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QUANTITATIVE DETERMINATION OF PROTEIN IN WHEAT VARIETIES VL-804 AND VL-832 BY DUMAN'S METHOD

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ABSTRACT The current investigation was carried out to evaluate the protein content from two wheat varieties VL-804 and VL-832 using Duman's cumbuston method. In accordance to our experiment the Nitrogen percentage was found high in the variety VL-804 (WHT-d and WHT-a) with 2.187 and 2.501 respectively. The Nitrogen to Protein calculation is noticed that the percentage of protein of variety VL-804 (WHT-d and WHT-a) is 12.668 and 12.502 respectively. On the other hand the Nitrogen percentage WHT-2 and WHT-3 of VL-832 variety found good with 1.973 and 1.998 respectively. The Nitrogen to Protein calculation is noticed that the percentage of protein of variety VL-832 (WHT-2 and WHT-3) is 11.949 and 12.015 respectively. Based on our results it was observed that all samples collected of both variety possessed subtle variations in the percentage of Nitrogen and Protein. However, WHT-c of VL-804 variety produced least Nitrogen percentage 1.668 and its nitrogen to Protein percentage is 11.770. Whereas, WHT-1 of VL-832 variety also produced least Nitrogen percentage with 1.076 and its Nitrogen to protein percentage is 11.71.

KEYWORDS : Duman's cumbuston, Nitrogen to Protein

1.1 INTRODUCTION

Wheat captures approx 25% of the world wide area in cereal production. According to FAO (2014), 729,012,175 tonnes of wheat is producing globally per year. This crop is grown mainly in tropical and sub tropical regions of the world. Wheat kernel is generally composed of endosperm containing high amounts of protein (Van-Hung et al., 2009). The quantity of wheat protein is directly proportional to the volume of the bread. Finney and Barmore (1948) showed the relationship between flour protein content and bread volume. Mostly, miller pay large amount especially for additional protein content when the wheat crop yield is average. Simultaneously, the determination of the protein content after the wheat arrives at the mill is a vital parameter considered. Rheological properties of wheat flour is also depends on quantity and quality of their existing proteins. In general the protein content and its bread making ability is measured as loaf volume (higher wheat protein and quality higher bread making performance).

Wheat protein most commonly utilized in the production of bread due to its high level of gluten protein (75-82%) on the dry basis. The crude wheat gluten or texturized wheat gluten, or pure form of gluten is extensively used in the production of various products. The Gluten protein function is majorly depends on the variety or type of wheat and also on the preparation process. Its various benefits, such as improved color stability, firmness, visco-elasticity and juiciness, and moisture retention have been adopted to produce different bakery products. The texturized wheat gluten is commonly used in the preparation of burgers, nuggets, etc, to increase physical property and the taste perception of reformed patties or nuggets. The quantity of wheat protein is a vital consideration in the baking industry for the production of especially pasta and noodles. The greater-protein of wheat possesses higher water-absorbing capacity and high loaf volume and better keeping quality. The protein content of wheat widely varies and depends on the type or variety of wheat, cultural conditions, and nitrogen inputs. The selection of wheat variety by the cultivar will always depend on the protein-content range. For instance, 7-11% of wheat protein is fixed in the production of cakes and pastries (protein content range 13.5% mb), while for the production of breads typically the protein requires 12% or higher than (protein content range 13.5% mb). The soil fertility and nitrogen fertilizers application are also play a key role on the protein content of wheat.

Different types of analytical methods have been developed world wide. However, only a few methods are commonly used because of its reliable and easy. Moreover, the lack of analytical infrastructure and high budget for conduction of certain methods is also added reasons for the selection of methods. For example, currently authors reported that 52% of all experiments on the protein content of seaweeds based on nitrogen determination included the nitrogen-to-protein conversion factor of 6.25. However, after may experiments with this protein conversion factor leads to overestimation of the protein content especially in plant foods [1-3]. Food protein analysis is an important procedure to determine the quality and quantity of protein. The heterogenic materials of food due lipids, carbohydrates and other micronutrients may reduce the protein accessibility and cause difficulties in the protein content determination. There are direct and indirect analytical methods are for determining protein content. Direct protein determination includes the analysis of amino acid residues and indirect protein determination includes the determination of the nitrogen content of the protein by methods such as Kjeldahl method, Lowry method, Bradford Method, Dumas method.

The current study was framed out to evaluate the wheat protein content of VL-804 and VL-832 by Duman's method.

2.0 MATERIAL AND METHODS

2.1 COLLECTION OF WHEAT VERITIES

The seed varieties of wheat were purchased from local market Warangal.

2.2 DUMA'S METHOD

The wheat samples were crushed and made fine powder. 20 µl containing 0.01 mg concentration of wheat is placed in the combustion tube at where the temperature raises up to 1800°C. During this the reduction of nitrogen oxides and removal of sulphur and excess oxygen by copper at 700°C takes place. the amount of nitrogen is caluculated and converted to protein ratio.

3.0 RESULT

In accordance to our experiment the Nitrogen percentage was found high in the variety VL-804 (WHT-d and WHT-a) with 2.187 and 2.501 respectively. The Nitrogen to Protein calculation is noticed that the percentage of protein of variety VL-804 (WHT-d and WHT-a) is 12.668 and 12.502 respectively. On the other hand the Nitrogen percentage WHT-2 and WHT-3 of VL-832 variety found good with 1.973 and 1.998 respectively.

The Nitrogen to Protein calculation is noticed that the percentage of protein of variety VL-832 (WHT-2 and WHT-3) is 11.949 and 12.015 respectively. Based on our results it was observed that all samples collected of both variety possessed subtle variations in the percentage of Nitrogen and Protein.

However, WHT-c of VL-804 variety produced least Nitrogen percentage 1.668 and its nitrogen to Protein percentage is 11.770. Whereas, WHT-1 of VL-832 variety also produced least Nitrogen percentage with 1.076 and its Nitrogen to protein percentage is 11.71. The results are represented in Table 1 and Fig. 1.

Table 1 N/P Evaluation in Wheat VL-804 and VL-832 by Dumans combustion method

Sample	Sample code	Sample quantity (mg)	Nitrogen %	Protein %
VL-804	WHT-a	231.05	2.501	12.502
	WHT-b	199.23	1.913	11.917
	WHT-c	208.10	1.668	11.770
	WHT-d	221.9	2.187	12.668
	WHT-e	219.7	1.886	11.890
Average ± SD =		**2.031±0.32**	**12.1±0.4**	
VL-832	WHT-1	109.5	1.076	11.71
	WHT-2	199.3	1.973	11.949
	WHT-3	202.10	1.998	12.015
	WHT-4	188.94	1.855	11.850
	WHT-5	189.32	1.888	11.863
Average ± SD =		**1.7255** ± 0.43	**11.8774** ± 0.11	
The results of Nitrogen and protein percentage is represented in Mean ± SD, n=3				

4.0 DISCUSSION

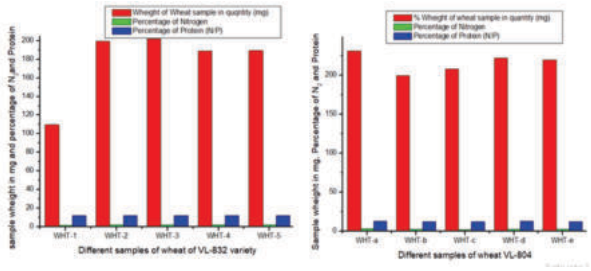

Figure 1Determination of percentage of Protein via Duman's method

The current study is focused on only the determination of protein content from two different types of wheat variety (VL-804 and VL-832). The protein determination is carried by Duman's method. According to our results the protein content is found more in the wheat variety VL-832 compare with that from VL-804. The samples were collected randomly from five different places of one village. The sample WHT-a contained high protein compared to all other samples. The review of literature revealed that the percentage protein in wheat with in arrange of 11-14% is used in the preparation of Hearth, French breads where the protein strength is medium and extensible (Pena, 2002). Whereas, the percentage of protein is >13% can be use to prepare the Pan-type buns where, the protein strength is strong and extensible. On the other hand, the protein percentage for the preparation of unleavened (flat) breads is ranged from 11-14% (Pena, 2002).

The wheat proteins are categorized into gluten proteins and non-gluten proteins. The gluten protein is generally considered of about 80 to 85% of total wheat protein. Whereas, the non-gluten proteins are present 15 to 20% is considered as total wheat protein. Wheat grain protein content generally ranges between 8 to 17 %, which depend on genetic make-up and as well as external factors where the crop is cultivated. A special character of wheat flour comes from its insoluble protein, when in contact with water. This protein mass is called as gluten. Wheat protein is complex with polymeric and monomeric proteins known as glutenins and gliadins, respectively (MacRitchie, 1994). Among these two glutenins gives elasticity, while gliadins give viscous flow. Science ancient time wheat has accompanied humans in their evolution and development from emmer wheat to the currently cultivated species. Now-a-days, modern wheat species such as hexaploid bread and tetraploid durum wheat i.e., *T. aestivum* L. and *T. turgidum* L. are gaining importance because of their different in genomic make-up and subsequently grain composition and quality attributes. Differences in the grain composition and quality of the wheat species are the depending factors to prepare type of wheat based food. The gluten proteins are the deciding factors in the quality and quantity of bread making from wheat flour. These proteins possess a special ability to undergo visco-elastic mass after post hydration. When this flour is mixed with water, these proteins allows the formation dough which is potential in holding gas which is produced during fermentation and result in fixed open foam structure of bread after baking. The dough rheological property is an essential element in bread making and determined by the interactions between the gluten protein matrix and other components present in the flour. This dough rheological property can be also be increased by the addition of reducing agents, oxidants or proteases which alter gluten proteins interactions. Molecular size distribution of flour is also considered as another important functional property in bread making. Based on some chromatography experiments it is revealed that the glutenin flour with longer period mixing possess greater average molecular weight compared to the glutenin flour with short mixing times. The short timed mixed flours produce dough which can break down rapidly easily extensible. Therefore short time mixing flours are generally not considered in bread making. On the other hand, longer period mixing time flour generates dough with high stability and resistance for its extension in bread making. However, very long mixing times flours are proven as not suitable in practical bakery conditions. Gluten proteins are less soluble in water and as well as dilute salt solutions. The low amino acid content with ionisable side and more number of non-polar amino acids are responsible for low solubility nature of gluten protein. In addition greater hydrogen-bonding potential also added factor its low solubility. The complete solubility this proteins can be achieved by destruction of its native structure.

CONCLUSION

Based on our results, here we conclude that, Duman's method of protein determination is a significant method for calculating nitrogen to protein percentage. We also conclude that the protein content of wheat depends on the climate conditions and nature of soil where the crop is growing.

According to International labeling regulations the determination of total protein content is directly proportional to quality of the protein. The analysis food protein content, there are two different primary methods has been developed. These are Kjeldahl's method and Dumas method. Since several years the Kjeldahl method has been used widely as standard method in the industry for the analysis of the total protein content from the food samples. This method includes the use of chemicals. Moreover, this method takes grater time to determine the protein content. The Kjeldahl method which uses of different catalysts and time of heating, different volumes of sulfuric acid and masses of test portion thus this method shows many experimental errors. In addition high labour costs, and use of toxic chemicals is also drawbacks of this method. Due to safety-based reasons and as well economic standpoint this method is replaced by the Dumas method.

Now-a-days, high temperature combustion method i.e., Dumas method of the protein determination is gaining importance in food industry. This method is rapid and as well reliability. the protein recovery percentage ≥ 99.5%, which is grater than Kjeldahl (0.001 mg N absolute vs. ≥ 0.1 mg N absolute).

The important advantages of the Dumas method are

- **Ease of operation** – the food sample directly packed in a foil and placed it into the analyzer
- **Operator safety** – As said above this method don't use dangerous chemicals which are reuired for Kjeldahl method
- **Reduced time of determination** – the determination of sample using Kjeldahl method takes from 2 h. whereas, the Dumas method takes only 4 min per sample.
- any waste is not generated using Dumas method.

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