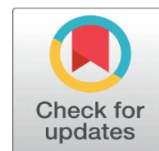


ANALYSIS OF PROTEIN IN WHEAT GRAIN

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ABSTRACT

Wheat is one of the most important cereal crops grown with highest demand. Nitrogen content that enables the protein in the grain is necessary for baking and processing quality. The importance of wheat protein and its functionality in bakery products are well discussed in the given topic. This Study was carried out to study the effect of nitrogen fertilizer supply and its implications on bread wheat variety in the baking process. Dumas's method is used for estimating the protein content in the bread variety that is grown in two different nitrogen fertilizers supply with 100kg/ha and 350kg/ha respectively. The results clearly depict the highest growth of bread wheat variety which was grown in 350kg/ha of nitrogen supply has high amount of protein content. Wheat is the best source of carbohydrates and the proteins present in are gliadin and glutenin which are responsible to provide viscoelastic properties in the bakery products. Functionality of the bakery products are well discussed in the introduction part. The result includes test values and graphs have been plotted based on the comparison of protein present in bread. In the discussion section the differences and effects of protein in processing of foods are analysed against similar other research works.

Received 1 September 2021
Accepted 15 September 2021
Published 30 September 2021

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DOI
[10.29121/granthaalayah.v9.i9.2021.4286](https://doi.org/10.29121/granthaalayah.v9.i9.2021.4286)

Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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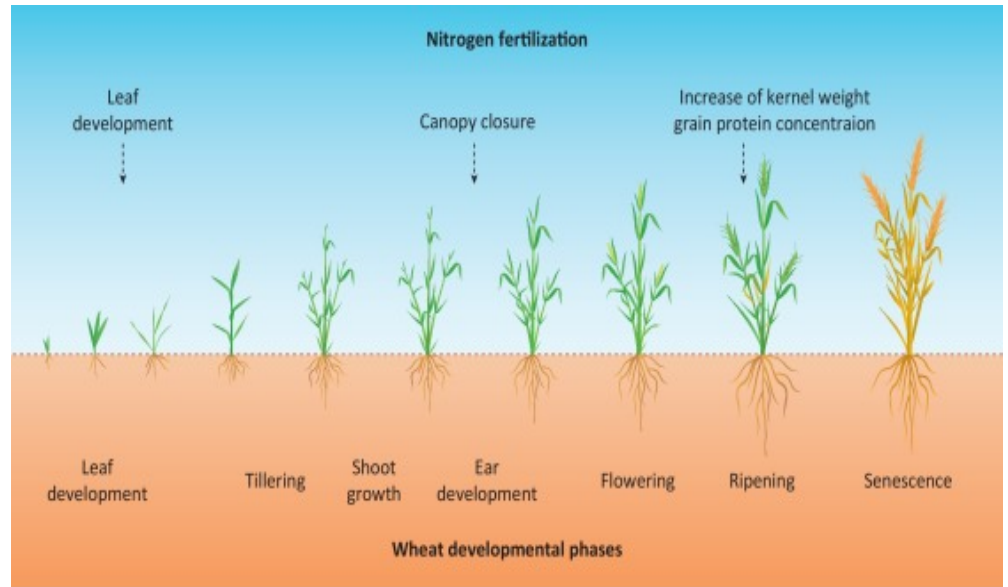
Keywords: Wheat Proteins, Dumas Method, Viscoelasticity Properties, Nitrogen Fertilizers

1. INTRODUCTION

Wheat (*Triticum aestivum*) is the fourth most staple crop consumed by world's population occupying 22% cultivated area [Shewry \(2009\)](#). Wheat cultivation has been started around 8500BC in Greece later reaching India and Cyprus in 6500BC, Egyptians are the first developers of the usage of oven and baking products into large food scale industries [Brennan \(2009\)](#). The wheat crop has three major phases which considers the demands of nitrogen ([Figure 1](#)). For wheat crops to obtain proper yields good amount of nitrogen fertilizer (60 kg N ha⁻¹) must be used additionally added [Zadoks et al. \(1974\)](#). Wheat crop contains 6mg of proteins in the kernel for the sufficient growth of the leaves ([Figure 1](#)). In the second stage nitrogen content is responsible for the growth of the tillers per plant [Bogard et al. \(2010\)](#). In the third stage the aim of the nitrogen is to build up the protein in the wheat grain [Pechanek et al. \(1997\)](#). In the modern day the percentage of the protein in the grain should be 12% dry matter; amino acids content must be high in vegetative tissues and are transported to newly forming grain where storage proteins are developed ([Figure 1](#)). The process is influenced by late application of nitrogen content where the activity of roots is declined. Hence if nitrogen content was decreased the plant cannot build up protein during the development of kernels.



Yu et al. (2017). However, single application of nitrogen showed same protein in the recent trials in southern Germany Schulz et al. (2015). Flag leaves are important for developing nitrogen metabolites which forms amino acids that are transported to kernels Barneix and Guitman (1993). However, it is not sufficient to reach the protein content; due to high temperatures, the nitrogen content will be decreased. Hence the protein in organic farming shows lower percentage (40%) compared to conventional agricultural systems Hellemans et al. (2018). Therefore, protein concentration will be varied even same genotype of wheat is used Zörb et al. (2006).



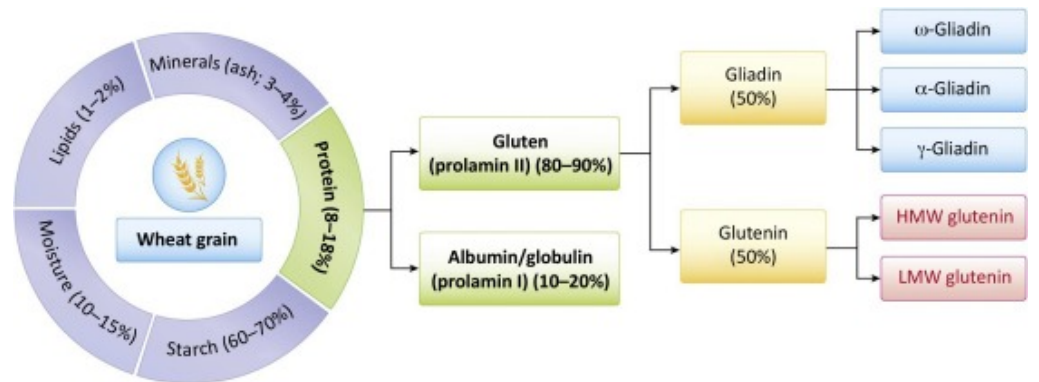
Trends in Plant Science

Figure 1 Development phase of wheat showing increased protein concentration in kernel Zörb et al. (2018)

Singh et al. (2016). However, grain quality can also be increased by increasing the storage proteins in modern varieties. In 1960, Germany has showed a high protein (12% to 16%) content by using improved nitrogen fertilization Laidig et al. (2016). Hence if farmers decided to make bread, he or she will use different varieties of nitrogen applications for obtaining high protein Munier and Salon (2005).

Transcriptome studies showed that 30,000 genes are present in developing wheat grain Wan et al. (2008). However, there is no influence of these components on the protein content of the wheat grain. Prolamin storage proteins that are related to gluten proteins have high impact on bread making (Figure 2) Shewry (2009). It is estimated to account 80% of protein in wheat grain of European wheat's Seilmeier et al. (1991). According to Osborne in 1907 the wheat proteins are classified into gluten and non-gluten groups Osborne and Clapp (1907). Gliadin provides viscosity and glutenin provides viscoelasticity properties to the dough Coulson and Sim (1965). By the absence of few allelic variants which as high molecular weight

subunits of glutenin will affect the quality of the bread [Wieser and Zimmermann \(2000\)](#). Water and environmental factors will also influence the quality of the proteins. However, cultivators choose high glutenin content for increasing the strength of the dough [Marchylo et al. \(1992\)](#). Hence nitrogen content and fertilizers will increase the quality of the grain protein.



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Figure 2 Functions of wheat grain [Zörb et al. \(2018\)](#)

Functionality of protein in bakery products

Main functional properties of protein are it provides viscoelasticity to the dough and are characterized by unusual amounts of residues of glutamine [Belton \(1993\)](#). They are important sources of plant protein which are related to conformational state and are affected by processing conditions. Certain properties such as colour, flavour formation, taste, and fat absorption capacities are obtained after baking process. Most of the protein is used in native form and small quantities are used in the form of concentrates, vital and non-vital wheat gluten isolates [Ortolan and Steel \(2017\)](#). The protein percentage in the wheat content ranges from 8% to 18% useful to make buns and bread for retaining gas and are helpful for rising of dough in the fermentation process and baking process [Lane \(1989\)](#). Cakes, biscuits, and cookies are generally made with low protein flour (8% to 9%) providing low water absorption, low damage to starch, good granulation of flour hence it is useful for making good biscuits [Bailey \(1914\)](#). Hence water absorption of the flour depends on the starch and protein content. So, the water content used to form dough should be minimum so flour of low water absorption will have low protein and less starch damage [Heinis \(2010\)](#). With the increase in the protein content of wheat grain there is a gradual increase of gluten content, hence several studies have suggested the non-gluten proteins will not play an important role in the making of bread [Kim and Lee \(2015\)](#). The differences in gliadin and glutenin will change the extensibility and elasticity properties of dough. Recent studies have shown that by addition of total gliadin and its subgroups will increase the quality of the bread therefore increasing the volume of the loaf [Shewry \(2009\)](#). Glutenin is useful to provide firmness in making of bread as it can increase the stability through a three dimensions network which is formed when sulphur cross linkages are developed during kneading process [Dhaka and Khatkar \(2015\)](#). Gluten proteins are the storage proteins of wheat and are very unique as they are also functional proteins, they do not possess any enzyme activity but they are only proteins which possess strong and cohesive dough to retain gases and produce light baked goods [Ortolan and Steel \(2017\)](#).

Molecular size distribution is an important functional property of the wheat flour [Tegge \(1985\)](#). Hence longer mixing of dough will increase the extensibility and improves the quality of the flour in bread making. A continuous gluten network will not be formed with the weak flour due it is quality characteristic. Hence the weak flour lacks strength and elasticity, but by the addition of good amount of sugar, fat, and less energy a greater number of biscuits can be prepared when the dough is exposed to high temperature with such properties [Belton \(1993\)](#). Hence the gliadin properties are very small and possess less interaction with the surface which makes it to act as a ball bearing action which is useful for extensibility and therefore desirable biscuits can be produced [Brockway \(1990\)](#).

Methods that are used to measure protein content in the food are kjeldahl method, dumas method, spectrophotometric methods, and physical methods. The concentration of protein is calculated by the amount of nitrogen content present in the food product. In the present Study dumas method is used for estimating nitrogen percentage in the wheat grain. It is calculated by thermal conductivity process. This method is faster compared to kjeldahl method. It does not use any harmful chemicals. Food samples are measured automatically in dumas method [Fieldner and Taylor \(1915\)](#).

2. RESULT

The result about the grain protein in the below [Figure 3](#) data on the protein showed that there was a significant difference in the flour quality used for bread making process. The percentage of the nitrogen has also significantly affected the protein quality of the grain. Two nitrogen doses were supplied to the crop, evidently the protein percentage has increased gradually with increase of nitrogen to 350kg/ha. The maximum protein was obtained highest supply of 350kg/ha. The minimum protein was obtained at lowest supply of 100kg/ha. The obtained results were agreed with the findings of [Chaudhry and Mehmood \(1998\)](#) who described that the protein content will be significantly affected by the nitrogen percentage. [Bänziger et al. \(1994\)](#) findings showed that that the genotypic variation in protein of grain may also affected by the supply of nitrogen in the soil. By the above results it can be concluded that bread grown with 350kg/h supply has high protein content in bread; protein rich and more yield can also be gained by application of proper amount of nitrogen.

The protein content of a bread sample that is grown in two different nitrogen supply was shown in the figure using IBM SPSS statistical analysis (SPSS survival manual: a step-by-step guide to data analysis using IBM SPSS, 2013). Hence, p value is less than 0.05, which is at 95% confidence level, we reject our null hypothesis. In other words, there is significant difference between the means of two groups

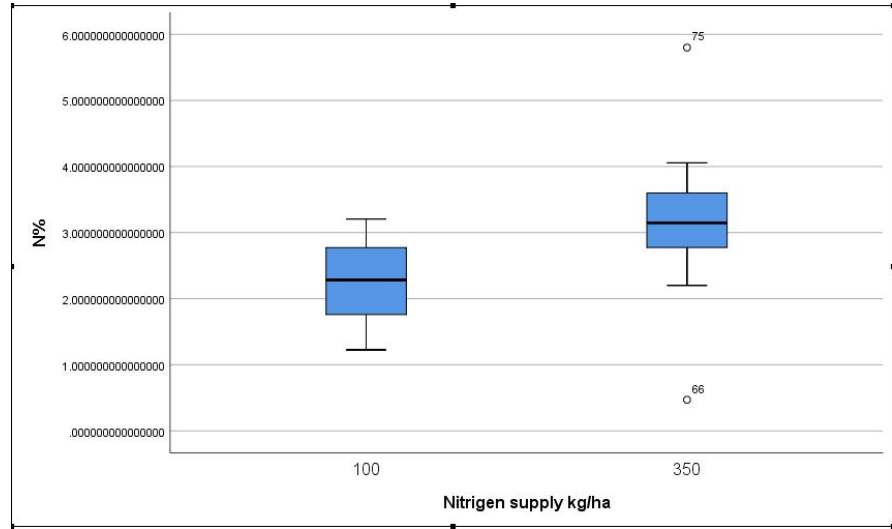


Figure 3 Comparison of a protein percentage in two different levels of nitrogen supply

The Smaller the standard error, the representative sample will be more. In our case the minimum standard error is 0.00485 which is of 1st group i.e. In the case of 100%

Group Statistics

	Nitrogen supply kg/ha	N	Mean	Std. Deviation	Std. Error Mean
Conversion factor	100	49	13.32098638	3.398024609	.4854320870
	350	49	18.45818549	4.403551621	.6290788030

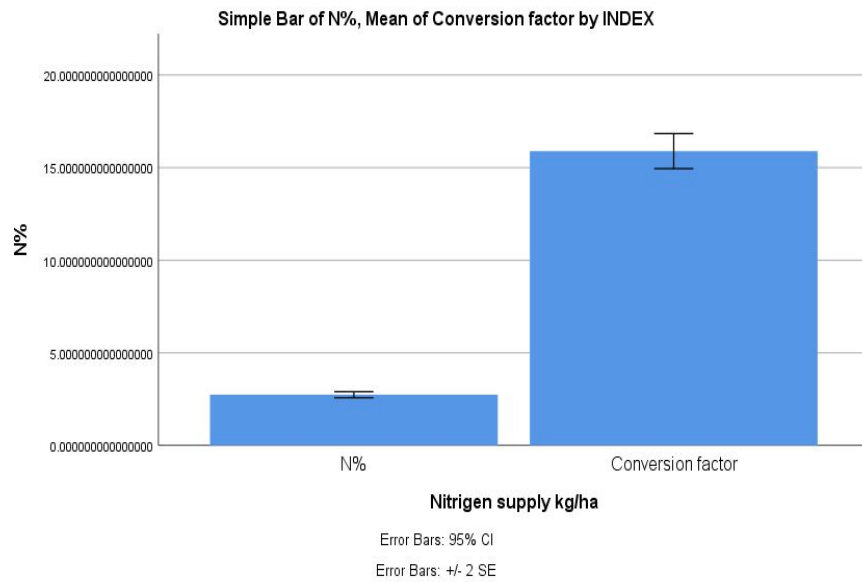


Figure 4 protein content of bread variety grown in two different levels of fertilizers; standard errors were displayed.

3. DISCUSSION

In the present study we discussed about the effect of nitrogen supply to the protein content of the wheat grain and the results obtained showed high percentage of protein supplied with 350kg/ha; three times more compared 100kg/ha. The result may depend on several factors such as supply of nutrition and problems during milling. Hence the topics are covered here in this section including the similar works done by other researchers.

Quantity and quality of the protein are very important for the protein development in the wheat crop. To obtain high quality of protein, plant should contain high molecular weight, long chain gluten proteins [Yu et al. \(2017\)](#). As we discussed above ([Figure 2](#)), the gluten proteins such as albumin, globulin and gliadin provides extensibility and processing properties to the dough. Nitrogen and sulphur are important and acts as a key nutrient for the growth of the grain protein. Nitrogen ensures the plant to grow with high protein percentage and time is also considered very important to attain good yield. Research, studies proved that effect of nutrients such as sulphur and nitrogen lead to changes in 1% grain protein for every 50kg/ha [Daniel and Triboi \(2000\)](#). Temperature and nitrogen supply will affect the protein and gliadin content. Proteins and gliadin in the flour will increase with increase of temperature where the quality of gliadin per grain will negatively affected by temperature and fertilization of nitrogen [Daniel and Triboi, \(2000\)](#)

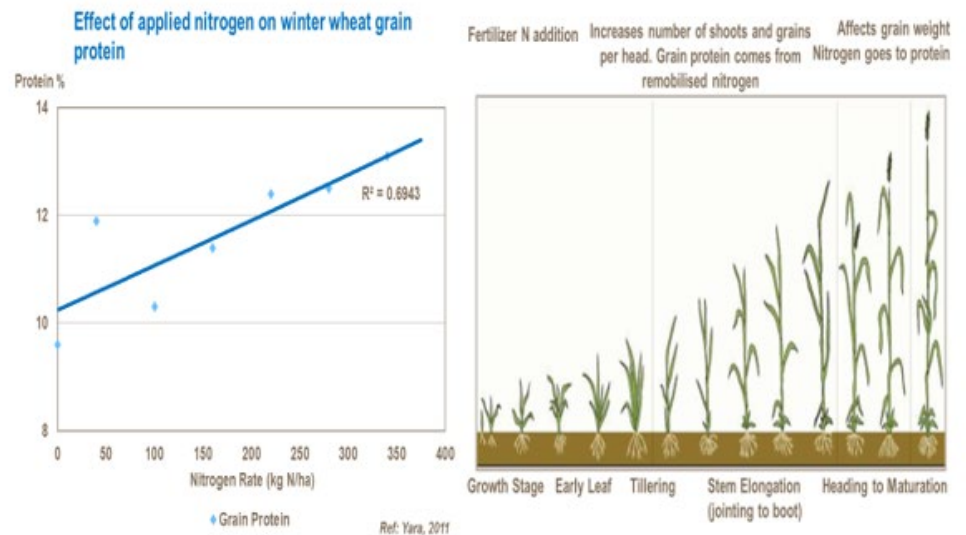


Figure 5 Depicts the research studies done on the effect of the nitrogen on wheat grain protein [Makowski et al. \(1999\)](#)

Grain protein with nitrogen in wheat should have 11%, however in making of the bread the protein should be 12%, with less amount of sulphur supply [Ortolan and Steel \(2017\)](#). The dough that is produced will get affected and amino acids such as methionine, cysteine and lysine will affect the baking properties. Micronutrients such as zinc and manganese will influence the metabolism rate of nitrogen. However, increased metabolism increases the nitrogen content in the developing proteins.

On the other hand, the milling process is a crucial stage affecting the concentration of the protein and nutrients in by products of the wheat grain. Aleurone layer and germ of wheat kernel is important and are rich in minerals and proteins. Conventional milling method will reduce the nutritional value of the wheat flour and concentrates the minerals in the milling residues [Cubadda et al. \(2009\)](#). White flour with 68% of mineral extraction means 32% of grain is not present in the wheat flour. Milling of wheat not only decreases the nutritional value but compared to the other flour it has high amount of nutrients even after the milling process. Nutritional value and antioxidant value will depend on the type of the soil, temperature and other climatic conditions [Adom et al. \(2003\)](#). According to [Cooper, and Blakeney \(1990\)](#) there was a significant difference identified between hard and soft wheat flours showing low protein percentage before and after milling process. Protein content is found to be 14.40% for hard wheat and soft wheat is 9.11% respectively. According to [Butt et al. \(2004\)](#) depending on the proportion of the bran the protein content will be increased showed in his experiment studies done on 20% and 25% bran supplements on bread making.

Bread making process is very important including three stages such as mixing, fermentation and baking process. Farinograph is used to identify the nature of the dough. Extensiograph is used to identify the extensibility which is used for gas retention during fermentation process. Fermentograph used to identify the volume of the loaf during fermentation process [Daniels and Fisher \(1976\)](#). The success of the tests directly depends on the flour behaviour in the mill and bakery. Protein changes and starch changes during baking can be observed due to variety of composition [Janssen et al. \(1996\)](#). Research studies suggested that ash content and dough time has increased after milling to 1.95% and 6 minutes respectively [Hrušková et al. \(2006\)](#). Research studies proved that the higher the resistance test the bread volume will be high and suitable for baking process [Cubadda et al. \(2009\)](#). Wheat protein content, fermentation gases, absorption of water has an important relationship between milling process and baking quality. Affectivity of milling will be based on parameter such as ash content and hardness which have influence on wheat flour during baking process. Fermentation is related to the content of the protein and the quality will changes the nature of the dough during proofing process ([Purlis, 2012](#)). Others studies have proven that Czech varieties have good behaviour at proofing and maturation resulting in good volume of bread shapes during baking process [Hrušková et al. \(2006\)](#).

Proper input of nitrogen is also very important for the crops to yield high protein content and good economic benefit. Some farmers due to lack of knowledge applies more nitrogen (180kg h⁻¹ y⁻¹) which causes environmental problems [Vitousek et al. \(1997\)](#). Major problems include soil and water acidification, contamination of ground and surface water, increase of depletion of ozone layer and injury of the crops. Due to nitrogen emission green-house gases will be increased, loss of biodiversity and production of airborne substances have been observed [Galloway et al. \(2002\)](#).

The movement of nitrogen in the soil system and plant till the final percentage of plant harvest was clearly shown in below ([Figure 6](#))

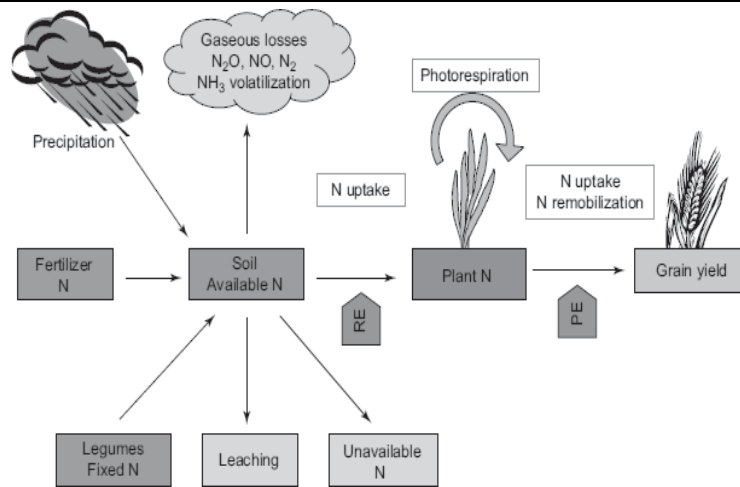


Figure 6 Presence of nitrogen in plants and environment [Good et al. \(2004\)](#)

In future water scarcity can also be seen. The cost of fertilizers will be increased. Variation in genetics will be observed in the plants. Increasing nitrogen content will increase the efficiency in drought conditions and leaching of soil [Foulkes et al. \(2009\)](#). Studies suggest that pollution can be caused in ground water, eutrophication of lakes and rivers, production of nitrogen oxides and denitrification of soil bacteria is observed [Chaves et al. \(2003\)](#). Therefore, nitrogen accumulation into the soil has increased the attention. At present the nitrogen use efficiency is low because the nitrogen fertilizer will greatly surpass the needs of the crop [Ju et al. \(2009\)](#). Hence the needs of the crop are related to ammonium levels in the soil. Therefore, the protein content will be increased with increase in nitrogen content. Studies suggested different nitrogen application rates on plants will apparently affect the chlorophyll and photosynthetic activity of flag leaves [Dahal et al. \(2014\)](#). Good nitrogen content will increase the root traits to increase the length of root density. Reduced grain nitrogen may increase NUE in wheat grain for making bread cultivars, more NUE will be linked to high nitrogen assimilation [Foulkes et al. \(2009\)](#).

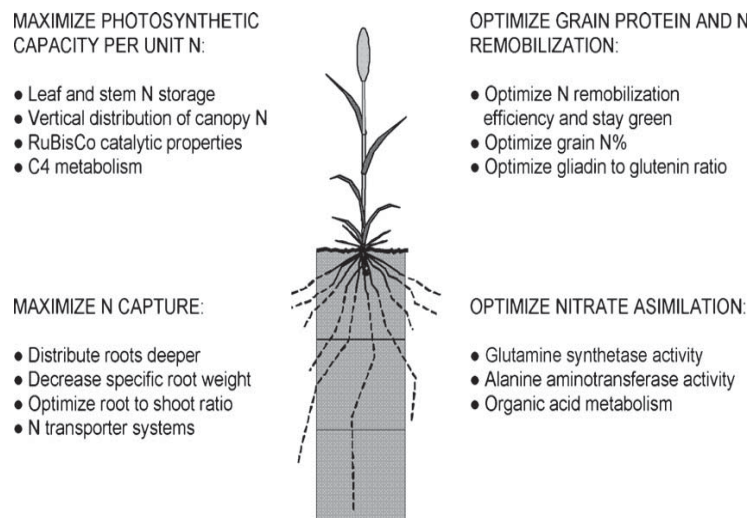


Figure 7 Management strategies for high nitrogen use efficiency [Foulkes et al. \(2009\)](#)

4. CONCLUSION

Wheat is an important crop consumed all over the world due to its nutritional properties. Wheat grain contains important proteins such as gliadin and glutenin which are responsible for baking properties. Gliadin and glutenin will together provide certain functions helpful to form dough. Hence factors' effecting the growth of crop is also important for the increase of protein in the dough. Several factors such as time, temperature, amount of fertilizer, milling process can affect the protein content. Therefore, nitrogen plays an important role in the growth of protein in the wheat grain. High input of nitrogen can lead to environmental pollution thus leading to damage of ecosystem. Hence proper nitrogen input is necessary for the wheat crop. The concentration of the nitrogen with different percentage will yields different results as we discussed above. The baking quality of the wheat flour is determined by the amount of protein present in the product. In other words, future for bakery products depends on the nitrogen input, nitrogen assimilation to the crop as it increases the protein content and further nutritional value of the product

5. ACKNOWLEDGMENT

I take pride to thank my professor Dr. Jibin He who encouraged me to work better in Teesside University. I wish to thank my parents and brother for their love and encouragement throughout my career.

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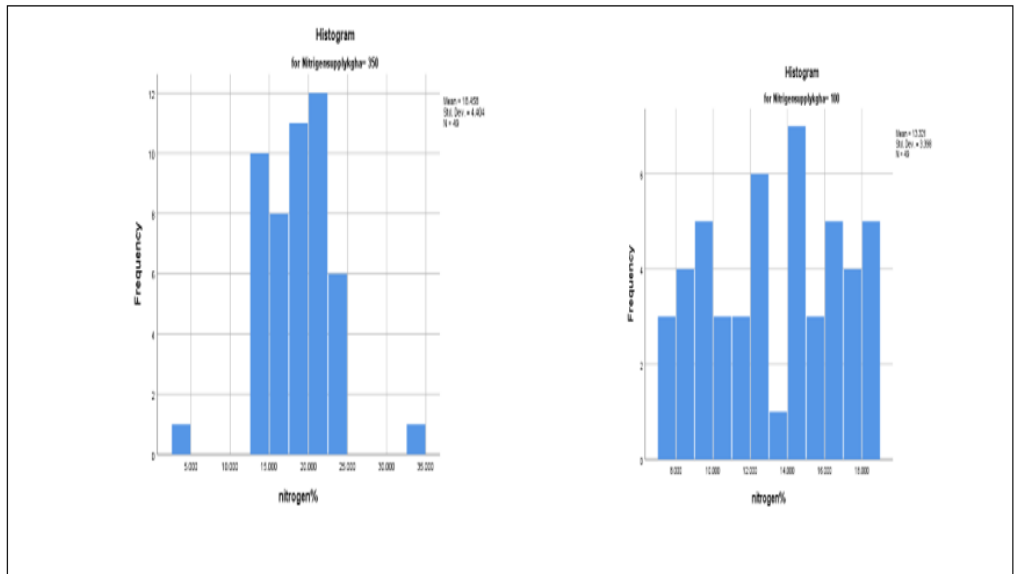
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APPENDIX

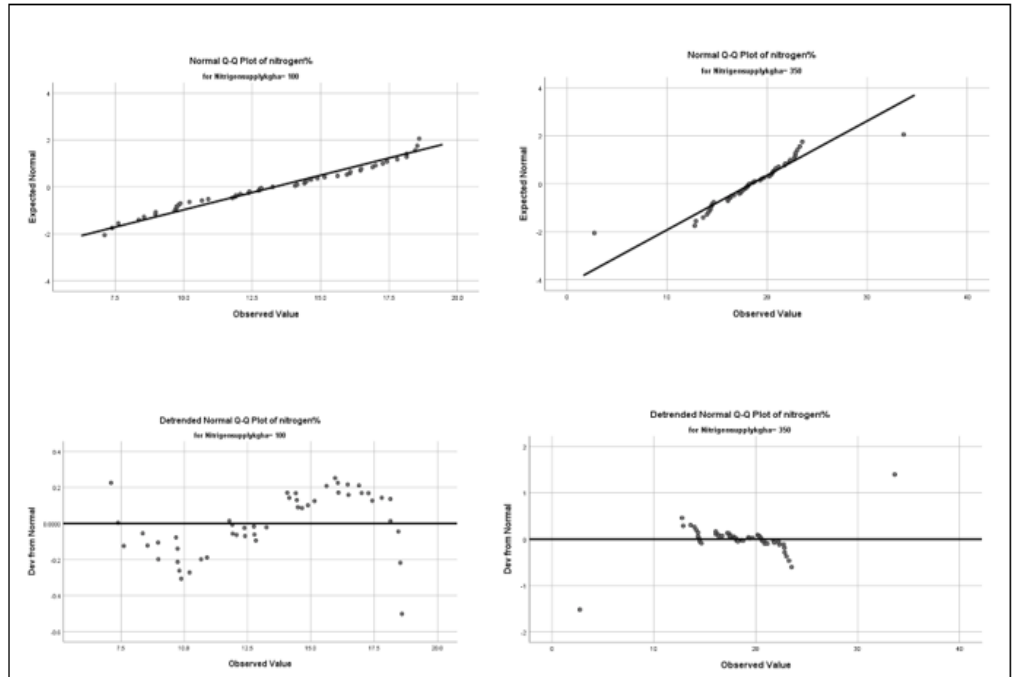
Independent Samples Test

		Levene's Test for Equality of Variances					t-test for Equality of Means		95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Conversion factor	Equal variances assumed	.264	.609	-6.465	96	.000	-5.13719912	.7945970372	-6.71446177	-3.55993646
	Equal variances not assumed			-6.465	90.201	.000	-5.13719912	.7945970372	-6.71575699	-3.55864125

APPENDIX- A



APPENDIX - B



APPENDIX - C