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IMPROVING THE ECONOMIC VALUE OF RICE BRAN OIL

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ABSTRACT

Cereal grains are usually considered to be sources of carbohydrate but they can also contain oil which is of considerable economic value. Rice oil is obtained from the bran produced by polishing brown rice. Key genes which control rice bran oil composition have been identified but oil quality is also affected by the rapid breakdown of the triglycerides following polishing. Several lipases in the bran have been identified by proteomic approaches. However, DNA sequence identity searches indicate that over a hundred putative lipase genes can be identified in the rice genome although it is not clear how many of these putative genes are expressed in the bran. A concerted research effort therefore is required to identify the lipases that are responsible for oil breakdown in the bran.

Key words: lipase, embryo, aleurone, cereal, grasses

INTRODUCTION

Cereals provide most of the world's calories through the starch that is present in the grain. The grain also contains lipids and these are of increasing importance, as ways to maximize the value of crops, are investigated. Furthermore grasses, which include cereals, are being considered as a source of biodiesel.

Oils from plants are primarily composed of triacylglycerols (TAGs), which have three fatty acid chains attached to the glycerol backbone through ester links. TAGs are stored in oil bodies which are surrounded by a monolayer comprising phospholipids and a diverse array of proteins, predominant among which are proteins termed oleosins. However, the main constituents of lipid bodies are TAGs (90 to 95%) (Ohlrogge and Browse, 1995).

Plant oils are an important component of our diet, as they serve 20-25% of the daily nutritional calorie intake in humans (Katan *et al.,* 1995). Plant oils are also used in chemical industries (e.g. in detergents, paints, inks, and plasticizers), food industries (e.g. in margarines, salad oils, fried foods) and also bio-based industrial formulations, like lubricants and drying oil (Lu *et al.,* 2011). Biodiesel production from plant oils is one of the main non-food applications (Emiliani and Pistocchi, 2006; Ramadhas *et al.,* 2005). Plant oil production has increased by nearly 50% overall over last decade to meet the increasing demand (Table 1). Naturally there is great demand for improvement and enhancement of plant oils.

Plant oil types

Plant oils are grouped into two major classes namely, essential and fixed oils. Most of the fixed oils are derived from fruit or seeds of plants (e.g. soybean, rapeseed, cottonseed, sunflower seed, groundnut, palm, copra, sesame, linseed & castor seed, maize and coconut oils) (http://www.fas.usda.gov/cots/oilseed). Apart from seeds and fruits, plant oils are also extracted from the flowers, leaves, stems, bark and roots of herbs, bushes, shrubs and trees through distillation. The remainder of the review will concentrate on oils from seeds.

Among major oilseed crops, soybean is biggest source of edible oils followed by canola, and sunflower (Wilcox, 2004). However, palm oil is largest source overall. Maize is the most widely used cereal for the production of oils. Rice oil production is much lower (Table 1). The production of oil from the other cereals is very minor.

Accumulation of seed oils

In the seeds, lipids accumulate in the embryo or endosperm, depending on plant species (Baud and Lepiniec, 2010).

For eudicots, which include important oilseeds like soybean (Glycine max), sunflower (Helianthus annuus), linseed (Linum usitatissimum), safflower (Carthamus tinctorius) and the Brassicaceae,

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Table 1. Production of the major plant oils

Oil	2002	2007	2012
Palm oil	26.1	39.7	50.1
Soybean	29	37.4	40
Rapeseed	13.3	17.9	23.5
Sunflower	7.8	11.1	15
Palm kernel	3.3	4.9	5
Maize oil	1.9	2.2	2.3
Estimated rice bran oil production	.7	.9	1.05

Quantities in millions of tonnes.
Source: FAOSTAT (http://faostat3.fao.org/faostat-gateway/go/to/browse/Q/*/E). Rice bran oil production is estimated based on rice production and proportion converted to oil from the bran currently.

embryonic tissues present between the integuments of the seed are major site of oil deposition. However, in castor bean, another important eudicot oilseed, the large endosperm tissue is the main oil storage tissue.

In cereals, which are monocots, both the embryo and the endosperm can be major sources of seed oil (Table 1).

Edible oils from monocot grasses

In cereals, which are monocot grasses like maize (*Zea mays*) and rice (*O. sativa*) the main product of the grain is starch. However, maize and oats are also good sources of plant oil as over 10% of the grain weight is oil (Morrison, 1977; Leonoval *et al.,* 2010). In other cereals such as rice, wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) oils constitutes only 2-3% of total dry weight of grain (Fincher, 1989) but can be obtained in much higher proportions (approximately 20%) in the bran fraction that results from processing of the grain. In most of the cereals, oil is mainly stored in embryo and the aleurone which are minor parts of the seed but in oats (*Avena sativa*) the majority of lipids are stored in the endosperm (Leonova *et al.,* 2010). In terms of oil production from cereals, maize and rice are the only cereals so used to any significant degree. Although oats is rich in oil, the production of oats is very low compared to these two cereals, amounting to only about 23 million tonnes in 2013 compared to about 720 million tonnes for rice and over 1 billion tonnes for maize (http://faostat3.fao.org/faostat-gateway/go/to/browse/Q/QC/E). Oil from rice and maize are discussed in greater detail below.

RICE

Rice grains contain a much smaller proportion of lipids than starch (Tokuşoğlu and Hall III, 2011). Milled rice from which bran layer is removed has about 0.2% of lipid content when compared to brown rice having about 1-4% of lipid content (Juliano and Bechtel, 1985). Lipids in rice grain are stored as spherosomes of <1.5μm in the aleurone layer, <1μm in the sub-aleurone layer and <0.7μm in the embryo. The distribution of lipids is quite uneven as the outer layers of rice kernels (that is in the bran including the germ) have larger amounts of lipid than the inner parts (that is in the core or inner endosperm) (Bechtel and Pomeranz, 1977; Bechtel and Pomeranz, 1978; Juliano, 1983). Moreover three dimensional distribution analyses of lipids in rice grain showed more lipid on the dorsal than the ventral side of rice grain (Ogawa *et al.,* 2002).

Rice bran oil

Rice bran oil (RBO) is in increasing demand a popular cooking oil in several Asian countries (Sugano and Tsuji, 1997; Ghosh, 2007). The proportion and composition of rice bran oil can vary somewhat depending on the type of rice that is milled. Lipid content is higher in purple/black rice (12-13%) as compared to brown rice where it ranges from about 3-4% on dry weight basis (Frei and Becker, 2005). There appears to have been no effort to breed rice lines with increased oil content because of the focus on increasing grain yield through increasing starch content.

RBO is mostly triglyceride but also contains compounds such as oryzanol and tocotrienes having antioxidant and hypocholesterolemic properties (Carroll, 1990; Orthoefer, 1996; McCaskill and Zhang, 1999) and it has the potential to reduce both total serum and low density lipoprotein cholesterol levels in those who consume it (Wilson *et al.,* 2000). The triglyceride component of rice bran oil consists largely of esterified palmitic, oleic and linoleic acids. Table 2 shows the composition of rice bran oil during development.

The content of phytochemicals like tocopherol, tocotrienol, and γ-oryzanol in rice bran has been thoroughly studied (Dykes and Rooney, 2007; Zhang *et al.,* 2010; Liu, 2007). Like any other phytochemical, the phenolic and flavonoid content also depends on quality traits (grain color, size and weight) of rice grain. Table 3 indicates that black rice has the highest content of phenolics and flavonoids and the greatest maximum antioxidant property followed by red rice and white rice (Zhang *et al.,* 2010).

The biosynthesis of the oil in the grain during development has not been studied recently. Early studies indicated that oleic and linoleic acids increase up to 16 days after flowering whereas palmitic acid remains constant (Choudhury and Juliano, 1980).

Table 2. Lipid accumulation in rice grain

Days after flowering	Total lipids (μg/grain)	Fatty acid accumulation of non-starch lipids (% of 20-day grain)	Fatty acid composition of non-starch lipids (wt% of total)			
			Palmitic (16:0)	Oleic (18:1)	Linoleic (18.2)	α-Linoleic (18:3)
4	74	10	19	14	39	26
8	272	50	20	22	44	12
12	460					
16	470					
20	472	100	21	28	43	4
24	464					
28	468					

(Adapted from Choudhry and Juliano, 1980)

Table 3. Comparison of phenolics, flavonoids contents and antioxidant capacity among white, red and black rice genotypes

	Phenolics[a]	Flavonoids[a]	Antioxidant capacity[a]
Total rice			
Mean±SD	197.5±144.8	134.7±19.8	0.413±0.696
CV %	77.3	14.7	168.63
Range	108.1-1244.9	88.6-286.3	0.012-5.533
White rice			
Mean±SD	151.8± 19.5	131.6±14.2	0.196±0.073
CV %	12.9	10.8	37.33
Range	108.1-251.4	88.6-170.7	0.012-0.413
Red rice			
Mean±SD	470.1±107.2	147.2±18.0	1.705±0.600
CV %	22.8	12.3	35.22
Range	165.8-731.8	108.7-190.3	0.291-2.963
Black rice			
Mean±SD	1055.7±176.2	240.6±38.1	4.484±1.095
CV %	16.7	15.8	24.41
Range	841.0-1244.9	187.6-286.3	2.527-5.533

[a]Phenolics content was expressed as mg GAE/100 g, flavonoids content was expressed as mg RE/100 g, and antioxidant capacity was expressed as mMTAEC. (Adapted from Shen *et al.*, 2009).

There are few recent and systematic studies to screen for differences in rice bran oil composition. Earlier studies showed an oleic acid content around 40% (Taira *et al.,* 1988). However, as the critical genes involved in determining the composition are known in other plants (e.g. in cotton seed, Liu *et al.,* 2002) transgenic approaches have been undertaken in rice to increase the proportion of oleic acid at the expense of both palmitic acid (a saturated fatty acid) and linoleic acid (two double bonds that can go to the trans arrangement upon heating) as these latter two can have negative health implications. By knocking out the FAD2 gene using an RNAi approach, rice grains with almost the double the proportion of oleic acid were produced recently (Zaplin *et al.,* 2013). However, whether this translates into double the proportion of oleic acid in RBO has not yet been tested.

Little work seems have been done in trying to increase the oryzanol content, largely because the genes involved are not well delineated. Clearly a survey of the variation in oryzanol and tocopherol content among different rices would be of use in this regard.

LIMITATIONS ON THE USE OF RICE OIL

The production of RBO involves two broad steps. The first step is the production of the bran and stabilization of the oil within the bran. The second step is the extraction of the oil from within the bran.

To improve the value of RBO it is important to focus on the action of lipases and other enzymes that lead to degradation of the oil bodies. The oil bodies consist of a triglyceride core contained within phospholipid layer that is it itself interrupted and protected by a selection of proteins, predominant among which are the oleosins (Frandsen *et al.,* 2001). The oleosins and the related proteins caleosins are 15-30 kDa in mass. The breakdown of the triglyceride at the core requires disruption of these protective layers. The process has not been clearly delineated although it has been suggested that the oleosins contain binding motifs for lipases as the plant requires regulated breakdown during germination. However, if breakdown is initiated after bran production, either by premature triggering of the germinative breakdown cascade or by adventitious lipases the release of fatty acids leads to poor oil quality and lowers the value of the product. The free fatty acids produced that can be further acted on by lipoxygenases to produce rancid flavor.

A large number of different types of lipases have been characterized and some of these have TAG lipase activity (Matos and Pham-Thi, 2009). It is not known what proportion of them would be present in the bran. The complementary approach, isolating lipases from the bran has been attempted by a number of researchers (Bhardwaj *et al.,* 2001, Funatsu *et al.,* 1971; Aizono *et al.,* 1976; Fujiki *et al.,* 1978), but the genes corresponding to these activities have only been identified in a few cases (Kim 2004; Vijaykumar and Gowda 2013). Vijaykumar and Gowda (2013), purified a lipase activity that was identical to that reported by Aizino *et al.* (1976) and could relate it to a cDNA for lipase available at NCBI. They expressed the cDNA as protein and demonstrated lipase activity. The cDNA sequence contained the canonical GxSxG motif of lipases. Furthermore, they followed the accumulation of transcripts for this sequence by real-time PCR. However, the initial purification was based on hydrolysis of tributyrin which may limit the potential lipases assayed to a subset of those available (Vijaykumar and Gowda 2012).

The genome sequence of rice has been available for some time and more than a hundred lipases have been annotated in the rice genome (http://mpss.udel.edu/rice/mpss_index.php?). Although some of these may turn out not to be functional or of limited importance in terms of rice bran oil preparation, this approach may provide insight into the number of the different lipase and protease activities present in the rice bran. An example of a putative rice lipase gene recently isolated is *This1* (Liu *et al.,* 2013) and it is ubiquitously expressed, including in the panicle. However, its presence in the bran has not yet been demonstrated.

Once the relative importance of the various lipases in the bran have been evaluated, steps can be taken to eliminate their expression in the bran. This could take the form of establishing markers for the targeted lipases and then breeding to exclude such markers. Alternatively, if these lipases have critical roles otherwise, an RNAi or micro RNA approach using promoters that drive expression during late grain development could perhaps be used. The seed-specific promoter used by Zaplin *et al.* (2013) to reduce the proportion of linoleic acid in the rice bran is an example of the type of promoter that could be used. No lines lacking specific lipases appear to have been reported yet.

Rice bran stabilization and extraction

Physical and chemical methods used for inactivating rice bran lipase activity for stabilizing rice bran include dry heating, wet heating, and extrusion (Sayre *et al.,* 1982). Refrigeration and addition of chemicals additions such as sodium metabisulfite have also been used to decrease lipase activity and promote stabilization of bran (Tao, 2001; Cheruvanky *et al.,* 2003). Rice bran enzymes have also been deactivated by altering pH, which helps to increase the shelf life of rice bran for three months (Escamilla Castillo *et al.,* 2005).

However, these methods which have had relatively little success to date (Raghavendra *et al.,* 2007; Tao, 2001) are not promising long-term solutions to increase the shelf -life of rice bran. Rice lines lacking some of the lipoxygenases have demonstrated significantly better storability (Zhang *et al.,* 2007; Suzuki *et al.,* 1999) but as indicated earlier lines lacking lipases do not appear to have been produced.

RBO is always a by-product of the production of polished rice grains. Oil yield from rice bran extraction can be increased if rice bran is enzymatically treated with cellulase and pectinase prior to oil extraction by hexane (Sengupta and Bhattacharyya, 1996). An overview of rice bran oil extraction is shown in Fig. 1. RBO yield is highest when extracted with hexane (20.21%) followed by CO2–ethanol (18.23%) and supercritical CO2 (17.98%) respectively (Orthoefer, 2005). Hexane is commercially used for oil extraction from oilseeds although it is considered to be an air pollutant (Rosenthal *et al.,* 1996).

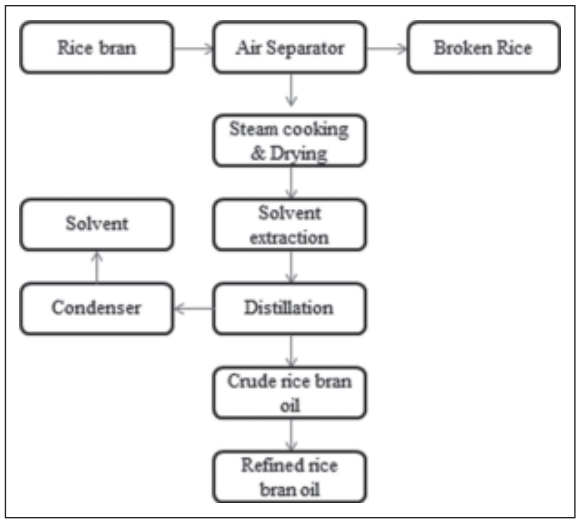

Fig. 1. Schematic diagram of rice bran oil extraction

OTHER CEREALS

Maize

Maize is often grown expressly for the oil and in such cases the corn is collected and processed by removing the germ (which contains about 85% of the oil) and oil content depends on its concentration and the area occupied by the embryo in the seed. However, corn oil can also be extracted from ground corn kernels (Hojilla-Evangelista *et al.,* 1992; Kwiatkowski and Cheryan, 2002) and corn fiber (Moreau *et al.,* 1996).

Maize oil is rich in polyunsaturated fatty acid (PUFA) content (65 to 85%) and thus fulfills the requirement of essential fatty acids in human nutrition (Goffman and Böhme, 2001). In maize oil, linoleic acid (18:2) alone comprises about 60% and monounsaturated fatty acid (MUFA) (oleic acid; 18:1) is about 24% of the total percentage of PUFA in maize oil. Among saturated fatty acids (SFA), palmitic acid (16:0) is almost 13% and stearic acid (18:0) is 1%. Thus maize thus has a high percentage of linoleic acid which is comparable to that of rice screw pressing and solvent extraction are two major methods of oil extraction; however these methods cannot be used to extract lipids present in the endosperm (MacRitchie and Gras, 1973). Commercially, corn oil is extracted either by hexane (Reiners, 1982; Stolp and Stute, 1982) or ethanol (Chien *et al.,* 1988; Chien *et al.,* 1990).

An overview of maize oil extraction is shown in Fig. 2.

Oats, wheat and barley

Oats apart from being a rich source of dietary soluble fiber beta-glucan (Glore *et al.,* 1994) also has a higher lipid content than other cereals as indicated previously, (see also Liu, 2011). The major fatty acids in oats oil are linoleic (18:2), oleic (18:1) and palmitic (16:0) (Welch, 1995). It also contains Vitamin E and antioxidant compounds which give oat oil cholesterol-reducing properties. (Youngs and Webster, 1986). The oleic content in

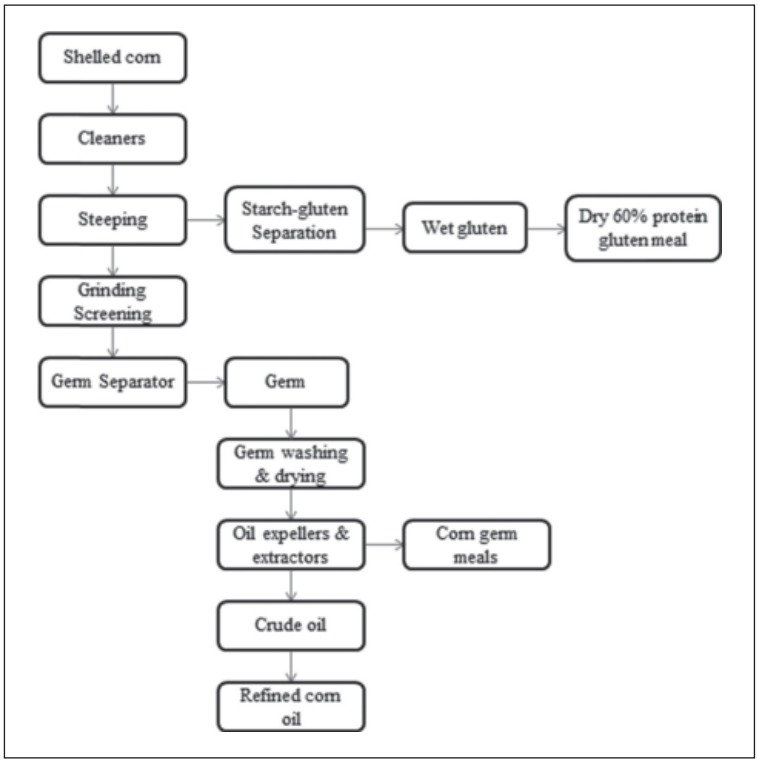

Fig. 2. Schematic diagram of corn oil extraction

oat oil is higher compared to commonly used soybean or sunflower oil but less than in canola and olive oils. The processing of oat oil is not economical and therefore, oat oil is not widely consumed or considered as edible oil.

The wheat embryo – also known as the germ contains approximately 11% of oil (Sonntag, 1979) comprising a large proportion of polyunsaturated fatty acids and vitamin E. It is one of the richest natural sources of γ-tocopherol, a compound known to have high vitamin E activity (Kahlon, 1989). Most of the fatty acids (57%) are present are as triglycerides (Kahlon, 1989). The most abundant is linoleic acid (18:2) (42–59% of the total triglycerides), followed by palmitic acid (16:0) and oleic acid (16:1) (Kahlon, 1989; Hidalgo and Brandolini, 2008). The characteristics of barley oil are similar to that of wheat (Liu, 2011). As with oats oil production from wheat bran does not yet appear to be economically attractive.

CONCLUSIONS

All the important cereals contain appreciable amounts of lipids in the grain. Maize oil has been most widely exploited but rice bran oil is a promising second. Much work needs to be done on investigating differences in the oil composition among different types of rice and on reducing the activity of the lipases. Such reductions in lipase activity should help in increasing the economic value of the oil. Clearly a combination of genomic and proteomic approaches are needed to investigate the lipase genes in rice grain, in order to understand their roles in lipid metabolism. This will help in devising approaches to improve the quality as well as quantity of rice bran oil. In addition, work needs to be initiated to increase the overall quantity of rice bran oil in the rice grain without affecting grain production.

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