

DEVELOPMENT OF VITAMIN A - RICH PASTA USING RICE BRAN FLOUR AS PARTIAL SUBSTITUTE TO WHEAT FLOUR*Lindsay D. Giagonia¹, Alexis Ailyn B. Reyes¹, and Christine O. Cruz, MSFS, LPT²*¹*Bachelor of Science in Nutrition and Dietetics, College of Business Administration and Accountancy*²*Faculty Member, Food Technology Area, College of Business Administration and Accountancy***ABSTRACT**

Micronutrient deficiencies are a continual lack of nutritional vitamins and minerals and constitute a huge public health problem. Vitamin A deficiency increases vulnerability to a variety of illnesses which includes diarrhoea, measles, and respiratory infections where it is common amongst children. In order to address this, great interest in rice bran has led in the discovery of various health benefits. With this, the study aimed to develop a pasta using the rice bran as partial substitute to wheat flour and to determine the Vitamin A content of the rice bran pasta in comparison to commercially available pasta. The rice bran has undergone dry heating method at 130°C for 20 minutes and was formed into dough. The sample pasta was then analyzed for proximate analysis which consists of moisture content, ash, carbohydrates, protein, fat and crude fiber. Vitamin A content analysis was also conducted. Moreover, aerobic plate count, yeast and mold count was observed for microbial activity. It was found out that the rice bran pasta is high in Vitamin A showing a high content of 188 µgRE/100g which is 47% of the recommended dietary allowance compared to the commercially available pasta. Researchers recommend developing a rice bran pasta that should be cut and shaped into different sizes, undergo further tests with different ratios of rice bran flour and can be used for supplementary feedings in the community. From this study, it can be concluded that rice bran is safe for human consumption and can be used as a food supplement.

Keywords: *Substitute, Proximate Composition, Vitamin A content, Micronutrient deficiency supplement, Rice bran pasta*

INTRODUCTION

Micronutrient deficiencies are a continual lack of nutritional vitamins and minerals and constitute a huge public health problem. Micronutrient deficiencies have severe health consequences and are mainly dangerous throughout the early childhood due to their impact on the physical and cognitive development. This problem affects 2 billion people globally according to the World Health Organization. According to WHO, worldwide nearly one in two preschool children suffer from anemia, many of them because of iron deficiency (McLean et. al, 2008), and nearly one hundred million preschool children suffer from Vitamin A deficiency (WHO, 2009). Severe and widespread deficiencies also prevail for iodine, zinc and a number of other micronutrients (Black et. al, 2008). Micronutrient deficiency are occasionally known as hidden hunger, as they are not as seen as malnutrition due to inadequate calorie and protein intake. They have severe health consequences and are mainly harmful during pregnancy and early childhood due to their impact on the bodily and cognitive development of children (Lozoff B and Georgieff MK, et. al, 2006).

Vitamin A is involved in immune function, vision, reproduction, and cellular communication (Johnson & Russell, 2010). Over the years, numerous studies have been conducted to identify the biological functions of vitamin A, the health consequences related with deficiency, and the mechanisms that explain these relationships. The Food and Nutrition Research Institute-Department of Science and Technology (DOST) conducted the 7th National Nutrition Survey (2007) and reported a decreasing trend in Vitamin A deficiency prevalence among preschool children 6 mos-5 years old (15.2%), pregnant (9.5%) and lactating women (6.4%). The current Food and Nutrition Survey shows that vitamin A deficiency amongst preschool children has elevated from 15.2% in 2008 to 20.4% in 2013. This is equal to about 2.1 million children. These children are at risk of getting sick owing to the weakening of their immune system; of going blind; and even of death from common childhood infections as diarrheal disease and measles. A number of pregnant women and nursing mothers are also vitamin A deficient.

Vitamin A deficiency is generally associated with decreased dietary intake of preformed vitamin A and its precursors, together with a high prevalence of infectious diseases, like measles, diarrhea, and respiratory tract infections. These are leading causes of mortality among children in low- and middle-income countries (Black RE, Allen LH, Bhutta ZA, Caulfield LE, de Onis M, Ezzati M, et al. 2008) where risk of infection and risk of mortality can be compounded by coexisting undernutrition. (Black RE, Allen LH, Bhutta ZA, Caulfield LE, de Onis M, Ezzati M, et al, 2008) Diets containing insufficient vitamin A lead to decreased serum vitamin A levels, resulting in various physiological implications, especially tissue development, metabolism, and resistance to infections. Severe Vitamin A deficiency leads to xerophthalmia, the most common cause of preventable blindness among children (World Health Organization, 2009).

Vitamin A is a necessary nutrient needed for the visual system, growth, development, and a healthy immune system of all ages especially children

ages 4-6 years old. Their diet should consist 1/3 or 33% of the 400µg requirement of Vitamin A. Carotenoids, substances like beta carotene that the body converts into vitamin A, are found in orange-colored fruits and vegetables and in dark-green leafy vegetables. Vitamin A also supports cell growth and differentiation, playing a critical role in the normal formation and maintenance of the heart, lungs, kidneys, and other organs (Ross, 2010). Different forms of vitamin A encompass β carotene, which is found in plants, and preformed vitamin A, which is found in animal sources. Vitamin A is an essential nutrient that cannot be synthesized so it ought to be acquired through diet. Preformed vitamin A (retinol, retinal, retinoic acid, and retinyl esters) is the most active in humans; it is typically used in dietary supplements in the form of retinyl esters. Chronic intakes of excess vitamin A lead to increased intracranial pressure, dizziness, nausea, headaches, skin irritation, pain in joints and bones, coma, and even death (Ross, 2010). Although hypervitaminosis A can be due to excessive dietary intakes, the condition is usually a result of consuming too much preformed vitamin A from supplements or therapeutic retinoids (Ross and Solomon, 2006) Periodic supplementation should not cause serious unfavorable effects.

Rice as the main staple food in the Philippines are widely grown and known as good sources of nutrients. Rice is a predominant staple food due to its high-satiety value and it gives a high nutritional content and promoting the daily energy for its consumers, most likely in carbohydrates.

Rice bran is the shell existing between the rice and the husk of the paddy and consists of embryo and endosperm of the seeds of *Oryza sativa*. It makes 8 percent of the total weight of the entire grain and houses most of the nutrients. Rice bran, a "little known", food is favorably nutritious and brings health supporting nutrients which is either thrown away or used as animal feed. Chemically, rice bran contains protein 11-17 %, fiber 10%, ash content 9%, fat 11-18% and nitrogen free extract 45-65%. It is a rich source of vitamins B and minerals such as copper, zinc, iron, potassium and phosphorus. It is also rich source of essential nutrients. Rice bran (smooth and non-smooth) is a healthy source of bioactive compounds like γ-oryzanol, tocopherols, and tocotrienols; which have health beneficial properties and antioxidant activity (Watchararujij et al., 2008; Moongngarm et al., 2012).

Pasta and noodles are staple food products in numerous nations (Li, Man & Zhu et. al, 2014). They differ from each other in numerous aspects, mostly in the raw-material utilized for their production. Pasta is usually produced from durum wheat semolina, though noodles are produced either from common wheat flour (and salt), or starches from different sources. Due to its unique proteins that will form a very strong and visco-elastic network, wheat is the preferred cereal to produce flour for pasta making. The enhancement of pasta-like products already started more than five decades prior with the addition of soy protein (Gopalakrishnan, et al, 2007). Since then, enrichment, mostly with vegetable and legume flours, is common only in pasta made from durum wheat semolina, and only a few studies were conducted on the enrichment of starch noodles. A few reasons for the enrichment of pasta have been pointed out in literature, such as nutritional improvement, use of local raw materials, use of cereal by-products,

production of gluten-free pasta or development of products with additional health benefits.

The traditional food regimen in populations with an excessive incidence of malnutrition consists predominantly of a starch-rich staple, such as a cereal (maize, rice) or tuber (cassava), with limited amounts of fruits, vegetables, legumes, and pulses, and little or no animal-source food. Such a food regimen is bulky, has a low density of energy and nutrients and a low bioavailability of minerals, and will result in impaired growth, development, and host defense to infections. In addition, introduction of such a diet too early or contamination of the food regimen will lead to generic infections, which will further impair nutritional status and, hence, extend the risk of infectious diseases. Young children are also likely to be more sensitive to the effect of antinutrients, e.g., high levels of phytate, which impairs the absorption of several growth-limiting minerals, such as zinc. Infants and young children are specially inclined to malnutrition because they have an excessive growth velocity and also high energy and nutrient needs. Thus, a diet with a high energy density could have an important role in preventing moderate malnutrition in such populations. When the need for other nutrients is expressed in relation to energy content, it is likely that the requirements will not differ much between children with moderate wasting and stunting (Golden MH, 2009; WHO, 2007).

To address these issues, the food industry is seeking new methods to supplement vitamins and minerals. Rice bran is making its way to the industry. Bran contributes a pleasing sweet, nutty flavour when added as a flour replacement in baked products and pasta. Bran improves the nutritional quality (fiber, proteins, vitamins and minerals) of the resultant product. Phytic acid, considered as the major constituent in bran and whole wheat, responsible for di-trivalent deficiency disorders in monogastric animals. Nutritionally, bran fractions produced by milling are rich in fibre, minerals, vitamin B6, thiamine, folate and vitamin E and some phytochemicals, in particular antioxidants such as phenolic compounds (Shewry, 2010).

The use of rice bran flour to enhance nutrient intake is an area of research that needs further attention. This may help children to reach their daily nutrient allowances in a way that is not costly and at the same time, enjoyable. This study will be carried out to develop a pasta out of locally available rice bran to be used as an alternative flour replacement and to identify the presence of nutrients after undergoing dry heating and forming it as a dough. The scope of the study will only focus on using the rice bran specifically, white rice bran which is a little known as favorably nutritious and brings health supporting nutrients that can be incorporated to food products. The study limits only to the development of rice bran pasta and identification of Vitamin A and its chemical composition.

MATERIALS AND METHODS

Collection and Heat- treated flour Process

The wheat flour was bought in a local baking shop in Quezon City while the commercial pasta was bought in a local market in Taft Ave., Manila. The rice bran was collected from a local rice mill supplier in Bocaue, Bulacan. The rice bran was subjected to dry heat method. A portion of rice bran (100g) was transferred into shallow pans and spread uniformly in a layer of about 0.5 cm thickness. The pans were then placed in a pre-heated oven at 130°C for 20 mins (Bagchi et al., 2014).

Preparation of Rice Bran Pasta

The flour mixtures of wheat flour and rice bran flour were prepared in ratios of 20%:80% respectively. This ratio is to assure that the required amount of vitamin A will be met.

The researchers placed the flour mixture on a board and made a well in the center and poured the water into it. Using the tips of the fingers, researchers mixed the water with the flour, incorporating a little at a time, until everything is combined. Add more water if the dough feels dry. Knead the pieces of dough together until it binds together. Wrap the dough in cling wrap tightly then rest for 60 mins. Roll and cut the pasta to desired thickness and length. Cook the pasta for 90 seconds and then drain (Jamie Oliver, 2018) and let it cool in room temperature for 5 mins and then pack it inside a sterilized polyethylene bag.

Table 1
Formulation of Pasta

Ingredients	Amount
Wheat Flour	400g
Rice Bran Flour	100g
Water	50ml

Proximate Analysis

The 100g of rice bran pasta and commercial pasta were brought to the SentrosaPagsusuri, Pagsasanay at Pangangasiwang Pang-Agham at Teknolohiya Corp. (SENTROTEK) at Pilar, Mandaluyong City to identify the proximate analysis according to the Official Methods of Analysis of A.O.A.C. International, 19th edition. The test methods used are: Ash, Moisture and Crude Fiber was determined by using Gravimetry, Fat using Acid Hydrolysis, Protein and Nitrogen was determined by using the Kjeldahl method and Total Carbohydrate was determined using computation method.

Vitamin and Mineral Analysis

The identification of Vitamin A was analyzed using the High-Performance Lipid Chromatography² (in-house) according to the Official Methods of Analysis of A.O.A.C. International, 19th edition by the SentrosaPagsusuri, Pagsasanay at Pangangasiwang Pang-Agham at Teknolohiya Corp. (SENTROTEK) at Pilar, Mandaluyong City.

Microbial Analysis

The 100g of rice bran pasta and commercial pasta were brought to the SentrosaPagsusuri, Pagsasanay at Pangangasiwang Pang-Agham at Teknolohiya Corp. (SENTROTEK) at Pilar, Mandaluyong City to identify the aerobic plate count and yeast and molds count using the pour plate methods according to the Bacteriological Analytical Manual.

Sensory evaluation

The sensory evaluation of RB pasta and commercially available pastas were undertaken by 33 children, 12 male and 21 female, ages 4-6 years old from the Barangay 672, Paco, Manila. The sensory attributes like appearance, color, flavor, texture and overall acceptability will be evaluated using 5-point hedonic score system. 100g serving of both pastas were given for the panelists. A 250 ml of distilled water were also given to the panelists for palate cleanser. Rice bran pasta and commercially available pasta were served without sauce, to achieve the desired parameters without any biases. The panelist will give score 5-1 to the product, ranging from 'strongly disliked' to 'strongly liked' to find out the most suitable composition of pasta (Shogren RL, and others, 2006). The researchers did a one-on-one question-interview to the panelist to obtain their opinions for each question.

Statistical Treatment of Data

The researchers used mean average, standard deviation, descriptive analysis and independent t-testing interpreting the results because mean average is the central value for a data being compared. The purpose of p-value is to determine if there's a significant difference, and it is used to know if the null hypothesis is true or not (Heymann and Lawless, 2010). It eliminates the random errors in the experiment and usually gives a more accurate value than a single experiment carried out. While standard deviation tells how closely an average represents the underlying numbers (Kallah, 2009).

RESULTS AND DISCUSSION

The nutritional components of the rice bran pasta make them the primary compositions of the beneficial dietary intake for the wellness and development of the children. It is of great importance to harness insight on the types of substances that supply energy and promote food digestibility that is present in the rice bran pasta before efficient formulation and production of market quality diets could be successfully achieved. Therefore, proximate parameters were determined to ascertain the dietary usefulness (Ajani, et al., 2016) and the result was as shown in Table 2 shows the proximate composition of the rice bran pasta. Vitamin A (188µg/100g), Total Carbohydrates (29.8g/100g), Protein (6.5g/100g), Total Fat (4.7g/100g), Ash (0.58g), Nitrogen (1.0g/100g), Crude Fiber (0.33%/ww), and Moisture (64.9g/100mL). The potential of the rice bran pasta as supplement is

exemplified by the proximate composition and presence of a few bacteria in the analyzed rice bran and commercially available pasta sample.

Table 2
Proximate Analysis of the Rice Bran Pasta

Parameters	Unit	Results (Rice Bran Pasta)	PDR1 2015
Vitamin A	µg/100g	188µg	400µg
Total Carbohydrates	g/100g	29.8g	-
Protein	g/100g	6.5g	-
Total Fat	g/100g	4.7g	-
Ash	g/100g	0.58	-
Nitrogen	g/100g	1.0g	-
Crude Fiber	% w/w	0.33%	-
Moisture	g/100mL	64.9g	-

Table 3 shows the comparison of nutrients between the Rice Bran Pasta and the Commercially Available Pasta. There are little differences in the nutritional content of the two pastas except for the total fat and the lack of Vitamin A in the commercially available pasta. The potential of the rice bran powder and rice bran pasta as a food supplement was depicted by the proximate composition and presence.

Also, in table 3, in accordance to the Food Fortification Guideline the amount of Vitamin A in the rice bran pasta constitutes to just above the 1/3 or 47% of the Recommended Dietary Intake for children ages 4-6. In addition, the rice bran pasta can be a probable substitute for commercially available ones.

Table 3
Proximate Composition and Vitamin A analysis of Rice Bran Pasta and Commercially Available Pasta

Parameters	Unit	Results (Rice Bran Pasta)	Results (Commercial)	PDR1 2015
Vitamin A	µg/100g	188µg	-	400µg
Total Carbohydrates	g/100g	29.8g	28.3g	-
Protein	g/100g	6.5g	5.9g	-
Total Fat	g/100g	4.7g	1.2g	-
Ash	g/100g	0.58	0.28g	-
Nitrogen	g/100g	1.0g	0.94g	-
Crude Fiber	% w/w	0.33%	0.12g	-
Moisture	g/100mL	64.9g	64.3g	-

The total plate count of the rice bran pasta was also evaluated. The result is shown in table 4. The Rice Bran Pasta showed a bacterial population within the ranges of 1.0×10^2 for the Yeast and Molds while 2.2×10^3 for the Total Plate Count. The heated rice bran powder and proper preparation of the pasta helped in achieving a low growth of bacteria and pathogens. In addition, according to the Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods, the standard limit for ready-to-eat food items is between 10^6 and 10^8 CFU/g while the minimum CFU/g of Aerobic Plate Count is $<10^5$ CFU/g and for yeast $<10^3$ CFU/g while in molds $<10^2$ CFU/g (Sciosia et al., 2016).

Table 4
Microbial Analysis of the Rice Bran Pasta

Parameters	Unit	Results	Minimum	m	M	c	Critical Value
Aerobic Plate Count	CFU/g	2.2×10^3	$<10^5$ CFU/g	10^3	10^5	2	$10^6 - 10^8$
Yeast and Molds	CFU/g	1.0×10^2	Yeast = $<10^3$ CFU/g Mold = $<10^2$ CFU/g	10^2	10^5	2	$10^6 - 10^8$

Legend:

m – acceptable level of microorganism determined by a specific method
M – level which when exceeded in one or more samples would cause the lot to be rejected as this indicates potential health hazard or imminent spoilage
c – maximum allowable number of defective or marginally acceptable units (DOH-FDA, 2013)

Table 5 shows the results in the sensory attributes of the product. For the appearance of rice bran pasta, results show strongly disliked with the mean average of 2.79, while in commercially available pasta, it shows 3.73 where it is above average. For the aroma of rice bran pasta, the acceptance rate was disliked with the mean average of 2.45 while in commercially available pasta, the mean average is 3.06 which is average. For the texture of rice bran pasta, the acceptance rate was neither liked or disliked with the mean average of 2.55 while in commercially available pasta, the mean average is 3.45 which is above average. For the flavor of rice bran pasta, the acceptance rate was liked by the panels with the mean average of 3.00 while in commercially available pasta, the mean average is 3.70 which is above average. For the overall acceptability of rice bran pasta, the acceptance rate was strongly liked by the panels with the mean average of 3.06 while in commercially available pasta, the mean average is 3.27 which is just average.

Table 5
Sensory Evaluation for Rice Bran Pasta

Attributes	Acceptance Rate	Mean RBP	Interpretation	Mean CAP	Interpretation
Appearance	Strongly Disliked	2.79	Average	3.73	Above Average
Aroma	Disliked	2.45	Average	3.06	Average
Texture	Neither	2.55	Average	3.45	Above Average
Flavor	Liked	3.00	Average	3.70	Above Average
Overall Acceptability	Strongly Liked	3.06	Average	3.27	Average

Table 5, Excellent = 4.20-5.00, Above Average 4.19 – 3.40, Average = 3.39 – 2.40, Below Average = 2.39 – 1.80, and Poor = 1.79 – 1.00. (Dayrit, Fernandez, Ymas Jr. 2017).

Table 5.1
Statistical Result of the Sensory Evaluation for Rice Bran Pasta

Attributes	Standard Deviation	P-Value	Interpretation
Appearance	0.77	2.09^{-8}	Significant
Aroma	0.80	8.4^{-4}	Significant
Texture	0.85	2.04^{-6}	Significant
Flavor	0.99	0.0019	Significant
Overall Acceptability	0.69	0.11	Non-Significant

If p is ($p > 0.05$), our group variances can be treated as equal. If $p < 0.05$, we have unequal variance

Table 5.1 shows the standard deviation, P-Value and Interpretation in the sensory attributes of the product. The mean values are expressed $P < 0.05$. For the appearance, aroma, texture, and flavor it showed in the table that it is statistically significant to $P < 0.05$. Meanwhile, for the overall acceptability, it showed that there is no significant difference.

CONCLUSION

The researchers have successfully met the desired objectives of the study which is to develop a pasta using rice bran that can be used as a food supplement and analyze both its chemical composition and sensory attributes. More importantly the developed pasta achieved a positive result with Vitamin A showing a high content of 188 µg/100g which is just above 1/3 based on Food Fortification Guideline or 47% of the recommended dietary allowance for children ages 4-6. While on the commercial pasta, there was no Vitamin A detected. Also, the developed pasta gained a positive remark in comparison with the commercially available pasta since there was no significant difference in terms of overall acceptability based on the

sensory evaluation and statistical results. In conclusion, rice bran utilization as food compound for human consumption and its feasible incorporation into pasta can be good for supplementation for children to improve their daily nutrient allowance. In addition, rice bran pasta has showed increase nutrients composition than commercial pasta. From this study, it can be concluded that rice bran is safe for human consumption and it can be a supplement for food production.

RECOMMENDATIONS

The researchers recommend developing a formulated wheat pasta using the standard recipe when doing the comparison of proximate and sensory evaluation instead of using a commercially available one because the latter already established the means to be generally acceptable by the public. The developed pasta should be cut and shaped into different sizes and also if it is possible to add a sauce when undergoing sensory evaluation as to achieve a much acceptable result. The rice bran pasta should undergo further tests with different ratios of rice bran flour to determine if it is a possible replacement for flour. The researchers also recommend tests for the dietary fiber, other vitamins and minerals such Iron, Calcium, Magnesium, Vitamin B-complex, and Zinc. A test for the shelf life of the developed pasta to determine how long can it be stored and be safe for human consumption. Lastly, the researchers hope that the developed rice bran pasta will be used for supplementary feedings in the communities where Vitamin A deficiency is rampant.

REFERENCES

- Ajani, O.O., Owoeye, T.F., Olasehinde, G.I., Audu, O.Y., Owolabi, F.E., Akinlabu, D.K., and EdoborOsoh, A., *Am. J. Food Tech.*, 11(6), 253 (2016). Characterization, Proximate Composition and Evaluation of Antimicrobial Activity of Seed Oil of *Bauhinia tomentosa*
- Bagchi, Robert & Rachel, Gallery & Gripenberg, Sofia & Gurr, Sarah & Narayan, Lakshmi & E Addis, Claire & Freckleton, Robert & Lewis, Owen. (2014). Pathogens and insect herbivores drive rainforest plant diversity and composition. *Nature*. 506. 10.1038/nature12911.
- Black RE, Allen LH, Bhutta ZA, Caulfield LE, De Onis M, Ezzati M, Mathers C, Rivera J. Maternal and child undernutrition: global and regional exposures and health consequences. *Lancet*. 2008;371:243–260. [PubMed]
- Dayrit, Benjamin C., Hernandez, Rogelio M, and Ymas Jr, Sergio E. 2017. *College Statistics, Revised Edition 2017*
- Department of Health – Food and Drug Administration (2013)
- Elisa Scioscia, Carmina Viola, Robert Colicchio, Maria Grazia Volpe, Michelle Di Stasio, Ettore Varricchio, Paola Salvatore, Caterina Pagliarulo. 2016. Microbiological Profile and Shelf life of Fresh Pasta subjected to several cycles of pasteurization.
- FAO. 2012. Rice and human nutrition. Food and Agriculture Organization of the United Nations.
- Food and Nutrition Research Institute- Department of Science and Technology (2013). 8th National Nutrition Survey
- Food and Nutrition Research Institute- Department of Science and Technology (2008). 7th National Nutrition Survey
- Gopalakrishnan, J., R. Menon, G. Padmaja, M.S. Sajeev, and S.N. Moorthy, Nutritional and Functional Characteristics of Protein-Fortified Pasta from Sweet Potato. *Food and Nutrition Sciences*, 2011. 2: p. 944-955.
- Golden MH. 2009. Proposed recommended nutrient densities for moderately malnourished children. *Food Nutr Bull*. 2009 Sep;30(3 Suppl):S267-342.
- Heymann, H. & Lawless, H.T. (2010). Sensory Evaluation in Food: Principles and Practice. 5999. 10.1007/978-1-4419-6488-5.
- Jamie Oliver, 2018. A basic recipe for fresh pasta.
- Johnson EJ, Russell RM. Beta-Carotene. In: Coates PM, Betz JM, Blackman MR, et al., eds. *Encyclopedia of Dietary Supplements*. 2nd ed. London and New York: Informa Healthcare; 2010:115-20.
- Li, Man & Zhu, Ke-Xue & Guo, Xiao-Na & Brijis, Kristof & Zhou, Hui-Ming. (2014). Natural Additives in Wheat-Based Pasta and Noodle Products: Opportunities for Enhanced Nutritional and Functional Properties. *Comprehensive Reviews in Food Science and Food Safety*. 13. 10.1111/1541-4337.12066.
- Lozoff B, Georgieff MK. 2006. Iron deficiency and brain development. *Semin Pediatr Neurol*. 2006 Sep;13(3):158-65.
- Mayo-Wilson Evan, Imdad Aamer, Herzer Kurt, Yakoob Mohammad Yawar, Bhutta Zulfiqar A. Vitamin A supplements for preventing mortality, illness, and blindness in children aged under 5: systematic review and meta-analysis *BMJ* 2011; 343:d5094
- McLean E, Cogswell M, Egli I, Wojdyla D, De Benoist B. Worldwide prevalence of anaemia, WHO vitamin and mineral nutrition information system, 1993–2005. *Publ Health Nutr*. 2008;12:444–454. –(PubMed)
- Official methods of analysis of AOAC International (19th ed.)
- Ross CA. Vitamin A. In: Coates PM, Betz JM, Blackman MR, et al., eds. *Encyclopedia of Dietary Supplements*. 2nd ed. London and New York: Informa Healthcare; 2010:778-91.
- Ross A. Vitamin A and Carotenoids. In: Shils M, Shike M, Ross A, Caballero B, Cousins R, eds. *Modern Nutrition in Health and Disease*. 10th ed. Baltimore, MD: Lippincott Williams & Wilkins; 2006:351-75.
- Solomons NW. Vitamin A. In: Bowman B, Russell R, eds. *Present Knowledge in Nutrition*. 9th ed. Washington, DC: International Life Sciences Institute; 2006:157-83.
- Shewry PR, Piironen V, Lampi AM, Edelmann M, Kariluoto S, Nurmi T, et al. Effects of genotype and environment on the content and composition of phytochemicals and dietary fiber components in rye in the HEALTHGRAIN diversity screen. *J Agric Food Chem*. 2010;58(17):9372–9383. [PubMed]
- Shogren R.L., Hareland G.A. and Wu Y.V. (2006). Sensory evaluation and composition of spaghetti fortified with soy flour. *Journal of Food Science*; 71(6): 5428-5432.
- Siddharth Kalla (Jan 13, 2009). Statistical Mean. Retrieved Oct 25, 2018 from Explorable.com: <https://explorable.com/statistical-mean>
- Solomons NW. All that glitters is not iron (deficiency): revisiting the question of why anemic individuals are anemic. *Nutr Rev*. 2002;60:91–96. [PubMed]
- United States. (1980). *Bacteriological analytical manual*. Washington, D.C: Association of Official Analytical Chemists.
- World Health Organization. Protein and amino acid requirements in human nutrition. Geneva: WHO, 2007.
- World Health Organization. Global Prevalence of Vitamin A deficiency in Populations at Risk 1995–2005. WHO Global Database on Vitamin A deficiency. Geneva: WHO; 2009.
- Watchararujij, K., Goto, M., Sasaki, M. and Shotipruk, A. 2008. Value-added subcritical water hydrolysate from rice bran and soybean meal. *Bioresource Technology* 99: 6207-6213.