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Research Article

Estimation of Resistant starch, Non-Resistant starch and Total starch of Unprocessed Foods Sourced within Nsukka Town

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Abstract: Resistant starch (RS) shows potential health benefits against certain metabolic diseases, hence, this research was aimed to determine the resistant starch contents of certain traditional foods using the Megazyme resistant starch assay kit (Megazyme, Bray, Ireland). The experimental sample was divided into four groups (cereals, legumes, stem tubers and root tubers). The RS contents measured from the pellet left after 16hr of α-amylase and amyloglucosidase digestion of samples, were greater than 10% for all the samples analyzed. White yam had the highest total starch (TS) (103.37 ± 23.43 %w/w), followed by maize (88.34 ± 8.51) and Eddoe (87.27 ± 2.32) and the least was Bambara nut (54.54 ± 2.33). Among the various groups, legume showed the least TS content on average. The resistant starch contents in the legume samples analyzed ranged from 31.60 ± 4.12 to 41.94 ± 0.43 (%w/w). On the other hand, the NRS contents in legume samples ranged from 17.30 ± 8.50 to 28.54 ± 7.57. Among the legumes analyzed, Pigeon pea had the highest RS content of 41.94 ± 0.43 and lowest NRS content of 17.30 ± 8.51. Bambara nut had quite low NRS of 17.19 ± 4.57 and relatively moderate RS content of 37.35 ± 2.93. The resistant starch contents in cereals ranged from 25.07 ± 4.19 to 31.59 ± 4.83 (%w/w). This result showed that among the various groups, cereals had the lowest RS but had the highest NRS contents on average. The NRS contents in cereals ranged from 30.12 ± 8.45 to 56.67 ± 7.51. There was a significant decrease, $p<0.05$, in the RS content of bitter yam (25.82 ± 10.81) when compared to all the other tubers. Conversely, there was a significant increase, $p<0.05$, in the level of RS content of Eddoe (54.85 ± 5.02) when compared to the bitter yam and Taro. White yam with 51.40 ± 18.29, was significantly high, $p<0.05$, in NRS content when compared to the other tubers. Among the stem tubers, the RS content of water yam, white yam and bitter yam was 44.69±3.95, 52.25 ± 16.96 and 25.82 ± 10.81 respectively. The NRS content in the same order was 17.53 ± 10.19, 51.40 ± 18.29 and 30.47 ± 12.58 respectively.

Keywords: Resistant starch, Cereals, Legumes and Tubers

INTRODUCTION

Most people from around the world today are suffering from more diet related diseases than ever before in history. Lifestyle such as wrong nutritional habits coupled with lack of regular exercise has been the leading cause of these ill-healths called metabolic disorder (Ann, 2008). The emergence of these disorders in recent times is due, partly, to the embrace with reckless abandon of western diet (Goldsmith, 2014). Thus, metabolic disorders such as cardiovascular diseases, obesity, diabetes, and cancer have recently become major threats to human health in many nations (Chen, 2010). Cardiovascular diseases and diabetes have been shown to be the leading cause of mortality in the world (Nigudkar, 2014). Resistant starch (RS), an important component of diet, shows the potential health benefits against these metabolic diseases (Nigudkar, 2014). Hence, it is one of the nature's most interesting bioactive compounds (Moongngarm, 2013). Resistant starch exerts its positive health benefits by escaping digestion in the small intestine and then serving as substrate for microbial fermentation in the colon which produces, beside other compounds, short chain fatty acids (SCFAs), such as butyrate, propionate and acetate (Dupuis, 2014). These short chain fatty acids play a number of roles in increasing metabolism, decreasing inflammation and formation of ATP (Topping and Clifton, 2001; Kolida and Gibson, 2007; Goldsmith, 2014). Therefore, resistant starch rich foods can decrease glycemic and insulin responses and reduce the risk of developing type II diabetes mellitus, obesity and cardiovascular diseases (Granfeldt *et al.*, 1995; Regina *et al.*, 2006; Zhu, *et al.*, 2017). Besides, resistant starch selectively stimulates the growth of beneficial bacteria which then prevent colonization and infection by potential pathogens (Kolida and Gibson, 2007;

Goldsmith, 2014). The escape of digestion by resistant starch is due, majorly, to the crystalline structure of starch which hinders enzymic interaction with the molecule (Nugent and Lockyer, 2016). Hence, any activity that disrupts the crystalline structure of starch such as gelatinization or that makes it accessible to the enzymes by releasing it from intact cell wall and bound molecules relieves its resistance to digestion (Karim *et al*., 2000). Thus, various food processing affect the resistant starch content (Nugent, 2005). Starch is one of the major components of legumes, cereals and tubers, thus, it is the major natural sources of resistant starch (Leszezynski, 2004). In addition, unripe banana, plantain and sweet potato have been shown variously to have high resistant starch contents. However, plant locations affect the nutritive composition and as such affect the resistant starch contents (Nugent and Lockyer, 2016).

Impact of Resistant Starch on Human Health

The normal human gut has hundreds of bacterial species, which are stimulated by RS in our intestine, helping to maintain a healthy balance of bacteria (Goldsmith, 2016). These good bacteria feed on RS and produce Short Chain Fatty Acids (SCFA), through fermentation, the most significant of which are butyrate, acetate and propionate by fermentation process - of these three SCFA, Butyrate is of particular importance due to its beneficial effects on the colon and overall health, and RS appears to increase butyrate production more when compared to the soluble fibers (Nugent, 2005). Butyrate is the preferred energy source of cells lining the colon, and it also plays a number of roles in increasing metabolism, decreasing inflammation and improving stress resistance. Also, butyrate functions to improve the integrity of our gut by decreasing intestinal permeability and therefore keeping toxins in the gut and out of the bloodstream (Chen *et al*., 2010). Butyrate that is not utilized by the colonic cells enters the bloodstream, travel to the liver, and spread throughout the body where they exert additional anti-inflammatory effect (Goldsmith, 2016).

RS have several beneficial effects that may contribute to weight loss, including decreased blood insulin spikes after meals as pointed out ab initio, decreased appetite, and decreased fat storage in fat cells. RS is also associated with decreased risk of colorectal cancer, thought to occur through several different mechanisms including protection and increased apoptosis of cancerous cells (Goldsmith, 2016).

MATERIALS AND METHODS

Sample Collection

Food samples (maize, millet, wheat, bambara nut, cowpea, pigeon pea, water yam, white yam, bitter yam, eddoes and taro) were purchased from food dealers at Ogige market in Nsukka, Enugu, Nigeria. They were grouped into cereals, legumes, stem tuber and root tuber.

Determination of moisture content

Moisture content of dry samples were determined using the method described by AOAC (1980)

Estimation of RS and NRS contents

Sample Preparation

Approximately 50 g of each of the samples was milled to pass a 1.0 mm sieve. It was then transferred to a wide-mouthed plastic jar and mix well by shaking and inversion.

The contents of RS, non-resistant starch and total starch were analyzed using the Megazyme resistant starch assay kit (Megazyme International Ireland Ltd, Bray, Ireland) by AOAC Method 2002.02; AACC Method 32-40.01 and Codex Type II Method.

In this determination, samples are incubated in a shaking water bath with pancreatic α-amylase and amyloglucosidase (AMG) for 16 h at 37°C, during which time non-resistant starch is solubilised and hydrolysed to D-glucose by the combined action of the two enzymes. The reaction is terminated by the addition of an equal volume of ethanol and the RS is recovered as a pellet on centrifugation. This is then washed twice by suspension in aqueous ethanol (50% v/v), followed by centrifugation. Free liquid is removed by decantation. RS in the pellet is dissolved in 2 M KOH by vigorously stirring in an ice-water bath over a magnetic stirrer. This solution is neutralised with acetate buffer and the starch is quantitatively hydrolysed to glucose with AMG. D-Glucose is measured with glucose oxidase/peroxidase reagent (GOPOD) and this is a measure of the RS content of the sample. Non-resistant starch (solubilised starch) is determined by pooling the original supernatant and the washings, adjusting the volume to 100 mL and measuring D-glucose content with GOPOD.

Statistical Analysis

All samples were analyzed in triplicates and data were expressed as means and standard deviations. Data obtained were subjected to one-way statistical Analysis of Variance (ONE-WAY ANOVA) using SPSS for Windows), version 16. The difference in means was determined by the Least Standard Deviation Multiple Range Test (LSD). Statistical significance was set at 95% confidence level

RESULTS

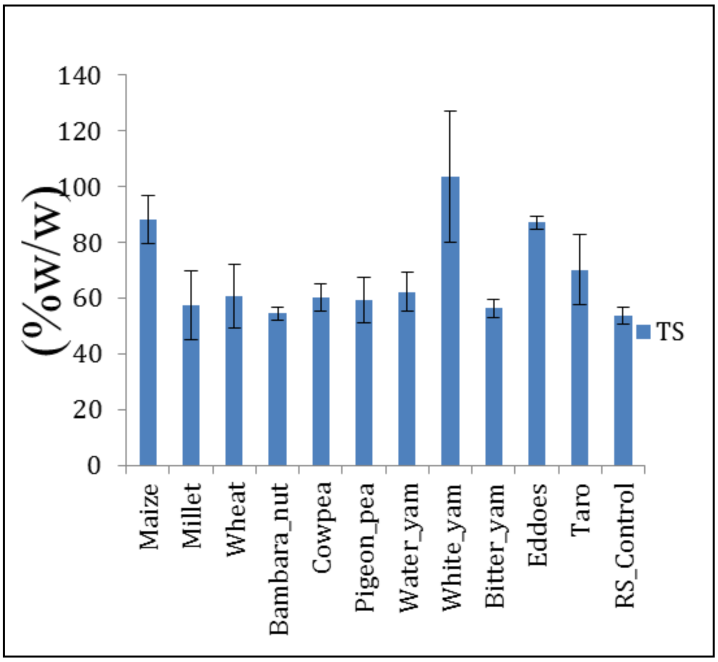

Fig. 1, Percentage (w/w) of total starch in food samples analyzed.
TS = Total Starch, n = 3

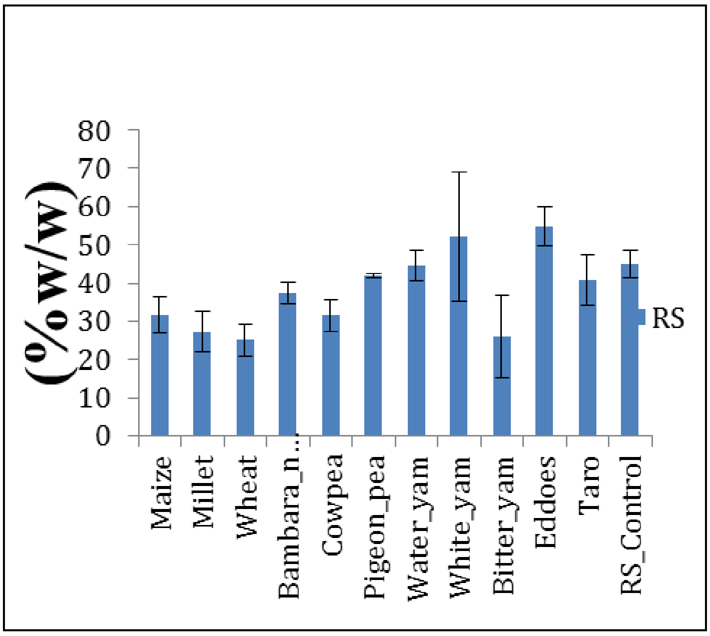

Fig. 2, Percentage (w/w) of the resistant starch contents in the food samples analyzed. These foods were purchased from Ogegi market at Nsukka in Enugu state, Nigeria.
RS = Resistant Starch, n = 3

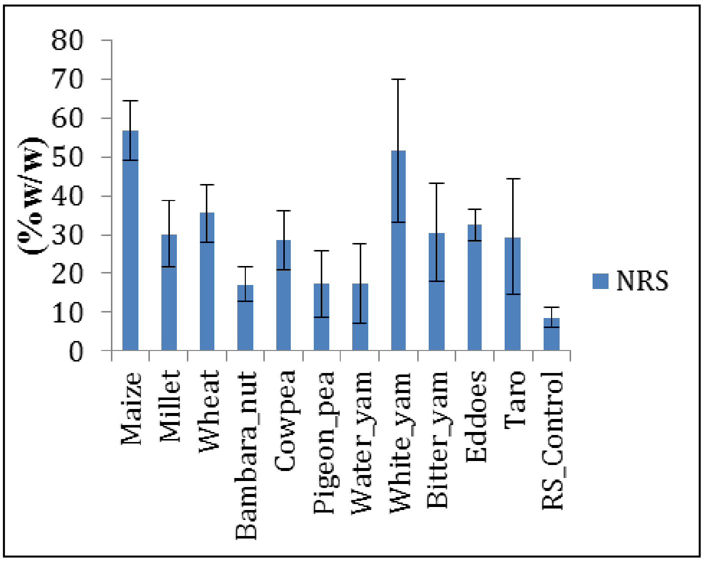

Fig. 3; Percentage (w/w) of the non-resistant starch contents in the food samples analyzed
NRS = Non-Resistant Starch, n = 3

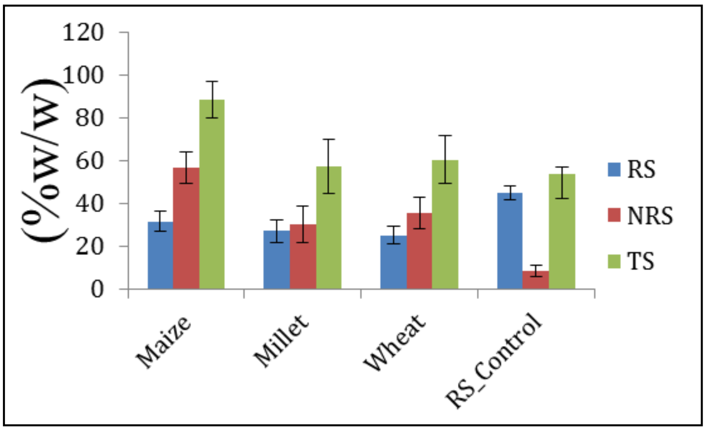

Fig. 4, Percentage weight per weight of RS, NRS and TS among the cereals
n = 3. RS = Resistant Starch. NRS = Non-Resistant Starch. TS = Total Starch

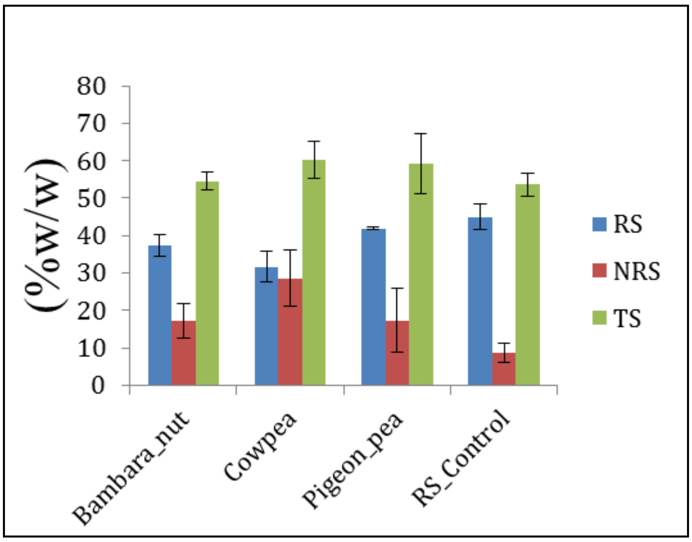

Fig. 5, Percentage weight per weight of RS, NRS and TS among the legumes
n = 3. RS = Resistant Starch. NRS = Non-Resistant Starch. TS = Total Starch

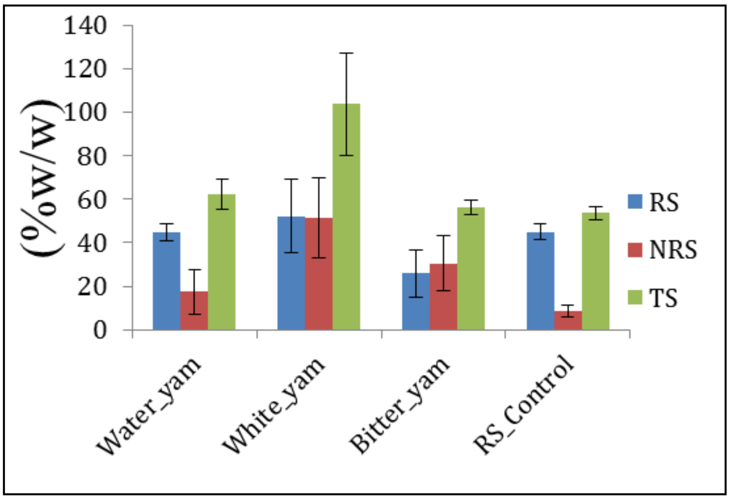

Fig. 6, Percentage weight per weight of RS, NRS and TS among stem tubers
n = 3. RS = Resistant Starch. NRS = Non-Resistant Starch. TS = Total Starch

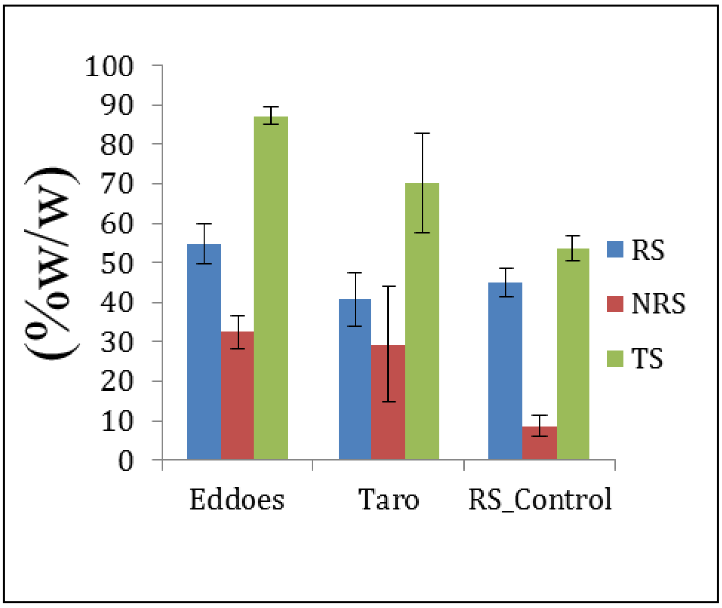

Fig. 7, Percentage weight per weight of RS, NRS and TS among root tubers
n = 3. RS = Resistant Starch. NRS = Non-Resistant Starch. TS = Total Starch

DISCUSSION

Traditional Nigerian diets include cereal, legume and tuber based foods. Maize, millet, and wheat were used as the representatives of cereal; bambara nut, Cowpea and Pigeon pea were used for legumes; White yam, Water yam and Bitter yam were used for stem tubers and Eddoe and taro for root tubers. The RS contents measured from the pellet left after 16hr alpha-amylase and amyloglucosidase activities on the samples, were greater than 10% for all the samples analyzed. Legumes belong to the group of starchy foods that are physically inaccessible to digestive enzymes due to the presence of intact cell walls (Goldsmith, 2014). The resistant starch contents in the legume samples analyzed ranged from 31.60 ± 4.12 to 41.94 ± 0.43 (%w/w) (mean ± SD) of the samples. This result indicates that legume starch maybe slowly digested as posited by Phillips (1993). On the other hand, the non-resistant starch contents in legume samples ranged from 17.30 ± 8.50 to 28.54 ± 7.57. This range was the lowest obtained among the whole samples, indicating that legumes had the lowest non-resistant starch contents among the food samples analyzed, further buttressing the slow digestibility of legumes as they may contain preponderance of RS. Thus, legumes are major sources of RS and they play role as therapeutic agents in the diets of persons suffering from metabolic disorders (Mahadevamma and Tharanathan, 2004). Among the legumes analyzed, Pigeon pea had the highest resistant starch content of 41.94 ± 0.43 and it had the lowest non-resistant starch content of 17.30 ± 8.51, but there was no significant difference (p>0.05) in the resistant and non-resistant starch contents within the group.

The resistant starch contents in cereals ranged from 25.07 ± 4.19 to 31.59 ± 4.83 (%w/w). This result showed that among the various food groups, cereals had the lowest RS and the highest NRS contents on average. The NRS contents in cereals ranged from 30.12 ± 8.45 to 56.67 ± 7.51. However, legumes showed opposite effect on the RS and NRS contents. Thus, cereals were the main sources of energy giving food (Shewry and Hey, 2015). Similarly, major starch sources are the cereals (Santana and Meireles, 2014; Alcazar-Alay and Meireles, 2015). The amount of TS in cereals ranged from 57.32 ± 12.38 to 88.34 ± 8.51. This could be the reason cereals have been described as major sources of energy giving foods. Among the three cereal food samples, TS content of maize was significantly high ($p < 0.05$) when compared to the others, wheat and millet, whereas there was no significant difference ($p > 0.05$) in the content of TS between wheat and millet. The significant increase ($p<0.05$) in the content of TS in Maize could have arisen from NRS, which was significantly high ($p<0.05$) when compared to the rest, as there was no significant difference ($p>0.05$) in the contents of RS in the three food samples. The high NRS in cereals indicated that they may not be appropriate to be used as they cannot be used as stated in the diets of persons suffering from metabolic disorders, unless they (cereals) are treated to reduce the NRS levels and possibly increase the RS levels.

A significant decrease ($p < 0.05$) in the RS content of Bitter yam was observed when compared to all the other tubers. Conversely, there was a significant increase ($p<0.05$) in the RS content of Eddoe when compared to the Bitter yam and Taro but it showed no significant difference ($p>0.05$) when compared to the other tubers in the samples. White yam with 51.40 ± 18.29 was significantly high ($p<0.05$) in NRS content when compared to the other tubers. However, all other tubers showed no significant difference ($p>0.05$) when compared to one another. The high content of NRS in White yam could be due to its high glucose response. Among the stem tubers, the RS contents of Water yam, White yam and Bitter yam were 44.69 ± 3.95, 52.25 ± 16.96 and 25.82 ± 10.81 respectively. The NRS levels in the same order were 17.53 ± 10.19, 51.40 ± 18.29 and 30.47 ± 12.58 respectively. This is in line with the result of Chen *et al.* (2010).

CONCLUSION

Cereals, legumes and tubers which are traditional Nigerian foods are composed of starch. The amounts of total starch, resistant starch and non-resistant starch component of these foods differ, with legumes having a moderate amount of total starch and resistant starch contents. However, cereals and tubers showed TS above 50 (%w/w) of the samples. Being that Pigeon pea, Water yam and Bambara nut have moderately high RS contents with corresponding low contents of NRS, it could suggest that they may help prevent or remedy metabolic disorders, whereas White yam and Maize which have relatively high NRS contents may not be ideal for use in managing metabolic disorders.

REFERENCES

1. A.O.A.C. (1980). Moisture content determination, official methods of analysis. *Association of Official Analytical Chemists*, Washington D. C., USA. Pp, 376 – 384.
2. Alcazar-Alay, S. C., & Meireles, M. A. A. (2015). Physiochemical properties, modification and application of starch from different botanical sources. *Food Sciences and Technology, 35*(2), 215 - 236.
3. Ann, N. R. (2008). Chronic diseases in developing countries, health and economic burdens. *New York Academy of Sciences*, 1136, 70 – 79.
4. Birt, D. F., Boylston, T., Hendrich, S., Jane, J. L., Hollis, J., Li, L., McClelland, J., Moore, S., Philips, G. J., Rowlinng, M., Schalinska, K., Scott, M., & Whitley, E. M. (2013). Resistance starch, promise for improving human health. *American Society for Nutrition*, 4, 587-601.
5. Chaphin, M. (2014). The use of enzymes in starch hydrolysis. *http//www.Isbu.ac.uk/water/euztech/starch.html*
6. Chen, L., Liu, R., Quin, C., Meng, Y., Zhang, J., Weng, Y., & Xu, G. (2010). Source and intake of resistant starch in the Chinese diet. *Asia Pacific Journal of Nutrition, 19*(2), 274 – 282.
7. Depuis, J. H., Liu, Q., & Yada, R. Y. (2014). Methodologies for increasing the resistant starch content of food starches, a review. *Journal of Food Sciences*, 13, 1219 – 1234.
8. Friche, M. C. and Prado, J. (2017). Intestinal microbiota in digestive diseases. *Arq Gastroenterol, 54*(3), 255-262.
9. Fuller, R. (2003). Probiotics – what they are and what they do. http//www.positivehealth,com/penuit/Artides/Nutrition/Fuller32.htm.
10. Gibson, G. R., & Reberfroid, M. B. (1995). Dietary modulation of the human colonic microbiota, introducing the concept of prebiotic. *Journal of Nutrition*, 125, 1401-1412.
11. Golddring, J. (2004). Resistant starch, safe intake and legal status. *Journal of the Association of Official Analytical Chemists, 87* (3), 733 – 739.
12. Goldsmith, F. R. (2014). The physiological effects of resistant starch on obesity and diabetes. *Louisiana State University Doctoral Dissertations*, USA. 3983
13. Granfeldt, Y., Eliasson, A., & Bjorck, I. (1995). An examination of the possibility of lowerting the glycemic index of oat and barley flakes by minimal processing. Journal of *Human Nutrition and Metabolism, 130*(9), 2207 – 2214.
14. Gray, J. (2006). Dietary fibre, definition, analysis, physiology and health. *International Life Science Institute, Europe Concise Monograph Series*, Belgium. P, 13.

15. Hen, L., Liu, R., Qin, C., Meng, Y., Zhang, J., Weug, Y., & Xu, G. (2010). Source and intake of resistance starch in the Chinese diet. Asia pac.
16. Hill, J. O., & Peters, J. C. (1998). Environmental contribution to the obesity epidermic. *Science, 280* (5368), 1371 – 1374.
17. Ihediohanma, N. C., Onuegbu, N. C., Peter-Ikechukwu, A. I., & Ojimba, N. C. (2012). A comparative study and determination of glycemic indices of three yam cultivars. *Pakistan Journal of Nutrition, 11* (6), 547 – 552.
18. Isiosio, U. E., Isiosio, I. O., & Emudianughe, P. T. (2015). Resistant starch and *in vitro* starch digestibility of five Nigerian foods. *Journal of Natural Sciences Research, 5* (18), 131 – 135.
19. Karim, A. A., Norziah, M. H., & Seow, C. C. (2000). Methods for the study of starch retrogradation. *Food Chemistry,* 71(1), 9 – 36.
20. Kolida, S., & Gibson, G. R. (2007). Prebiotic capacity of inulin-type fructans. *Journal of Nutrition*, *137*(11), 2503S – 2506S.
21. Langworthy, C.F., & Deuel, H.J. (1913). Digestibility of raw corn, potato, and wheat starches. *A Dictionary of Applied Chemistry,* London. *179*, 27 – 40.
22. Lavanya, D., Kulkarni, P. K., Dixit, M., Raavi, P. K., & Krishna, L. N. V. (2011). Source of cellulos and their applications-A review. *International Journal of Drug Fomulation and Research, 2*(6), 19-38.
23. Leszezynski, W. (2004). Resistant starch-classification, structure, production. *Polish Journal of Food and Nutrition Sciences, 13*(54), 37-50.
24. Mahadevamma, S., & Tharanathan, R. N. (2004). Processing of legumes, resistant starch and dietary fiber contents. *Journal of Food Quality,* 27, 289-303.
25. Masakuni, T., Tamaki, T., Teruya, T., & Takeda, Y. (2014). The principles of starch gelatinization and retrogradation. *Food and Nutrition Sciences* 5, 280 - 291.
26. Moongngarm, A. (2013). Chemical compositions and resistant starch content in starchy foods. *American Journal of Agricultural and Biological Sciences,* 8(2), 107 – 113.
27. Nigudkar, M. R. (2014). Estimation of resistant starch content of selected routinely consumed Indian food preparations. *Current Research in Nutrition and Food Science, 2*(2), 13-83.
28. Nugent, A. (2005). Health properties of resistant starch. *Nutrition Bulletin,* 30, 27 – 54.
29. Nugent, A. P., & Lockyer, S. (2016). Health effects of resistant starch. *British Nutrition Foundation,* London, UK. Pp, 1 – 32.
30. Ottenhof, M., & Farhat, I. A. (2004). Starch retrogradation. *Biotechnology and Genetic Engineering Reviews, 21*(1), 215 – 228.
31. Phillips, R. D. (1993). Starchy legumes in human nutrition, health and culture. *Plant Food for Human Nutrition, 44*(3), 195-211.
32. Purwani, E. Y., Purwadaria, T., & Suhartono, M. T. (2012). Fermentation RS3 derived from sago and rice starch with *Clostridium butyricum* BCC B2571 or *Eubacterium rectal* DSM 17629. *Anaerobe,* 18, 55 – 61.
33. Regina, A., Bird, A., Topping, D., Bowden, S., Freeman, J., & Barsby, T. (2006). High-amylose wheat generated by RNA interference improves indices of large-bowel health in rats. *Proceedings of the National Academy of Sciences, 103*(10), 3546 – 3551.
34. Roberfroid, M., Gibson, G. R., Hoyles, L., McCartney, A. L., Rastall, R., Rowland, I., ... & Guarner, F. (2010). Prebiotic effects: metabolic and health benefits. *British Journal of Nutrition*, *104*(S2), S1-S63.
35. Row land, I., Gilbson, G., Flieken, A., scott, k., Swann, J., Theile, I., & Touhy, K. (2017). Gut microbiota functions, metabolism of nutrients and other food components. *European journal of nutrition.*
36. Santana, A. L., & Meirels, M. A. A. (2014). New starch are the trend for industrial application, a review. *Food and Public Health, 4*(5), 229-241.
37. Schrezenmeir, J., & Vrese, M. (2001). Probiotics, prebiotics and symbiotic, Approaching a definition. *American Journal of Clinical Nutrition* 73, 3615-3645
38. Shewry, P. R., & Hey, S. J. (2015). The contribution of wheat to human diet and health. *Food and Energy Security,* 4, 178 - 202.
39. Slavin, J. (2013). Fibre and prebiotics; mechanism and health benefits. *Nutrition,* 5,1417-1435.
40. Tako, M., Tamaki, Y., Teruya, T., & Takeda, Y. (2014). The principles of starch gelatinization and retrogradation. *Food and Nutrition Sciences,* 5, 280 – 291.
41. Thursby, E., & Juge, N. (2017). Introduction to the human gut microbiota. *Biochemical Journal,* 474, 1823-1836.
42. Topping, D. L., & Clifton, P. M. (2001). Short-chain fatty acids and human colonic function, role of resistant starch and non-resistant starch polysaccharides. *American Physiological Society, 81*(3), 1031 – 1064.
43. Wang, J., Hu, P., Chen, Z., Liu, Q., & Wei, C. (2017). Progress in high-amylose cereal crops through inactivation of starch branching enzymes. *Frontiers in Plant Science, 8*(469), 1 – 10.
44. Wang, S., Niu, Q., & Wang, S. (2015). Starch retrogradation, A comprehensive review. *Comprehensive Reviews in Food Science and Food Safty, 14*(5), 568-585.
45. Warren, F. J., Zhaug, B., waltzer, Gidley, M. J., & Dhital, S. (2015). The interplay of α-amylase and amyloglucosidase activities on the digestion of starch *in vitro* enzymic systems. *Carbohydrate Polymers, 6*(117), 192-200.

46. Zaman, S. A., & Sarbini, S. R. (2016). The potential of resistant starch as a prebiotic. *Critical Reviews in Biotechnology*, 36, 574 – 584.
47. Zhu, D., Fang, Y., Gao, K., Shen, J., Zhong, T. P., & Li, F. (2017). Vegfa impacts early myocardium development in zebrafish. *International Journal of Molecular Sciences, 18*(2), 57 – 73.
48. Zhu, F. (2014). Structure, physicochemical properties, modifications and uses of sorghum starch. *Comprehensive Reviews in Food Science and Food Safety, 13*, 597 – 609.