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Quantification of Structural, Physical, Phyto-nutrient and Rheological Traits in Selected Minor Millets

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ABSTRACT

Background: Minor millets like Browntop (*Urochloa ramosa* L.), Barnyard (*Echinochloa crusgalli* L.) and Kodo (*Paspalum scrobiculatum* L.) hold immense nutritional and functional promise. Yet, detailed characterization of their traits remains limited, making such studies vital for advancing processing and product development.

Methods: Three minor millets browntop, barnyard and kodo were selected for detailed morphological analysis. Linear dimensions were measured and further evaluated for geometric, spatial, physical, phyto-nutrient and rheological properties. Antioxidant activity was assessed using the DPPH method, while total phenols, tannins, flavonoids and oxalate contents were quantified. The pasting profile of each millet was determined to evaluate gelatinization, viscosity and stability under heat and mechanical stress.

Result: Browntop reported greater slenderness ratio among three, kodo with high GMD, AMD, sphericity, SA, volume. Spatial properties were calculated for the same and kodo has maximum projected area. Kodo millet has highest thousand grain weight and volume whereas barnyard has high hydration index and capacity along with swelling index, swelling capacity and porosity. Browntop has more bulk and true density whereas kodo reported to be the hardest grain among the three. The present study investigated anti-oxidant activity (DPPH method), total phenols, tannins, total flavonoids content and oxalate content in three minor millets. Kodo millet exhibited highest anti-oxidant activity (89.34%). Browntop millet showed high levels of total phenols (1.77 mg GAE/g), oxalates (38.0 mg/100 g) and flavonoids (21.40 mg RE/g), while barnyard millet (0.13 mg TA/g) had the highest tannin content. The pasting profile revealed that browntop millet requires high temperature to get gelatinized, barnyard millet exhibited high peak viscosity indicating a strong gelling ability. Kodo millet demonstrated high trough viscosity, holding strength and final viscosity along with low breakdown. Kodo grain, despite being tough and thick coated, forms a paste that was stable and its starch structure can effectively withstand heat and mechanical stress, ensuring consistent performance even under challenging conditions.

Key words: Bioactive, Grain structure, Morphological, Nutraceutical, Small millets, Viscoelastic.

INTRODUCTION

Minor millets, the gluten-free, nutri-dense, resilient, sustainable grains of nature, were not able to show their true potential due to certain complex situations in yesteryears, now reclaim their endurance due to increased awareness of their nutritional benefits and ability to thrive in harsh climatic conditions. These grains are indeed nutrient powerhouses loaded with phyto-nutrients such as anti-oxidants, polyphenols and dietary fibre along with essential minerals, bolstering a healthful diet. Among minor millets brown top millet (*Urochloa ramosa* L.), barnyard millet (*Echinochloa frumentacea* L.) and kodo millet (*Paspalum scrobiculatum* L.) hold a distinctive position due to their exceptional qualities in every aspect.

Despite numerous benefits, they remain unexploited which warrants further discussion. The underutilization of these grains might be attributed to several factors: insufficient knowledge of their applications in various products, limited research and development efforts, inadequate pre-cleaning equipment tailored for these grains, lack of quantification of their phyto-nutrient properties and minimal understanding of their starch profile for broader applications (Komara *et al.,* 2022, Nithyashree *et al.,* 2020). A comprehensive study of grains dimensions is necessary for keen observation and understanding of grain structure. This includes assessing hydration properties, the hardness of grains with husk, the phyto-nutrient and pasting profile. To date, no study has thoroughly examined the physical nature of grains across these many aspects for three different grains, including these detailed considerations (Pawase *et al.*, 2019).

Hence, the present study aimed to quantify the linear dimensions, geometric parameters, physical properties, phyto-nutrient characterisation and rheological properties of uncorticated browntop, barnyard and kodo millet flour.

MATERIALS AND METHODS

The selected minor millets (browntop millet, barnyard millet and kodo millet) purchased from local market of Madhurai,

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were cleaned from unwanted materials in Millet Processing and Incubation Centre, Professor Jayashankar Telangana Agricultural University, Rajendranagar, Hyderabad in the year of 2023. Ideal moisture content was maintained throughout the experiment, as it is crucial for primary processing of minor millets to achieve reliable insights.

Dimensional properties

Randomly selected whole grains (5 no.) free from crevices or cracks were chosen to evaluate the physical properties like length (mm), breadth (mm) and thickness (mm) using vernier calipers (least count of 0.01 mm) to avoid any physical distortions. Based on physical dimensions geometric properties and spatial properties were calculated using following equations.

Geometric mean diameter

The geometric mean diameter (GMD) also called as equivalent diameter, was calculated by using the method recommended by Sahay and Singh (2001).

$$GMD \text{ in mm} = (LWT)^{\frac{1}{3}}$$

Arithmetic mean diameter

The arithmetic mean diameter (AMD) of grain sample was calculated by the procedures of Mpotokwane *et al.* (2008) using below equation.

$$AMD \text{ in mm} = \frac{L + W + T}{3}$$

Where,
L = Length in mm.
W = Width in mm.
T = Thickness in mm.

Aspect ratio (AR)

The aspect ratio (%) of grain was calculated using below mentioned formula as per method of Vanrnamkhasti *et al.* (2008) as follows:

$$\text{Aspect ratio (in \%)} = \frac{W}{L} * 100$$

Slenderness ratio (SR)

The slenderness ratio (the ratio of grain length to width) was determined by the following equation (Bagheri *et al*., 2011).

$$SR = \frac{l}{w}$$

Sphericity (S)

Sphericity is the ratio of volume of solid to the volume of circumscribed sphere that has a diameter equal to the longest diameter of the solid so that it can be circumscribe the solid sample (Mohsenin, 1986). Sphericity was obtained from equation (Sahay and Singh, 2001).

$$\text{Sphericity } (\varphi) = \frac{2BL^2}{2L - B}$$

Where,
L- Length of grain, mm.
W- Width of grain, mm.
T- Thickness of grain, mm.

Surface area (SA)

The surface area (mm^2) of the grain was calculated based on method suggested by (Karababa and Coskuner, 2013 and Jagbir Rehal *et al.*, 2019).

$$SA = \frac{2BL^2}{2L - B}$$

Grain volume (V)

Volume (mm^3) of single grain was calculated through the equation suggested by Jain and Bal (1997) and Karababa and Coskuner (2013) as:

$$\nu = \frac{\pi B^2 L^2}{6\,(2L - B)}$$

$$\text{Where } B = (WT)^{1/2}$$

Projected area to largest/maximum area (mm^2), intermediate (mm^2) and small dimensions (mm^2), critical projected area (mm^2), radius minimum (mm) and maximum (mm) were calculated using following equations:

$$P \text{ (Max)} = \frac{\pi \times L \times W}{4}$$

$$P \text{ (Int)} = \frac{\pi \times W}{4}$$

$$P \text{ (s)} = \pi \times L \times W$$

$$CPA = \frac{Pm + Pi + Ps}{3}$$

$$R \text{ min} = \frac{W}{2}$$

$$R \text{ (Max)} = \left[W^2 + \frac{L^2}{4}\right] / 2W$$

Physical properties

Similarly physical properties of grain such as 1000 weight of grain (g), grain volume (ml), hydration capacity, hydration index, swelling capacity, swelling index, bulk density, true density, porosity and grain hardness(N).

Thousand grain weight and volume

1000 randomly picked grains without any distortions were chosen and weighed (0.001 g) for 1000 grain weight later transferred to graduated measuring cylinder to measure volume of the same.

Hydration index and hydration capacity calculated before and after grain weight after soaking for 24 hrs. Swelling capacity and swelling index was calculated based on differences in volume of the grain before and after soaking for 24 hours (Williams *et al.,* 1983).

Bulk density, true density and porosity was determined for all the grain samples (Shepherd *et al.,* 1986).

Bulk density (B_d) of grains was determined by taking the weight of grain in fixed volume:

$$BD\ (g/ml) = \frac{\text{Weight of the grains}}{\text{Volume of the grains}}$$

The True density (g/ml) (T_d) is defined as the ratio of mass of grain to the solid volume occupied. It is determined using toluene displacement technique.

Porosity was calculated as ratio of the difference in the grain and bulk densities to grain density and expressed in percentage.

$$\text{Porosity } (\%) = 1 - \left[\frac{BD}{TD}\right] \times 100$$

Grain hardness

Grain hardness (N) of millet samples measured using grain hardness tester. The grain sample was kept in seed resident area and grain pressed with the help of rotating screw head till it ruptured and the experiment was repeated at least five times to determine grain hardness.

Pasting properties

Pasting properties were determined using Rheometer (Make: Anton Paar, Model: MCR 32). The flour sample (5 g) was dissolved in water to form (1:3 ratio) without any lumps, made into slurry and transferred to canister, then canister was placed within holder, locked in place to remain stable during test. This locking mechanism keeps the canister stationary during rheological measurements and avoid any undesired movements that could impact the precision, tested for pasting properties of the sample. The heating range was from 50°C to 95°C and flour viscosity profile was tested throughout heating and cooling phase. The parameters recorded were peak viscosity, pasting temperature, holding time, breakdown and setback viscosity.

Phyto-nutrient evaluation

Quantifying anti-nutrient properties in minor millets is critical to establish the contour of the nutritional and therapeutic potential. In the present experiment estimation of certain phyto-nutrients were examined. Total phenol content (TPC) of the millet samples were determined by the Folin-Ciocalteu method as described by Singleton and Rossi (1965). Gallic acid was used as standard and the results were expressed as milligram of Gallic acid Equivalent (mg/100 mg GAE). Total tannins content estimated as per Nisaar *et al.* (2017) using Folin Denis reagent. Tannic acid was used as standard and results expressed as milligram of tannic acid equivalent. Total antioxidant activity was determined by using DPPH. Total phenol content (TPC) of the millet samples were estimated by using aluminium chloride assay using rutin as standard as described by Meda *et al.* (2005). Total oxalates content were estimated by titration method (Nissar, 2017).

All data were expressed as the mean±SD of triplicate measurements.

RESULTS AND DISCUSSION

As per the present study three minor millets *i.e.* browntop,barnyard and kodo millets were quantified for their dimensions, geometrical, spacial, physical and hardness initially then samples were further analysed for rheological and phyto-nutrient properties.

Dimensional characterisation of grains

Scaling of grain dimensions is crucial in research and development, enhancing efficiency of grain handling equipment thus improving market potential of the same. Farmers and breeders relies on plant varieties that yield optimal dimensions for efficient processing. The present study carried out to measure linear measurement, geometric values and spatial values for three minor millets (Fig 1). The mean values of length (mm), width (mm) and thickness (mm) for twenty grains were measured using digital vernier callipers. The average values of three grains were presented in Table 1. The length of browntop was greater (3.44 mm) compared to kodo (2.69 mm) and barnyard (2.58 mm), kodo millet was the widest and thickest grain with 2.42 mm. Slenderness ratio was more for browntop millet (2.09) and sphericity was more for kodo millet. Based on their dimensions, browntop millet has elongated form, while kodo millet is more rounded and

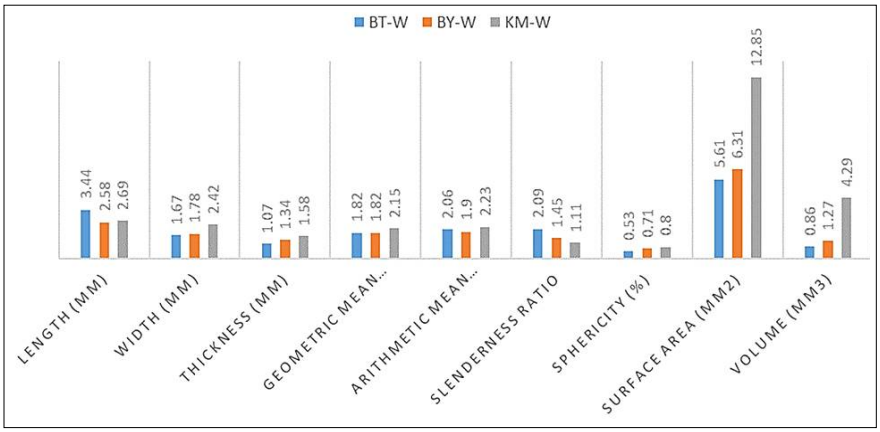


Fig 1: Dimensional characterisation of grains.

spherical. Geometric mean diameter and arithmetic mean diameter ranges from 1.82 mm to 2.15 mm and arithmetic mean diameter were in between 1.90 mm to 2.23 mm. The surface area of grain varied in between 5.61 mm^2 to 12.85 mm^2 and the volume ranged from 0.86 mm^3 to 4.29 mm^3. Grain varieties, environment, farming conditions, moisture in grain highly impact bulk density, true density, kernel weight, grain magnitude and largest projected area (Konak *et al.,* 2002; Aydin, 2002).

Calculation of maximum projected area, intermediate, smaller dimensions, critical projected area, radius minimum and maximum were effective in planning more precise design for equipment or sieves ensuring they were tailored to fortify existing equipment and enhance grain handling effectively.

As prefigure 2 maximum and critical projected area of the above mentioned grains from largest to smallest were kodo (5.12 mm^2 and 3.34 mm^2)> brown top (4.53 mm^2 and 2.42 mm^2) > barnyard (3.61mm^2 and 2.29 mm^2). Projected intermediate and minimum dimensions were minimum in browntop (1.31 mm^2 and 1.41 mm^2) and maximum in kodo millet (1.90 mm^2 and 3.01 mm^2). Radius minimum was for kodo millet (1.21 mm) and maximum for browntop (1.74 mm). These results were in agreement with (Abhishek *et al.,* 2021). Assessment of maximum and lower limitation of radii is fundamental in fabricating hoppers and material transportation channels and these valuation help the grains glide smoothly without getting stranded.

Physical properties of grain

1000 grain weight of and volume were calculated to determine seed quality and presented in Table 1. These parameters helps in calibrating and optimizing processing machinery. Kodo millet recorded highest 1000-grain weight and volume *i.e.* 59.73 g and 9.73 ml than other two grains. The 1000-grain weight of browntop millet was more but 1000-grain volume of barnyard millet was high compared to browntop millet. This type of quantification play vital role in outlining machinery design and hopper size.

Hydration capacity and hydration index explicates moisture absorption tendency of grain and to what extent grain can hold on to the absorbed moisture content. Swelling capacity and swelling index refers to the measure of grain volumetric expansion after moisture absorption. Among three grains hydration index and hydration capacity was high for barnyard millet and low for kodo millet. Similarly, swelling index and swelling capacity was high for barnyard millet and low for kodo millet. This is due to thick-coated husk of kodo millet, made it less permeable to water affecting it's ability to imbibe water efficiently. Similar results were reported by (Muragod *et al.,* 2019; Reddy *et al*., 2019 and Roopa *et al.,* 2020).

The maximum force required to crush the grain is the measure of grain's physical strength and termed as grain hardness. This property is crucial in determining stramina of the grain and it's resistance to get compressed. Harder grains need more breaking force. This property helps in

Table 1: Physical characterization of grains.

Grains	1000 wt (in g)	1000 volume (in ml)	HI (g/1000 seeds)	HC (%)	SW.I (ml/1000 seeds)	SW.C (%)	Bulk density	True density	Porosity	Grain hardness (N)
BT-W	3.59±0.15	5.83±0.05	1.59±0.13	59.27±13.19	1.27±0.08	26.88±7.83	0.61±0.00	5.22±0.06	87.85±0.04	19.27±3.12
BY-W	3.12±0.08	6.10±0.28	1.75±0.08	75.34±8.30	1.43±0.06	43.48±6.21	0.55±0.01	5.00±0.22	89.07±0.18	18.68±1.19
KM-W	59.73±0.21	9.73±0.05	1.55±0.01	54.84±1.12	1.24±0.01	23.63±0.74	0.59±0.01	5.17±0.24	88.23±0.75	39.30±8.58

designing dehulling equipment for the grains and the required force to separate the hull and the endosperm.

As per the results kodo millet was hardest among three millets and was intact till 39.30N and the other grains *i.e* browntop (19.27N) and Barnyard (18.68N) were lesser hard compared to kodo millet.

Phyto-nutrient content

One of the most prominent component in millets is their phyto-nutrients contour. Compounds that are biologically active and have potential health and nutritional benefits when consumed in appropriate measures are called as phyto-nutrients. Millets are notably loaded with phenolic compounds, flavonoids, oxalates and tannins. Anti-oxidant activity of the millets exhibit many health benefits. The present study investigated anti-oxidant activity of the millets (DPPH method), total phenols, tannins, total flavonoids content and oxalate content in three minor millets and presented in Table 2.

DPPH radical scavenging activity

Anti-oxidant activity can be quantitatively measured using DPPH (2,2-diphenyl-1-picrylhydrazyl), a stable free radical that reacts with anti-oxidant molecule by donating electron to DPPH radical and reducing its absorbance. Anti-oxidant activity in three millet flours was in the range of kodo> browntop>barnyard millet. Among three flours kodo flour exhibited highest anti-oxidant activity. The higher the value of inhibition greater the value of anti-oxidant activity (Malgorzata, 2021; Bechini Romayssa *et al.,* 2024).

Phenol content

Results revealed that total phenol content in barnyard millet as 0.51 mg gallic acid equivalents (GAE)/g, brown top millet with 1.77 mg gallic acid equivalents (GAE)/g, highest among three millet flours and kodo in between the two with 0.89 mg gallic acid equivalents (GAE)/g. Millet flour exhibits 1.2 to 1.208 µmol ferulic acid per gram of total phenolic substances. The phenolic compound which play a key role in the nutritional and anti-oxidant properties of grains, are indeed influenced by numerous factors such as geological conditions, ecological factors, post-harvest measures, storage conditions and processing techniques (Ansheef *et al.,* 2022).

Tannins

Total tannins content in these millets varied from 0.11 mg/g of tannic acid to 0.13 mgTA/g. Tannins content in the present research for kodo millet were similar to Sharma *et al.* (2021), values were higher for Barnyard millet Rajeswari *et al.* (2021) and Browntop millet Harichandana *et al*. (2023) compared to the present study. Different varieties of grains have varying levels of tannins which can affect the grain's nutritional profile, taste and potential health benefits (Hahn and Rooney, 1986).

Flavonoid content

The flavonoids in general are the naturally occuring compounds exhibiting chemopreventive properties along with regulating proper cellular functions. The present investigation revealed that total flavonoids content was very high in browntop millet (21.4 mgRE/g) compared to barnyard (3.95 mgRE/g) and kodo millet flours (1.70 mgRE/g). These values can be attributed to several factors that can greatly influence the stability of flavonoids, which can lead to eventual thermal or oxidative breakdown, become photosensitive or can be reactive in extreme pH conditions (Bridle *et al.,* 1997).

Oxalate content

Oxalates, one of the anti-nutrients present in flour that has the ability to form hard complexes with magnesium or

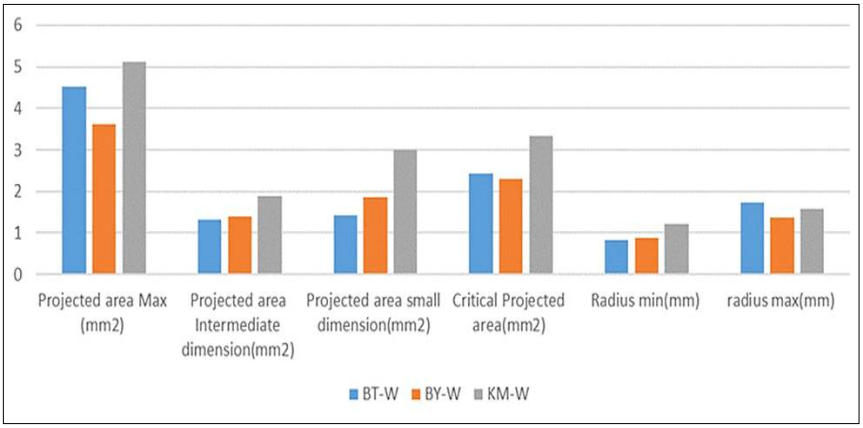


Fig 2: Critical characterisation of grain's morphology.

Table 2: Phyto-nutrient content of grains.

	Total phenols (mg/GAE/g)	Total tannins (mg/TA/g)	TAOX (%)	Total oxalate (mg/100 g)	Total flavonoids (mg/RU/g)
BT-W	1.77±0.16	0.11±0.04	57.16±3.28	38.0±0.00	21.4±1.18
BY-W	0.51±0.08	0.13±0.07	48.22±1.61	24.0±0.01	3.95±0.08
KM-W	0.89±0.33	0.11±0.07	89.34±1.08	13.0±0.01	1.70±0.16

Table 3: Rheological characterisation of grains.

	PST (°C)	PT (min)	PV (Pa-Sec)	TV (Pa-Sec)	HS (Pa-Sec)	FV (Pa-Sec)	BDV (Pa-Sec)
BT-W	75.19±0.88	7.93±0.17	7.59±0.36	5.22±0.08	2.82±0.16	8.04±0.01	4.76±0.18
BY-W	73.62±0.32	6.83±0.82	16.32±0.09	5.05±0.04	3.11±0.01	8.11±0.08	13.32±0.03
KM-W	74.20±0.23	7.73±0.14	14.35±0.17	7.61±0.30	4.39±0.02	12.17±0.24	9.96±0.13

calcium causing major nutritional concerns and one such complication is formation of calcium oxalate stones (kidney stones) in human body (Saiener *et al.,* 2021). Oxalate content was high in browntop millet (38 mg/100 g) followed by barnyard (24 mg/100 g) and kodo millet (13 mg/100 g). Though the oxalate content in millets was moderate, it could be reduced through the application of various processing technologies, whether thermal, non-thermal, or moisture-dependent (Suhan *et al.,* 2024).

The results indicated that anti-oxidant profile of grains is not solely determined by its highest values, but rather by a combination of other compounds which collectively contribute to it's overall effect.

Pasting properties

Pasting properties of flour indicates the gelling and starch forming ability. The expression of flour behavior in terms of viscosity and swelling kinetics is essential mandate in technology applications and evaluation of flour is necessary to observe it's behavior during processing and cooking. Pasting temperature is the point at which flour on heating absorb water, swell, thickens and exhibits rise in viscosity.

The pasting temperature (PST) profile of cereal flour typically ranges from 65°C to 85°C based on flour type and its functional properties as discussed in Table 3. Among three millet flours, brown top has highest pasting temperature (75.19°C) preceded by kodo (74.20°C) and barnyard millet (73.62°C). The high pasting temperature in the browntop flour revealed the fact that it requires high temperature to swell making the flour suitable for bakery products and formulating snack foods. Application of this functionality in making biscuits and cookies where smooth dough is required is preferred rather than breads and buns making. Similar results were observed by Renu *et al.* (2022) for Kodo flour.

Peak viscosity is the maximum viscosity attained by starch granules present in flour and this phase indicates flour strong gelling and paste forming ability. High viscosity is observed in barnyard>kodo>browntop flours and values were between 7.59Pa-s to 16.32Pa-s which means that barnyard millet have thick paste forming ability than remaining flours hence can be used in sauces and soup preparations. In a recent 2024 study by Nazni *et al.* (2024) formulated soup using barnyard and kodo millet. Kodo millet flour in the study exhibited high trough viscosity, strong holding strength, high final viscosity and low breakdown viscosity compared to barnyard and browntop flours. This type of behavior in flour indicates that kodo millet flour forms a very stable, thick paste that does not break down much during heating. The variation observed in these three millet flours and kodo millet superiority among paste forming ability primarily stem into structural and compositional peculiarities. Kodo millet flour has a higher amylose content compared to barnyard and browntop millet, kodo millet's large and polygonal starch granular structure with higher swelling power and less retrogradation power contribute to starch stability and clarity (Yue Wu *et al.*, 2014; Renu *et al.,* 2022; Akshitha *et al.,* 2022; Drugkar Shubham Gopal and Bhuvana, 2021; Kamaljith Kaur, 2018).

CONCLUSION

The physical, dimensional, geometrical, phyto-chemical and rheological properties of three minor millets-Browntop, barnyard and kodo were evaluated thoroughly. As per dimensional characterization browntop millet was long and slender whereas kodo millet quantified as wider and round grain with more surface area, volume, maximum projected area and critical projected area as per dimensions. Barnyard millet has more swelling index and hardest grain among these three was kodo millet. Total phenol, oxalate and flavonoid content was high for browntop whereas anti-oxidant activity reported high for kodo and tannin content high in barnyard millet. As per rheological evaluation barnyard has high paste forming ability, browntop require more temperature for cooking and kodo had highest polygonal starch complexes which made it superior in paste forming ability.

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Conflict of interest

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