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Title

Phospholipids in rice: Significance in grain quality and health benefits: A review

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Abstract

Phospholipids (PLs) are a major class of lipid in rice grain. Although PLs are only a minor nutrient compared to starch and protein, they may have both nutritional and functional significance. We have systemically reviewed the literature on the class, distribution and variation of PLs in rice, their relation to rice end-use quality and human health, as well as available methods for analytical profiling. Phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and their lyso forms are the major PLs in rice. The deterioration of PC in rice bran during storage was considered as a trigger for the degradation of rice lipids with associated rancid flavour in paddy and brown rice. The lyso forms in rice endosperm represent the major starch lipid, and may form inclusion complexes with amylose, affecting the physicochemical properties and digestibility of starch, and hence the cooking and eating quality of rice. Dietary PLs have a positive impact on several human diseases and reduce the side-effects of some drugs. As rice has long been consumed as a staple food in many Asian countries, rice PLs may have significant health benefits for those populations. Rice PLs may be influenced both by genetic (G) and environmental (E) factors, and resolving G x E interactions may allow future exploitation of PL composition and content, thus boosting rice eating quality and health benefits for consumers. We have identified and summarised the different methods used for rice PL analysis, and discussed the consequences of variation in reported PL values due to inconsistencies between methods. This review enhances the understanding of the nature and importance of PLs in rice and outlines potential approaches for manipulating PLs to improve the quality of rice grain and other cereals.

Keywords:

Rice, phospholipid, lysophospholipid, lecithin, starch lipid, amylose-lipid complex, rice quality, glycaemic index, health benefit.

1. Introduction

Rice has been consumed for almost 5000 years and currently feeds almost half the human population. Botanically, cultivated rice belongs to the Poaceae family and includes two species: *Oryza sativa* L. (commonly known as Asian rice) and *O. glaberrima* Steud. (commonly known as African rice) (Linares, 2002). Since *O. sativa* accounts for the majority of world rice production, and hence research effort, rice in this review and many other papers refers to *O. sativa* only, unless otherwise specified. *O. sativa* is comprised of two subspecies, *indica* and *japonica*.

Rice grains are mainly composed of starch, including amylose and amylopectin. According to starch composition, rice is often classified into waxy and non-waxy varieties. Compared with non-waxy rice, waxy rice is especially sticky when cooked and contains no, or negligible amounts of, amylose. Rice grains contain a much smaller proportion of lipids than starch, however, these lipids may make a significant contribution to processing and nutritional properties (Moazzami, Lampi & Kamal-Eldin, 2011). For instance, rice bran oil is a popular cooking oil in several Asian countries which has a direct impact on human nutrition and health (Ghosh, 2007)

Phospholipids (PLs), consisting of covalently bound phosphate and lipid, are a major class of lipid in rice, comprising up to 10% of total grain lipid content (Table 1 and 2). These amphiphilic lipids, found in plant and animal cell membranes, are critically important to all cellular organisms. Dietary PLs have beneficial effects on a range of

human diseases and conditions, such as coronary heart disease, cancer or inflammation (Kullenberg, Taylor, Schneider & Massing, 2012). Since rice is the single most important staple food in the world, it is likely to represent a significant source of dietary PLs for a large proportion of the world's human population. Although there have been a large number of isolated reports describing the nature and importance of these compounds, much of the information, such as the amount and distribution of PLs in different rice varieties, their effects on rice storage and eating quality, and implications for human health, is dispersed, with little consensus.

Natural PLs can be classified into two major categories, glycerophospholipids (GPLs, e.g. phosphatidylcholine or lecithin in egg yolk) (Table 1) and sphingophospholipids (SPLs, e.g. sphingomyelin, in brain and neural tissue). To date, only GPLs have been identified in rice grain. GPLs consist of fatty acids (FAs) esterified to a glycerol backbone, a phosphate group and a hydrophilic residue (such as the amino acid choline). PLs are a major component of lipids in the oil rich rice embryo (includes scutellum, plumule, radicle, epiblast) and bran (includes pericarp, seed coat, nucellus, aleurone layer) (Fig. 1) (Yoshida, Tanigawa, Yoshida, Kuriyama, Tomiyama & Mizushina, 2011b). Lysophospholipids (LPLs) are an important subcategory of GPLs with a free alcohol in the *sn*-2 position, and are the major (~50%) starch lipids in the rice endosperm (Choudhury & Juliano, 1980a). The PLs in rice bran and endosperm make a significant contribution to the quality of rice, affecting properties such as the rancidity of paddy or brown rice (Aibara, Ismail, Yamashita, Ohta, Sekiyama & Morita, 1986) and the physicochemical properties of starch (Pérez & Bertoft, 2010), each of which requires careful evaluation.

89 Previously, different extraction and analytical methods have been employed for the
90 analysis of PLs in rice and other cereals, which can result in significant apparent variation
91 for similar samples. We have also experienced unexpected incomplete extraction when
92 extracting cereal lipids in our recent research (Liu, 2009). The incomplete extraction may
93 arise from the natural starch-lipid complex in cereal grains. This starch-lipid complex
94 may also decrease the efficiency of human digestive enzymes *in vivo* and hence
95 contribute to strategies for controlling glycaemia and reducing the risk of Type 2 diabetes
96 mellitus (Liu, Deseo, Morris, Winter & Leach, 2011). There is scope to improve the
97 content and composition of beneficial PLs in cereal grains, by modification of component
98 genetic and agro-environmental factors. As rice has a smaller and less complex genome
99 than other cereals such as wheat and maize, it provides an excellent research model to
100 explore and attribute genetic and environmental contributions to PL variation. Our
101 objective in preparing this review was to enhance the understanding of the variation and
102 distribution of PLs in rice and their contribution to rice quality and human health, and so
103 provide a context for prioritising future research directions for crop improvement and
104 utilisation of rice and other cereals.

105 **2. Phospholipids in rice grain**

106 Rice lipids are classified according to their chemical structure into acylglycerols, free
107 fatty acids, wax esters (such as policosanols), PLs, glycolipids and unsaponifiables (such
108 as γ-oryzanols, tocopherols/ tocotrienols and squalene) (Moazzami et al., 2011). Lipids
109 are primarily concentrated in the rice bran (19.4-25.5%) and germ (34.1-36.5%) fractions
110 rather than in the milled rice which is around 0.8% of the endosperm fraction (Table 2)
111 (Juliano, 1983). Among these lipids, PLs (~10% of total lipids in brown rice) are

considered to be polar compared to the neutral lipids such as triacylglycerols (TAG) (Mano, Kawaminami, Kojima, Ohnishi & Ito, 1999). Rice PLs have diverse hydrophilic heads, comprised of fatty acids distributed in the rice grain, which may reflect their different biosynthetic pathways and biological functions.

2.1 Class, composition, location and concentration of PLs in rice grain

As with PLs in many other organisms, rice PLs contain components such as fatty acid ester(s), glycerol(s), phosphate(s), and choline, ethanolamine, inositol or serine. The PLs found in rice include phosphatidylcholine (lecithin) (PC), phosphatidylethanolamine (cephalin) (PE) and phosphatidylinositol (PI), phosphatidylserine (PS), lysophosphatidylcholine (LPC), lysophosphatidylethanolamine (LPE), lysophosphatidyl-inositol (LPI), N-acyl phosphatidylethanolamine (NAPE), N-acyl lysophosphatidyl-ethanolamine (NALPE), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG, cardiolipin) and phosphatidic acid (PA) (Table 1) (Choi, Takahashi, Inatsu, Mano & Ohnishi, 2005; Glushenkova, Ul'chenko, Talipova, Mukhamedova, Bekker & Tolibaev, 1998; Lam & Proctor, 2004; Yoshida, Tanigawa, Kuriyama, Yoshida, Tomiyama & Mizushina, 2011a). Among them, PI, PG, DPG, NAPE, NALPE are considered to be the acidic glycerophospholipids (Choi et al., 2005).

Generally, PC, PE and PI are the principal PLs in the oil rich rice bran and germ and constitute ~80% of total PLs (Yoshida et al., 2011b). Besides the PLs within the cell membranes, it is believed that PLs also form a single layer membrane bounding the spherosomes or subcellular lipid bodies, which are mainly composed of TAGs (Fig. 1) (~98.6%) (Huang, 1996). Spherosomes are prominent within the cells of oil storage tissues such as the aleurone layer (Bechtel & Pomeranz, 1977; Bechtel & Pomeranz,

1978a). PLs are also important components of organelle membranes such as the mitochondrial and endoplasmic reticulum (He, Wang, Li & Liu, 2007). However, to the best of our knowledge, there has been no targeted research on these PLs within rice grain.

It is important to note that the lipids in the endosperm are present in different forms compared to those in the bran and germ. Based on their relative association with starch (stabilised with van der Vaals contacts), rice lipids are often classified as starch and non-starch lipids (Morrison, 1995). Non-starch lipids such as TAGs are primarily located in the lipid bodies of rice bran (aleurone layer) and germ (embryo) fractions, while starch lipids are associated with starch granules in the rice endosperm (Fig. 1). Apart from free fatty acids, the major starch lipids are LPC and LPE, which account for about 50% of the starch lipid in non-waxy rice (Table 2) (Juliano, 1985).

With the exception of the sub-aleurone layer, no distinct lipid bodies have been found in the starchy endosperm (Bechtel & Pomeranz, 1978b). Lipids in the endosperm are composed of (a) non-starch lipid, present outside of starch granules, and (b) internal starch lipid (as 'true' starch lipid), forming inclusion complexes with amylose in starch granules (Fig. 1) (Moazzami et al., 2011; Morrison, 1981; Tester, Karkalas & Qi, 2004). Compared to other cereals such as maize and triticale, rice has small starch granules. Recent research has also shown that in the former, non-starch PLs may also be found in the channels within the starch granules (Naguleswaran, Li, Vasanthan & Bressler, 2011). However, this has not been investigated for rice starch granules which could be too small to have the 'channels' such as those found in the larger starch granules.

Internal (or integral) starch lipids, including LPLs (Azudin & Morrison, 1986), can only be extracted under rigorous conditions such as hot aqueous alcohol (Morrison &

158 Coventry, 1985). The amylose-lipid complex was initially suspected to be an artefact
159 formed during starch isolation. However, ^{13}C-cross-polarisation/magic angle spinning
160 nuclear magnetic resonance (^{13}C CP/MAS-NMR) analysis has confirmed the amylose-
161 lipid inclusion complexes exist *in vivo* in native rice starch granules (Morrison, Law &
162 Snape, 1993). It is believed that the cavity of amylose in single-helical (V) conformation
163 is a hydrophobic tube, and the hydrocarbon chain of the lysophospholipids lies stably
164 within the amylose helix, leaving the polar ends of the lipids outside of the helix cavity
165 (Fig. 2) (Godet, Tran, Delage & Buléon, 1993).

166 As each of the PLs may contain a different combination and distribution of fatty acid
167 esters, this could result in a large number of distinct PLs in rice. In general, rice bran PLs
168 contain palmitic (16:0, ~20%), oleic (18:1n-9, ~35%) and linoleic (18:2n-6, ~35%) acids
169 as the principal fatty acid components. The unsaturated fatty acids predominantly locate
170 at the *sn*-2 position and saturated fatty acids primarily occupy the *sn*-1 or *sn*-3 position
171 (Yoshida et al., 2011b). For the starch PLs in the endosperm, palmitic (48-63%) and
172 linoleic (25-42%) acids are predominant, with minor contributions from oleic (~5%) and
173 myristic (14:0, ~5%) acids (Maniñgat & Juliano, 1980).

174 The variety of different methods used for rice PL analysis is reflected in the
175 considerable degree of variance found amongst the reported values. Some reports also
176 suggest that PLs and other rice lipids may be associated with proteins such as oleosin or
177 nonspecific lipid transfer proteins in their native state, but this information is still limited
178 (Cheng, Cheng, Peng, Lyu & Sun, 2004; Chuang, Chen, Chu & Tzen, 1996; Huang, 1996;
179 Noda & Ikegami, 1966).

2.2 Phospholipid biosynthesis

Although understanding relevant biosynthetic and modification pathways and their regulation is critically important in order to modulate PL content, this has only been extensively studied in animal and yeasts, and we are far from having a clear understanding in plants including the model species *Arabidopsis thaliana* (Bessoule & Moreau, 2004; Eastmond, Quettier, Kroon, Craddock, Adams & Slabas, 2010). In rice, only a limited number of enzymes involved in PL biosynthesis pathways have been purified, or their corresponding genes cloned (Suzuki, 2011; Suzuki, Takeuchi & Shirasawa, 2011). The PLs in rice grain discussed here are primarily based in the context of the biosynthetic pathways summarised by Kinney (1993) (Fig. 3).

Rice PLs may be synthesised via two pathways: (a) cytidine diphosphate (CDP)-diacyglycerol and (b) 1,2-diacylglycerol (DAG) pathways (Fig. 3) (Kinney, 1993). Both CDP-DAG and DAG are derived from phosphatidic acid (PA), which are synthesised by the sequential acylation of glycerol-3-phosphate by acyltransferases.

Within the CDP-DAG pathway, CDP-DAG is produced from the reaction between PA and CTP. Sequentially, PS and PI are yielded by displacement of cytidine monophsophate (CMP) from the CDP-DAG with the hydroxyl group of serine or inositol. PS may be decarboxylated to PE by PS decarboxylase, and the PE may later be methylated to PC by the action of *N*-methyltransferases. PG is synthesised by a two-step reaction. Firstly, CDP-DAG reacts with glycerol phosphate to yield phosphatidylglycerolphosphate (PGP) and CMP. Secondly, the PGP is dephosphorylated to produce PG. The PG then reacts with CDP-DAG to yield DPG.

Within the DAG pathway, DAG is produced by dephosphorylation of PA catalysed by phosphatidate phosphohydrolase. PE and PC are produced by displacement of CMP from CDP-ethanolamine or CDP-choline with the hydroxyl group of DAG. In addition, PLs may also derive from head group exchange reaction with other PLs (Kinney, 1993).

The minor PL, N-acyl phosphatidyl ethanolamine (N-acyl-PE) may be yielded by reaction of PE with free fatty acid or acyl-CoA (Coulon, Faure, Salmon, Wattelet & Bessoule, 2012). As the main starch lipids, lysophospholipids (LPLs) are likely the hydrolysis products (by phospholipase A2, Fig 3) of diacylphospholipids. Another minor rice PL, phosphatidic acid (PA) is the precursors of other PLs and, at the same time, may also be a degraded product of PLs by the action of phospholipase D (PLD, Fig 3) (D'Arrigo & Servi, 2010).

During rice grain development, the level of total non-starch PLs plateaus (about 5mg/100grains) at 8 days after flowering (DAF) (Choudhury & Juliano, 1980b), which is likely due to the early synthesis of membrane lipids. Interestingly, the content of non-starch LPE and LPC increases up to 8 DAF, but subsequently decreases to the point that non-starch LPC is not detected beyond 16 DAF. In contrast, within the mature rice grain, the major internal starch lipids, LPLs (29 μg/grain), are significantly more abundant than their non-starch counterparts (4 μg/grain) (Choudhury et al., 1980b). This indicates that LPLs might be transferred from the amyloplast membrane (non-starch lipids) to the sites of starch synthesis, and so become starch lipids (Morrison, 1988). It has also been suggested that LPLs may form inclusion complexes with amylose which protect amylose from branching, perhaps by inhibiting branching enzymes, or breakdown, but not prevent its elongation (Morrison, 1988; Vieweg & Fekete, 1976). Therefore, starch LPLs and

their relationship with starch synthesis during rice grain development warrants further research.

2.3 Genetic and environmental influence on rice PLs

It is difficult to make meaningful comparisons of the values for PL composition and content presented in different reports, since there is no standardisation in terms of tissue sample, storage, pre-processing, and degree of milling. In order to identify and understand genotype by environment interactions (G×E) for rice PLs, a concerted research effort is required to assign components of variance to either genotype or environment.

Generally, non-starch PL content appears to be less variable between rice varieties than starch LPL content. Genetic differences between waxy and non-waxy varieties may dictate the level of starch LPLs in the grain (Azudin et al., 1986; Choudhury et al., 1980a; Morrison, Milligan & Azudin, 1984). Starch lipids and LPLs in particular, are consistently found to be significantly higher (10 times) in non-waxy rice varieties than waxy varieties, where on occasion these are hardly detectable (Azudin et al., 1986; Choudhury et al., 1980a; Morrison et al., 1984). The low content of starch LPLs in waxy rices could be attributed to the absence of amylose content. However, there is no clear correlation between amylose and starch LPL content in the non-waxy varieties (Morrison et al., 1984). A similar trend is also observed in other cereals such as maize, barley, sorghum and millet (Morrison et al., 1984). Environmental factors such as climate or soil, may also affect starch LPL content of non-waxy rice, but those factors have not been identified and evaluated (Morrison, 1988; Morrison & Azudin, 1987). The observed variation in starch LPL content could be attributable to the genetic composition of waxy

and non-waxy varieties, and closely associated with the pattern of *waxy* gene allelic variation.

Although non-starch PL content is relatively uniform (Mano et al., 1999; Yoshida et al., 2011a) *japonica* rice cultivars appear to contain PLs with higher polyunsaturated fatty acid (PUFA) content than *indica* cultivars (Mano et al., 1999). The PLs of *japonica* rice harvested from a cold growing environment have higher PUFA contents compared to a mild environment (Mano et al., 1999), suggesting that temperature during grain ripening may affect PUFA content. Similar environmental effects on the degree of unsaturation have also been identified for rice acidic glycerolphospholipids apart from cardiolipin (Choi et al., 2005). This higher unsaturation may be explained by membrane fluidity associated with cell functions required by the plant seed to survive in the colder environment. However, Nakamura et al. (1995) found higher PL PUFA contents in cold sensitive rice varieties (as measured by percentage seed sterility) (Nakamura, Ohnishi, Kojima, Mano, Inazu & Ito, 1995), suggesting the involvement of other mechanisms.

Within a recent report evaluating the unintended effects of transgenic rice containing an insect resistant gene, the growing environment was found to have a greater effects than genetic variation on levels of rice PLs such as LPC, LPE and lysophosphatidylglycerol (Chang et al., 2012). This effect had not been detected in previous studies, and it was suggested that the difference may be caused by abiotic or biotic stresses present within the different trials. However, as a non-targeted metabolomic approach was employed to evaluate the unintended effect, the rice samples had not been prepared specially for PLs analysis (detailed in Section 4). Therefore, the observed variation, which, based on the extraction method, is likely to have been in non-starch LPLs, may have been caused by

partial hydrolysis of diacylglycerolphospholipids catalysed by the native phospholipases in rice. A more concerted and targeted research effort is required to establish whether growing environment has a significant influence on rice PL content.

3. Phospholipids and rice quality

The quality of rice is determined by its suitability for specific end uses, and evaluation is based on a combination of subjective (consumer) and objective (quality testing) factors. Many factors affecting processes such as milling, cooking and eating quality have been reviewed and correlated with rice grain composition (Juliano, 2003; Zhao, 2009; Zhou, Robards, Helliwell & Blanchard, 2002). However, the role and relative importance of PLs as a factor in rice quality has been overlooked due to the emphasis on conventional factors such as starch composition. In this section we systematically discuss the relationship between degradation of PLs in the bran and rice storage stability, as well as the relationship between starch LPLs and starch physiochemical properties and digestibility that affect cooking and eating.

3.1 Phospholipids and rice storage

Following long term storage, paddy rice can develop an unacceptable stale flavour or smell, caused by the oxidation and decomposition of free unsaturated fatty acids, primarily oleic and linoleic, in the rice bran. These free fatty acids are released from TAGs, which are originally confined to the spherosomes, but ooze out due to the alteration of the spherosome (PL) membrane (Fig. 1). The major component of this membrane, phosphatidylcholine, may degrade to phosphatidic acid, catalysed by

phospholipase D. This deterioration of the PL membrane is considered to be a trigger for the degradation of rice lipids and associated rancid flavour (Aibara et al., 1986).

Freshly harvested rice grains are usually dried to reduce water content to <15% in order to suppress respiration, mould growth, and the multiplication of microorganisms. Natural or hot-air drying conventionally used for this post-harvest treatment may accelerate the breakdown of the PL membrane, and subsequently bring about the degradation of neutral lipids and associated stale flavour. By contrast, dehumidified-air drying may preserve the integrity of the PL membrane and suppress the deterioration of lipids during storage (Ohta, Aibara, Yamashita, Sekiyama & Morita, 1990). A recent report on the storage of paddy rice suggested that freshly harvested rice could be stored at low temperature (<20^{o}C or <15^{o}C) directly after harvest in order to preserve the freshness (Li et al., 2006), possibly by protecting the integrity of spherosomes.

The PL membrane may also be physically damaged during dehusking and milling. When paddy rice is dehusked to brown rice and further milled to white rice, the individual cells and spherosomes in the bran and germ are disrupted. TAGs leak from the damaged spherosomes, and come into contact with previously dormant but highly reactive lipases in the aleurone and germ tissues, significantly limiting the shelf life of nutritionally valuable rice bran and brown rice (Fig. 1) (Champagne, 1994; da Silva, Sanches & Amante, 2006). Some of these lipids (PL and TAG) and lipases may also be deposited on the surface of polished rice kernel, thus reducing the storage stability of white rice (Lam & Proctor, 2003; Lam et al., 2004). Compared to the well-milled white rice, under-milled rice has some residue of the bran layer and better nutritional quality.

However, also due to the additional residual and exposed bran lipid on the kernel surface, under-milled rice has very poor storage stability (Piggott, Morrison & Clyne, 1991).

Storage at low temperature (e.g. 9^{o}C) could reduce the rate of PL degradation and development of rancidity in polished and brown rice (Lam & Proctor, 2002; Tang, Zhang, Li, Tang & An, 2001; Yasumatsu & Moritaka, 1964). However, in order to avoid the degradation of rice lipids effectively, brown rice or rice ban has to be stabilised immediately after processing, either by using heat, such as microwave or parboil methods, to deactivate the lipase, or by removal of the kernel surface lipid with organic solvents, such as petroleum ether or ethanol (Champagne, 1994; da Silva et al., 2006; Ramezanzadeh, Rao, Windhauser, Prinyawiwatkul, Tulley & Marshall, 1999; Zhang, Zhou & Feng, 1998). As phospholipase D (PLD) is the most important enzyme involved in the degradation of spherosome membranes, a rice variety with PLD deficiency could significantly improve storage stability. A PLD-deficient rice mutant which arises from a phospholipase D null allele has recently been identified and a single-nucleotide polymorphism (SNP) marker for PLD deficiency developed (Suzuki, 2011; Suzuki et al., 2011). This strategy may also be applied to other cereal crops to solve similar storage problems. However, it is important to establish an exhaustive understanding of the storage stability and associated chemical properties in such mutant-derived materials.

Compared to non-starch PLs, internal starch LPLs are unlikely to be hydrolysed or oxidised during rice storage (Zhang et al., 1998). This resistance to degradation may be due to the protective structure of the amylose-lipid complex (Fig. 2). The specific effects of internal starch LPLs on rice storage should be investigated further in order to understand better what is required to maintain rice quality during storage.

337 3.2 Phospholipids and starch physicochemical properties

338 Monoacyl lipids, such as the internal rice starch LPLs, form a complex with amylose
339 and so change the physicochemical properties of starch (Putseys, Lamberts & Delcour,
340 2010), resulting in modified rice cooking and eating quality. Generally, PLs in rice may
341 reduce the iodine-binding capacity and swelling (Gelders, Goesaert & Delcour, 2006;
342 Morrison, 1995), although the increase in gelatinisation temperature, gel viscosity and
343 pasting temperature (Kaur & Singh, 2000; Maniñgat et al., 1980; Singh, Dartois & Kaur,
344 2010; Zhang et al., 1998) may not be completely isolated from other components in rice.

345 Iodine-binding capacity is a conventional colorimetric assay for measuring amylose
346 content. The presence of starch lipids (e.g. LPLs) interferes with iodine binding,
347 suggesting the existence of two types of amylose, namely lipid-complexed amylose
348 (LAM) and lipid-free amylose (FAM). FAM is equivalent to the apparent amylose
349 measured by iodine binding, while LAM corresponds to the difference between total
350 amylose and apparent amylose (Morrison, 1995).

351 Gelatinisation is a macro-molecular phenomenon during starch hydrothermal
352 processing and an important indicator of rice cooking quality. Studies of defatted rice
353 starch flour indicate that the presence of internal starch lipids (e.g. LPLs) may increase
354 starch gelatinisation temperature (GT) (Maniñgat et al., 1980). As the relationship
355 between amylose content and the amylose-lipid complex or LPLs in the rice grain is still
356 not clear, there is scope to investigate this relationship and perhaps manipulate starch
357 physiochemical properties including GT. Although the non-starch lipids remaining on the
358 brown, under-milled or milled rice kernel could also increase starch gelatinisation
359 temperature (Champagne, Marshall & Goynes, 1990; Marshall & Normand, 1990; Zhang

et al., 1998), the contribution of non-starch PLs compared to the major neutral lipids is unknown.

3.3 Phospholipids and starch digestibility

The global increase in the incidence of human Type 2 diabetes has stimulated research on the digestibility of the starch in high carbohydrate food, and low GI is now considered an important factor in rice quality. Rice grain is well known for its high starch content and has sometimes been considered a relatively high glycaemic index (GI) food, which may rapidly increase human blood glucose following consumption. However, it has been documented that consumption of rice with higher amylose levels gives significantly lower (*in vivo*) serum glucose and insulin responses (Miller, Pang & Bramall, 1992). This is consistent with the response to other cereal foods. The lower serum glucose response may be partially due to the amylose-lipid complexes, which reduce susceptibility of rice starch to hydrolysis by digestive enzymes such as α-amylase or α-glucosidase (also called glucoamylase) (Goddard, Young & Marcus, 1984; Shu, Jia, Ye, Li & Wu, 2009; Singh et al., 2010).

Although the association of amylose with LPL is yet to be proven, amylose complexed with monoacyl lipids, such as lysolecithin, has substantially lower susceptibility to α-amylase *in vitro* (Guraya, Kadan & Champagne, 1997; Holm et al., 1983). The complexed amylose may still be hydrolysed by an excess of enzyme. However, compared to free amylose, the digestion and adsorption of the complexed amylose would be slower *in vivo* (Holm et al., 1983). Rice amylose complexed with LPL also has strong *in vitro* resistance to the action of glucoamylase, which converts starch completely to glucose (Kitahara, Suganuma & Nagahama, 1996; Kitahara, Tanaka,

383 Suganuma & Nagahama, 1997). The adsorption of glucoamylase on the amylose
384 complexed with LPL is very low (Kitahara et al., 1996). Since it appears that enzyme
385 resistance of the amylose-lipid complex could be utilised to manipulate the GI for a range
386 of rice food products (Jaisut, Prachayawarakorn, Varanyanond, Tungtrakul &
387 Soponronnarit, 2008; Putseys, Derde, Lamberts, Ostman, Bjorck & Delcour, 2009), this
388 is worthy of further investigation. The bioavailability of the amylose-LPL complexes in
389 the human gastrointestinal tract is discussed further in Section 5.

390 **4. Quantification and analysis of rice PLs**

391 Due to its biological importance, methods for PL analysis from various organisms
392 have been well developed and reviewed (Brouwers, 2011; Christie & Morrison, 1988; Di
393 Stefano et al., 2012; Kasemsuwan & Jane, 1996; Kim, Wang & Ma, 1994; Peterson &
394 Cummings, 2006). Although classical thin layer chromatography (TLC) and gas
395 chromatography (GC) methods are still used in some recent PL research (Yoshida et al.,
396 2011a), liquid chromatography, especially high performance liquid chromatography
397 (HPLC) coupled with mass detector (MS), has gradually been recognised as a more
398 efficient and accurate method to analyse PLs and characterise food quality (Brouwers,
399 2011; Christie, 1985; Di Stefano et al., 2012). A considerable amount of experience and
400 knowledge has been accumulated from the analysis of PLs in cereals, and rice in
401 particular (Morrison, 1988; Morrison, Mann & Coventry, 1975; Morrison, Tan & Hargin,
402 1980). The procedures developed in these studies, such as deactivation of phospholipase
403 in the bran and use of hot aqueous alcohol for extraction of starch PLs, must be
404 considered prior to any experimental design, even when using the most advanced
405 analytical instruments. If not taken into account, there is a danger that artefacts of PLs,

406 contamination between the extractions or incomplete extraction will occur and lead to
407 confusing results (Chang et al., 2012; Liu, 2009; Liu et al., 2011). This section provides a
408 commentary on both the classical and more recent analytical procedures for the analysis
409 of PLs in rice and other cereals. For more detailed review of PL analysis in general, the
410 systematic methodology reviews of Brouwers (2011) and Christie (1985) are
411 recommended.

412 4.1 Extraction of PLs

413 Since the nature of starch and non-starch PLs is significantly different, those in the
414 rice bran and endosperm are usually extracted and analysed separately. For non-starch
415 lipid, it has been emphasised that inactivation of lipolytic enzymes prior to extraction is
416 important to avoid lipolysis and formation of artefacts (Morrison, 1982). Lipolytic
417 enzymes are present and active in cereal grains, but generally dormant before germination.
418 Processes such as milling and solvent extraction disturb the native structure of cells in the
419 germ and aleurone layer, and release lipolytic enzymes (D'Arrigo et al., 2010; Morrison,
420 1982; Morrison et al., 1980). For example, phospholipase D is often activated during
421 milling and extraction, and is able to catalyse the transformation of phosphatidylcholine
422 (PC), phosphatidylethanolamine (PE) and phosphatidylinositol (PI) to phosphatidic acid
423 (PA) (Liu, 2009).

424 Inactivation of lipolytic enzymes can be achieved by boiling samples in water for 8-
425 10 min (Morrison, 1982) or steaming for 2-3 min (100^{o}C) (Fujino, 1978). However, we
426 suspect that these hydrothermal processes may also transform some of the non-starch
427 lipids to starch lipids, which requires further investigation. Extraction at low temperature
428 has also been used to avoid the action of lipolytic enzymes. The rice bran lipids,

429 including PLs, may be safely extracted using chloroform/methanol (2:1, v/v) at 0^{o}C with
430 addition of 0.01% butylated hydroxytoluene (BHT) to inhibit the oxidative degradation of
431 lipids during analysis (Yoshida et al., 2011b). However, whether this cold solvent
432 extraction could exhaustively extract the non-starch lipid is currently unknown. Other
433 solvents such as diethyl ether, petroleum ether and water-saturated butanol (WSB) have
434 also been used to extract non-starch lipids (Choudhury et al., 1980b; Zhou, Blanchard,
435 Helliwell & Robards, 2003). However, petroleum ether and diethyl ether are relatively
436 non-polar solvents and may not give an efficient extraction of relatively polar PLs
437 (Yoshida et al., 2011b). Conversely, WSB at 20-30^{o}C is relatively polar and can easily
438 extract the non-starch lipids, but tends also to extract some of the starch lipids from the
439 starch granules damaged by milling (Choudhury et al., 1980b).

440 To measure the native non-starch PLs accurately, we recommend that the rice grain
441 should be stored at 4^{o}C directly after harvest to avoid degradation of PLs during storage.
442 The rice bran fraction or brown rice should be analysed immediately after dehusking or
443 milling, as the PLs are exposed and vulnerable to lipolysis. The decision as whether to
444 use heat treatment or cold solvent extraction should be carefully evaluated, and applied
445 according to the purpose of research. Since rice PLs contain polyunsaturated fatty acids
446 (linoleic and linolenic), they are liable to undergo autoxidation. PL extracts or fractions
447 should be stored at a low temperature (e.g. -20^{o}C) and kept under nitrogen, ideally, with
448 addition of antioxidants such as BHT (Christie, 1985).

449 Since rice starch LPLs are protected by the impermeable starch granules and form
450 inclusion complexes with amylose (Fig. 1 and 2), they are relatively stable in the grain,
451 and not readily extracted by organic solvents at room temperature. Interestingly,

compared with the classic "defatting" by lipophilic organic solvents, efficient extraction of internal starch LPLs requires both water and heat to swell (or gelatinise) the native starch granule to permit the alcohol to penetrate and extract the lipids. Although cold WSB (2-4^{o}C) is able to extract some of the starch lipid it takes a very long time, estimated 2.5x10^{5}hr, to complete the extraction (Morrison, 1981). When there is insufficient water to swell the starch (e.g. using 90% methanol), the extraction of internal starch lipids is as ineffective as using cold or room temperature WSB (Choudhury et al., 1980b; Morrison et al., 1985; Morrison et al., 1975). Other aqueous alcohols such as ethanol, n-propanol and isopropanol have also been tested for extraction of starch lipids. It has been suggested that the ideal conditions for complete extraction of rice starch lipids are two 2-hr exactions and one 1-hr extraction at 100^{o}C, with not less than 16ml of 75% n-propanol per gram of starch (Morrison et al., 1985).

Starch LPLs may be extracted and measured after removal of the non-starch lipids (Morrison et al., 1980). However, as lipids on rice protein bodies are not as readily removed as other non-starch lipids, a protein fraction with the non-starch lipid attached is also sometimes removed before the extraction of internal starch lipids. Rice starch can be suspended in aqueous 1.2 or 2% sodium dodecylbenzenesulfonate to remove lipoprotein adhering to the surface of the starch granules (Ito, Sato & Fujino, 1979; Maniñgat et al., 1980). The protein can also be removed through proteolytic digestion with amylase-free protease (Azudin et al., 1986; Morrison, 1985.; Morrison et al., 1984).

4.2 Column chromatography, TLC and GC analysis of PLs

The classical method of rice PL analysis involves separating and identifying PLs using a silica column or TLC (silica), followed by analysis of the fatty acid composition

using GC (Fig. 4). Such methods have been routinely used over the past 30 years (Miyazawa, Yoshino & Fujino, 1977; Morrison et al., 1980; Yoshida, Tomiyama, Mizushina & Yoshida, 2012). However, these methods are time- and solvent-intensive and lack accuracy and sensitivity. These classic techniques such as multi-dimensional development for TLC (Fig. 4) seem to be simple, but require experienced and highly-skilled operators to produce reliable results.

Silica columns can be used to separate rice PLs effectively from other lipids (Christie, 1985; Fujino, 1978; Glushenkova et al., 1998; Hemavathy & Prabhakar, 1987; Miyazawa et al., 1977; Vasanthan & Hoover, 1992). For example, after loading total lipids, the silica column is eluted sequentially with chloroform (simple lipid fraction), acetone (glycolipid fraction) and methanol (PL fraction). The PL fraction may be further separated into different classes such as PE, PC and PI by separation on another silica column with stepwise elution: PE can be eluted with chloroform/methanol (8:2 and 7:3. v/v), PC with chloroform/methanol (7:3 and 2:1, v/v) and PI with chloroform/methanol (2:1 and 1:2, v/v) (Miyazawa et al., 1977).

Currently, silica TLC is still widely used to separate and analyse rice PLs (Fig. 4) (Fujino, 1978; Glushenkova et al., 1998; Ito et al., 1979; Vasanthan et al., 1992; Yoshida et al., 2011b; Yoshida et al., 2012). For example, PLs can be separated from other rice lipids using preparative TLC with n-hexane/diethyl ether/acetic acid (80:20:1, v/v/v) as the mobile phase. Separated PLs can be further fractionated by another TLC into PE, PC and PI with chloroform/methanol/acetic acid/ deionised water (170:30:20:7, v/v/v/v) as the mobile phase and detected by iodine vapour. Two-dimensional silica TLC can be used to analyse the PLs in detail on a single plate, for instance, developed by

498 chloroform/methanol/ammonia (65:35:5, v/v/v, 1st) and chloroform/acetone/methanol/
499 acetic acid/water (10:4:2:2:1, by volume, 2nd) (Fig. 4). All the major PL species in Table
500 1 are able to be visualised through two dimensional TLC (Fig. 4) (Aibara et al., 1986;
501 Fujino, 1978).

502 Following separation by column chromatograph or TLC, the composition of fatty
503 acids in PLs is normally analysed using a standard "free fatty acid methyl ester (FAME)"
504 method by GC. For example, PLs may be scraped from the TLC plate into a test tube and
505 heated for 20min at 80°C in BF_3/methanol to produce the FAME for GC analysis
506 (Yoshida et al., 2012). To identify the positional distribution of the fatty acids, a lipase-
507 catalysed hydrolysis has been used to partially deacylate the PLs, by selectively removing
508 the fatty acid esterified to the *sn*-1 (or *sn*-3) position on the glycerol backbone. The
509 partial hydrolysates are then separated and converted to FAME for GC analysis to
510 elucidate the fatty acid distribution (Liu et al., 2011; Miyazawa et al., 1977).

511 4.3 HPLC-MS analysis of PLs

512 HPLC-MS is the fastest growing sector among analytical methodologies in food
513 analysis due to its superior sensitivity and reproducibility. While reports for food analysis
514 using GC-MS have doubled in the last decade, those using HPLC-MS have increased
515 nearly tenfold in the same period (Di Stefano et al., 2012). Advanced analytical
516 instruments such as HPLC, UHPLC (ultra HPLC) and nano-HPLC, coupled with MS and
517 MS/MS have become more accessible and popular in the agrifood sector. Many PL
518 classes of biomembrane can be separated by HPLC and detected by MS or MS/MS
519 detectors, with better precision and reproducibility (Brouwers, 2011; Chang et al., 2012;
520 He et al., 2007; Liu et al., 2011; Peterson et al., 2006). Using HPLC-MS to analyse intact

PLs in rice can significantly shorten the pre-cleaning steps and avoid the derivatisation procedures required by GC or GC-MS analysis (Fig. 4).

In the 1980s, due to the limited development of HPLC stationary phase, a silica gel column (normal phase) was often used for the separation of PLs (Christie et al., 1988; Demandre, Tremolieres, Justin & Mazliak, 1985; Jungalwala, Evans & McCluer, 1984; Palacios & Wang, 2005). Several mobile phase systems have been developed to separate PLs on silica gel columns. These include (1) gradients of hexane-isopropanol-water (Demandre et al., 1985), (2) isocratic elution with acetonitrile-methanol-sulphuric acid (Christie, 1985) and (3) a complex ternary gradient election containing mixture of hexane, butan-2-one, acetic acid, chloroform, isopropanol, serine, and ethylamine (Christie et al., 1988). Ultraviolet (UV) detectors have been used for detection of PLs. As PLs have very limited chromophores, UV detection has to be operated at 200-205 nm, which is usually complicated by strong background noise from the solvents and significantly large peaks caused by traces of oxidised PLs (Christie, 1985).

During the last 15 years developments of U-HPLC and advanced stationary phase, reverse phase HPLC coupled with MS or MS/MS detectors has shown its superiority for the analysis of PLs (Brouwers, 2011; Chang et al., 2012; He et al., 2007). In PL research for animal sources, this method has identified 163 PLs in plasma and 400 PLs in liver (Hu et al., 2008; Retra, Bleijerveld, van Gestel, Tielens, van Hellemond & Brouwers, 2008). The MS/MS detector can help to identify the PL fatty acids and their distribution in a single HPLC run. Recent metabolomic research of transgenic rice has identified significant variation in lysophosphatidylethanolamine levels using HPLC-MS/MS (Chang et al., 2012). The HPLC-MS/MS method should be further refined to analyse the rice PLs

544 and other cereals in a greater detail, which will help to reveal the biosynthetic pathways
545 and processing of PLs in rice and the relative contribution of genetic and environmental
546 factors.

547 **5. Health benefits of PLs in rice**

548 Dietary glycerophospholipids have been extracted from soybeans, egg yolk, milk and
549 marine organisms such as fish and krill, and have been sold commercially as supplements
550 for decades. Although the mechanisms for the beneficial mode of action remain unclear,
551 the beneficial health effects have been well publicised, and thus accepted by the public
552 (Kullenberg et al., 2012). The potential health benefits include: a) regulating the
553 inflammatory reaction, e.g. arthritis (Hartmann et al., 2009); b) inhibiting tumour growth
554 and metastasis (Jantscheff et al., 2011; Sakakima, Hayakawa, Nagasaka & Nakao, 2007);
555 c) lowering cholesterol and cardiovascular risks (Wójcicki, Pawlik, Samochowiec, Kaldo
556 ń ska & Myśliwiec, 1995); d) enhancing learning and memory (Nagata, Yaguchi &
557 Nishizaki, 2011); e) improving immunological functions (Jannace, Lerman, Santos &
558 Vitale, 1992); f) treating hepatic disorders (Gundermann, Kuenker, Kuntz & Drozdzik,
559 2011). However, most of the health benefit claims flow from *in vitro* or in animal models
560 and so more *in vivo* human study is needed (Kullenberg et al., 2012).

561 Although rice bran and brown rice are considered nutritious and healthy foods
562 (Lamberts et al., 2007), the contribution of rice PLs to this enhanced quality has not been
563 highlighted or investigated, which may be partially due to the lower concentration of PLs
564 in rice (about 0.3% in brown or white rice) (Juliano, 1985) compared with other rich
565 sources (e.g. 10% egg yolk) (Palacios et al., 2005). However, as rice is a staple food

(208-578g/day) in many Asian countries (Abdullah, Ito & Adhana, 2006), the contribution of PLs from consuming rice could reach up to 1.8 g/day in these populations, representing a significant contribution to their normal dietary intake of PLs (2–8 g per day) (Cohn, Wat, Kamili & Tandy, 2008). The PLs represent 10% (in the bran) to 50% (in non-waxy white rice) of total lipids in rice, higher than PLs in the total daily fat intake (1-10%) (Cohn et al., 2008). Thus, rice could be considered a more accessible food source for dietary PLs. Rice non-starch PLs may also be concentrated and purified as a nutraceutical product arising as a side-product of the degumming process for rice bran oil, and so could add value to the large quantity of rice bran produced each year (Ghosh, 2007).

To date, most rice is still consumed in its "whole milled rice" form, as white rice. A recent study indicates that higher consumption of white rice is associated with a significantly increased risk of Type 2 diabetes, especially in Chinese and Japanese populations (Hu, Pan, Malik & Sun, 2012). The combination of the type of rice and processing and cooking methods may affect the starch structure and chemistry of the cooked rice, resulting in variation of rice GI and thus the risk of Type 2 diabetes. Rice starch LPLs may play an important yet unknown role in this system.

Although the amylose-LPL complex in rice may have little or no effect on the bioavailability of both starch and LPLs in the human gastrointestinal tract (Chung, Liu, Wang, Yin & Li, 2010), it may slow their rate of digestion and adsorption (Holm et al., 1983). Starch (e.g. amylose) alone is hydrolysed by the enzymes, such as salivary α-amylase, pancreatic amylase and brush border glucoamylase of the intestine, into glucose and absorbed in the small intestine (Singh et al., 2010). LPLs alone are solubilised by bile

589 salts and directly absorbed in the small intestine (Goodman, 2010). The consumption of
590 high amylose rice reduces the intensity of serum glucose and insulin responses (Goddard
591 et al., 1984). Goddard et al. (1984) speculated that the reduction could be caused by
592 differential enzymatic hydrolysis of amylose and amylopectin, and the presence of starch-
593 lipid complexes in the amylose-containing rice. This delay of digestion and/or absorption
594 of carbohydrate may be explained by reduced susceptibility of amylose-lipid complexes
595 to hydrolysis by the digestive enzymes (as discussed in Section 3.3) (Kitahara et al.,
596 1996). Consumption of the natural starch-LPL complex in rice may be more beneficial to
597 diabetics than simply coingestion of more fat with carbohydrate because the latter only
598 reduces the serum glucose response but not the serum insulin response (Collier & O'Dea,
599 1983).

600 Relatively “sticky” rice, with lower amylose content, is more preferable in China and
601 Japan than in South Asian Countries, such as India (Champagne et al., 2010). Significant
602 research effort has been dedicated to developing low or intermediate amylose rice
603 varieties to satisfy this preference. It is well known that the GI of low-amylose rice is
604 significantly higher than the high-amylose rice (Miller et al., 1992). The preference for
605 eating this low-amylose rice, with little or no starch LPLs, may partially contribute to the
606 increased risk of Type 2 diabetes in Chinese and Japanese rice consumers. Interestingly,
607 the high-amylose rice varieties with similar amylose content differ significantly in starch
608 digestibility and glycaemic response in humans (Panlasigui, Thompson, Juliano, Perez,
609 Yiu & Greenberg, 1991), which has been attributed to the different physicochemical
610 properties of the grain from each rice variety. As discussed in Section 3.2, starch lipids,
611 including LPLs, can influence the physiochemical properties of rice starch. Therefore, we

612 suggest that this differential glycaemic response of rice with similar amylose content may
613 in part be caused by different content, species or unsaturation of the starch LPLs
614 complexed with the amylose (Kitahara et al., 1996). As such, this warrants further
615 investigation.

616 **6. Conclusion**

617 Phospholipids in rice grain represent only a minor proportion of available nutrients
618 compared with starch and protein, but significantly influence rice quality, especially
619 when complexed with amylose. To date, most understanding of rice PLs has been based
620 on thorough studies carried out from the mid-1970s to the mid-1990s by a few dedicated
621 researchers (e.g. Morrison, W. R. and Juliano, B. O.), based on the technology and
622 instruments available at that time. Following the development and application of new
623 technologies and analytical instrumentation, such as UHPLC-MS/MS and next-
624 generation sequencing, the efficiency of chemical and genetic analysis has improved by
625 several orders of magnitude in the last decade, which could be harnessed to re-assess rice
626 PLs in greater detail. This may help us to understand the relationship between
627 biosynthesis of amylose, amylopectin and PLs, and reveal the biological function of LPLs
628 in rice starch formation.

629 Based on this review, it is plausible that both starch and non-starch rice phospholipids
630 may have significant impacts on the traditional (storage, cooking and eating) and modern
631 (GI) concepts of rice qualities, but much more research is needed. For non-starch PLs,
632 future investigation should focus on the optimisation of postharvest conditions and
633 processing methods to stablise PLs for better rice storage. For starch LPLs, research
634 priority should be given to establishing the contribution of genetic variation and

cultivation environment to the content and composition of LPLs complexed with amylose. The mechanism and contribution of starch LPLs to low GI should be given particular attention.

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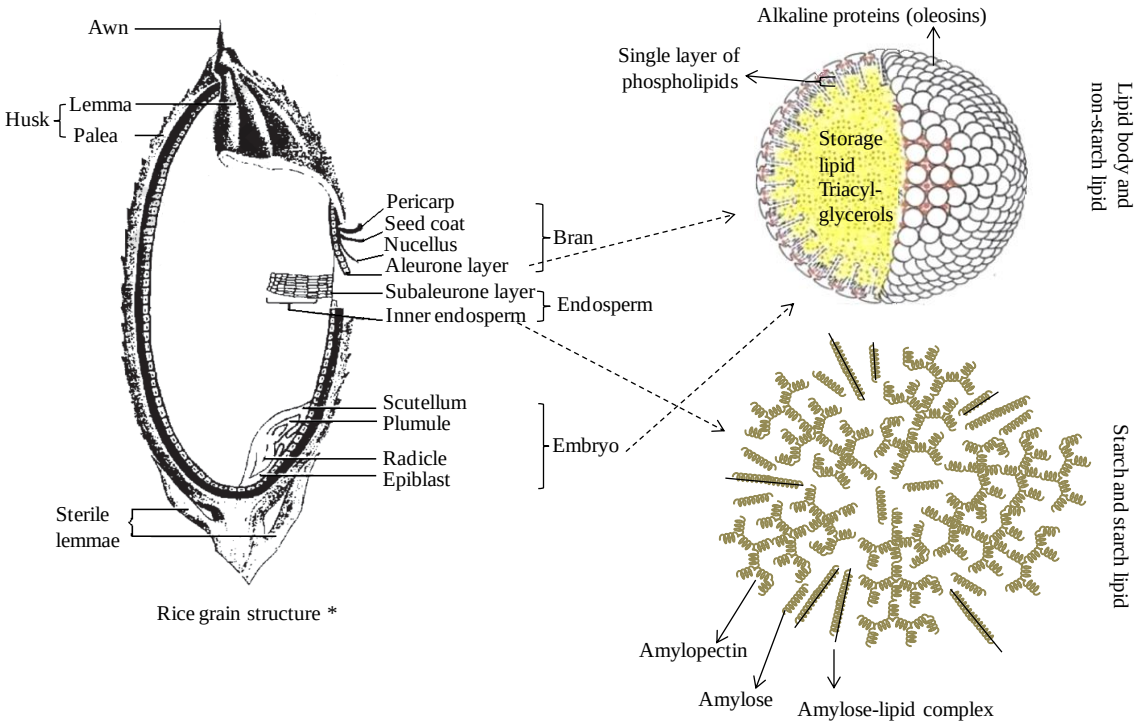
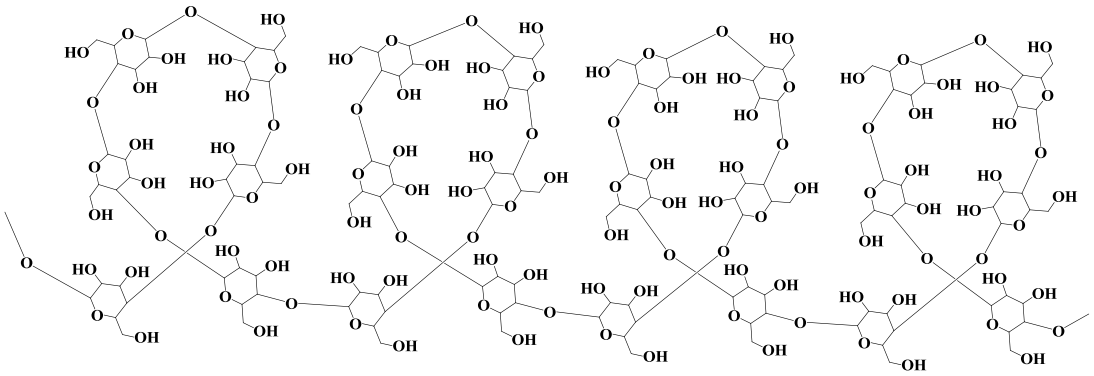
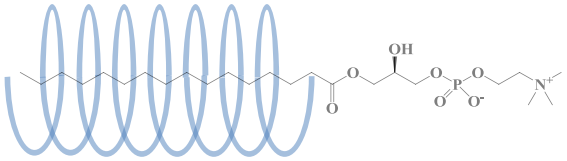


970

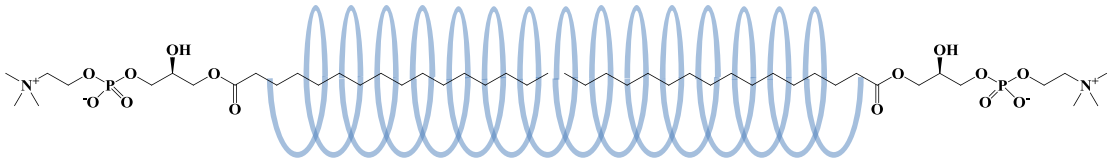
971 **Figure 1. Longitudinal section of the rice grain and distribution and illustration of**
972 **starch and non-starch lipids.**
973 *Rice grain structure redrawn based on Bao (2012)
974



(a) Illustration of an amylose helix (left-handed)*



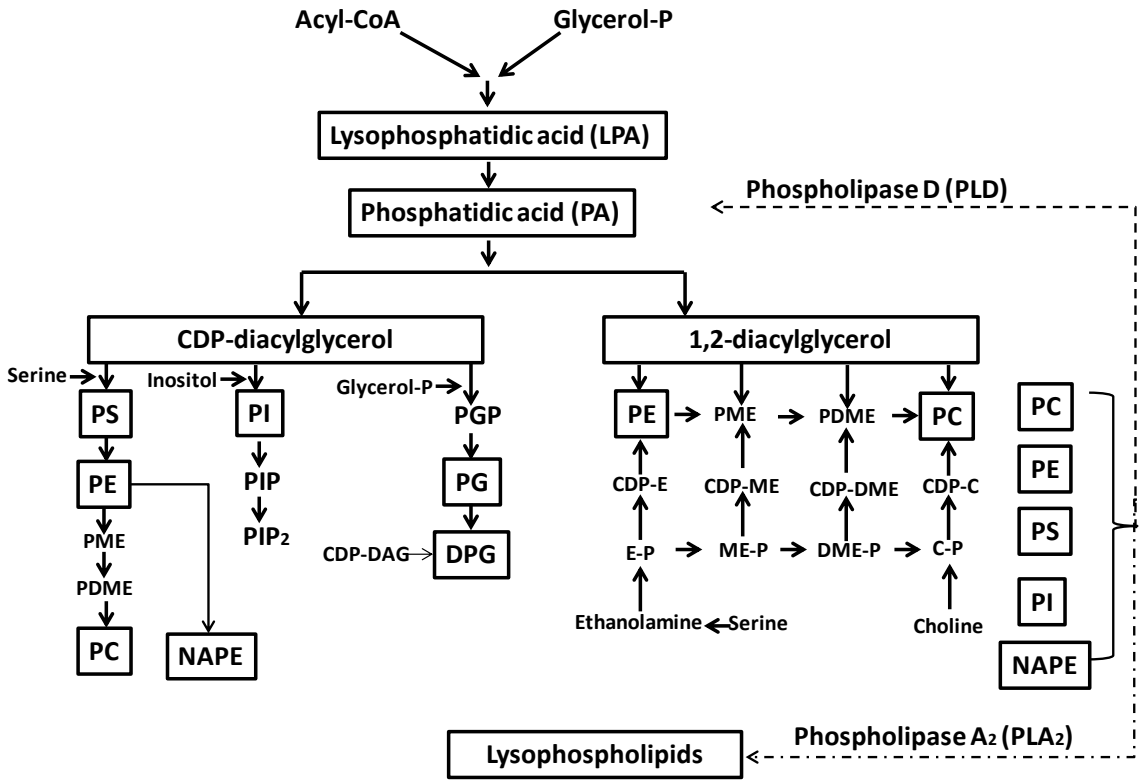
(b) Schematic representation of a complex of amylose with one lysophosphatidylcholine*



(c) Schematic representation of a complex of amylose with two lysophosphatidylcholines*

Figure 2. Illustration of an amylose helix (a) and schematic representation of two amylose-lysophosphatidylcholine complex (b) and (c).

*(a) redrawn based on Gessler et al. (1999); (b) redrawn based on Juliano (1985); (c) redrawn based on Copeland, Blazek, Salman and Tang (2009).



982

983 **Figure 3. Potential biosynthetic pathways of phospholipids***

984 *Redrawn based on (D'Arrigo et al., 2010; Kinney, 1993); E: ethanolamine; M: methyl; DM: dimethyl; C: choline; P: phosphate; CDP:
985 cytidine diphospho; PE: phosphatidylethanolamine; NAPE: N-acyl phosphatidylethanolamine; PME: phosphatidylmethylethanol-
986 amine; PDME: phosphatidyl dimethylethanolamine; PC: phosphatidylcholine; PS: phosphatidylserine; PI: phosphatidylinositol; PIP:
987 phosphatidylinositol phosphate; PIP2: phosphatidylinositol bisphosphate; PGP: phosphoglycerol phosphate; PG: phosphatidylglycerol;
988 DPG: diphosphatidylglycerol.

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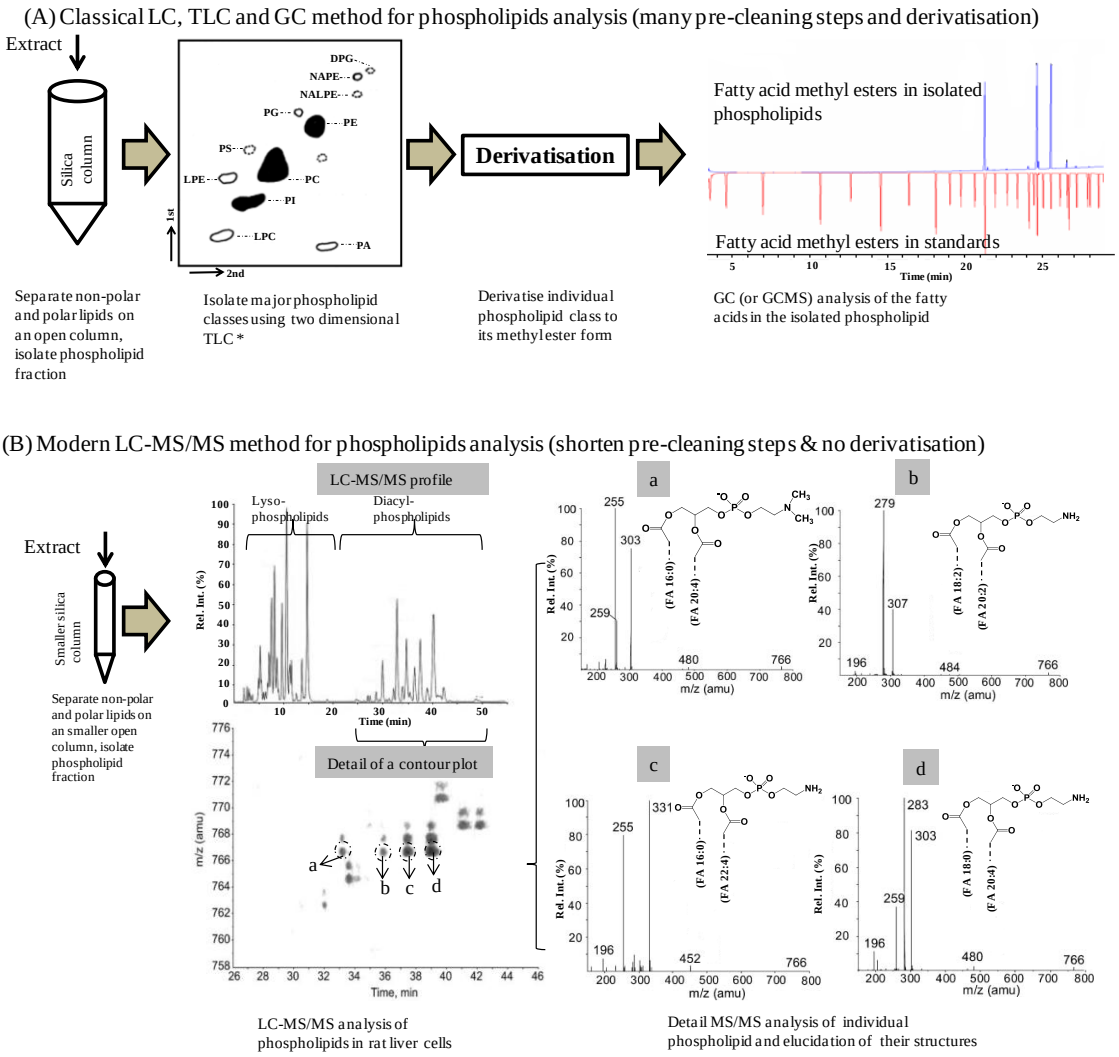
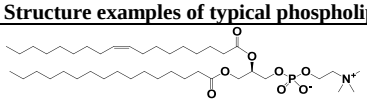
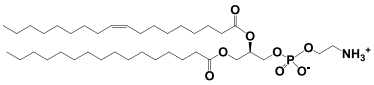
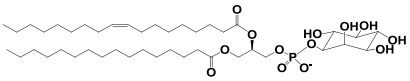
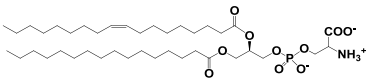
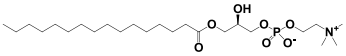
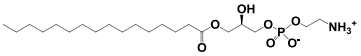
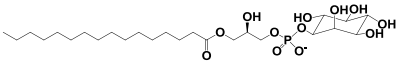
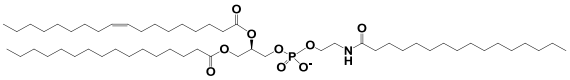
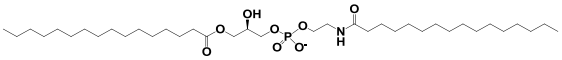
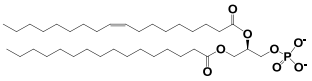
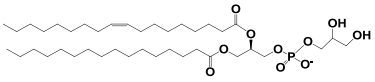
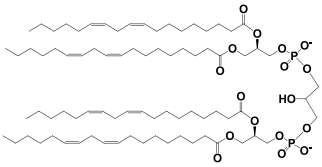


992

993 **Figure 4. Illustration of classical TLC, GC method and modern HPLC-MS/MS**
994 **method for phospholipid analysis.**
995 * TLC and HPLC-MS/MS redrawn based on Fujino (1978) and Retra et al. (2008); Phospholipid abbreviations are same as in Fig 2.
996

997 **Table 1. Major phospholipid classes in rice and their structures**

Glycerophospholipids (GPL)	Structure examples of typical phospholipids
Phosphatidylcholine (or lecithin, PC)	 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine
Phosphatidylethanolamine (or cephalin, PE)	 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine
Phosphatidylinositol (PI)	 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoinositol
Phosphatidylserine (PS)	 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoserine
Lysophosphatidylcholine (LPC)	 1-palmitoyl-*sn*-glycero-3-phosphocholine
Lysophosphatidylethanolamine (LPE)	 1-palmitoyl-*sn*-glycero-3- phosphoethanolamine
Lysophosphatidylinositol (LPI)	 1-palmitoyl-*sn*-glycero-3-phosphoinositol
N-acyl phosphatidylethanolamine (NAPE)	 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho(N-palmitoyl) ethanolamine
N-acyl lysophosphatidylethanolamine (NALPE)	 1-palmitoyl-*sn*-glycero-3-phospho(N-palmitoyl)ethanolamine
Phosphatidic acid (PA)	 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphate
Phosphatidylglycerol (PG)	 1-palmitoyl-*sn*-glycero-3-phosphor-*rac*-(1-glycerol)
Diphosphatidylglycerol (or cardiolipin, DPG)	 1,3-*bis*(1', 2'-dilinoleoyl-*sn*-3'-phosphatidyl)-*sn*-glycerol

998

999 **Table 2. Starch and non-starch lipids in rice[a]**

Property	Brown rice[n]	Bran	Embryo	Polish	Subaleurone layer	Inner endosperm[o]
Wt. % of brown rice	100	5.9-6.4	1.3-1.5	4.1-4.4	4.9-5.2	82.5-83.8
NSL[b] content (% dry basis)	2.9-3.4	19.4-25.5	34.1-36.5	10.2-15.0	5.6-8.5	0.41-0.81
PL[c] in NSL (%)	8-9	7-8	6-7	8-9	8-12	12-17
NSL-PL[d] classes (%)						
NSL-PC[e]	4	3-4	3-4	3-4	4	3-5
NSL-PE[f]	3-4	3	3-4	3	3-4	3-5
NSL-LPE[g]	<1	<1	<1	<1	1-2	2-4
SL[h] content (% dry basis)	0.21-0.76	-	-	-	-	0.12-0.57
PL in SL (%)	37-54	-	-	-	-	24-56
SL-PL[i] classes (%)						
SL-PC[j]	5	-	-	-	-	3-5
SL-PE[k]	3-4	-	-	-	-	3-4
SL-LPC[l]	18-23	-	-	-	-	13-24
SL-LPE[m]	15-21	-	-	-	-	12-23

1000 [a] Values summarised from Juliano, B. O. (1983); [b] NSL for non-starch lipids; [c] PL for phospholipid; [d] NSL-PL for non-starch
1001 phospholipids, [e] NSL-PC for non-starch phosphatidyl choline; [f] NSL-PE for non-starch phosphatidyl ethanolamine; [g] NSL-LPE for
1002 non-starch lysophosphatidyl ethanolamine; [h] SL for Starch lipid; [i] SL-PL for starch phospholipid; [j] SL-PC for starch phosphatidyl
1003 choline; [k] SL-PE for starch phosphatidyl ethanolamine; [n] SL-LPC for starch lysophosphatidyl choline; [m] SL-LPE for starch
1004 lysophosphatidyl ethanolamine; [n] Starch PLs in brown rice were summarised based on data from of three rice varieties with different
1005 amylose content: 2% for IR4445-63-1 (waxy), 24% for IR480-5-9, and 29% for IR 42; [o] Starch PLs in 82-84% milled rice of the same
1006 varieties as above.