

Chemical Composition, Nutritive value and Health benefits of edible clam *Meretrix casta* (Chemnitz) from West Coast of India

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Abstract

The present study was undertaken with a view to determine the nutraceutical value of the commonly consumed edible clam, *Meretrix casta* (Chemnitz), based on the identification of its organic chemical constituents particularly lipids and carbohydrates. Electrospray ionization tandem mass (ESI-MS/MS) analysis of the bivalve indicated maltodextrins to be the major carbohydrate constituent. Triacylglycerols (TAGs) (0.88%, dry weight) were rich in C14:0, C16:0 to C18:0 (6-11%) saturated and monounsaturated palmitoleic (C16:1n9c; 11.76%) and oleic fatty acids (C18:1n9c; 14.53%). Though the clams contained PUFAs which are known to be beneficial in lowering the risk of cardiovascular diseases, they were devoid of docosahexaenoic acid (C22:6n3, DHA). Maltodextrins being less digestible than glucose beneficially affects the host by selectively stimulating the growth of gut microflora particularly *Lactobacillus* (LAB) and *Bifidobacteria*. These microflora inhibit colonization of pathogens by producing butyrate. The profile of sterols (1.67%, dry wt.) showed it to be a complex mixture of C26, C27, C29 and C30. To our knowledge no reports are available in the literature on the identification of maltodextrins and of positional distribution of PUFA's at the *sn*2 position of TAGs in *M.casta*. The results of this study demonstrated the positive attributes of the bivalve for human consumption.

Keywords: *Meretrix casta*; Bivalve; Maltodextrins; Triacylglycerols; ESI-MS; GC-MS, Sterols

Highlights

ESI-MS/MS identification of lipids and carbohydrates in *Meretrix Casta* and their beneficial effects on human health.

Present study is the first report of positional distribution of FAs in triacylglycerols and of maltodextrins as carbohydrate constituents of the clam.

The possible symbiotic / dietary origin of these constituents is also discussed.

Introduction

Shelled molluscs particularly clams, mussels and oysters, belonging to commercially important group of benthic organisms act as food resource comprising of proteins, lipids and minerals. Being filter feeders, relatively immobile and possessing the ability to accumulate pollutants from the surrounding, molluscs are also known to be among the best bio-indicators for monitoring environmental pollution in coastal waters.

The knowledge on biochemical composition of any organism is extremely important since the nutritive value is reflected in its biochemical contents. Molluscs have been recognized as a high quality nutritious food source and many species are considered as culinary delicacies. The nutritional quality of mollusc-flesh lies not only with the high quality of proteins, but also in its relatively low lipid content and high proportion of PUFAs (Mooney et al. 2002). The economically important species of marine bivalves are green mussel (*Perna viridis*), estuarine oyster (*Crossostrea madrasensis*), giant oyster (*Crossostreagryphoides*) and clams (*Meretrix casta*, *M. meretrix*, *Paphia malabarica*, *Villorita cyprinoides*).

The lamellibranch bivalve, *Meretrix casta* (Chemnitz) (Bivalvia, Veneridae), is an edible mollusc commonly consumed by the coastal population of India. It is abundant in estuaries, backwaters and bays along both the coasts of India. *M. casta* has been analysed earlier for its total amino acids, fatty acid, protein, carbohydrates, vitamins (Srilatha et al. 2013), and mineral content (Shoba & Senthikumar 2014). Nevertheless, this species with anti-oxidative (Nazeer et al. 2013), antibacterial (Mariappan et al. 2010), and antiviral properties (Chatterjee et al. 2002) is yet to be explored for its nutraceutical value based on the detailed identification of its organic chemical constituents.

The aim of the present investigation was to identify organic chemical constituents particularly carbohydrates and lipids and to assess the clam for its health benefits for human consumption. The origin of the metabolites identified has also been discussed. Since electrospray ionization tandem mass spectrometry (ESI-MS/MS) is the method of choice for the identification of minor constituents in complex biological materials, this technique was employed in the present investigation.

Materials and methods

Instrumentation

Nuclear Magnetic Resonance (NMR) spectra were recorded in deuterated chloroform (CDCl₃) on a Bruker Avance 300 MHz spectrometer (Switzerland) with tetramethyl silane (TMS) as an internal standard. The mass spectrometer used was ESI-QTOF MS/MS instrument from Applied Biosystem instrument (Canada) equipped with the Analyst Software application. The instrument was operated in

positive as well as negative ionization mode. GC-EIMS analyses were performed on a Shimadzu QP2010 gas chromatograph (Tokyo Japan) connected to a QP2010 mass spectrometer and equipped with flame ionization detector. MS was operated at 70eV and mass range of m/z 40-600. The fatty acid methyl esters were identified by comparison with standard mixture of fatty acid methyl esters.

Biological material and preparation of methanol extract: The clams, *M. casta* were collected from a non polluted coastal belt off Candolim, Goa, during premonsoon season. Twenty mature clams measuring approximately 15.2 to 32.5 mm and weighing 2.5 to 15.1 g. were de-shelled and the soft tissue was rinsed twice with distilled water and lyophilized. Known amount of this lyophilized material was pulverized and the powder was used for various parameters. Methanol extract was prepared from this dried tissue using 16 ml of methanol: water (9:1, v/v).

Carbohydrates

The total carbohydrate content in samples was determined using phenol-sulphuric acid method of Dubois et al. (1956) and measured at 490nm on a spectrophotometer (UV-240 IPC, Shimadzu, Kyoto, Japan) with D-glucose as standard. In brief, 5 mg of pulverized tissue was taken in a test tube to which 1ml each of phenol and sulphuric acid were added in quick succession and left for half an hour at room temperature. Optical density (OD) of the colour developed was measured at 490 nm against the blank. To identify the nature of carbohydrates present, methanol extract of the tissue was subjected to positive ESI-MS (Fig. 1x) and ESI-MS/MS (Fig.1a-e).

Separation of triacylglycerols (TAGs) and steroids

Methanol extract of the bivalve (3g, dry wt.) was partitioned successively with chloroform and n-butanol. Silica chromatography of chloroform fraction using hexane: ethyl acetate gradient elution yielded neutral lipids. Fractions containing TAGs, distinguished as purplish spots and sterols as bluish spots, on spraying with 5% H₂SO₄ in MeOH, were monitored by thin layer chromatography (TLC) on alumina backed sheets (Silica gel F₂₅₄, 0.25 mm thick, Merck) using petroleum ether: ethyl acetate (95:5 v/v) as mobile phase to afford TAG rich fraction PF1 (26.5 mg; R_f 0.93, 0.88%) and steroids fraction PF2 (50 mg; R_f 0.6, 1.65%). A part of the TAG fraction thus obtained was subjected to spectral data [Fig. 2x, Fig. 2(a-d) and Online Resource 1 and 2] and the remaining (20 mg) was used for saponification.

Methyl esters of fatty acid constituents of TAGs were obtained by the addition of 0.1 ml of 2N KOH in methanol to a solution of PF1 (20 mg) in 5 ml of hexane. The mixture was dried over anhydrous sodium sulphate and left for 25 minutes at room temperature. After phase separation the upper n-

hexane layer containing fatty acid methyl esters (FAME) was processed and subjected to GC-MS analysis.

Results

Results of ESI-MS/MS analysis indicated maltodextrins to be the main carbohydrate (5.35%) constituents of the bivalve, *M. casta*. TAGs were dominant in saturated C14:0 and C16:0 to C18:0 (6-11%) fatty acids and monounsaturated palmitoleic (C16:1n9c; 11.76%) and oleic acids (C18:1n9c; 14.53%). PUFAs particularly linoleic acid (C18:2n6, LA), linolenic acid (C18:3n3, ALA), eicosapentaenoic acid (C20:5n3,EPA) and arachidonic acids (C20:4n6,ARA) were present in considerable amounts. Docosahexaenoic acid (C22:6n3, DHA) which also belonged to PUFA was not detected in the present investigation. Sterol (1.67%) profile showed it to be a complex mixture of C26, C27, C29 and C30 sterols.

Carbohydrates:

ESI-MS/MS of selected signals (Fig 1a-e) from positive ESI-MS of the methanol extract (Fig. 1x) indicated the presence of maltodextrins oligosaccharides as sodium and potassium adducts in the mass range of m/z 100-1050. These dextrin have been identified, on the basis of fragmentation observed (Fig.1a-e and Fig.4b) and by comparison with literature as sodium adduct of glucose [m/z 203]; sodium and potassium adducts of maltose [m/z 365, 381], maltotriose [m/z 527, 543], maltopentaose [m/z 851, 867] and sodium and water adducts of maltotetraose [m/z 689, 707] and maltohexaose [m/z 1013, 1031] (Cappiello et al. 2007).

Nomenclature developed by Domon and Costello (1988) has been used to explain the formation of the fragments in the MS/MS spectra of these neutral oligosaccharides (Fig.1a-e/ Fig.4b). As shown in Fig.4b, fragmentation of ions at m/z 851, 689, 527 and m/z365 observed in the MS/MS spectrum of maltohexose correspond to C/Y type glycosidic cleavage fragment ions while those at m/z 833, 671, 509, 347 and m/z185 are the result of glycosidic cleavages leading to B/Z –type ions. Fragments of the type $[M+Na-60]^+$ are attributed to the presence of $^{0,2}A_n$ type fragments probably formed by loss of $C_2H_4O_2$ as suggested by Cancilla et al. (1999). This fragmentation pathway is well in agreement for cationized α (1-4) linked oligohexoses (Zhou et al., 1990). It is therefore evident from ESI-MS of the methanolic extract of *M. casta* and MS/MS of individual molecular species, that the extract contains maltodextrins maltose, maltotriose, maltotetraose, maltopentaose and maltohexaose.

Spectral analysis of Triacylglycerols

^1H and ^{13}C NMR spectral pattern (Online Resource 1 and 2) of fraction PF1 was typical of triglycerides with the glycerol sn1 and sn3 protons signals being evident at δ 4.1 (dd, $J= 6\text{Hz}, 12\text{ Hz}$) and δ 4.29 (dd, 4.5 Hz, 7.5 Hz) respectively and sn2 protons appeared as a multiplet at δ 5.27. Corresponding ^{13}C NMR signals were observed at 62.02 ppm (t) and 68.8 ppm(d). Absorption due to both saturated as well as unsaturated fatty acyl chain was evident with the ester carbonyl absorption as quartets at δ 173.29 and 172.84ppm.

ESI-MS profile of PF1 (Fig.2x) was indicative of each signal representing not a single triacylglycerol but a mixture of TAGs as protonated or sodium adducted molecular ion, with that particular molecular mass. Tandem mass spectrometry (Fig. 2a-d) was used to determine the fatty acyl composition of each molecular species. No distinction is made between the sn1 and sn3 position. The lower abundance of fragment ions produced by the loss of a fatty acid from the secondary versus primary position has been utilized to differentiate between regioisomers of TAGs (Lin and Arcinas 2008). Since electrospray is a very soft ionization technique, fatty acid fragment ions and monoacyl glycerol fragments were not observed in the ESI spectrum. For example, the CID spectrum of TAG molecular species with $[\text{M}+\text{H}]^+$ at m/z 855 (Fig.2d) produced diacylglycerol (DAG) fragment at m/z 551, 573, 577 and m/z 599 formed due the neutral loss of fatty acid from primary sn1/sn3 and secondary sn2 position of glycerol. Based on the abundance of the signals for DAG^+ ions, TAG regioisomers with $[\text{M}+\text{H}]^+$ m/z 855 were identified as LnOP and PArP as it is known that elimination of fatty acid from sn2 results in the lower abundance of DAG^+ fragment ion as compared to the loss of fatty acid from primary sn1/sn3 position. Thus, fragmentation of TAG molecular species of $[\text{M}+\text{H}]^+$ at m/z 855 indicated it to be a mixture of two regioisomers LnOP and PArP where the intensity observed for DAG^+ signal were $\text{PO}^+>\text{LnO}^+>\text{LnP}^+(577>599>573)$ and for the isomer PArP signal intensities observed were $\text{PAr}^+>\text{PP}^+$ i.e. $599>551$.

In this purified fraction, 14 pairs of regioisomers of different TAGs and 5 TAGs with identical FAs at sn1 and sn2 were detected containing thirteen different fatty acids (Table1). The analysis of this data shows that PUFAs are the most common fatty acids at the sn2 position of glycerol.

Fatty acid composition

The signals in negative ESI-MS (Online Resource 3) of the clam tissue extract at m/z 227, 241, 255, 269, and 283 were indicative of the presence of free C14:0 -C18:0 saturated fatty acids and unsaturated FAs at m/z 251 (C16:2) and m/z 281 (C18:1).

The fatty acid composition of purified TAGs fraction, as evident from tandem mass spectrometry data as well as GC/MS after saponification, is listed in Table 2. ESI-MS/MS (Table1; Fig. 2a-d) showed palmitic acid (C16:0) to be common to practically all TAGs in *M. casta* and it occupies mainly external positions (sn1 and sn3). There are only two saturated fatty acids stearic (C18:0) and nonadecanoic (C19:0) each one comprising of one triacylglycerol (TAG). Among unsaturated acids, linoleic acid was absent but linolenic and oleic acids were found to be the most dominant, other unsaturated acids being present in considerable amounts contained 3 or more than 3 double bonds. Among long chain PUFAs, it was found to be rich in EPA but totally devoid of DHA. It has been observed that low environmental temperatures generally increased the degree of unsaturation for the fatty acid in aquatic organism (Wang et al. 1990).

GC/MS analysis of FAs of saponified TAG fraction (Table2), as corresponding methyl esters, showed dominance of C14:0 (6.18%), C16:0 - C18:0 (6.42-11.6%), among the saturated fatty acids and was rich in monounsaturated palmitoleic (C16:1n9c; 11.76%) and oleic (C18:1n9c; 14.53%) acids. Monounsaturated C16:1 and C18:1 acid are reported to be abundant in triacylglycerols of clams *Tapes decussates* and *T. Philippinarum* (Beninger and Stehpan 1985) while, C18:1 was found at the level of 14.6% in *Donax cuneatus* (Idayachandiran et al. 2014). Though TAGs of *M. casta* contained PUFAs like LA, ALA, EPA, ARA, hexadecatrienoic acid (C16:3) and stearidonic acid (C18:4), no PUFAs were detected by GC/MS except for minor amounts of LA (0.28%). Most of free PUFAs and their oxidative metabolites are known to be heat unstable and have low boiling points (Sajiki and Yonekubo 2002). Martin et al. (1993) reported that long chain PUFAs such as EPA, DHA, and ARA are resistant to lipase hydrolysis when linked to outer positions of glycerol molecule. As evident by ESI-MS/MS of triglycerides (Fig.2a-d), in the present study, PUFAs occupying sn2 position were not released after saponification.

Steroids

ESI-MS profile of the steroidal fraction PF2 (Fig. 3) and NMR spectra (Online Resource 4 and 5) showed it to be a complex mixture of C26, C27, C29 and C30 sterols. Tandem mass spectra (Fig. 3a-f) of some selected steroids from the ESI-MS indicated that it contained not only monooxygenated steroids but also polyoxygenated ones (Fig. 4a, Online Resource 6).

Discussion

The biochemical constituents in animals are known to vary with the physiological state of the animal, availability of food and environmental factors. Biochemical components such as proteins, carbohydrates and lipids are essential for body growth and maintenance. Literature published on the

nutritional composition of the bivalve of present investigation shows that, except identification of amino acids and vitamin constituents, it has been analysed for its total fatty acid, protein, carbohydrates, (Srilatha et al. 2013; Venugopal and Gopakumar 2017) and mineral content (Shoba and Senthikumar 2014).

In the present investigation, *M. casta* has been studied for its nutritional quality in the light of its main biochemical constituents particularly fatty acids (free and bound as TAGs), sterols, and carbohydrates. The possible symbiotic / dietary origin of these constituents is also discussed.

In general, carbohydrate contents including dietary fibre in shellfish tissue are low (Venugopal and Gopakumar, 2017). In *M. casta*, the clam of the present study, the soluble carbohydrate content was 5.35%. Earlier reports on soluble carbohydrates in *M. lusoria* showed to vary from 0.32- 7.80% (Karnjanapratum et al. 2013) and in *M. meretrix* from 4.14-8.3% (Kang et al. 2008). No report is available on identification of constituent sugars in bivalve except that mussels (*Mytilus* spp.) contained mytilan, a noncovalently linked complex of 95% polysaccharide and 5% protein and another polysaccharide, a (1-4)-D-glucan (Grienke et al. 2014). An anti -HIV sulphated B-galactan with a β -(1 \rightarrow 3) glycosidic linkage is also known as a metabolite of the marine clam *M. petechialis* (Amornrut et al. 1999). Present study identifies maltodextrins (MDs) as the main carbohydrate constituents which is, till recently unreported from clams in general, and *M.casta* in particular.

Glycogen, an oligosaccharide of glucose, is our body's backup source of fuel which is stored in liver and muscles to be used under extreme stress conditions in metabolic pathways and biochemical reactions. All shellfish contain more than 1% of their body weight of glycogen while short-neck clam contain glycogen in abundance as a characteristic property of bivalves (Yamanaka 1988). Glycogen is enzymatically degraded by phospholysis or hydrolysis under anaerobic conditions during storage to yield free sugars including glucose and maltose. It is therefore clear that glycogen is another source of maltodextrins which are possibly formed as degradation products of glycogen by enzyme amylase. Tsao et al. (2004) reported amylase designated as AI-1 and AI-2 which could digest amylase starch into glucose and maltose and AII could digest amylase starch and pullulan into glucose from viscera of hard clam *Meretrix lusoria*. All the three amylases hydrolyse amylase, amylopectin and glycogen and moderately hydrolyse maltopentaose, maltotetraose and laminarin.

As mentioned, maltodextrins (MDs) have been identified as the main carbohydrate constituents in *M.casta*. MDs are considered to be a good source of energy but are not as easily digestible and absorbed in small intestine as oral glucose. While glucose will be immediately available for absorption upon arrival in the small intestine, MDs are partly converted into maltose by salivary α -amylase, due to the

relatively short time that MDs reside in the mouth. Subsequent to arrival in the stomach, the gastric contents need to be transferred into the small intestinal duodenum for digestion and further transit in the gut. Pancreatic amylase, secreted in the small intestine, plays a final role in complete hydrolysis of MDs to maltose. Maltose is either taken up by the gut epithelium directly or further broken down by brush border maltase, resulting in free glucose which is absorbed into blood (Hofman et al. 2016). MDs being less digestible, beneficially affects the host by selectively stimulating the growth and/or activity of gut microflora particularly *Lactobacillus* (LAB) and *Bifidobacteria* and thus inhibit colonization by pathogens with the production of butyrate (Olano-Martin et al. 2000). Slower digestion and absorption also results in MDs being less harmful than glucose due to lower glycaemic response.

Lipids are highly efficient as source of energy and they contain more than twice the energy of carbohydrates and proteins. Molluscs are known to contain a wide variety of PUFAs, some of which are considered as essential fatty acids that humans cannot synthesize and must be obtained from food (Russo 2009). Molluscs too cannot synthesize these ω -3 and ω -6 fatty acids and must be acquired from their food intake, such as phytoplankton or algae (Foster and Hodgson 1998). In this study, FA composition of *M. casta*, indicated the dominance of C14:0, C16:0 to C18:0 saturated FAs and was rich in monounsaturated palmitoleic (C16:1n9c) and oleic (C18:1n9c) acids. It was devoid of DHA but contained appreciable amount of other PUFAs. Except LA which was present in free form all other PUFAs were present in bound form as TAGs. Regio-specific locations of acyl chains affects the human absorption of TAGs in food and is thus a valuable nutritional source in food industry.

Among the fatty acids, long chain PUFAs, EPA and DHA have beneficial effects in prevention of human coronary heart disease by lowering plasma lipids and decrease platelet aggregation (Russo 2009). Childs et al. (1990) reports lowering of VLDL (very low density lipoproteins), TAGs, LDL and total cholesterol on consumption of Manila clam which is low in cholesterol and high in n-3FAs by normolipidemic men. Studies on the lipids of marine organisms have shown that fish and shellfish could be unique sources of these long chain ω -3 polyunsaturated fatty acids (Wang et al. 1990). The content of PUFA in *M. casta* is persistent to meet future energy requirements and TAGs may also act as a temporary reservoir of physiologically important PUFAs which could be transferred to the structural lipids or directed to specific metabolic pathways. Accordingly, *M. casta* TAGs may be an excellent source of polyunsaturated ω -3 and ω -6 fatty acids for the mollusc as well as a good source of MUFA and PUFA for the human diet.

Glucose and glycerol must have been derived from symbiotic microalgae, commonly known as zooxanthellae, which form the feed of most bivalve mollusc (Ishikura et al., 1999). These symbiotic microalgae are also rich in EPA and DHA (Beninger and Stehpan 1985). EPA is predominant in diatoms (Pond et al. 1998) and has been used as a benthic diatom marker in food webs, while DHA is known to be dinoflagellate marker. In marine invertebrate-microalgae symbiosis, the hosts are known to depend nutritionally on photosynthesis of their symbiotic algae. Photosynthetic products, especially low molecular weight compounds, particularly glucose and maltose, are the major release products (Venn et al. 2008). There are reports that in the presence of host tissue homogenate, zooxanthellae *Symbiodinium* sp. isolated from a coral *Pocillopora damicornis* and a giant clam *Tridacna crocea*, release a large part of their photosynthates as glycerol (Muscatine, 1967).

Thai researchers (Khowhit et al. 2012) have reported the presence of phytoplankton like *Coscinodiscus* sp. (70.46%), *Cyclotella* sp. (18.29%) *Oscillatoria* sp. (4.79%), *Paralia sulcata* (3.71%), *Protoperidinium* sp.(1.41%), *Thalassiosira* sp.(0.44%), *Synedra* sp.(0.44%) and *Skeletonema costatum* (0.44%) in *M. casta*.

Though the sterol composition, particularly cholesterol of selected marine bivalve is well documented, there are limited reports on the sterol content of genus *Meretrix*. The fat of clam *Meretrix meretrix* is known to be a source of brassicasterol, poriferasterol, clionasterol and cholesterol (Toyama et al. 1953). A recent publication reports cholesterol content of 0.07-0.21% wet weight in *Meretrix lusoria* (Karnjanapratum et al. 2013). Present investigation showed a concentration of 1.67% (dry weight) in *M. casta* and though belonging to the same genus its sterol profile was found to contain complex mixture of C26, C27, C29 and C30 sterols besides cholesterol. The absence of brassicasterol, poriferasterol, clionasterol and presence of other sterols (as shown in Online Resource 6) were elucidated from ESI-MS/MS analysis and comparison with literature. The only C₂₆ sterol identified from *M. Casta* was 24 nor cholest-5,22-dien-3-ol. Besides cholesterol, which is a major common constituent of molluscs, cholest-4ene-3,6-dione and cholest-4ene-20,24-diol were the other two C₂₇ sterols found to occur in the fat of *M. casta*. C₂₈ sterols identified included 24-methylene cholesterol, 4-methyl-cholest-4ene 3, 6, diol and 4-methyl-cholest-4- $\Delta^{7,8}$ -diene-3,6,20-triol. Dinosterone (4,23,24-trimethyl-cholest-22-ene-3one) was the only C₃₀ sterol identified in the clam. Among the above listed sterols, C₂₆ sterol had been reported earlier from clams (Phillips, et al. 2012).

Dinosterone is reported to be a biomarker of the heterotrophic dinoflagellate *Pfiesteria piscicida* (Leblond and Chapman 2004). Therefore, except for cholesterol, remaining sterols seems to have originated from the diet of bivalve as these steroids are reported from zooxanthellae (Leblond and Chapman 2004) and as mentioned, zooxanthellae are the feed of most bivalve molluscs.

Polyoxygenated sterols are being reported for the first time in a clam. Molluscan shellfish are low in cholesterol with 1/3 of total sterol being cholesterol and the percentage of non cholesterol sterols being high is potentially beneficial as they might decrease LDL cholesterol on prolonged feeding.

Conclusion

In conclusion, this study describes the importance of *Meretrix casta* on the basis of its chemical constituents such as lipids and carbohydrates. In addition, we believe this to be the first report of identification of positional distribution of PUFA's at the *sn2* position of TAGs and maltodextrin carbohydrates in the clam. Further, since it is well known that n-3 and n-6 fatty acids cannot be synthesized by molluscs, they must have been acquired from the ingested phytoplankton. Similarly, the bivalve contained considerable amount of non cholesterol sterols, also from dietary origin and are known to lower absorption of cholesterol in the system. *M. casta* has thus been evaluated for its health benefits on the basis of its chemical constituents. This bivalve hence, may be considered as a healthy food source, similar to other edible molluscs. Further research should be carried out to identify factors influencing variation in the chemical composition and investigate methods to improve the concentration of beneficial components.

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Figure Legends

Fig. 1 Positive ESI-MS of methanol extract of *Meretrix casta*

Fig 1(a-e) ESI-MS/MS spectra of protonated $[M+H]^+$ ions at m/z 1013.3075 identified as Maltohexaose (a); (m/z 851.2671) Maltopentaose (b); (m/z 689.21) Maltotetraose (c); (m/z 407.11) Maltotriose (d); m/z 365.1126 maltose(e)

Fig. 2 Positive ESI-MS profile of purified Triacylglycerol fraction (PF1) from *M. casta*

Fig 2 (a-d): MS/MS of triacylglycerol molecular species with $[M+H]^+$ ion at m/z 875(a); m/z 801(b); m/z 827(c); m/z 855(d)

Fig. 3 Positive ESI-MS profile of purified sterol fraction (PF2) from *M. casta*

Fig 3(a-f) : MS/MS of $[M+H]^+$ ion at m/z 372 identified as 24 nor cholest-5,22-dien-3 ol(a); (m/z 399) 24-methylene cholesterol (b); (m/z 420) cholest-5-ene-3,20,24-triol(c) ; (m/z 416) 23,24-dimethyl-5 α -cholest-5ene- 3-ol(d); (m/z 431) 4-methyl-cholest- $\Delta^{7,8}$ -ene 3,6,20-triol (e) (m/z 428) 4,23,24-trimethyl-5 α -cholest-22-ene-3one (f)

Fig. 4 Structures of steroids identified in *Meretrix casta* (a); Fragmentation of ions as observed in the MS/MS spectrum of maltohexose corresponding to the C/Y and C/Z type glycosidic cleavage (b).

Electronic Supplementary Material

Online Resource 1 1H NMR spectrum of fraction PF₁ (TAG).

Online Resource 2 ^{13}C NMR signals of PF₁ (TAG).

Online Resource 3 Negative ESI-MS spectrum of the shellfish *M. casta* extract

Online Resource 4 1H NMR signals of the fraction PF₂ (Steroids).

Online Resource 5 ^{13}C NMR signals of fraction PF₂ (Steroids).

Online Resource 6 Structural assignments and ESI-MS/MS data of selected sterols found in *M.casta*.

Captions to Tables

Table 1: TAGs molecular species identified in the extract of *Meretrix casta*

Table 2: Fatty acid methyl esters profile of TAGs as evident by GC/MS of the hydrolysed TAGs and free FAs present in methanol extract of *Meretrix cast*

Table 1: TAG molecular species identified in the extract of *Meretrix casta*

Serial No.	Triacylglycerol	CN:DB ^a	[M+H] ⁺	DAG ⁺	Relative abundance
1	PHtP	48:3	801	PP ⁺ →551 PHt ⁺ →545	551 < 545
2	LnMyP	48:3	801	PMMy ⁺ →523 LnMy ⁺ →545 LnP ⁺ →573	523>545>573
3	St PP	50:4	827	PSt ⁺ →571 PP ⁺ →551	551>571
4	LnOMy	50:4	827	LnO ⁺ →599 MyO ⁺ →549 LnMy ⁺ →545	549>599>545
5	PMMyAr	50:4	827	PMMy ⁺ →523 ArMy ⁺ →571 PAr ⁺ →599	571>523>599
6	LnPPo	50:4	827	LnP ⁺ →573 LnPo ⁺ →571 PPo ⁺ →549	549>571>573
7	PPoP	48:1	804+Na	PPo→549 PP ⁺ →551	549>551
8	LnOP	52:4	855	LnO ⁺ →599 PO ⁺ →577 LnP ⁺ →573	577>599>573
9	PArP	52:4	855	PAr ⁺ →599 PP ⁺ →551	599>551
10	PHnP	53:1	875	PHn ⁺ →619 PP ⁺ →551	619>551
11	NOP	53:1	875	NO ⁺ →619 NP ⁺ →593 OP ⁺ →577	619>593>577
12	PLnE ₅	54:8	875	PE ₅ ⁺ →597 PLn ⁺ →573 LnE ₅ ⁺ →619	619>573>597
13	PE ₅ P	52:5	875	PP ⁺ →551 PE ₅ →619	551<619
14	E ₅ MyS	52:5	875 [M+Na]	SMy ⁺ →573 SE ₅ →647 E ₅ My→593	573>593>647

^aCN, carbon number, DB, double bond, [M+Na]⁺, sodium adduct of molecular ion, [M+H]⁺, protonated molecular ion, My, myristic acid (C_{14:0}); P, palmitic acid (C_{16:0}); Po, palmitoleic acid (C_{16:1}); S, stearic acid (C_{18:0}); O, oleic acid (C_{18:1n9c}); L, linoleic acid (C_{18:2n6}); Ar, arachidonic acid (C_{20:4n6}); E₅, eicosapentaenoic acid (C_{20:5n3}); Ln, linolenic acid (C_{18:3n3}); Ht, hexadecatrienoic acid (C_{16:3}); St, stearidonic acid (C_{18:4}); N, nonadecanoic acid (C_{19:0}); Hn heneicosenoic acid (C_{21:1})

Table-2: Fatty acid methyl esters profile of TAGs as evident by GC/MS of the hydrolysed TAGs and free FA present in methanol extract of *Meretrix casta*

GC/MS		% A	Negative EI-MS [RCOO] ⁻
Fatty Acids	Trivial name		
Saturated Fatty Acids (SFA)			
C _{12:0}	Lauric	0.2	
C _{14:0}	Myristic	6.18	227.09
C _{15:0}	Pentadecylic	2.86	241.23
C _{16:0}	Palmitic	9.69	255.27
C _{17:0}	Margaric	6.42	269.26
C _{18:0}	Stearic	11.6	
C _{19:0}	Nonadecylic	0.25	297.32
C _{20:0}	Arachidic	0.95	
C _{22:0}	Behenic	0.26	
C _{24:0}	Lignoceric	0.35	
3-OH-C _{18:0}	3-Hydroxy-octadecanoic	0.05	
Monounsaturated Fatty Acids (MUFA)			
C _{16:1n-9c}	palmitoleic	11.76	
C _{18:1n-9c}	oleic	8.07	281.24
C _{18:1n-10c}	oleic	0.11	
C _{18:1n-11c}	oleic	6.35	
C _{19:1n-10}	Nonadecenoic(undifferentiated)	0.08	
C _{20:1n-11}	Eicosenoic (undifferentiated)	1.02	
	10-Octadecynoic acid (undifferentiated)	0.27	
	2-Hexadecenoic acid, 2,3-dimethyl (undifferentiated)	0.81	
Polyunsaturated Fatty Acids (PUFA)			
C _{18:2n-6}	linoleic	0.28	
C _{16:3}	Hexadeca-6,9,12-trienoic acid		249.09

Fig. 1X Positive ESI-MS of methanol extract of *Meretrix casta*

Fig 1(a-e) ESI-MS/MS spectra of protonated $[M+H]^+$ ions at m/z 1013.3075 identified as Maltohexaose (a); (m/z 851.2671) Maltopentaose (b); (m/z 689.21) Maltotetraose (c); (m/z 407.11) Maltotriose (d); m/z 365.1126 maltose(e)

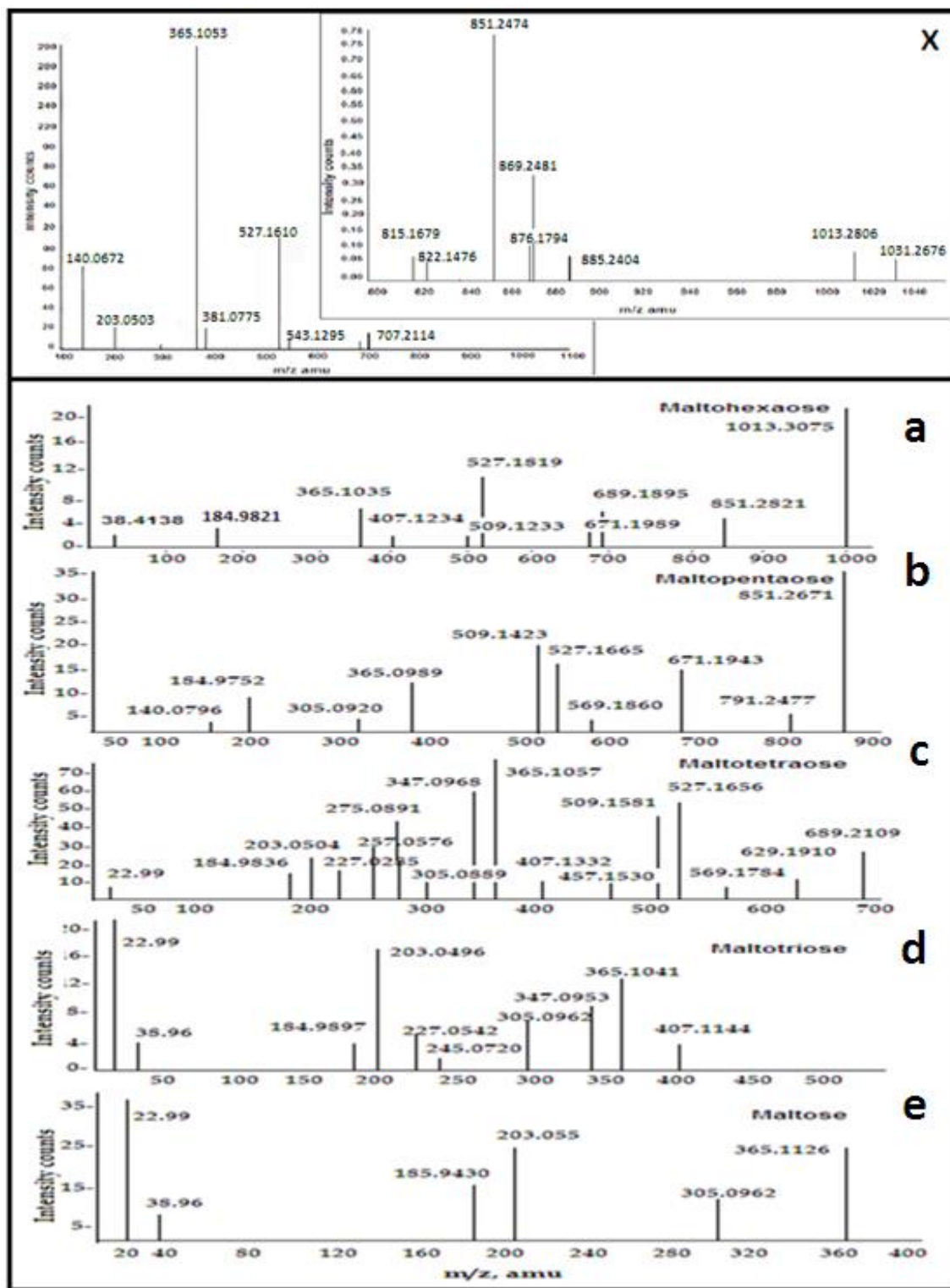


Fig. 2X Positive ESI-MS profile of purified Triacylglycerol fraction (PF1) from *M. casta*

Fig 2 (a-d): MS/MS of triacylglycerol molecular species with $[M+H]^+$ ion at m/z 875(a); m/z 801(b); m/z 827(c); m/z 855(d)

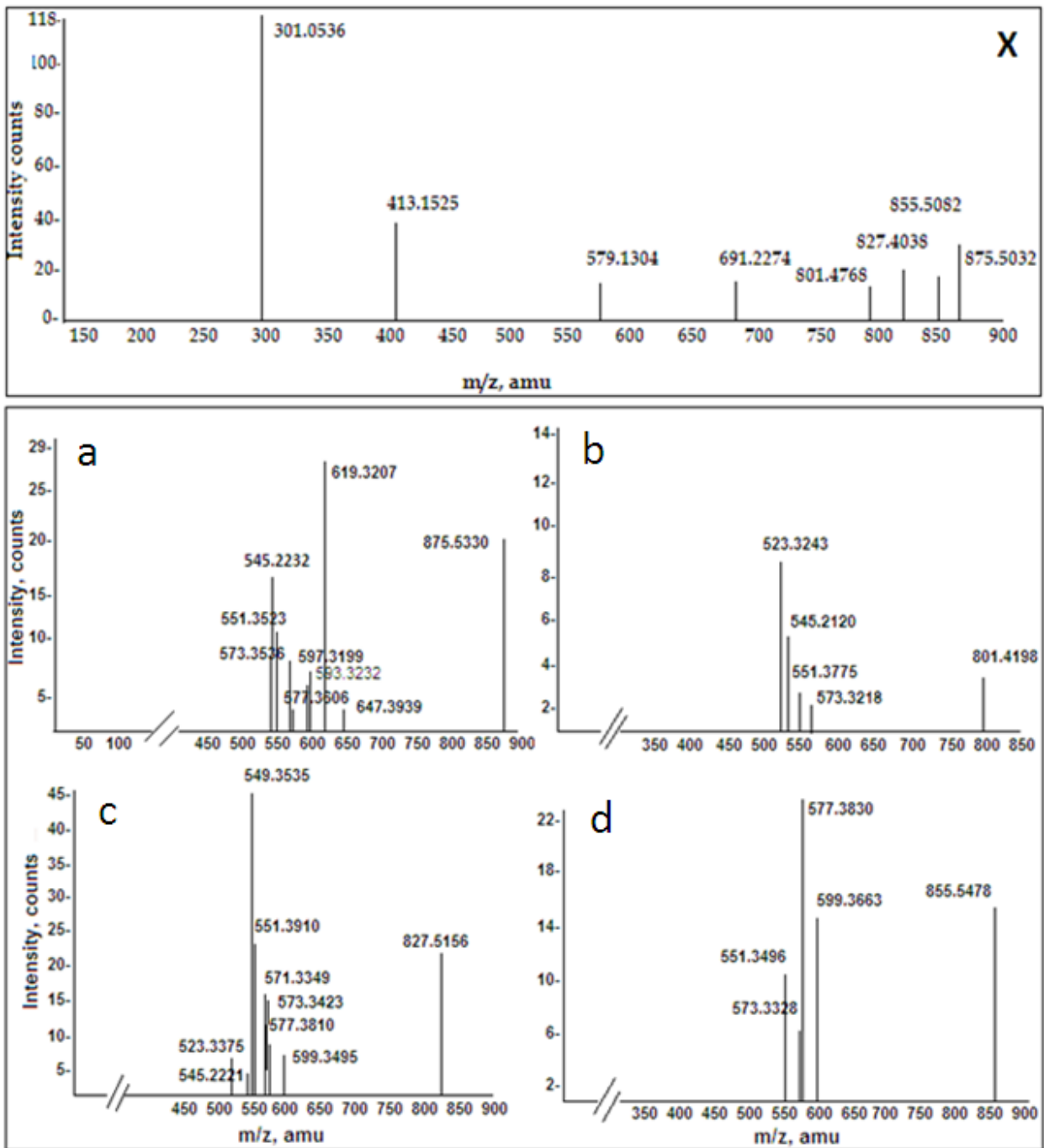


Fig. 3 Positive ESI-MS profile of purified sterol fraction (PF2) from *M. casta*

Fig3(a-f) MS/MS of $[M+H]^+$ ion at m/z 372 identified as 24 nor cholest-5,22-dien-3 ol(a); (m/z 399) 24-methylene cholesterol (b); (m/z 420) cholest-5-ene-3,20,24-triol(c) ; (m/z 416) 23,24-dimethyl-5 α -cholest-5ene- 3-ol(d); (m/z 431) 4-methyl-cholest- $\Delta^{7,8}$ -ene 3,6,20-triol (e) (m/z 428) 4,23,24-trimethyl-5 α -cholest-22-ene-3one (f)

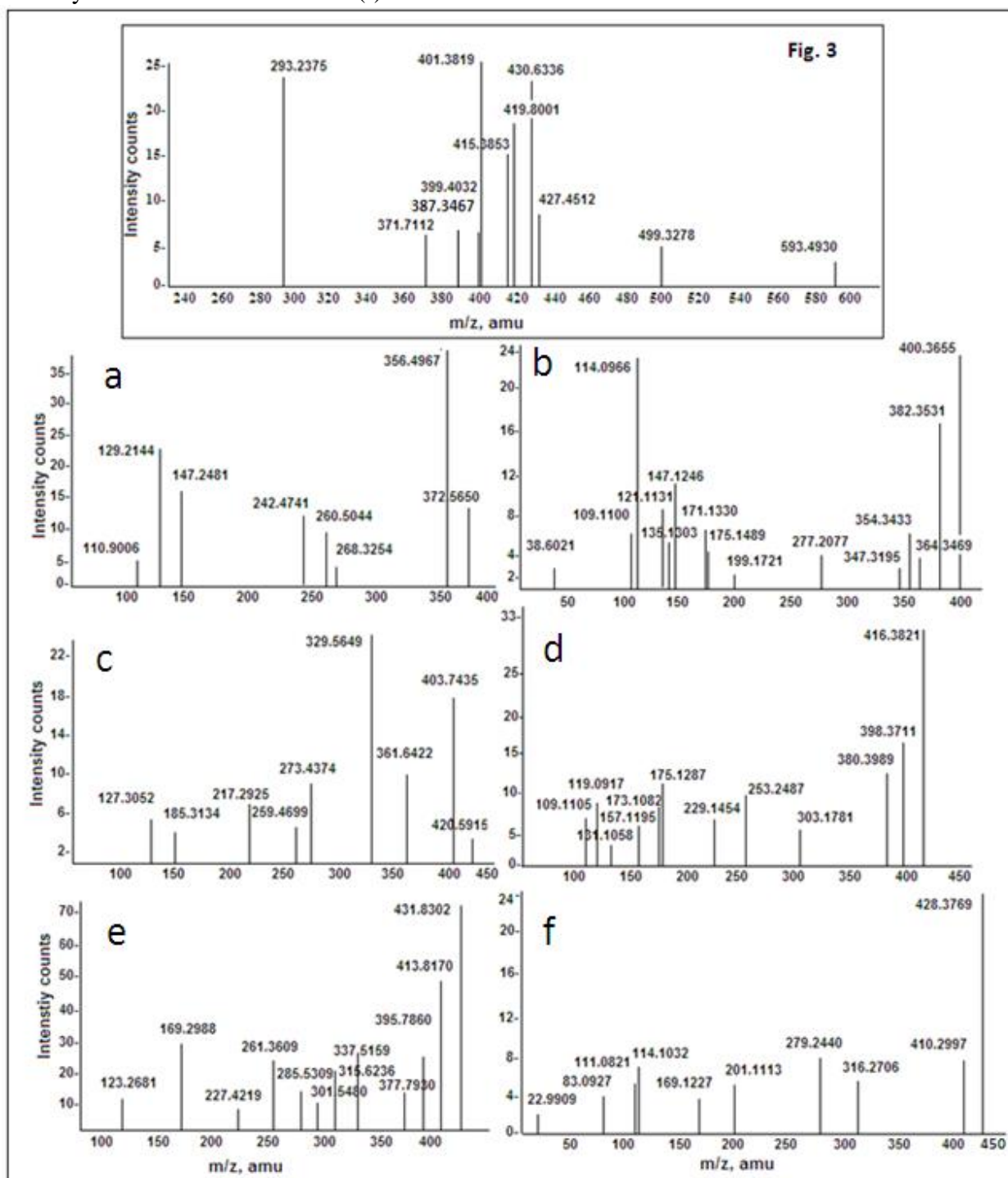


Fig. 4 Structures of steroids identified in *Meretrix casta* (a); Fragmentation of ions as observed in the MS/MS spectrum of maltohexose corresponding to the C/Y and C/Z type glycosidic cleavage (b).

