

**Chapter 1 : Variation in Fatty Acid Distribution of Different Acyl Lipids in Rice (*Oryza sativa* L.) Brans**

*Lipids in Cereal Technology provides a comprehensive review of cereal lipids and their role in cereal processing and products. Topics range from acyl lipids and non-saponifiable lipids in cereals, such as barley and maize, to lipid metabolism in germinating cereals, physical state of lipids and their technical effects in baking, the effect of storage on the lipids and breadmaking properties of.*

This article has been cited by other articles in PMC. To the best of our knowledge, there is no single solvent system currently known that efficiently extracts all non-starch lipids from all flours without the risk of chemical, mechanical or thermal damage. This paper compares nine ambient solvent systems monophasic and biphasic with varying polarities: Seven ambient extraction protocols were further compared for their ability to extract total non-starch lipids from three alternative samples: **RESULTS** For wheat flour the original BD method and those containing HCl or NaCl tended to extract the maximum lipid and a significant correlation between lipid extraction yield especially the glycolipids and phospholipids and the polarity of the solvent was observed. **CONCLUSION** In general, BD-based methods showed better extraction yields compared to methods without the addition of water and, most interestingly, there was much greater method dependence of lipid yields in the starches when compared to the flour samples, which is due to the differences in lipid profiles between the two sample types flours and starches. Gas bubble retention is affected by the presence of surface-active compounds, which include lipids, at the gas bubble surface. Their functionality is further highlighted in previous studies, 1 , 2 which have shown that in a model system the addition of polar lipids to defatted flour can increase the loaf volume of baked products e. In the cereal grain, triacylglycerols are the main storage lipid and are contained in small stable subcellular organelles termed oil bodies, 11 , 12 triacylglycerols can also be found to a lesser extent in the starchy endosperm. Storage and shelf life also have a direct effect on flour lipids; the most common example being the development of free fatty acids over storage by enzymatic lipase and oxidative lipid degradation pathways. The functional properties of starch are strongly determined by the presence of non-starch lipids on the granule surface, 15 especially during gelatinization. The importance of non-starch lipids is more evident when dealing with purified starches such as maize and tapioca, where other free lipids are absent; furthermore, tapioca originates from cassava root, so it does not contain endosperm lipids. This method will act as the starting point for a more thorough investigation of the various factors affecting flour quality e. While there is some excellent work that has been carried out over the last 10 years, 19 – 22 there is still discussion over which extraction method would ensure the most representative extraction of non-starch lipids, including free lipids and bound lipids, in a single type of extraction, without thermal or mechanical damage; 20 , 23 , 24 this is unsurprising given the wide variation of polarity, 25 accessibility and location of lipids in flour, and the extremely low concentration of surface lipids in cereal and tuber starches. Compendium methods, such as those provided by the AACC 26 for crude lipid extraction, make use of non-polar solvents under reflux e. Soxhlet and Goldfish ; however, non-polar solvents are well known for their inability to extract polar lipids, and the need for refluxing limits the solvent choice to single low-boiling-point solvents or azeotropes. There have been a number of attempts to increase the extraction yield of non-starch lipids by using solvent mixtures, especially those containing water, as it appears to help break the associations between lipids and the flour matrix. In general, non-polar solvents e. Unfortunately, there is no single recommended method for the extraction of all lipid classes present in flour and starch with a single extraction solvent and, therefore, the overall aim of this work was to find a fast and robust method for the measurement of flour non-starch lipids by quantification of their associated fatty acids with a single-solvent system i. This was to be achieved by comparing and evaluating a range of ambient solvent systems for their ability to extract non-starch lipids from two types of flour: All reagents used were of analytical grade. Extraction procedures The following extraction procedures were evaluated for their capacity to extract non-starch lipids at ambient temperature: Bligh and Dyer BD. Samples mg were extracted as follows: The aqueous phase was discarded as no lipids were detected and the pellet and interfacial layer were re-extracted twice by following the same procedure described above. Samples were extracted following the

same protocol described above for BD, but using 0. Samples were extracted as per BD, although extraction water was replaced with 0. Extraction by solvents of different polarities. The organic phase was collected by aspiration, and the sample was extracted three consecutive times. The composition and Snyder polarity 31 of extraction solvents were as follows abbreviation and Snyder polarity are shown in parenthesis: Snyder polarity is calculated based on the ratio of solvents in the mixture multiplied by the polarity of that pure solvent. Fractionation of lipid classes Lipid isolates were fractionated by solid-phase extraction according to the method of Ohm and Chung, used recently by Hobbard et al. Lipid extract 1 mL in chloroform was then loaded on to the SPE column and the eluting solvents were tested for the absence of lipids, to confirm total retention of lipids by the solid phase. Neutral lipids were eluted with 10 mL chloroform:acetone 4:1. The elution rate was adjusted to 0. Quality of separation was evaluated using authentic standards of different lipid classes. Triplicates of all samples were extracted three times by each extraction protocol three sequential extraction steps, at ambient temperature. Experimental design and statistical analysis All experiments were conducted with a fully balanced experimental design, with randomized analysis order and three sample replicates. Probability values lower than 0. The remaining extraction solvents showed lower yields compared to BD methods; hexane was the solvent system with the lowest extraction yield. For external comparison, it should be noted that total lipid values correspond to the sum of the five major fatty acids and therefore do not accommodate non-fatty acid lipid components.

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Published online Apr This article is an open-access article distributed under the terms and conditions of the Creative Commons Attribution license <http://creativecommons.org/licenses/by/4.0/>: This article has been cited by other articles in PMC. Abstract The lipids extracted from rice brans were classified by thin-layer chromatography into eight fractions, and their fatty acid FA compositions were investigated among five different Japanese cultivars. The lipids of these rice brans comprised mainly triacylglycerols TAG; The PL components included phosphatidyl choline FA distribution of TAG among the five cultivars was characterized as: These results suggest that the rice bran lipids may be well incorporated into our daily diet to improve nutritional value of the Japanese diet.

**Introduction** Rice *Oryza sativa* L. Japan is self-sufficient in rice. Rice grain quality is an important economic trait that influences rice production in many rice-producing areas. Although the fat or oil in rice grain is low i. For instance, the surface lipid content has been thought to be an indication of the degree of milling [ 3 ]. In addition, rice lipid, frequently forming complexes with starch granules [ 4 ], was shown to affect starch gelatinization, water availability to starch, and rice swelling and thus influenced rice eating and cooking quality [ 5 ]. Rice brans also contain a number of nonfiber constituents in the nonsaponifiable oil fraction that have been implicated as factors in lowering cholesterol, including oryzanol [ 6 ]. Besides dietary consumption, the unique health benefits of rice fat, which includes many unsaturated fatty acids, have drawn much attention [ 7 ]. A number of studies have shown that rice bran oil reduces the harmful cholesterol LDL without changing good cholesterol HDL [ 8 ]. On the other hand, some reports showed that the hydrolysis and oxidation of rice fat are responsible for rice aging and deterioration of grain flavor during storage, and low-oil rice cultivars are more suitable for grain storage [ 9 ]. Although many extensive studies have been made on rice bran oil, little is known regarding the rice bran lipids including complex lipids. Some of these lipids are thought to be associated with protein in the native state, particularly in germ, but concrete evidence has yet to be presented. To the best of our knowledge, many investigations on lipid fractions of rice brans have been published. However, research dealing with a comparison study between different rice bran cultivars is limited. Therefore, the aim of the present study is to compare the lipid components and fatty acid FA distribution of different acyl lipids obtained from five different rice bran cultivars.

**Rice Seeds** Commercially obtained mature rice seeds *Oryza sativa* var japonica used in this study were from five different Japanese cultivars; Koshihikari, Haenuki, Akitakomachi, Hitomibore and Sasanishiki. These seeds were harvested at Akita prefecture in Japan on September of

**Reagents and Standards** All chemicals and solvents used were of analytical grade Nacalai Tesque, Kyoto, Japan , but diethyl ether was further purified to remove peroxides. Lipase from porcine pancreas was obtained from Sigma Chemical Co. Louis, MO, USA , and used after purification with acetone and then diethyl ether as described previously [ 10 ]. Glycerol-sn-1,3-myristate-snoleate Sigma Chemical Co. These solvents contained 0. Extraction was repeated three times, then 20 mL aqueous KCl 0. Helium was used as the carrier gas, at a flow rate of 1. All samples were dissolved in n-hexane for injection. FA was identified by comparison of the retention times with those of standard FAME and the results are reported as a weight percentage of the lipid. The other GC conditions were as previously described [ 15 ]. PL classes were detected by iodine vapor and were consistent with the authentic standards.

**Enzymatic Hydrolysis of Lipids** TAG hydrolysis was carried out in vitro as previously reported [ 10 ]. A 30 min reaction was selected based on the preliminary results using the standard TAG glycerol-sn-1,3-myristate-snoleate: The reaction products were separated by TLC as previously reported [ 10 ].

**Statistical Analyses** All preparations and determinations were carried out in triplicate, and the results were subjected to one-way analysis of variance ANOVA [ 16 ].

**Results and Discussion**

**3. Lipid Components in the Rice Brans** The compositional analyses carried out in this work included determination of the lipid classes and the FA compositions of the lipids. For all of the five cultivars, the original amount of total lipids was a range of mg per 20 g brans. Profiles of the different lipid classes in

the rice brans are shown in Figure 1 ; the data for Koshihikari and Sasanishiki were omitted as their patterns were very similar to those of Haenuki, Akitakomachi and Hitomebore. Predominant components were TAG

Figure 1 Open in a separate window Lipid components in the oils obtained from rice brans of three Japanese cultivars. Each value represents the average of three determinations, and vertical bars depict the mean and standard deviation. Lipid Components of Major Phospholipids in the Rice Brans To clarify the distribution of individual PL in the rice brans, further separation of the PL fraction into several fractions, such as phosphatidyl ethanolamine PE , phosphatidyl choline PC , phosphatidyl inositol PI and others was carried out on TLC in the presence of authentic standards. Other PL included diphosphatidyl glycerol, phosphatidic acid, phosphatidyl glycerol, lysophospho-lipids and lysoglycolipids. Comparisons were made between the profiles of PE, PC, PI and the others of all five cultivars Figure 2 ; the data for Hitomebore and Sasanishiki were omitted as their patterns were very similar to those of Koshihikari, Haenuki and Akitakomachi. For all the five cultivars, the original amounts of each PL were in a range of mg These PL are known to be essential components of the cell membranes in plants. Since membrane lipids are involved in such fundamental cell processes as ion transport, energy generation and biological reactions, they are highly conserved in terms of both quality and quantity [ 20 ]. Figure 2 Open in a separate window PL components in the oils prepared from rice brans. FA Composition of Major Lipids in the Rice Brans FA composition expressed in terms of the esters by weight of total lipids, FFA and PL in the rice brans were compared among all five cultivars Figure 3 ; the data for Haenuki and Hitomebore were omitted as their patterns were very similar to those of Koshihikari, Akitakomachi and Sasanishiki. The principal FA components are generally palmitic The samples presented significant amounts of total unsaturated FA, which consisted mainly of oleic With a few exceptions, the percentage of palmitic The percentage of oleic The data for FA distribution of minor lipid components, such as SE 1,3- and 1,2-DAG Figure 1 , were not included in Figure 3 as these lipid components were too small to obtain reliable results for these lipids. For abbreviations, see Figure 1. With a few exceptions, however, oleic acid Taken together, the regiospecific distribution profiles for the FA of TAG were very similar to the results obtained from other plant seed lipids such as soybeans, corn [ 22 ] or broad beans [ 15 ]. Each value represents the average of three replicates, and vertical bars depict the mean and standard deviation. For abbreviations, see Figure 1 and Figure 2. Figure 5 shows typical FA distributions for the PE, PC and PI fractions among all five cultivars; the data for Koshihikari and Akitakomachi were omitted as their patterns were very similar to those of Haenuki, Hitomebore and Sasanishiki. The major FA in the three PL were commonly palmitic The data showed that the percentage composition of linoleic However, PI was unique in that it had the highest saturated FA Particularly, the percentage of palmitic The data for FA distributions of minor lipid components, such as diphosphatidyl glycerol, phosphatidic acid, phosphatidyl glycerol, lysophospho-lipids and lysophosphoglycolipids, were omitted from Figure 5 because these PL components could not be isolated perfectly from each other. Therefore, we would like to consider them in our future work. FA distribution of TAG among all five cultivars was characterized as: The distribution patterns in the different acyl lipids and their FA profiles in rice brans were very similar to each other among the five cultivars. Therefore, the lipid composition suggests that these rice bran lipids could be a good source of nutraceuticals with positive health benefits. The rice bran lipids may be well incorporated into our daily diet to improve the nutritional value of the Japanese diet. Identification of quantitative trait loci for seed storability in rice *Oryza sativa* L. Composition and functional properties of rice. Milling characteristics of rice cultivars and hybrids. Lipids in Rice and Rice Processing. Lipids in Cereal Technology. Swelling and gelatinization of cereal starches. Effects of amylopectin, amylopse, and lipids. Effects of gamma-oryzanol on hyperlipidemic subjects. Effectiveness of natural versus synthetic antioxidants in a rice bran oil-based structured lipid. 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