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Original Article

Effects of differences in manufacturing methods and raw materials on the mineral composition of bran-derived phytin

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Summary

Phytic acid is usually deposited in dormant seeds bound to minerals in the form of phytin. Bran is a byproduct of grain processing and used as a raw material for phytin production. In this study, commercially available rice bran and wheat bran were used as raw materials, and phytin was extracted and prepared by four typical production methods: alkali addition, calcium salt addition, magnesium salt addition, and ethanol precipitation. In addition to phosphorus, the raw bran contained magnesium, calcium, manganese, zinc, and iron, and these minerals were also recovered in all phytin products. The phytin products produced by alkali addition showed a mineral composition that was almost identical to the proportions found in the bran. The addition of alkali metal salts increased the corresponding metal content in the phytin products, suggesting that magnesium and calcium may be antagonistic in their binding to phytic acid. Ethanol precipitation resulted in lower calcium and magnesium recoveries and a relatively higher iron content. Among the phytate products obtained, those with a high iron ratio were amber in color, while those with a high manganese ratio were pink. The mineral composition in phytin is affected and complicated by the type of raw material and method of production, and the mineral composition in phytin and phytic acid must be noted when interpreting the results of animal studies on phytin and phytic acid.

Grains and legumes are major sources of energy, protein, and minerals. Minerals in the seeds exist bound to phytic acid, an inositol hexakisphosphate, and such a mineral-phytic acid complex in seeds is referred to as phytin[1]. Phytin is the primary raw material used to produce inositol and is applied in various fields such as pharmaceuticals, nutraceuticals, and food additives.[2] Under physiological conditions, the negatively charged phosphate groups of phytic acid bind to metal cations such as calcium (Ca), iron (Fe), magnesium (Mg), manganese (Mn), and zinc (Zn), making them insoluble and inhibiting their absorption in the gut[3]. In monogastric animals, the limited ability to digest phytic acid may result in phosphorus deficiency and growth inhibition when phytic acid is used as a phosphorus source[1]. The inhibitory effects of phytate on mineral absorption have been extensively studied. These studies have used various grades of phytate, ranging from purified phytate to naturally occurring phytin[4-8]. In a previous study, we found that the phytin in rice bran is mainly magnesium phytate, with varying amounts of Zn and Mn[9]. This is in contrast to the previous view that phytin was mainly a mixture of calcium phytate, potassium phytate, and magnesium phytate[10]. Although the physicochemical properties of phytates have been studied under laboratory conditions[11-13], few studies on the mineral composition of natural and commercial phytin can be found.

Phytin in seeds is mainly concentrated in the external covering layers of the seed pericarp and aleurone layer, with lower levels in the germ[14]. These parts are removed during the refining process of grains, referred to as bran. In Japan, rice bran, an abundant byproduct of rice polishing, is used as a raw material for producing phytate and inositol[2,15]. Taking advantage of the fact that phytic acid is soluble in acids but insoluble in organic solvents and the insolubility of divalent metal salts of phytic acid, several methods using organic solvents and alkaline metal salts have been used in the industrial production of phytin and purified phytate from bran[2,15]. However, the diversity of phytin production methods leads to variation in the mineral composition of the produced phytin. Indeed, we ana-

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lyzed a rice bran-derived phytin labeled as calcium phytate and found that it contained Mg, Zn, and Mn, but not Ca[9]. Since phytin is often used in nutritional studies on phytic acid, clarifying the relationship between the mineral composition of phytin and method of production is important for the accurate interpretation of nutritional studies on phytic acid.

In this study, in order to clarify the effect of manufacturing methods on the mineral composition of phytin, commercially available fresh rice bran and wheat bran were used as raw materials, and the contained phytin was extracted, precipitated, and recovered according to several typical industrial manufacturing methods, and the mineral composition of the obtained phytin was investigated.

Materials and Methods

Materials

Domestic wheat bran and rice bran were purchased by mail order. Three samples of each type of bran produced in the same batch were randomly selected for mineral analysis, while the remaining bran samples were used as raw materials for phytin preparation. Sodium phytate was purchased from FUJIFILM Wako Pure Chemical Co. (Osaka). Ferric phytate and manganese phytate were prepared as follows: 1 M $FeCl_3$ or 1 M $MnCl_2$ solution was mixed with an equal volume of 0.2 M sodium phytate, and the resulting precipitates were collected as ferric phytate or manganese phytate, respectively.

Preparation of phytin

Four different preparation methods were applied in this experiment, including the alkali addition method, alkaline earth metal (Ca or Mg) addition method, and ethanol precipitation method[2, 15]. In the alkali addition method or alkaline earth metal addition method, 5 g (precisely weighed) of wheat bran or rice bran was placed in a 50-mL triangular flask with 50 mL of 1% HCl and extracted overnight at 30 ℃ with constant shaking in a water bath, and the supernatant was collected as an acid extract. The acid extract was neutralized to pH 4.0 using 3 M NaOH (alkali addition method) or a solution containing 3.6 M $Ca(OH)_2$ and 0.9 M $CaCl_2$ (calcium salt addition method) or a solution containing 2 M $Mg(OH)_2$ and 1 M $MgCl_2$ (magnesium salt addition method), followed by pH adjustment to 7.0 using 1 M NaOH. Each mixture was filtered, and the resulting precipitate was collected, washed with pure water, and then dried to obtain the three phytin samples. For the ethanol precipitation method, 50 mL of the acid extract was prepared from the defatted wheat or rice bran. Anhydrous ethanol (50 mL) was added to the acid extract for the first sedimentation. After separation by centrifugation, the clear supernatant was subjected to second sedimentation with 100 mL of anhydrous ethanol, and the resulting precipitate was obtained by centrifugation. The two precipitates were combined, washed with ethanol, and then dried to obtain the fourth phytin sample.

Metal analysis

A portion of the bran or phytin samples was accurately weighed, placed in a Kjeldahl flask, a mixture of HNO_3 and $HClO_4$ (5/1, v/v) was added, and the mixture was heated until the solids disappeared and white fumes appeared. After cooling to room temperature, ultrapure water was added and heated again to remove any remaining acid until the white fumes disappeared, indicating that digestion was complete.

The digests were diluted with 0.1 M HCl and filtrated through a 0.45-μm filter. The standard metal element solutions and sample solutions were analyzed using a flame atomic absorption spectrophotometer (AA-7000, Shimadzu, Kyoto, Japan). The inorganic phosphorus determination was performed using the vanadomolybdate method[16].

Results and Discussion

Table 1 shows the mineral composition of the bran used in this study compared with the values listed in the Food Standard Composition Tables[17] or US food database[18]. The quantitative results for each mineral in rice bran were very close to the values in the Standard Tables of Food Composition in Japan[17]. In contrast, the mineral content of the wheat bran used was slightly lower in phosphorus and Mg than the values listed in the U.S. food database[18]. This is probably due to the fact that the wheat bran used in this study and the wheat bran from which the U.S. values were derived differ in the type of wheat (e.g., hard, ordinary, and soft wheat) as well as the region of origin. Still, in both brans, the Mg content was much higher than the Ca content and small amounts of Zn, Mn, and Fe were contained. Rice bran was high in phosphorus and Mg contents, and its total mineral content was markedly higher than that of wheat bran. Thus, rice bran, a byproduct of rice polishing, has been widely used for the production of phytic acid and inositol because of its high phosphorus content[2,15]. On the other hand, wheat bran, a byproduct of wheat flour production, does not contain much phosphorus and has not been widely applied for phytic acid production.

Table 2 summarizes the mineral composition of phytin prepared from rice or wheat bran by different production

Table 1 Mineral composition in bran

Minerals	Measured value (mg/g)*		Reported value (mg/g)	
	Rice bran	Wheat bran	Rice bran**	Wheat bran***
P	21.2 ± 0.2 (66.5)	7.10 ± 0.40 (69.8)	20.0 (68.6)	10.1 (58.6)
Mg	9.84 ± 0.15 (30.8)	2.19 ± 0.08 (21.5)	8.5 (29.2)	6.11 (34.8)
Ca	0.49 ± 0.01 (1.5)	0.55 ± 0.02 (5.4)	0.35 (1.2)	0.73 (4.2)
Mn	0.21 ± 0.01 (0.7)	0.15 ± 0.02 (1.5)	0.15 (0.5)	0.11 (0.6)
Zn	0.08 ± 0.01 (0.2)	0.06 ± 0.01 (0.6)	0.06 (0.2)	0.07 (0.4)
Fe	0.08 ± 0.01 (0.2)	0.12 ± 0.01 (1.2)	0.08 (0.3)	0.11 (0.1)
Total	31.9 ± 0.3	10.2 ± 0.5	29.14	17.23

Values in parentheses are percentages (%) of the total (P+Mg+Ca+Mn+Zn+Fe) amount
* Values are means ± SD (n=3).
** Listed value in the Standard Tables of Food Composition in Japan (eighth revised edition)[16].
*** Quoted from US food database[17].

Table 2 Mineral composition of phytin produced from rice bran or wheat bran by several methods

Minerals	Produced from rice bran (mg/g)				Produced from wheat bran (mg/g)			
	Alkali addition	Calcium salt addition	Magnesium salt addition	Ethanol precipitation	Alkali addition	Calcium salt addition	Magnesium salt addition	Ethanol precipitation
P	84.4 ± 7.5	118.5 ± 1.5	148.6 ± 9.9	97.3 ± 3.8	80.5 ± 6.6	108.9 ± 0.5	140.3 ± 3.3	101.1 ± 16.2
	(61.8)	(40.1)	(54.9)	(77.1)	(56.8)	(36.3)	(51.8)	(94.5)
Mg	47.3 ± 4.3	7.29 ± 0.40	117.3 ± 3.4	24.9 ± 1.4	35.8 ± 2.5	3.13 ± 1.47	113.5 ± 4.3	1.05 ± 0.14
	(34.7)	(2.5)	(43.1)	(19.7)	(25.2)	(1.0)	(42.2)	(1.0)
Ca	2.64 ± 0.26	167.4 ± 12.6	2.42 ± 0.07	1.91 ± 0.27	17.5 ± 1.0	178.7 ± 0.6	10.3 ± 0.6	1.76 ± 0.16
	(1.9)	(56.8)	(0.9)	(1.5)	(12.3)	(59.7)	(3.8)	(1.6)
Mn	1.25 ± 0.13	1.17 ± 0.06	0.80 ± 0.07	0.77 ± 0.07	4.30 ± 0.36	6.37 ± 0.40	1.79 ± 0.02	0.16 ± 0.02
	(0.9)	(0.4)	(0.3)	(0.6)	(3.0)	(2.1)	(0.7)	(0.1)
Zn	0.43 ± 0.03	0.44 ± 0.03	0.61 ± 0.01	0.23 ± 0.07	1.49 ± 0.13	1.02 ± 0.05	1.35 ± 0.16	0.05 ± 0.01
	(0.3)	(0.1)	(0.2)	(0.2)	(1.1)	(0.3)	(0.5)	(< 0.1)
Fe	0.45 ± 0.06	0.22 ± 0.04	1.32 ± 0.12	1.08 ± 0.04	2.21 ± 0.16	1.53 ± 0.20	2.42 ± 0.07	2.78 ± 0.89
	(0.3)	(0.1)	(0.5)	(0.9)	(1.6)	(0.5)	(0.9)	(2.6)
Total	136.5 ± 12.3	294.1 ± 12.8	271.2 ± 9.8	126.2 ± 5.2	141.8 ± 10.3	300.0 ± 5.5	269.9 ± 0.8	106.9 ± 17.2

For each mineral, the upper values are the means ± SD (n=3) of the content (mg/g), and the lower values in parentheses are the percentages (%) of the total (P+Mg+Ca+Mn+Zn+Fe) amount.

methods. The composition and percentage of minerals in phytin prepared by the alkaline addition method using only NaOH is almost the same as that of the raw bran. This indicates that when only NaOH, the most common neutralizing agent, is used, the cations precipitate in approximately the same proportion as in the acid extract. In other words, the phytin prepared by this method is considered to be the pre-extracted form of phytin, with magnesium phytate as the main component and various metals in the form of phytates.

In addition to the use of NaOH, the addition of alkaline earth metals such as Ca and Mg to produce phytin has been effective in improving the yield of phytin[2,15]. However, when these alkaline earth metal salts were added, the total mineral concentration of the obtained phytin was clearly higher than that of other methods, as shown in Table 2. This indicates that the phytin precipitate obtained by alkaline earth metal addition contains not only calcium phytate or magnesium phytate, but also some of the added alkaline earth metal salts that have become insoluble and precipitated. Thus, the introduction of Ca or Mg resulted in a significant difference in the metal ratio of the resulting phytate precipitate from that of the raw bran. Furthermore, when calcium salts were used as precipitants, the ratio of Mg in the precipitates was much lower, and when magnesium salts were used, the ratio of Ca was even lower than in the original bran. This means that Ca and Mg inhibit each other's binding to phytic acid, i.e., there is an antagonistic relationship between the binding of calcium and magnesium ions to phytic acid in solution. On the other hand, adding large amounts of Ca and Mg did not significantly affect the recovery of Mn, Zn, and

Fe, irrespective of whether the source material was rice or wheat bran.

Phytin is known to be insoluble in organic solvents such as alcohols, but soluble in acids[2,15]. In our experiment, the addition of ethanol could also precipitate phytin. The addition of ethanol to the acid extract did indeed decrease the solubility of phytate, but the hydrogen ion concentration of the solution was not markedly changed, suggesting that a large amount of phytin was not precipitated. Compared with other methods, the phytin obtained by ethanol precipitation had lower percentages of Ca and Mg, and a higher percentage of Fe. This difference is attributed to the fact that calcium or magnesium phytates remain soluble at pH 4 to 5, whereas ferric phytate is insoluble under even lower pH conditions[19]. Therefore, the phytate product obtained by the ethanol precipitation method is considered to have a high percentage of free phytic acid or alkali metal salts of phytic acid.

Figure 1 shows images of phytin from different sources and methods, and ferric phytate and manganese phytate, along with Fe and Mn content in each phytin product. Ferric phytate is amber and manganese phytate is pink.

Phytin obtained by ethanol precipitation showed an amber color derived from ferric phytate, regardless of the raw material. Since the ethanol precipitation method also produces less calcium phytate and magnesium phytate (Table 2), which are white in color, the amber color of ferric phytate, which has a relatively high content ratio, may have determined the color tone of the obtained phytin. On the other hand, phytin obtained from wheat bran by the alkali addition or calcium salt addition method showed a pink color derived from manganese phytate. This may reflect the high ratio of Mn in these phytin products. The remaining phytate products were white in color, reflecting low ratios of Fe or Mn and high ratios of calcium or magnesium phytate.

The above findings indicate that coloration of the phytin products is due to Fe and Mn. However, wheat bran contains many brown pigments such as flavonoids and lignin[20,21], and it cannot be ruled out that these may be incorporated into the final phytate precipitation and contribute to coloration of the product. Commercially available phytin and phytic acid vary in color from colorless or white to light brown[2,22]. This variation is most likely due to differences in raw materials, methods, and even the degree of purification among different manufacturers.

Although the methods for extracting phytin from bran are relatively similar, different precipitation methods can have a significant impact on the composition of the resulting phytin product. Phytic acid possesses six strong acidic groups and six moderately weak acidic groups, with pKa values ranging from 1.9 to 9.5[11]. In the present experiment, to fully extract phytin from the raw material, an extended period is required under strongly acidic solutions ($pH < 1$). At this stage, phytin in the bran can be completely dissociated into phytic acid. Subsequently, an alkaline neutralizing agent or a precipitating agent is added to adjust the pH to the desired level. As the pH gradually increases, the metal ions present in the acidic extraction solution interact with the added metal ions and progressively bind to the acidic groups of phytic acid. In

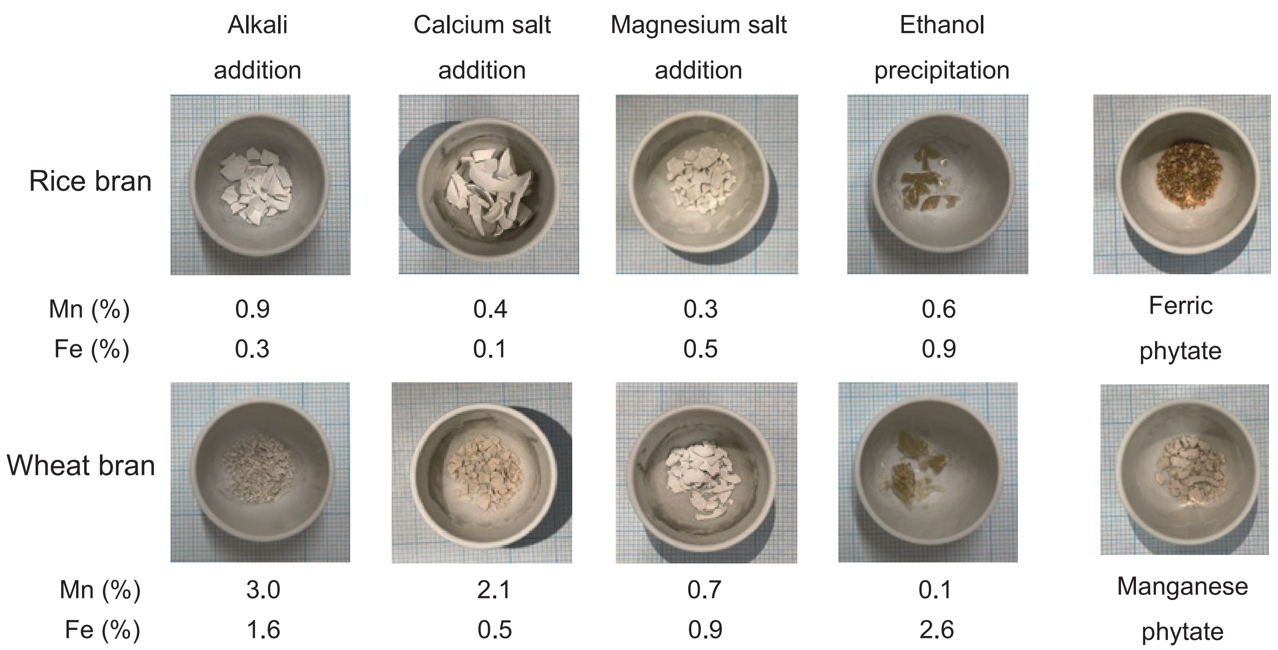

Fig. 1. Phytin products produced by different sources and method

other words, the positions of the weak acidic groups of phosphate connected to the inositol hexaphosphate ring are occupied by the cations with the highest affinity, followed by the positions of the strong acidic groups. The affinity sequence between phytic acid and multivalent cations is $Zn^{2+} > Ni^{2+} > Co^{2+} > Mn^{2+} > Fe^{3+} > Ca^{2+}$[12]. According to the previous discussion, we can infer that the affinity of Mg for phytic acid should be like that of Ca. Finally, after all 12 acidic group positions are fully occupied, precipitation occurs, and the product can be recovered as phytin products. The binding mechanism between phytic acid and cations in solution is complex and influenced by factors such as ion strength, pH, and temperature. However, it is clear that without thorough ion exchange, phytin products derived from natural sources will invariably contain certain amounts of elements such as Mn, Fe, Zn, as indicated by our experimental results. Accordingly, the type and origin of the grains used might significantly influence the composition of phytate products, as the types and concentrations of minerals in bran can vary markedly[3].

Phytic acid, as an antinutrient factor inhibiting the absorption of trace elements and leading to trace element deficiencies, has been investigated in various studies using natural phytin-containing substances or phytin[4-7]. In these studies, some experiments have analyzed the elemental composition of the experimental materials, while others have not. Nevertheless, many experiments lack a detailed understanding of the composition of phytin in the materials, which is critical for discussing minerals. This is because natural phytin-containing substances have abundant and diverse minerals, which can also significantly impact the minerals status within the body. In one of our previous animal experiments using rice bran-derived phytin, we found that the Zn contained in phytin could potentially be utilized by rats, and that the high levels of Mn contained in phytin may even interfere with Fe absorption[9]. In nutritional studies on phytic acid, the presence of trace elements such as Mn, Fe and Zn in phytates may significantly affect the results of research on the inhibitory effect of phytic acid on the absorption of these elements, as organisms may be able to absorb even very small amounts of these trace elements depending on their nutritional status. In daily life, phytic acid is typically consumed in its natural form from grains and legumes, and even commercial phytin can exhibit variations in composition due to various factors. Therefore, the composition of minerals in phytin and effects of cations in phytin should also be further discussed in future nutritional studies on phytates and minerals.

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References

1) Schlemmer U, Frølich W, Prieto RM, Grases F (2009) Phytate in foods and significance for humans: Food sources, intake, processing, bioavailability, protective role and analysis, Mol Nutr Food Res 53: S330–S375.
2) Kimura G (1967) Review on the phytic acid, Yukigosei Kagaku Kyokaishi (J Syn Org Chem Jpn) 25: 167–179.
3) Brouns F (2021) Phytic acid and whole grains for health controversy, Nutrients 14 (1): 25, doi: 10.3390/nu14010025.
4) Tsuchiya S (1953) Studies on the phytic-acid from the standpoint of the nutrition, Eiyo to Shokuryo (J Jpn Soc Food Nutr) 6: 120–126.
5) Davies NT, Olpin SE (1979) Studies on the phytate: zinc molar contents in diets as a determinant of Zn availability to young rats, Br J Nutr 41: 591–603.
6) McCance RA, Widdowson EM (1942) Mineral metabolism of healthy adults on white and brown bread dietaries, J Physiol 101: 44–85.
7) Ebisuno S, Morimoto S, Yoshida T, Fukatani T, Yasukawa S, Ohkawa T (1986) Rice-bran treatment for calcium stone formers with idiopathic hypercalciuria, Br J Urol 58: 592–595.
8) Jin Z, Hosomi R, Fukunaga K, Yoshida M (2021) Effect of phytic acid administration on the zinc concentration, uric acid biosynthesis, and serum lipid components in rats, Metallomics Res 1 (1): reg26–reg32, doi: 10.11299/metallomicsresearch.MR202101.
9) Jin Z, Hosomi R, Fukunaga K, Yoshida M (2022) Effect of sodium phytate and phytin on the absorption and organ concentration of several minerals in rats, Trace Nutr Res 39: 1–7.
10) A. Kumar K, Singh B, Raigond P, Sahu C, Mishra UN, Sharma S, Lal MK (2021) Phytic acid: Blessing in disguise, a prime compound required for both plant and human nutrition, Food Res Int 142: 110193, doi: 10.1016/j.foodres.2021.110193.
11) Evans WJ, McCourtney EJ, Shrager RI (1982) Titration studies of phytic acid, J Am Oil Chem Soc 59: 189–191.
12) Graf E, Eaton JW (1990) Antioxidant functions of phytic acid, Free Radic Biol Med 8: 61–69.

13) Yamada F, Kaneko Y, Iwasaki H (1989) Synthetic condition for calcium phytate to obtain hydroxyapatite powder, Nippon Kagaku Kaishi (J Jpn Chem Soc) 1989: 1712–1717.

14) Stevenson L, Phillips F, O'sullivan K, Walton J (2012) Wheat bran: its composition and benefits to health, a European perspective, Int J Food Sci Nutr 63: 1001–1013.

15) Kimura G, Inaba Y (1963) Review on the industrial utilization of rice bran. II. Utilization of defatted rice bran, Yukagaku (J Jpn Oil Chem Soc) 12: 69–78.

16) Koenig R, Johnson C (1942). Colorimetric determination of phosphorus in biological materials. Ind Eng Chem Anal Ed 14: 155–156.

17) Ministry of Education, Culture, Sports, Science and Technology, Japan (2023) Standard Tables of Food Composition in Japan, Eighth Revised Edition, Updated and Enlarged Version 2023, https://www.mext.go.jp/a_menu/syokuhinseibun/mext_00001.html (accessed 20 Aug, 2023).

18) U.S. Department of Agriculture (2022) Wheat bran, in Food Data Central, https://fdc.nal.usda.gov/fdc-app.html#/food-details/2343972/nutrients (accessed on 24 August, 2023).

19) Kumar V, Sinha AK, Makkar HPS, Becker K (2010) Dietary roles of phytate and phytase in human nutrition: A review, Food Chem 120: 945–959.

20) Goufo P, Trindade H (2014) Rice antioxidants: phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, γ-oryzanol, and phytic acid, Food Sci Nutr 2: 75–104.

21) Saini P, Kumar N, Kumar S, Mwaurah PW, Panghal A, Attkan AK, Singh VK, Garg MK, Singh V (2021) Bioactive compounds, nutritional benefits and food applications of colored wheat: a comprehensive review, Crit Rev Food Sci Nutr 61: 3197–3210.

22) Anderson RJ (1914) A Contribution to the chemistry of phytin: I. Composition of barium phytate and phytic acid. II. A study of properties of phytic acid and its decomposition products. Eighth paper on phytin, J Biol Chem 17: 171–190.