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Standardization of Methodology to Determine Nitrogen, Total Reducing Sugar, Crude Lipid and Urea Content in Panchgavya Formulation

Soma Maji[1], Priyanka Singh Rao[2], Ramu N.[1], Sumit Arora[1] and Vivek Sharma[1]

[1]Dairy Chemistry Division, ICAR- National Dairy Research Institute, Karnal, Haryana (132 001), India
[2]Dairy Chemistry & Bacteriology Section, ICAR- National Dairy Research Institute Southern Regional Station, Bengaluru, Karnataka (560 068), India

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Corresponding vivek.vishk12000@gmail.com

0000-0002-6287-3419

ABSTRACT

The present study was conducted ICAR-National Dairy Research Institute, Karnal, Haryana, India during the year 2016–2020 to standardize the methodology to analyse the composition of panchgavya. Direct raw ingredients (milk urine and dung) were collected from live stock research centre, NDRI Karnal while indirect raw ingredients curd and ghee were prepared in laboratory. Panchgavya was prepared in three ratios i.e. RA, RB and RC by changing the level of urine and dung in final formulation. The methods for determination of nitrogen, crude lipid, reducing sugar and urea in panchgavya formulation were standardized. For all the analysis first theoretical value was calculated based on their percent in raw materials and experimental value was compared with it. Effect of sample weight and different methods to analyse composition was compared. In conclusion, it was found that Kjeldahl method for nitrogen content, Mojonnier acid digestion method for crude lipid, colorimetric method for lactose and DMAB (p- dimethyl amino benzaldehyde) for urea can be used as standard methods in the panchgavya formulation.

KEYWORDS: Colorimetric, DMAB, Kjeldahl, Mojonnier method, Panchgavya, standardization

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1. INTRODUCTION

The Sanskrit word Panchgavya means "mixture of five cow products" and it has been used in traditional Indian rituals throughout history (Rai et al., 2022). It is a formulation of the five ingredients of indigenous cow i.e. cow dung, cow urine, milk, curd and cow ghee. The three direct constituents are cow dung, urine, and milk, the two derived products are curd and ghee. In Ayurveda the importance of panchgavya is in the treatment of various human diseases has been documented (Bajaj et al., 2022, Sathiyaraj et al., 2022, Khan et al., 2015). Panchgavya has also been used as fertilizer and pesticide in agricultural operations (Bajaj et al., 2022, Sathiyaraj et al., 2022, Khan et al., 2015, Yadav and Lourdraj, 2006). A recent literature also suggested the use of panchgavya in the management of cancerous conditions (Bhojraj and Sawarkar, 2020). Application of panchgavya as an antioxidant has been mentioned in the management of oxidative stress-related disorders (Sathiyaraj et al., 2022). Panchgavya is also claimed to be useful against liver disorders, fever and inflammations and has a hepato-protective effect (Kishor et al., 2019) and immunostimulant activity with herbs (Fulzele et al., 2002, Fulzele et al., 2003). Panchgavya's use, especially as an organic bio-fertilizer and bio-pesticide in the field has been reported to increase yield, improve soil fertility and crops (Pathak and Ram, 2013, Devakumar et al., 2014). Panchgavya based bio-fertilizers are free from health-hazardous chemicals which can be the best replacement of the chemical fertilizer and pesticides used in agricultural practices. Panchgavya has many virtues and is being promoted as a promising agricultural formulation in the years to come. The use of panchgavya is gaining popularity these days. Literature also suggests that traditionally its use is more in South India and particularly in Tamil Nadu and Kerala (Raut and Vaidya, 2018). Depending on the different ratio of the basic components (cow milk, cow ghee, cow curd, cow urine, and cow dung), additive ingredients (banana, tender coconut water, toddy), and fermentation period, there are different methods of panchgavya preparation cited in the literature (Rai et al., 2022, Yadav and Lourdraj, 2006). Although few studies have been done on the bioactivity, physicochemical composition, safety, and acceptability of panchgavya and other cow products (Ram et al., 2020, Prajapati et al., 2022). Now a days, Panchgavya based products are coming into the market and being promoted in agricultural sector as biofertilizer, vermicompost, and biopesticide, which improves the soil fertility and results an increase in yield and quality of the agricultural produce. These market formulations of panchgavya have different compositional claims but information related to standardized methodology to establish their claimed composition is lacking or very scarce. Moreover, panchgavya is a complex matrix and existing gap in the knowledge of a well define methodology of its testing is a concern. Hence, the present study was conducted to standardize a protocol/ methodology for the compositional analysis of panchgavya, which can be adopted as a standard methodology.

2. MATERIALS AND METHODS

2.1. Collection of Raw material

The collection of milk, urine and dung was done during the milking time. All the collected raw samples were stored at 4°C in the refrigerator immediately.

2.2. Preparation of indirect ingredients for panchgavya formulation

To prepare a panchgavya formulation the ingredients were milk, urine and dung, whereas, the indirect ingredients were curd and ghee. Dahi was prepared by using mix dahi culture - 167, procured from National Collection of Dairy Cultures (NCDC), NDRI, Karnal and ghee by direct cream method (De, 2005).

2.3. Preparation of Panchgavya formulation to standardize the methodology of analysis

Panchgavya was prepared according to method of Rai et al. (2022) with slight modifications. The flow chart is given below (Figure 1).

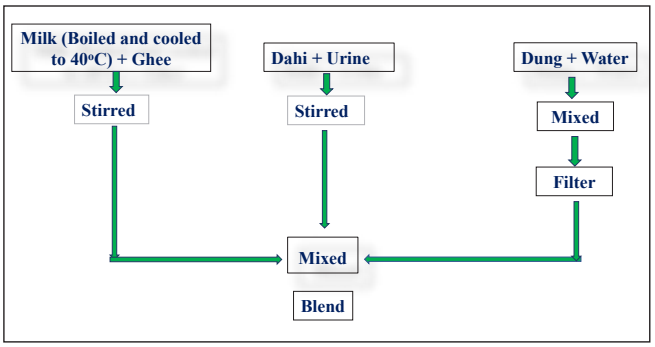

Figure 1: Preparation method of panchgavya

2.4. Preparation of panchgavya formulations for the Validation of the standardized methodology

To see the effect of matrix on the standardized methodology of analysis, three formulations of panchgavya were prepared using different ratios of the five basic ingredients. The formulation RA, RB and RC having urine: dung: curd: ghee: milk in ratios 1:1:1:1; 2:1:1:1:1; 1:2:1:1:1, respectively.

2.5. Calculations of experimental and theoretical values during standardization of methodology

Two terms have been used in the present research article i.e. (i) Experimental value and (ii) theoretical value as follows:

2.5.1. Experimental value

Experimental values were the content of crude lipid,

nitrogen, urea and total reducing sugars experimentally determined in the panchgavya formulation. For example, nitrogen content determined from 2.0 g of panchgavya, gave the value 'x%'. So, 'x%' was the practical value of nitrogen content in panchgavya. Same scheme was followed for other components like urea, lipids etc.

2.5.2. Theoretical value

Theoretical value was calculated mathematically.

2.6. Nitrogen estimation in panchgavya formulation

Two methods i.e. Micro -Kjeldahl method 991.20 (Anonymous, 2005) and Anonymous 20B: (1993) were evaluated to estimate nitrogen content of panchgavya formulations.

2.7. Standardization of methodology to estimate reducing sugar in panchgavya formulation

Reducing sugar content of panchgavya formulations was essentially determined by Lane Eynon and Colorimetric method specified for milk samples (Anonymous 1479-part II, 1961) with slight modifications. The modifications employed were in sample preparation. The following approaches were explored to obtain a clear filtrate.

2.7.1.1. Acid precipitation

Mixed 10 ml of panchgavya sample with 2 ml of 10% acetic acid followed by holding for 10 min, and then filtration through Whatman no 42 filter paper.

2.7.1.2. Centrifugation

Centrifugation of 10 ml panchgavya sample at 4000 rpm for 20 min at room temperature followed by filtration through Whatman no 42 filter paper.

2.7.1.3. Dilution

Mixing of 10 ml of sample with distilled water followed by making up of volume to 100 ml and then filtration through Whatman no. 42 filter paper.

2.7.1.4. Combination

Mixing of 10 ml of sample with 40 to 50 ml of lukewarm distilled water and 2 ml of acetic acid (10%), followed volume making to 100 ml and thorough mixing. The mixture was cooled to 4°C and was centrifuged at 4000 rpm for 20 min. After the removal of the upper-fat layer, filtered it through Whatman no. 42 filter paper.

The clear filtrate obtained from the fourth approach (combination step) was used and calculated using the below formula

Reducing sugar (in g) in panchgavya by colorimetric method=12.5×y ... (1)

Where, y=Corresponding concentration from the standard curve

2.8. Standardization of methodology to estimate Crude lipid content in panchgavya formulation

2.8.1.1. Mojonnier and Folch method

On the basis of the total solid content of panchgavya formulation, approximately 4.0 g liquid panchgavya sample was taken in a clean dry beaker and kept in a hot air oven for drying at 70°C for 24 h, so that the weight of panchgavya solids after drying was 1.0 g.

2.8.1.2. Soxhlet method

Approximately 20 to 25 ml of well-mixed panchgavya formulation was kept for drying in a hot air oven at 70°C for 24 h., so that that weight of panchgavya solids after drying was 5.0 g.

2.9. Standardization of methodology of Urea estimation in panchgavya

DMAB method (Bector et al., 1998) and kit-based method. panchgavya sample processing was carried out as mentioned below:

2.9.1. Dilution

10 ml of panchgavya sample was diluted with 10 ml of distilled water and filtered through Whatman no. 42 filter paper.

2.9.2. TCA precipitation

10 ml of panchgavya sample was added with 10 ml of 24% TCA and filtered through Whatman no. 42 filter paper.

2.9.3. Centrifugation

10 ml of panchgavya sample was added with 10 ml of 24% TCA and centrifuged at 4000 rpm for 20 min and filtered through Whatman no. 42 filter paper.

2.9.4. Dilution and centrifugation

10 ml of panchgavya was diluted with 10 ml of distilled water and centrifuged at 4000 rpm for 20 m and filtered through Whatman no. 42 filter paper.

2.9.5. Combination

1 ml of panchgavya sample was diluted with 9ml of distilled water and added with 10 ml of 24% TCA and mixed. The mixture was cooled to 4°C and centrifuged at 4000 rpm for 20 m. The upper-fat layer was removed and filtered through Whatman no. 42 filter paper.

The clear filtrate obtained from the fifth approach (combination step) was used and calculated using the below formula for DMAB method,

Urea (%) in panchgavya=400×*y* …(2)

Where, y=Corresponding concentration from the standard curve for urea

For kit-based method,

$$\text{Urea content in panchgavya} = \frac{(\text{Absorbance of sample})}{(\text{Absorbance of standard})} \times 40 \times \text{Dilution factor} \quad \text{.....(3)}$$

2.10. Statistical analysis

All measurements were performed in triplicate and reported as the mean±standard deviation. One-way analysis of variance (ANOVA) with Tukey's Multiple Comparison Test was performed whereas two-way ANOVA with Bonferroni post-test was performed. All statistical analyses were performed at 95% confidence interval with Graph Pad Prism software (version 5.01 for Windows), San Diego California, US.

2.11. Validation of the standardized methods

The critical evaluation of the reliability of the developed methods was carried out through systematic statistical analysis. All the methods were evaluated for their precision (Smith, 1994), recovery analysis (Gomez et al., 2003) in the quantitative estimation of different chemical parameters.

3. RESULTS AND DISCUSSION

The standardization of methodology to determine nitrogen, reducing sugar, crude lipid and urea content in panchgavya is described as follows-

3.1. Standardization of Methodology of Nitrogen Estimation in panchgavya samples

As mentioned in methodology section, two candidate methods were evaluated to estimate nitrogen content of panchgavya formulation as well as its ingredients (milk, curd, dung and urine). Notably, ghee is not a significant source of nitrogen, hence, it was not analysed for the nitrogen content. The nitrogen content of the individual ingredients as determined by using anonymous, 20B: 1993 and anonymous (2005) official method 991.20 has been presented in table 1. It is evident from the data that both the used methods gave almost similar results in case of individual ingredients of the panchgavya. Hence, the theoretical values were calculated on the basis of the percent contribution of those ingredients in the panchgavya formulation.

It is evident from the data (Table 1) that the experimental value of nitrogen content determined in panchgavya formulation by using anonymous, 20B: 1993 and anonymous (2005) official methods were 0.43±0.01 and 0.45±0.01, respectively. The nitrogen content in the same panchgavya formulation as calculated theoretically from the experimental values (Table 2) in the basic ingredients of the formulation estimated by both the methods was 0.44±0.03and 0.46±0.02%, respectively. The results revealed that difference in the experimental and theoretical values was not significant (p>0.05), whether estimated by anonymous, 20B: 1993 or anonymous (2005) methods. Therefore, both the methods were found suitable to determine nitrogen content in panchgavya. However, anonymous (2005) official method was finally chosen for its subsequent validation in the panchgavya formulations prepared by altering the ratio of basic ingredients. The advantage of the chosen method was its leverage of diluting the digested sample to 100 ml and keeping it for the multiple distillations in the later.

Table 1: Theoretically calculated values of individual ingredients of panchgavya in different method for nitrogen, reducing sugar, crude lipid and urea

Ingredients	Nitrogen content (%)		Reducing Sugar content (g 100 ml^{-1})		Crude lipid content (percent dry matter basis)	Urea content (mg 100 ml^{-1})	
	Anonymous 20B: (1993) method	Anonymous (2005) method	Lane Eynon method	Colorimetric method	Mojonnier acid digestion method	DMAB method	Kit based method
Milk	0.56±0.02	0.55±0.03	5.29±0.04	5.69±0.94	4.51±0.35	37.86±6.36	44.22±13.48
Curd	0.53±0.02	0.54±0.03	5.20±0.04	4.98±1.10	4.45±0.30	29.51±3.23	32.53±14.19
Urine	1.07±0.07	1.19±0.07	NA	NA	NA	1417.26±17.19	1466.53±76.08
Dung	0.15±0.05	0.17±0.07	ND	1.08±0.98	3.23±0.08	87.27±19.26	349.74±23.73
Ghee	NA	NA	NA	NA	99.71±0.39	NA	NA

Data represents as Mean±SD, NA: Not applicable and ND: Not detectable

Table 2: Theoretically and experimentally determined nitrogen content in panchgavya formulation

Methods	Nitrogen content (%)	
	Experimental value	Theoretical value
Anonymous, 20B: (1993) method	0.43±0.01^{aA}	0.44±0.03^{aB}
Anonymous, (2005) method	0.45±0.01^{bA}	0.46±0.02^{bB}

Data represents as Mean±SD; n=4, ^{a,b}: different superscript row-wise and ^{A,B}: different superscript column-wise shows no significant difference (p<0.05)

To check the effect of matrix, the selected method (Anonymous, 2005) was applied in the panchgavya formulations (RA, RB and RC) prepared by altering the ratio of basic ingredients. It is evident from the graph (Figure 2) that there was no significant difference in the nitrogen content calculated theoretically by summing up the

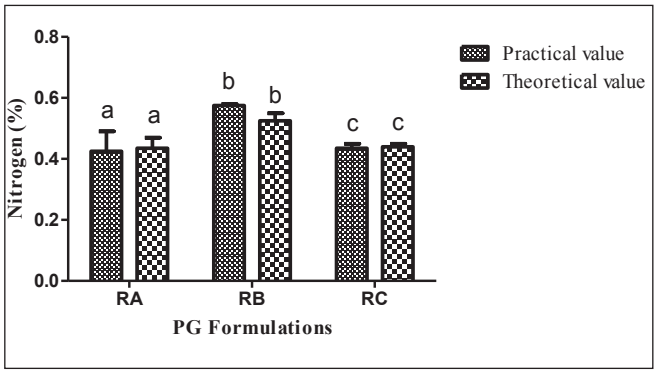

Figure 2: Comparison of theoretical and practical values of nitrogen content in three formulations of panchgavya.

nitrogen content in five ingredients used in the 3panchgavya formulations and the experimental values determined in the said formulations. Hence, it was concluded that the nitrogen estimation method (Anonymous, 2005), 991.20 can be used for the nitrogen estimation in panchgavya formulations having variable ratios of its basic ingredients and matrix had no adverse effect on the method of estimation. The detailed procedure is described in figure 3. The coefficient of variation (CV) was found to be 2.44% which is in the statistical range (within 5%), showed that the method had good repeatability. The findings of the present investigation are in accordance with the findings of earlier researchers (Ali et al., 2011), who used similar methodologies to determine the nitrogen content in panchgavya formulation.

Each bar represents mean ± SD, n=3, a–c- different letters represents no significant difference between the same ratio ($p<0.05$)

3.2. *Standardization of methodology of reducing sugar estimation in* panchgavya *samples*

Reducing sugar estimation either by Lane-Eynon or colorimetric method needs a clear filtrate to avoid interference in the reaction or spectrophotometric measurements. Hence, the protocol of reducing sugar estimation in milk anonymous 1479- part II (1961) specifies that milk is to be treated with acetic acid followed by filtration to obtain clear filtrate which is used further in Lane-Eynon and colorimetric methods. However, in case of panchgavya formulation which contains free fat, milk and fibrous material of dung, it becomes essential to remove these interfering components before the estimation of reducing sugars. Therefore, the four approaches (i–iv) as mentioned in the methodology section of the present manuscript were evaluated. It was observed that out of four evaluated approaches only approach (iv) i.e. the combination of acid precipitation and centrifugation resulted into the clear filtrate. Hence, the said approach was selected to process the panchgavya formulation before the estimation of reducing sugars by the Lane-Eynon and colorimetric

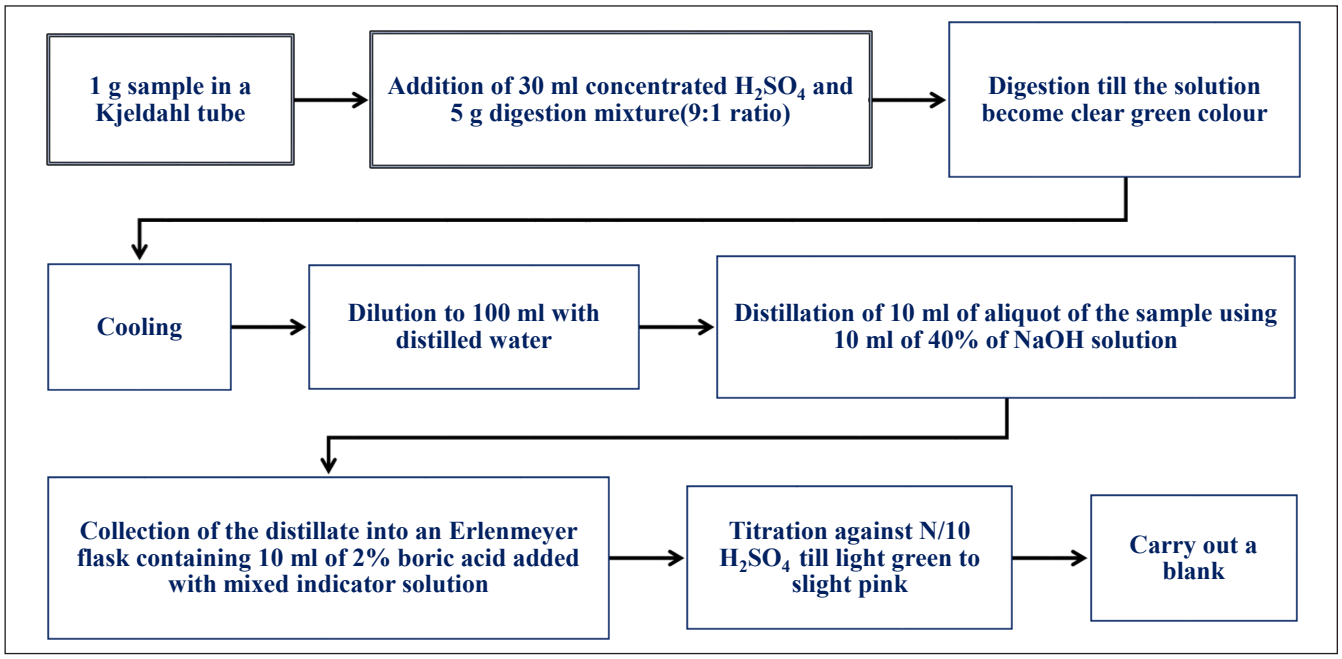

Figure 3: Flow diagram of the standardized method for estimation of nitrogen content in panchgavya by Kjeldahl method by anonymous, (2005)

methods.

The average value of reducing sugar content determined by Lane Eynon and the Colorimetric methods are given in table 2. The result (Table 2) revealed that there was a significant difference ($p<0.05$) between the practical and theoretical value of reducing sugar measured in panchgavya by the Lane-Eynon method, whereas, the difference was non-significant in the case of the colorimetric method. The perusal of the data (Table 3) clearly revealed that experimental value of reducing sugar in panchgavya formulation as determined by Lane- Eynon method was found to be 2.53±0.28, which was higher than the value (1.91±0.02) calculated theoretically from the reducing sugar data of basic ingredients (Table 1) determined by the same method. The difference observed in theoretical and experimental value of reducing sugars of panchgavya formulation in Lane-Eynon method can be attributed to the lower values (Table 1) determined in cow dung despite of the presence of main reducing sugars i.e. glucose, xylose, xylitol, cellobiose, arabinose, pentose and galactose in cow waste (Mtui, 2009).

Table 3: Theoretically and experimentally determined reducing sugar content in panchgavya formulation

Methods	Reducing sugar content (g 100 ml^{-1})	
	Experimental value	Theoretical value
Lane- Eynon method	2.53±0.28aA	1.91±0.02bB
Colorimetricmethod	2.67±0.34aA	2.31±0.29aB

Data represents as Mean±SD, n=5, a–b-different superscript row-wise and $^{A-B}$-different superscript column-wise shows no significant difference ($p<0.05$)

The possible reason was the difficulty faced in observing the sharp endpoint of the reaction in pure dung sample while determining the reducing sugar by the Lane-Eynon method as the method is a titrimetric one and needs comparatively high concentrations of sugar (200–600 ppm) in the solution to monitor the endpoint. It was also reported that the detection limit of the Lane-Eynon method was low compared to any colorimetric method (Jayawardhane et al., 2016).

On the contrary in the case of colorimetric method, the experimentally determined reducing sugar content in the panchgavya formulation was 2.67±0.34, which was close to the content (2.31±0.29) calculated theoretically for the same panchgavya formulation using the reducing sugar data of basic ingredients (Table 1) determined by colorimetric method. It was also observed that reducing sugar content in cow dung was easily measurable by colorimetric method wherein spectrophotometer was used, hence the results were comparable in this case.

Further, this method was validated in panchgavya formulations (RA, RB and RC) prepared by altering the ratios of its ingredients. It is evident from the graph (Figure 4), that there was no significant difference in the theoretical and practical values of reducing sugar content in panchgavya samples (RA, RB and RC), determined by the colorimetric method. This showed that the colorimetric method of reducing sugar estimation was not affected by the matrix (Figure 5).

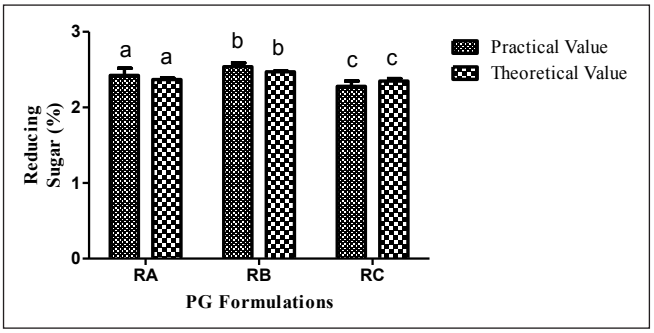

Figure 4: Comparison of the theoretical and practical values of reducing sugar content in three formulations of panchgavya.

Each bar represents mean ± SD, n=3, a–c- different letters represents no significant difference between the same ratio ($p<0.05$)

Recovery was checked by spiking of lactose in the panchgavya sample at a concentration of 0.125 mg ml^{-1}, 0.25 mg ml^{-1} and 0.45 mg ml^{-1}. Data of recovery trials (Figure 6) showed that recovery was 92–99%. Relative standard deviation (RSD) was found to be 1.46% which was in the statistical range (within 5%), showed that the method had good repeatability in successive measurements. Therefore, it was concluded that the colorimetric method can be used as a standard method for the determination of reducing sugar in panchgavya formulations.

3.3. Standardization of methodology of crude lipid estimation in panchgavya *samples*

To extract the crude lipid content from panchgavya, three methods viz., Mojonnier acid digestion method, Soxhlet method, and Folch method were evaluated. Literature suggested that for the efficient lipids extraction, samples must be dried before the extraction process, since non-polar solvents cannot permeate samples with more than 8% moisture. Samples with more moisture or endogenous water frequently results a false increased crude fat content (Anonymous, 2014). Therefore, the dried panchgavya samples were used for the extraction of crude lipid. The sample processing steps have been discussed in materials and methods section of the manuscript.

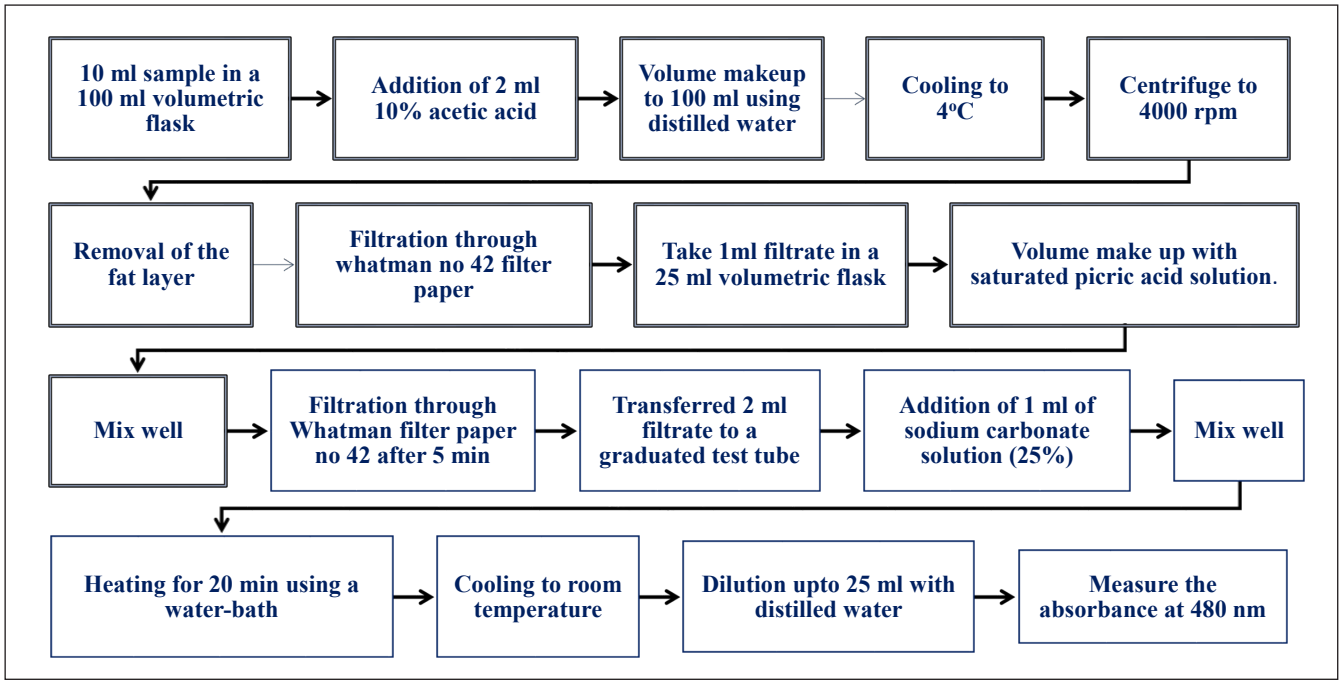

Figure 5: Flow diagram of the standardized method for estimation of reducing sugar content in panchgavya by modified colorimetric method

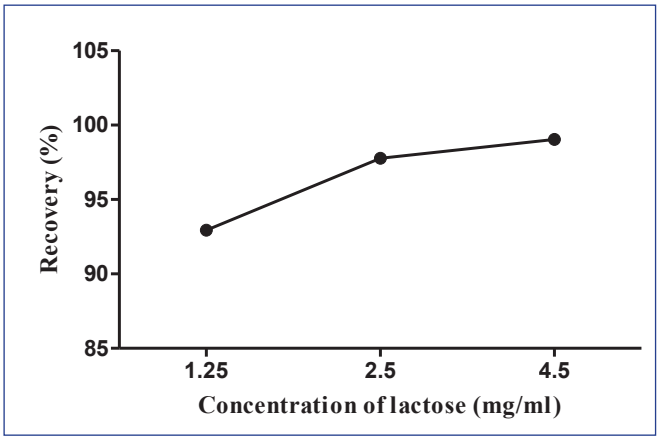

Figure 6: Percent recovery of lactose in panchgavya sample prepared with equal ratio of raw ingredients

The perusal of the data (Table 4) revealed that significantly lower amount of crude lipid was extracted from panchgavya formulation by the Folch method compared to Mojonnier acid digestion and Soxhlet method. The crude lipid content extracted by Folch method, Mojonnier acid digestion method, and Soxhlet method was 66.52±0.74, 75.94±0.46, and 75.85±0.28% on dry matter basis, respectively. It was also observed that there was no significant ($p<0.05$) difference in the crude lipid content of panchgavya formulation extracted by the Soxhlet and Mojonnier acid digestion methods. However, the Mojonnier acid digestion method was opted in the present study due to its less extraction time compared to the Soxhlet method.

Table 4: Comparison of methods for extraction of crude lipid from panchgavya on dry matter basis

Methods	Crude lipid content (percent dry matter)
Mojonnier acid digestion method	75.94±0.46[a]
Soxhlet method	75.85±0.28[a]
Folch method	66.52±0.74[b]

Data represented as Mean±SD, n=3, a-b – different superscript column wise differ significantly ($p<0.05$)

It is also evident from the data (Table 5) that the experimental and theoretical value of crude lipid content of panchgavya was 79.46±0.20 and 80.75±0.15%, respectively on dry matter basis. There was no significant difference ($p>0.05$) between the experimental value of panchgavya

Table 5: Comparison of theoretically calculated and practically extracted crude lipid content in panchgavya samples

Method	Crude lipid content (percent Dry matter basis)	
	Experimental value	Theoretical value
Mojonnier acid digestion method	79.46±0.20[a]	80.75±0.15[a]

Data represented as Mean±SD, n=4,[a]- superscript row-wise differ non-significantly ($p>0.05$)

formulation determined by the Mojonnier acid digestion method and theoretical value of crude lipid content of the same formulation calculated from the crude lipid data (Table 1) of its ingredients.

Further this method was also validated to check the effect of matrix in panchgavya formulations (RA, RB and RC) prepared by altering the ratios of its ingredients. It is evident from the graph (Figure 7), that there was no significant difference between the theoretical and experimental values of crude lipid content in three types of panchgavya (RA, RB and RC), extracted by Mojonnier acid digestion method. Therefore, based on the above discussion it can be concluded that 1 g dried panchgavya sample followed by Mojonnier acid digestion method is suitable and can be used as a standard method for the determination of crude lipid in panchgavya formulations of unknown composition. The detailed method of the Mojonnier acid digestion method is given in Figure 8.

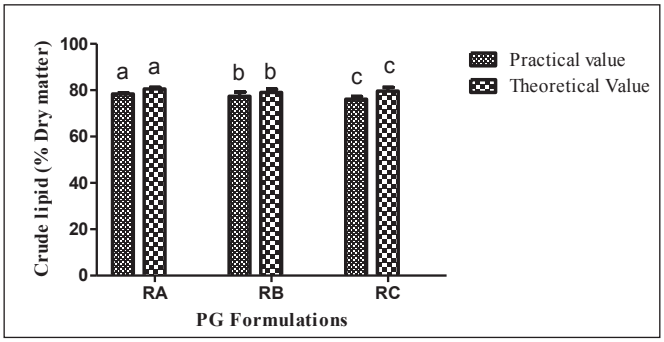

Figure 7: Comparison of practical and theoretical values of crude lipid content in three formulations of panchgavya.

Data represented as Mean ± SD, n=3, a–c– different letters represent no significant difference between the same ratio ($p<0.05$)

Further the repeatability of the Mojonnier acid digestion method was checked by relative standard deviation (RSD) and was found 0.30% which was within 5%. That indicated, the method was well within the limits.

3.4. Standardization of methodology of urea estimation in panchgavya samples

To estimate urea content in panchgavya samples, a clear filtrate was the pre-requisite, hence different approaches were tried to obtain the clear filtrate. As discussed in materials and methods section, the clear filtrate was obtained by the combination approach. The clear filtrate obtained was used to evaluate the DMAB reagents method as well as kit-based method, which is generally used for urea estimation in milk and human urine, respectively.

The average experimental and theoretical value of urea content by DMAB method and Kit-based method has been depicted in Table 6.

Table 6: Comparison of theoretically calculated and practically extracted urea content in panchgavya samples

Methods	Urea content (mg 100 ml^{-1})	
	Experimental value	Theoretical value
DMAB Method	268.21±9.40^{aA}	294.70±5.37^{aC}
Kit-Based method	397.10±80.51^{bB}	362.96±20.01^{bD}

Data represented as Mean±SD, n=4, a–b: different superscript row wise and A-D: different superscript column wise differ significantly ($p<0.05$)

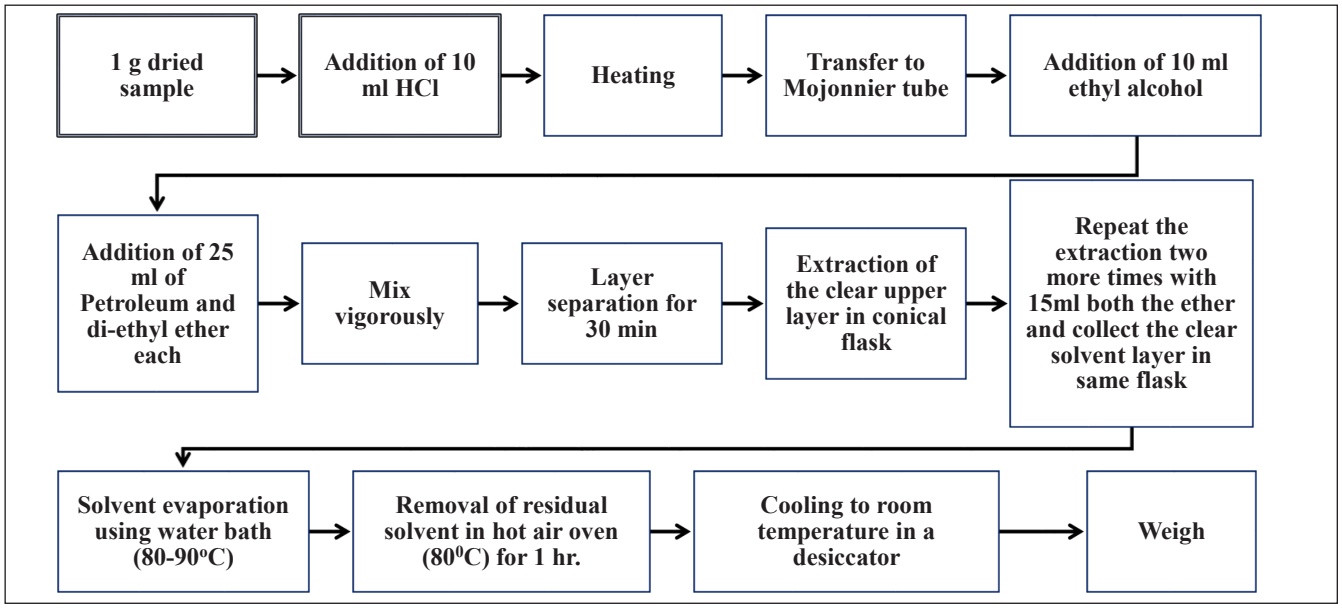

Figure 8: Flow diagram of the standardized method for estimation of crude lipid content in panchgavya by modified Mojonnier acid digestion method

Data revealed that the urea content in panchgavya as determined by kit- based method was significantly higher ($p<0.05$) than the urea content determined by the DMAB method. It was observed that the experimental value of urea as measured by the kit-based method was significantly higher (397.10±80.51) compared to the experimental value determined by the DMAB method (268.21±9.40). A similar trend was observed in the case of the sum of the values of urea content of individual ingredients as determined by Kit based method, which was significantly higher (362.96±20.01) than the DMAB method (294.70±5.37). These differences in the kit based and DMAB method can be attributed to the fact that the basic principle of kit- based method was to determine the total amount of released ammonia from the sample after the reaction of urea with water in presence of the urease enzyme. Literature suggested that cow dung contains higher content of undigested protein, nitrous oxide and microbial nitrogen in addition to the small amount of unabsorbed urea (Saatkamp et al., 2016, Mcdonald et al., 2002, Maynard and Loosli, 1956). Literature also suggested that the nitrogenous compound present in the dung is attacked by the dung microbes and release ammonia (Tamminga, 1992) and this ammonia might have contributed to the urea content. Higher content of urea in dung was also reported by Fischer et al. (2016). On the contrary, in the case of DMAB method, the change of colour solely depends on the amount of urea present in the sample and ammonia present in the dung does not contribute to the measured urea unlike Kit- based method. It is also evident from the data that in case of kit- based method the variation in results was high in comparison to DMAB method. During the study it was also experienced that the repeatability of kit -based method was an issue hence, DMAB method was selected to determine urea content in panchgavya samples.

The DMAB method was also validated in panchgavya formulation (RA, RB and RC) prepared by altering the ratios of its ingredients, mentioned in materials and methods. It is evident from the graph (Figure 9), that there was no significant difference between the theoretical and practical values of urea content in three types of panchgavya

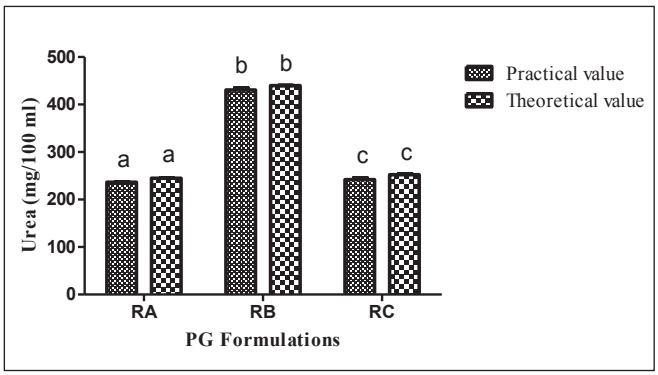

Figure 9: Comparison of the theoretical and practical values of urea content in three formulations of panchgavya.

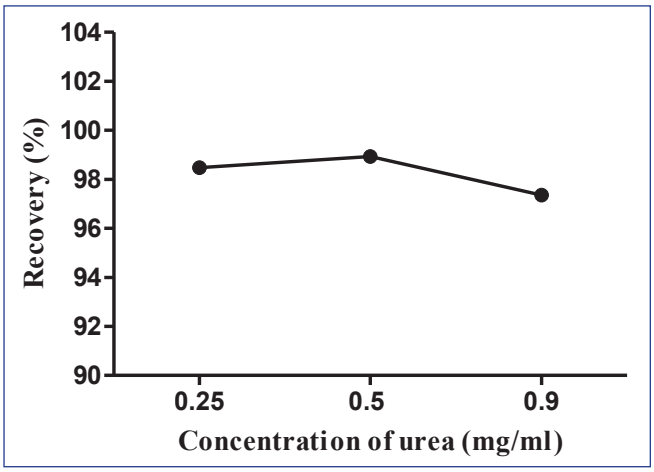

Figure 10: Percent recovery of urea in panchgavya sample prepared with equal ratio of raw ingredients

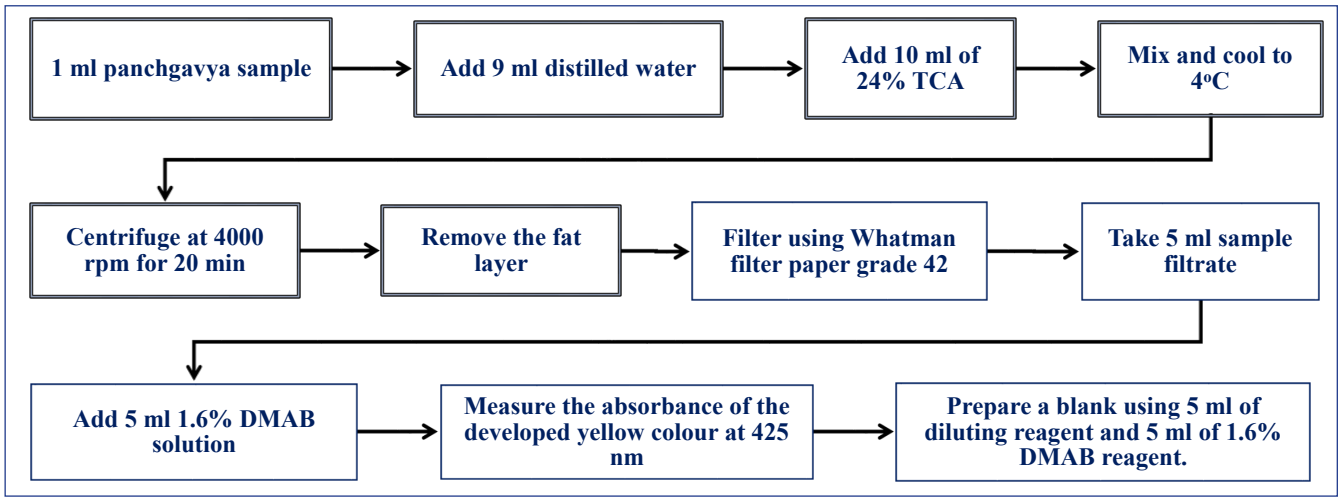

Figure 11: Flow diagram of the standardized method for estimation of urea content in panchgavya by modified DMAB method

(RA, RB and RC), determined by DMAB method.

Further, the recovery study was done to check the efficiency of the method. Recovery was checked by spiking of urea in panchgavya at a rate of 0.25 mg ml^{-1}, 0.5 mg ml^{-1} and 0.9 mg ml^{-1}. It is evident from the graph (Figure 10), that the recovery of the method was in the range of 97–98%. Relative standard deviation (RSD) or co-efficient of variation (CV) was 0.30% which was within 5%, indicated that the method was repeatable and recovery was well within the limits. Therefore, based on the above discussion DMAB method was finalised to determine the urea content in panchgavya and the detailed flow diagram is depicted in Figure 11.

4. CONCLUSION

A wide variation in the content of nitrogen, reducing sugar, crude lipid and urea content of panchgavya was observed in literature due to the use of different chemical methods. Here, the methodology was standardised to determine the content of the above analyte in the panchgavya formulations. To determine the nitrogen, reducing sugar, crude lipid and urea content in panchgavya formulation, the Kjeldahl, modified colorimetric method, modified Mojonnier acid digestion method and modified DMAB method, respectively were finalised.

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