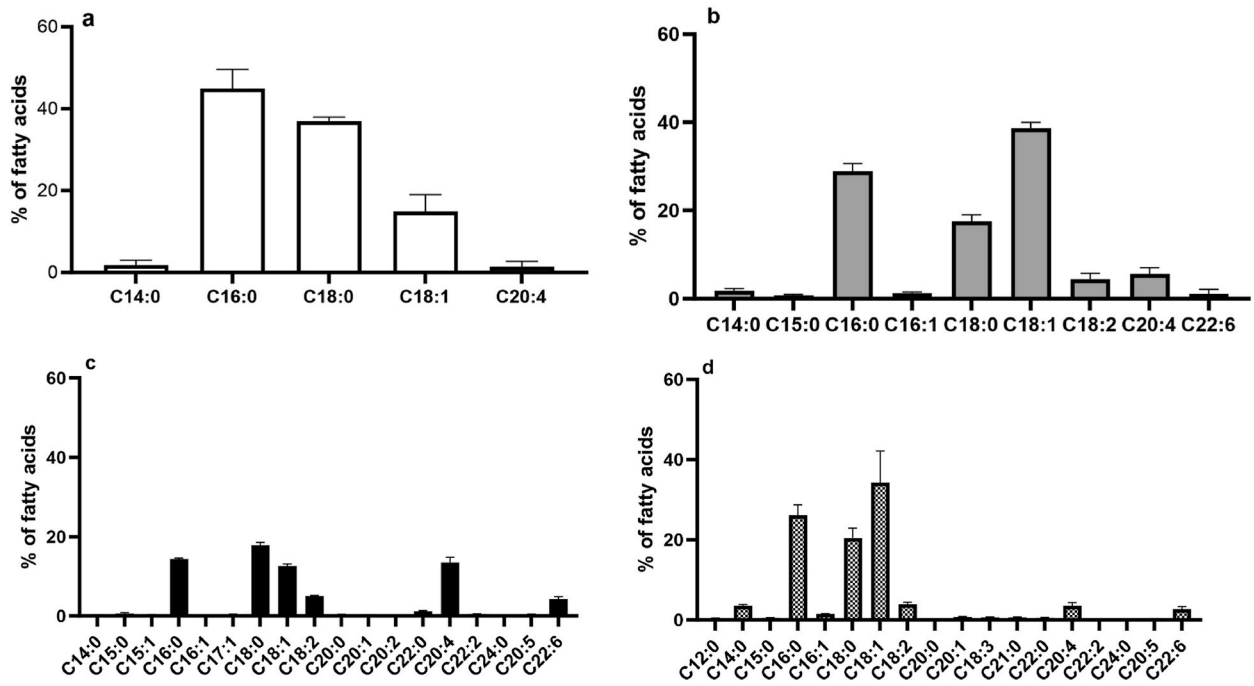

Fig. 1. Fatty acid composition in the entire eye of the Arabian sheep *(O. aries)* and its various compartments, including the vitreous humor (a), cornea (b), retina (c) and whole eye (d). Results are expressed as a percentage of total FA and presented as ±SEM of five values.

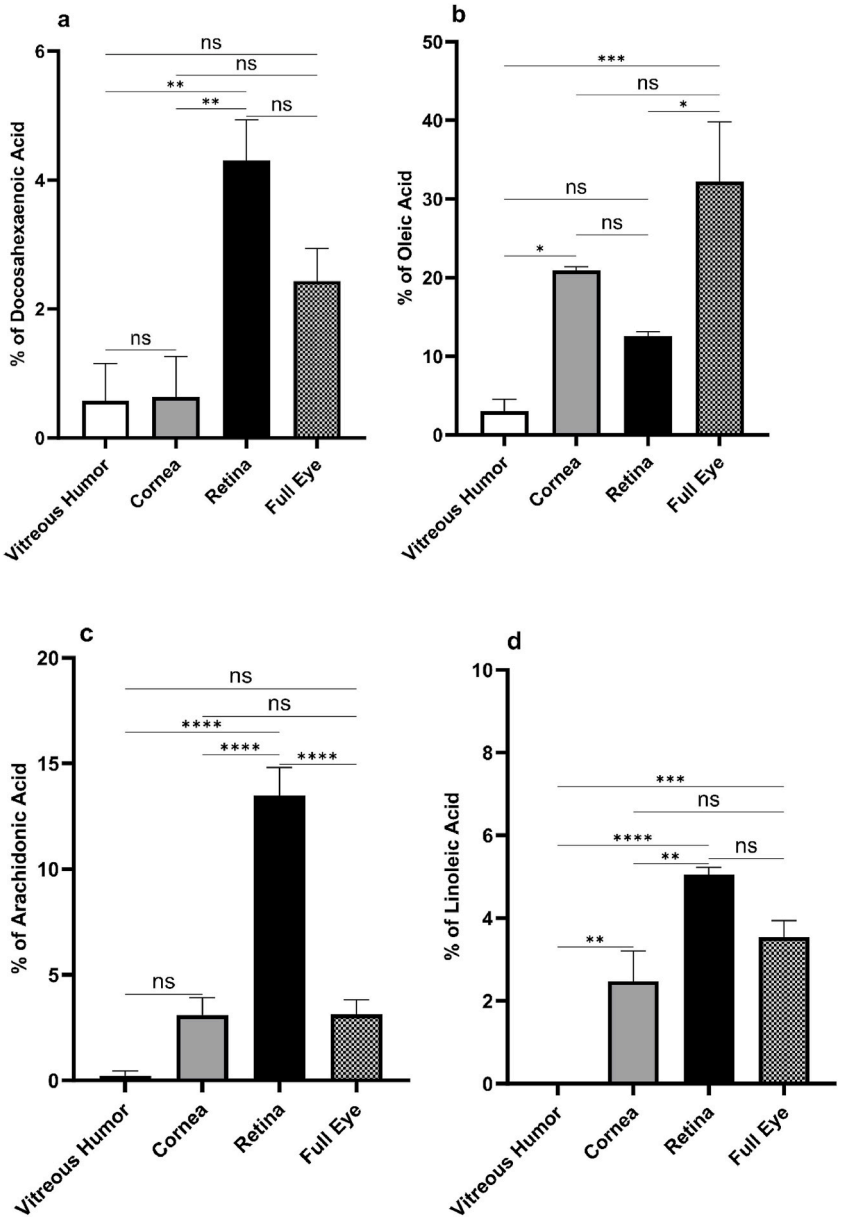

Fig. 2. Main unsaturated FAs detected in the vitreous humor, cornea, retina, and globe of the eye of Arabian sheep (*O. aries*), including DHA (a), OA (b), AA (c), and LA (d). Results were expressed as a percentage of DHA and presented as ±SEM of five values. Groups were compared by analysis of variance (ANOVA) followed by the Bonferroni post-hoc test. ns: not significant; *: $p < 0,05$; **: $p < 0,01$; ***: $p < 0,001$; ****: $p < 0,0001$.

To assess similarities between different ocular compartments in terms of relative FA composition, a Principal Component Analysis (PCA) was performed. The PCA results showed clear groupings in terms of FA relative composition of the different compartments (Fig. 3). The vitreous humor and cornea showed similar relative FA compositions (Fig. 2a, 2.b) and appeared grouped, while the retina and whole eye samples exhibited distinct FA profiles, particularly containing more PUFAs (Fig. 2c, 2.d), resulting in their separation in the PCA (Fig. 3). The axis loads indicated that PC1 was primarily driven by long-chain FAs, while PC2 was influenced by short-chain saturated FAs (Supplementary Fig. 3).

3.2. Fatty acid prolife of Arabian camel (*C. dromedarius*)'eyes

When investigating the FA profile in camel eyes and its different isolated sections, C18:0 was the predominant FA in vitreous humor, accounting for 41.9 ± 4.83 % of total FA content followed by C16:0 at 35 ± 1.85 % (Fig. 4a). In the cornea, C18:1 was the most abundant FA, comprising 33.03 ± 1.11 % of the total FA, followed by C16:0 at 25.03 ± 0.77 %. DHA was also detected in the cornea at

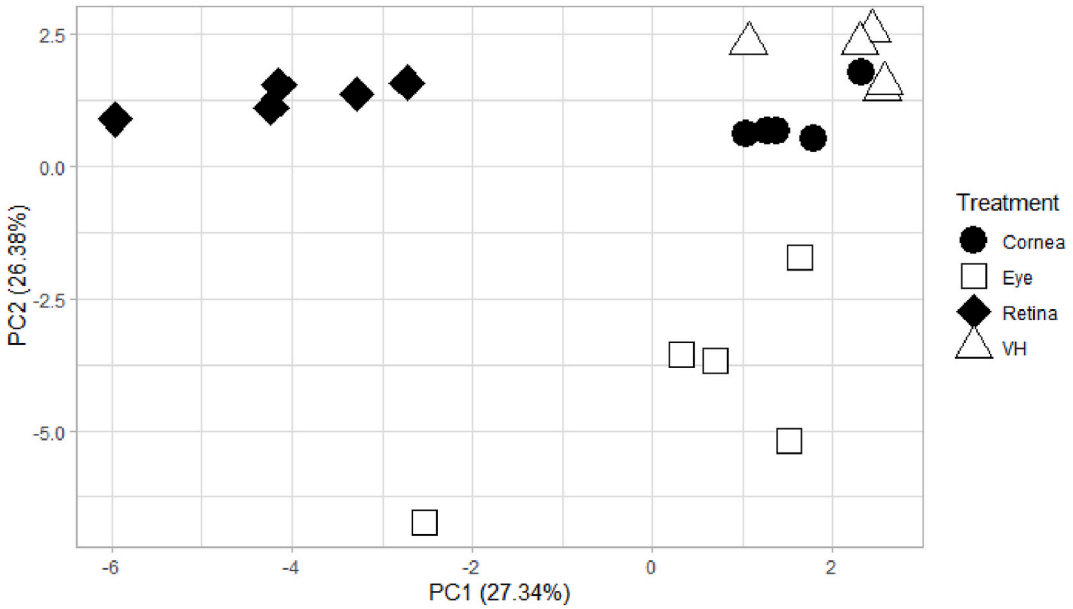

Fig. 3. Principal Component Analysis of the relative FA compositions of the cornea, retina, vitreous humor (VH), and whole eyes of the Arabian sheep *(O. aries)*. Results were presented for five values.

0.41 ± 0.2 % (Fig. 4b). In retina, C16:0, C18:0, and C18:1 were the most prevalent FAs, representing 22.77 ± 0.25 %, 20.71 ± 0.92 %, and 18.09 ± 1.13 %, respectively. AA and DHA are also present in the retina, with levels of 15.38 ± 0.71 % and 7.7 ± 1.75 % (Fig. 4c). In the wholel camel eye, C16:0 and C18:1 were the two predominant FAs, accounting for 33.67 ± 3.81 % and 29.08 ± 2.93 % of the total FA, respectively (Fig. 4d).

When comparing the composition of key unsaturated FAs, including DHA, OA and AA, DHA was not observed in the vitreous humor but was present in all other components, including the retina (7.7 ± 1.74 %) and cornea (0.41 ± 0.28 %), as well as the whole eye (2.49 ± 0.77 %) (Fig. 5a). The retina had the highest DHA content compared to all other samples, with significant differences between the retina and whole eye (**: $p < 0{,}01$) and cornea (***: $p < 0{,}001$) (Fig. 5a).

OA was detected in all analyzed samples, with the highest levels detected in the cornea (33.03 ± 1.11 %) and whole eye (29.07 ± 2.92 %). No significant differences were observed between the vitreous humor (16 ± 2.44 %) and retina (18.09 ± 1.12 %) (Fig. 5b).

AA was detected in the vitreous humor, cornea, retina, and whole eye, with the highest content observed in the retina (15.38 ± 0.71 %). Significant differences were found between the retina and whole eye (**: $p < 0{,}01$), as well as between the retina and cornea (*: $p < 0{,}05$), while no significant differences were noted between vitreous humor and cornea (Fig. 5c).

The PCA of the FA composition in the eyes of the Arabian camel *(C. dromedarius)* FA revealed clear groupings among the different ocular compartments (Fig. 6). The vitreous humor and cornea were similar in terms of relative FA composition along PC1 but showed dissimilarities along PC2 (Fig. 6, Supplementary Fig. 4). The retina was distinctly separated from the other structures and contained a higher proportion of PUFAs compared to other eye parts (Fig. 6, Supplementary Fig. 4). The whole eye was positioned at the centre of the plot. The axis loads indicated that PC1 was mainly driven by PUFAs, while PC2 reflected a greater influence from short-chain saturated FAs (Supplementary Fig. 4).

3.3. Comparison between FA composition in the eyes of the Arabian sheep (O. aries) and Arabian camel (C. dromedarius)

DHA was significantly higher in camel retina compared to the sheep retina ($p < 0{,}05$). DHA was not detected in the vitreous humor of either mammal. No significant differences were observed between the corneas of both mammals, nor between their whole eyes (Fig. 7a).

Regarding OA, the vitreous humor of camel eyes had higher levels of OA compared to Arabian sheep ($p < 0{,}05$). No differences were noted in the cornea, retina, or whole eye (Fig. 7b). Finally, no significant differences were observed in the accumulation of AA across the various ocular compartments in both species (Fig. 7c).

The PCA of the relative FA contents in camel and sheep showed that the whole eyes of the two species and sheep were sufficiently dissimilar to be clearly separated along PC1, while PC2 was primarily driven by differences within the sheep eye samples (Fig. 8 a, Supplementary Fig. 5). However, the individual eye compartments were similar enough between species, in terms of relative FA composition, to group together in the analysis regardless of the species (Fig. 8 b, Supplementary Fig. 5) The cornea (dotted line), retina (dash dot), and vitreous humor (long dash) forming distinctive groups in the plot. The axis loads show that the PC1 is driven by the presence of high levels of PUFA in the retina while PC2 is mostly driven by the dominance of saturated FA in the vitreous humor (Supplementary Fig. 5).

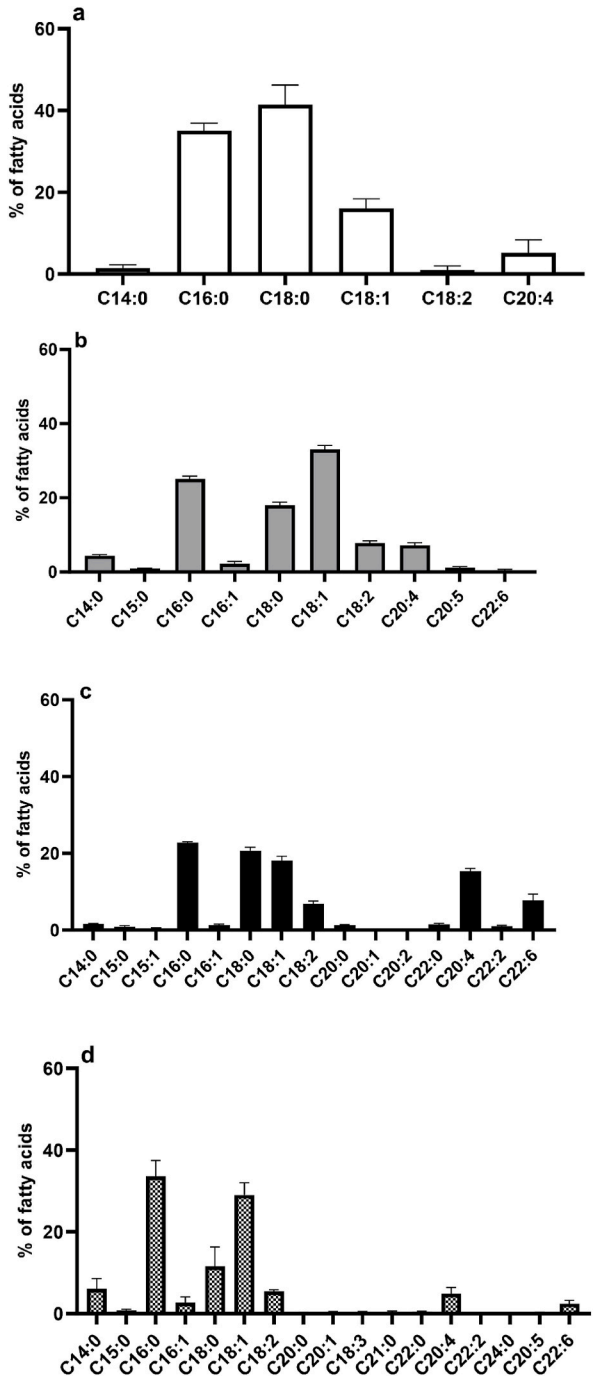

Fig. 4. Fatty acid composition of the *Arabian camel (C. dromedarius)* eyes and its various compartments, including the vitreous humor (a), cornea (b), retina (c), and whole eye (d). Results are expressed as a percentage of total FA content and presented as ±SEM of five values.

4. Discussion

Food waste recycling, through the efficient recovery of valuable biomolecules, is an emerging biotechnological strategy that can positively influence the economy and the environment [24]. This issue is particularly concerning in the case of beef, where only 44 % is considered "meat" and the remaining 56 %, often discarded, includes non-edible materials (e.g., specified risked materials) and useable parts (e.g., eyes) [25]. Tedeschi et al. (2021) proposed that upcycling slaughterhouse waste can be achieved through enzymatic hydrolysis. The authors tested the proteolytic activity of four enzymes - papain, trypsin, pancreatin, and bromelain - on

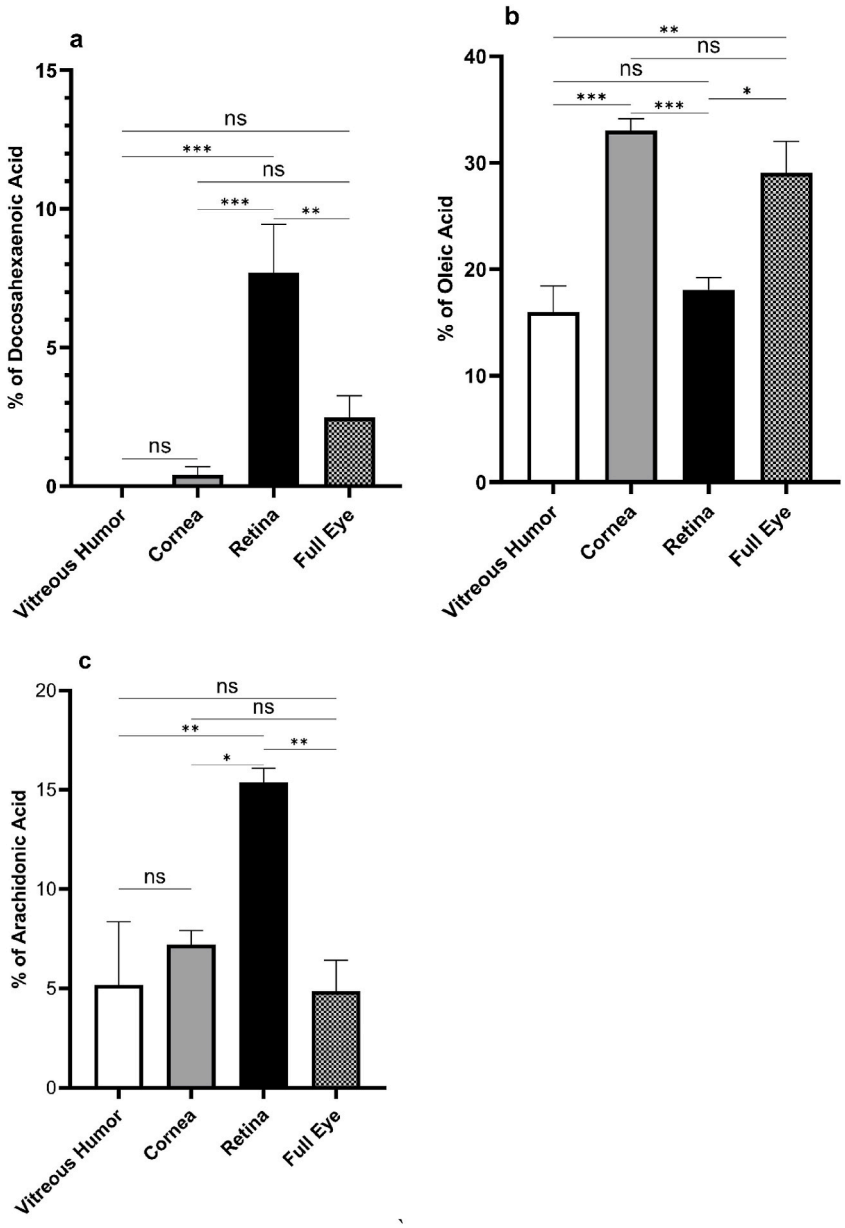

Fig. 5. Key unsaturated FAs detected in the vitreous humor, cornea, retina, and, whole eye of Arabian camel *(C. dromedarius)*, including DHA (a), OA (b), and AA (c). Results were expressed as a percentage of DHA and presented as ±SEM of five values. Groups were compared by analysis of variance (ANOVA) followed by the Bonferroni post-hoc test. ns: not significant; *: $p < 0,05$; **: $p < 0,01$; ***: $p < 0,001$; ****: $p < 0,0001$.

slaughterhouse waste and analyzed the FA profiles after treatment. They found that monounsaturated FAs were the most abundant in lipids in all samples, except for those with treated with pancreatin, where PUFAs were predominant [24].

As mentioned earlier, the purpose of the present study was to investigate the FA composition in the eyes of common mammals, including Arabian Sheep *(O. aries)* and Arabian camel *(C. dromedarius),* collected from slaughterhouse waste in Abu Dhabi. The findings revealed that the FA composition of the vitreous humor in both species was quite similar, being mainly dominated mainly by Palmitic acid (C16:0) and Stearic acid (C18:0). However, Linoleic acid (C18:2n-6) was detected in the camel's eyes but absent in the sheep. This dominance of saturated FAs in the vitreous humor has also been previously reported for rabbits [26].

When examining the cornea, a similar pattern to that of the vitreous humor was observed in booth species, with elevated levels of C16:0 and C18:0, and C18:1 being the most abundant. Regarding differences in FA profile, Eicosapentaenoic acid (C20:5n-3) was present in the camel but absent in the sheep. These findings are consistent with observations in the corneal layers of oxen and rabbits, where C18:1 was dominant [18,21]. This is also consistent with observations from another study on the rabbit corneal epithelia, where C18:1 comprises 57 % of the total FAs of phospholipids, followed by C:16:0 at 18.7 % [27]. Furthermore, the predominant FAs in the

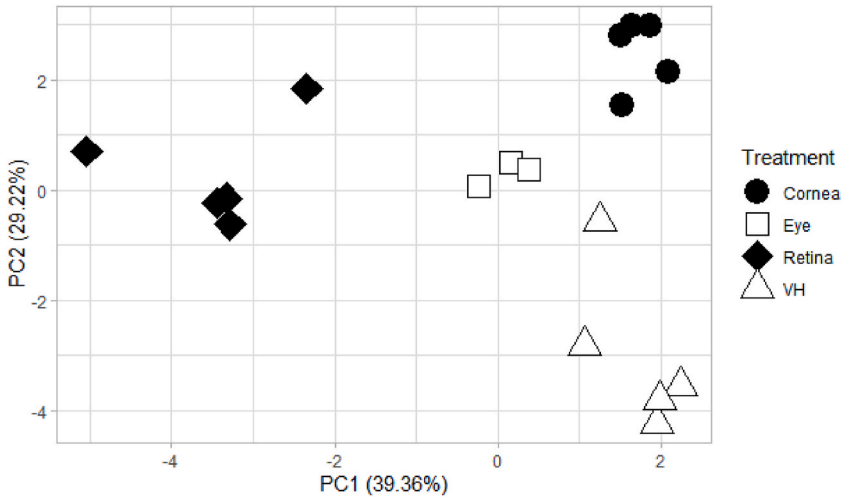

Fig. 6. Principal Component Analysis of the FA composition of the cornea, retina, vitreous humor (VH) and whole eye of the Arabian camel *(C. dromedarius)*. Results were presented for five values.

human cornea - C16:0, C18:0, and C18:1 – are remarkably consistent with those in sheep and camel [28]. It has been reported that FAs play a role in corneal wound healing [27], despite being composed mostly of saturated and monounsaturated FAs. In clinical studies, dietary supplementation of omega-3 FA has shown beneficial effects in reducing symptoms of dry eye disease and improving meibomian gland dysfunction [27]. The authors suggest that these beneficial effects could be due to the action of C18:3n3 and to the elongation and desaturation products of EPA and DHA [27].

Another ocular compartment studied was the retina, which is one of the most important, given its role in photoreception and phototransduction. Several studies have focused on its FA composition. In terms of its FA profile, the retinas of both sheep and camels were similar, though the sheep exhibited slightly higher FA diversity, with FAs like C17:1 and C24:0, present in the sheep but absent in camel eyes. While the retina contains saturated fatty acids, it also includes PUFAs, notably AA and DHA. AA was the most dominant PUFA in both camel and sheep retinas with approximately 15 % of the total FA. This characteristic presence is well-documented. For instance, AA accounts for roughly 10–40 % of retinal FA in rabbits and mature bovines [19]. A similar abundance of AA and DHA has also been observed in human retinas, where they represent about 11 and 15 %, respectively [29].

In mice retina, the major FAs are C16:0, C18:1, C18:0, AA, and DHA [30,31], while in the retinal outer segment membranes of rabbits, the main FAs are 16:0 (13–22 mol%), 18:0 (15–27 mol%), and DHA (28–50 mol%) [32]. Another study reported that the retinal rod outer segments of bovine, frog and rat retinas have DHA levels of 50.7 ± 10.4 %, 50.9 ± 0.6 %, and 46,2 ± 3.6 %, respectively (Stone et al., 1979). Furthermore, it has been shown that retina tissue is responsive to dietary changes. In rats, omega-3 supplementation induced spatial organization changes in DHA in the photoreceptor layer around the optic nerve [33]. In an study investigating the effects of an oxidant on rat retinal neurons, DHA supplementation reduced oxidation-driven photoreceptor apoptosis by almost half [34]. In another study on rabbits, retinal phosphatidylethanolamine demonstrated high sensitivity to a fish oil diet, with DHA levels in newborn rabbits increasing from 10 % (control diet) to 43 % of total FAs [35].

In general, the most abundant FAs in sheep and camel eyes, in relative terms, are C16:0, C18:0, and C18:1, comprising about a 20–40 % of the total FA. This corresponds well with observations in rabbits and mature bovines [19] where these FAs represented approximately 30–40 % of the FA profile, and as well in the eyes of the oxen, where they represented around 17-30 % of the total FA [18].

5. Limitations of the study

This study represents the first known attempt to systematically extract and purify PUFAs from the eyes of specific desert-adapted livestock, employing an approach designed to enhances yield, purity, and suitability for pharmaceutical-grade materials. A novel method for extracting PUFAs from the eyes of Arabian sheep and camels was introduced, featuring a sustainable protocol for isolating and purifying fatty acids. This method specifically targets high-value components like DHA and AA, which are known to play critical roles in ocular health.

6. Conclusion

Sheep and camel eyes, currently treated as slaughterhouse waste, could serve as a valuable source of PUFAs. The biotechnological and sustainable production of both DHA and AA from slaughterhouse waste presents a pioneering prospect in the fields of nutrition, pharmacy and healthcare. Further experiments are needed to examine the distribution of these FAs among different lipids classes, including phospholipids, triglycerides, and cholesterol as well as to refine the purification of major PUFA. Such studies would provide

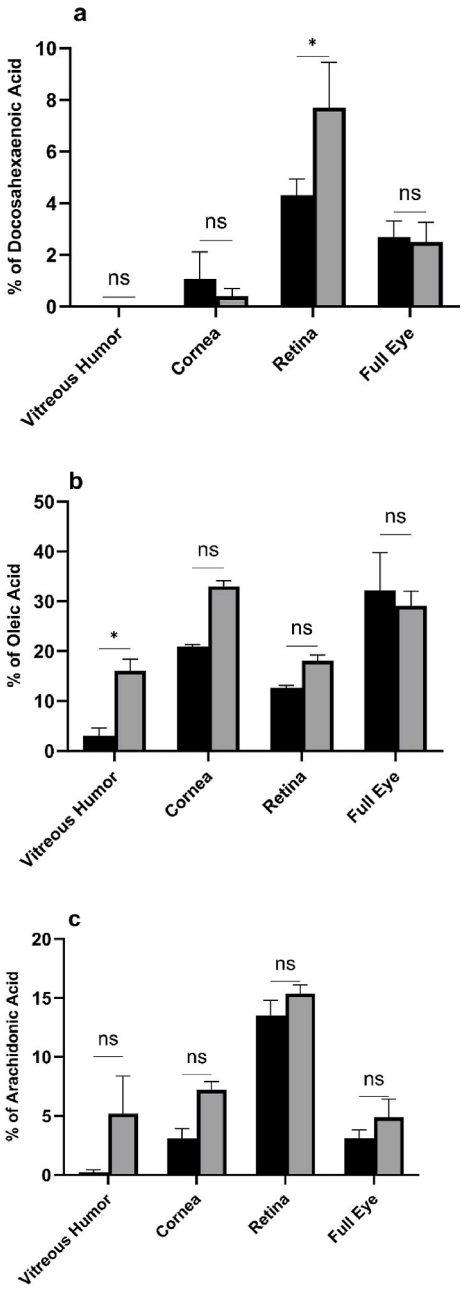

Fig. 7. Comparison of Main Unsaturated Fatty Acids detected in vitreous humor, cornea, retina and whole eye including DHA (a), OA (b) and AA (c) between (▬) Arabian sheep (*O. aries*) and (■) camel (*C. dromedarius*). Results were expressed as percentage of DHA and presented as ±SEM of five values. Groups were compared by analysis of variance (ANOVA) followed by Bonferroni test. ns: not significant; *: $p < 0,05$; **: $p < 0,01$; ***: $p < 0,001$; ****: $p < 0,0001$.

stronger support for the valorization of this waste, contributing to circular bioeconomic advantages.

Data availability statement

The datasets generated during and/or analyzed during the current study are not publicly available. Dr Mayssa Hachem can provide data from this study upon request.

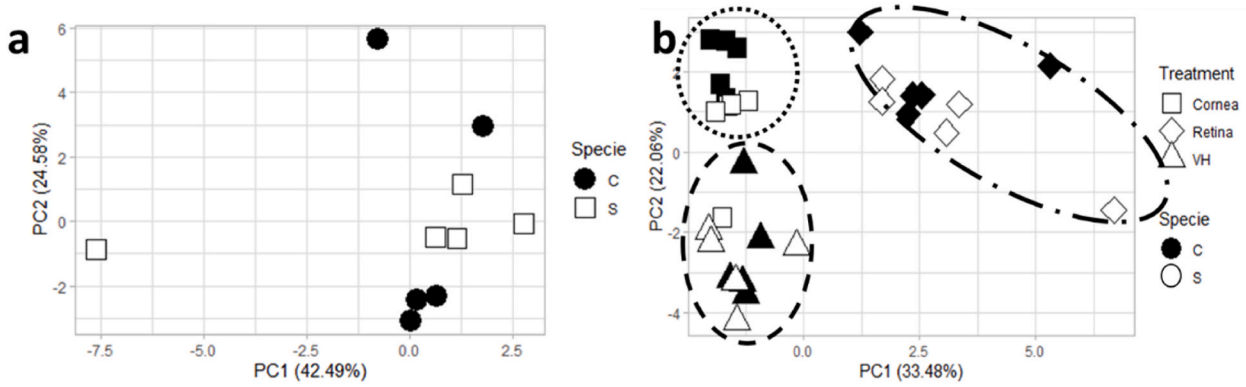

Fig. 8. PCA analysis of the relative FA content of a) the entire eye of camel (C) and sheep (S), b) the cornea, retina, vitreous humor (VH), and whole eyes of Arabian camel (black, C) and sheep (white, S). Results were presented for five values.

CRediT authorship contribution statement

Mayssa Hachem: Writing – review & editing, Project administration, Funding acquisition. **J. Rafael Bermudez:** Writing – original draft. **Abdelmoneim H. Ali:** Formal analysis. **Fiza F. Murtaza:** Formal analysis. **Mohan Rommala:** Formal analysis. **Peter R. Corridon:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e38148.

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