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Rheological dynamics, bioactive compounds and antioxidant properties of underutilized Assam lemon based functional jelly

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Abstract
Assam lemon (*Citrus limon* Burm.f) is underutilizing citrus fruit highly regarded for their exceptional nutritional value. It is good source of vitamin C and bioactive compounds. The additional functional ingredients (lemongrass, ginger, and mint extract) made it more functional and mask slight bitterness of lemon juice. This study aims to develop jelly from blends of Assam lemon juice with herbal extract and analyse various physicochemical, phytochemical, rheological and sensory properties. The developed jelly found to have total phenol content (72.17±0.76 mg GAE/100g), total flavonoid content (23.55±1.48 mg QE/100g), DPPH (30.90±5.11%), FRAP (290.81±8.22 µg AA/g). The rheological dynamics of functional jelly showed non-newtonian, shear thinning properties. The functional jelly had lower and more stable modulus. Moreover, the functional jelly tailored for softer or more spreadable textures. The functional jelly prepared with herbal extract was found to be highest acceptability on the basis of sensory evaluation.

Keywords: Assam lemon, bioactive compound, rheological, dynamics, functional jelly

Introduction
Citrus fruits are cultivated globally and highly regarded for their exceptional nutritional value. Numerous scientific studies have highlighted the health benefits of citrus based food products, demonstrating their potential in mitigating degenerative diseases such as cancer, hypertensions and cardiovascular diseases (Wedmulla *et al*, 2022) [1]. The family *Rutaceae* includes over 140 genera and approximately 13000 species of citrus plants (Ghosh *et al*, 2017) [2]. Among them is Assam Lemon (*Citrus limon* Burm.f), commonly referred to as acid lime or *Kagzi Nemu*. This species is predominantly cultivated in the northeastern region of India and is harvested year-round with peak seasons occurring in April-May, August-September and November-December.

Assam lemon is widely grown in the northeastern region of India but it is often underutilized due to limited awareness and market access. A significant portion of the harvest goes to waste due to inadequate storage and processing infrastructure. Transforming underutilized Assam lemon into value added products like jelly increases its market value, provides better income opportunities for local farmers, producers and helps in utilizing surplus produce.

Jelly is popular product that appeals to various age groups, making it an effective medium to introduce the health benefits of functional jelly from Assam lemon, lemongrass, mint and ginger (Tazi *et al*, 2024; Pinto *et al*, 2021; Singletary, 2010) [3, 4, 5]. Incorporating lemongrass, mint and basil leaves extracts into Assam lemon jelly enhances its functional, sensory and preservation properties, creating a product that is not only nutritious and appealing but also aligned with current consumer trends for natural and health promoting foods. The combination of Assam lemon with lemongrass, mint and ginger creates nutrient dense products rich in antioxidants, vitamins and bioactive compounds. These extracts work together to provide multiple health benefits such as improved digestion, reduced inflammation, enhanced immunity and protection against oxidative stress. The inclusion of these extracts positions the jelly as a premium functional food product catering to the growing demand for health oriented and innovative food items.

Materials and Methods

The freshly harvested Assam lemon was collected from the experimental farm of ICAR-Central Citrus research Institute, Nagpur. The fruits were washed manually to remove any dust or foreign particles on their surface. The herbal plants required for jelly such as lemongrass, mint and basil leaves were collected from botanical garden of Institute. The primary ingredients used for product preparation including sugar, citric acid and pectin were procured from the local market in Nagpur.

Product formulation

The different formulations of jelly were made which were named as control jelly and Functional jelly. The quantity of Assam lemon juice in each variant was 38%. The control jelly was prepared with Assam lemon juice only. Preliminary studies (textural and sensory) were done to optimize the proportion of lemongrass, ginger, and mint extract, and it was found that the proportion of 3% lemongrass, 2% mint, 2% ginger extract and 38% Assam lemon juice of highest acceptability. The 50% quantity of sugar was added in the jelly. The quantity of pectin was constant in all the jelly formulations i.e. 0.2%.

Preparation of jelly

For the preparation of jelly, along with lemongrass, mint leaves and basil leaves extracts and sugar was placed in heavy- bottomed stainless steel pan and heated on an induction cooktop (Prestige TTK Prestige Ltd, Bangalore) set to 100 ºC. The mixture was brought to a boil and pectin powder (0.2 g) was gradually added while stirring continuously to prevent clumping. The temperature was then increased to 120 ºC on the induction cooktop, although the actual temperature of jelly during cooking stabilized at 103 ºC. This temperature (103 ºC) was maintained for several minutes to facilitate quick cooking. The endpoint of the jelly preparation was determined by cooling a small sample and measuring its total soluble solids (TSS) using a refractometer. Once the consistency reached to 64ºBrix TSS, the heat was turned off. The prepared jelly was immediately poured into clean, pre-sterilized glass bottles, allowed to cool to ambient temperature and subsequently store in a refrigerator (Garg *et al*, 2018) [6].

Determination of physico-chemical and phytochemical properties of jelly

Total soluble solids (TSS) of the jelly were determined by keeping the sample on the prism of the calibrated refractometer (Erma hand refractometer, Tokyo, Japan) and the result was expressed in ºBrix. The pH of the jelly was measured by digital pH meter (Entech Instruments, Singapore). The colour of the jelly were measured by a colorimeter (ColorFlex E2, Hunter Lab, Virginia, USA) and the results was recorded in CIE (L*, a*, b*) system. The water activity of jelly samples were analysed by using water activity meter (HC 2 AW, Rotronic measuring solutions, Switzerland). The total phenol content (TPC), Total flavonoid content (TFC), Antioxidant activities (DPPH and FRAP) were carried out as per the procedure suggested by Bansode *et al* (2024) [7].

Determination of rheological properties of jelly

The rheological properties of jelly are critical for determining its texture, consistency and behaviour under different conditions, which significantly affect its quality and consumer acceptance. The rheological properties in Rheometer (MCR 102e, Anton Par, Germany) using pp50 and 1 min gap between the plates and temperature 25±1ºC (Sonkar *et al*., 2023) [8]. The relation between viscosity vs shear rate, Storage modulus vs Angular viscosity, Storage modulus vs Shear strain were carried out.

Sensory evaluation

The sensory evaluation of control and functional jelly samples were carried out on 9 point hedonic scale (Tauati *et al*., 2014) [9]. The mean score of minimum 25 semi trained judges for each quality parameters *viz*., colour and appearance, taste, flavour, consistency, transparency and overall acceptability was recorded.

Statistical analysis of jelly

All the experimental data were subjected to statistical analysis using SPSS (statistical package for the social sciences, IBM corporation, Armonk, NY, USA) version 22 for windows. The results were expressed as mean ± standard deviation. The T-test was performed to detect nay statistically significant difference ($p< 0.05$).

Results and discussion

Physico-chemical properties, bioactive compounds and antioxidant properties of jelly

The physico-chemical properties, bioactive compounds and antioxidant properties of control and functional jelly were mentioned in Table 1. The control jelly had a slightly higher TSS (67.2±0.50) compared to the functional jelly (66.3±0.20). This indicates that functional jelly may have a marginally reduced sugar concentration, which would result from the inclusion of functional additives. The pH of the functional jelly (2.92±0.05) was slightly higher than that of the control jelly (2.63±0.03), making it less acidic. This difference may be attributed to the incorporation of additional ingredients with buffering capacity in the functional jelly. The obtained results are similar with Bansode *et al* (2020) [10]. The functional jelly exhibited higher water activity (0.43±0.02). This could result from the higher moisture content in the functional jelly.

The colour measurements (L*, a*, b*) showed notable variation between control and functional jellies reflecting changes in the visual appearance. The functional jelly had L* values (5.41±0.54) compared to the control jelly (4.72±0.09), indicating lighter appearance. The a* value decreased in the functional jelly (10.22±0.52) compared to the control jelly (11.13±0.07) suggesting a slight reduction in red intensity. The functional jelly exhibited a less negative b* value (-0.23±0.03) compared to the control jelly (-1.27±0.04).

The functional jelly demonstrated significantly enhanced bioactive compounds and antioxidant properties compared to the control jelly. The functional jelly had a higher TPC (72.17±0.78 mg GAE/100 g) compared to the control jelly (50.45±0.52 mg GAE/100 g), reflecting an increased concentration of phenolic compounds due to the added functional ingredients. Similarly, the functional jelly exhibited a higher flavonoid content 23.55±1.48 mg QE/100g) compared to the control jelly (18.10±0.05 mg QE/100g). The DPPH radical scavenging activity was greater in the functional jelly (30.90±5.11%) than in the control jelly (25.66±3.27%) indicating antioxidant potential.

The functional jelly exhibited a significantly higher FRAP value (290.81±8.22 µg AA/g) compared to the control jelly (200.15±10.47 µg AA/g), indicating greater ferric reducing antioxidant power. The similar were observed in banana peel jelly (Lee *et al*, 2010) [11].

Table 1: Physico-chemical properties and bioactive compounds of jelly

Properties	Control jelly	Functional jelly
Physico-chemical properties		
TSS (°Brix)	67.2±0.50^{a}	66.3±0.20^{a}
pH	2.63±0.03^{a}	2.92±0.05^{a}
Water activity (a_w)	0.43±0.02^{a}	0.49±0.01^{a}
Moisture (%)	27.54±0.15^{a}	28.36±0.27^{b}
Colour		
L*	4.72±0.09^{a}	5.41±0.54^{b}
a*	11.13±0.07^{a}	10.22±0.52^{a}
b*	-1.27±0.04^{a}	-0.23±0.03^{b}
Bioactive compounds		
Total phenolic content (TPC) (mg GAE/100g)	50.45±0.52^{a}	72.17±0.78^{b}
Total Flavonoid content (mg QE/100g)	18.10±0.05^{a}	23.55±1.48^{a}
DPPH (%)	25.66±3.27^{a}	30.90±5.11^{b}
FRAP (µg AA/g)	200.15±10.47^{a}	290.81±8.22^{b}

Values in a row with different alphabets are significantly different ($p < 0.05$), GAE: Gallic acid equivalent, QE: Quercetin equivalent

Rheological properties of control and functional jelly

The impact of shear strain on storage modulus of control and functional jelly was shown in Figure 1. The control jelly demonstrates significantly higher storage modulus values throughout the shear strain range compared to the functional jelly. The sharp decline in the control jelly's modulus suggests it is more susceptible to deformation. Whereas, the functional jelly despite being weaker overall, maintains a more stable modulus across the strain range. This graph underscores the contrasting rheological properties of the jelly formulations. The control jelly's higher initial storage modulus reflects a stronger gel network, but it is rapid decrease indicates fragility under strain. Conversely, the functional jelly, with its lower and more stable modulus, may have been engineered for applications requiring greater deformation tolerance, possible due to added ingredients altering its gel network dynamics (Javanmard & Endane, 2010) [12].

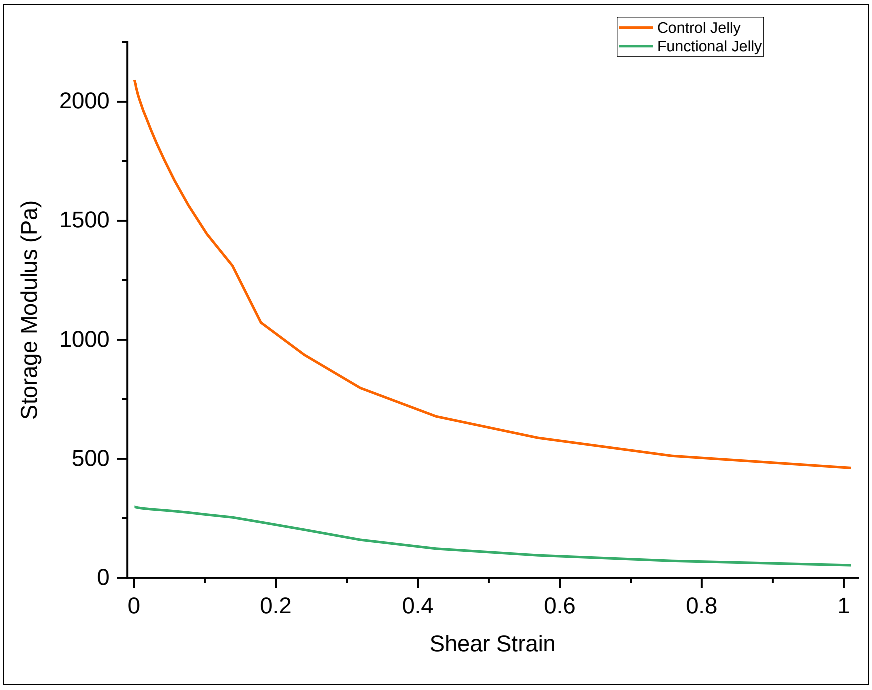

Fig 1: Shear strain vs storage modulus

The relationship between shear rate and viscosity was mentioned in Figure 2. Both the control and functional jelly exhibit a pronounced decrease in viscosity with increasing shear rate. This indicates that both jellies possess non-newtonian, shear thinning properties, which are characteristic of gel like materials. Shear thinning occurs at the internal gel network is progressively disrupted under applied shear, allowing for easier flow. Such behaviour is desirable in many food applications, as it enables the jelly to maintain a firm structure at rest while becoming more spreadable under applied stress. Control jelly exhibit significantly higher viscosity across all shear rates, reflecting a denser and more robust gel structure. This is likely attributed to stronger intermolecular interactions within the gel matrix. In case of functional jelly demonstrates a markedly lower viscosity at al shear rates, suggesting a weaker gel network. The addition of herbal ingredients may have interfered with the formation of strong gel structure leading to reduced viscosity.

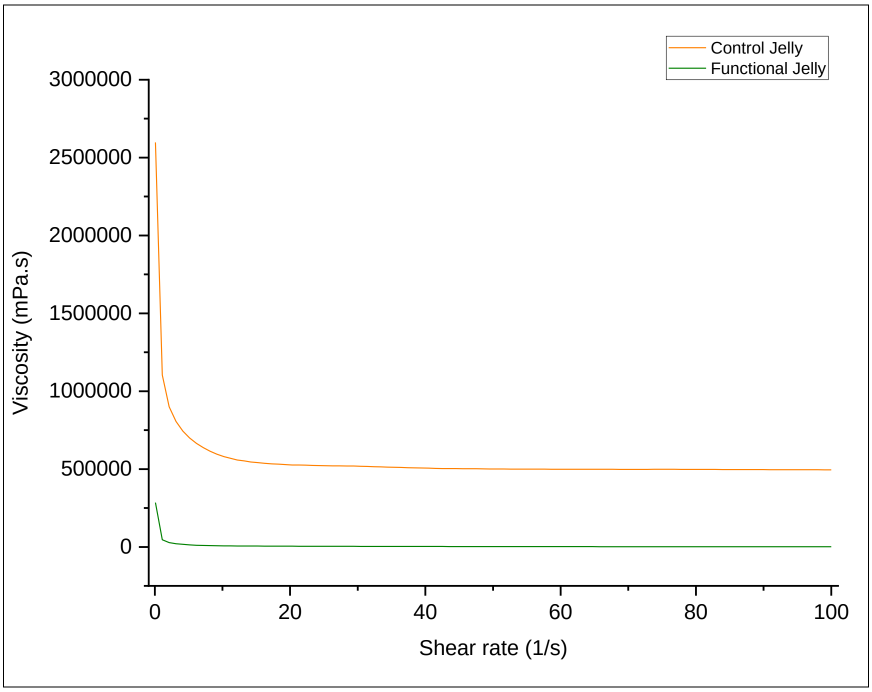

Fig 2: Shear rate vs Viscosity

The relationship between storage modulus and angular frequency for both control and functional jelly is depicted in Figure 3. Both jellies showed an increase in storage modulus with angular frequency, which are characteristics of viscoelastic materials. This indicates that the elasticity of the jellies becomes more pronounced at higher frequencies. The significant difference in storage modulus values between the control and functional jelly highlights the impact of formulation on gel strength. The control jelly's higher storage modulus suggests it is more suitable for applications requiring a firmer, more elastic texture, while the functional jelly may be tailored for softer or more spreadable textures. The obtained results are good agreement with Tiwari *et al*. (2016) [13].

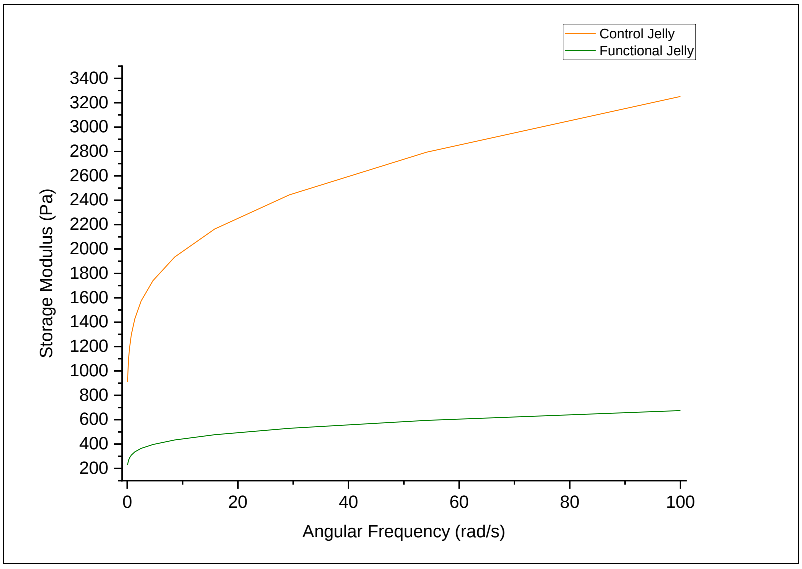

Fig 3: Angular frequency vs Storage modulus

Sensory evaluation of jelly

The various sensory attributes measured for jelly were colour, taste, texture and overall acceptability and scores are depicted in Figure 2. Since both jellies were prepared using the same method, the inclusion of additional functional ingredients (ginger, mint and lemongrass extract) in the functional jelly might have altered its texture and colour. The control was found to be best in colour and texture. The overall acceptability of functional jelly was found to be highest as compared to the control jelly.

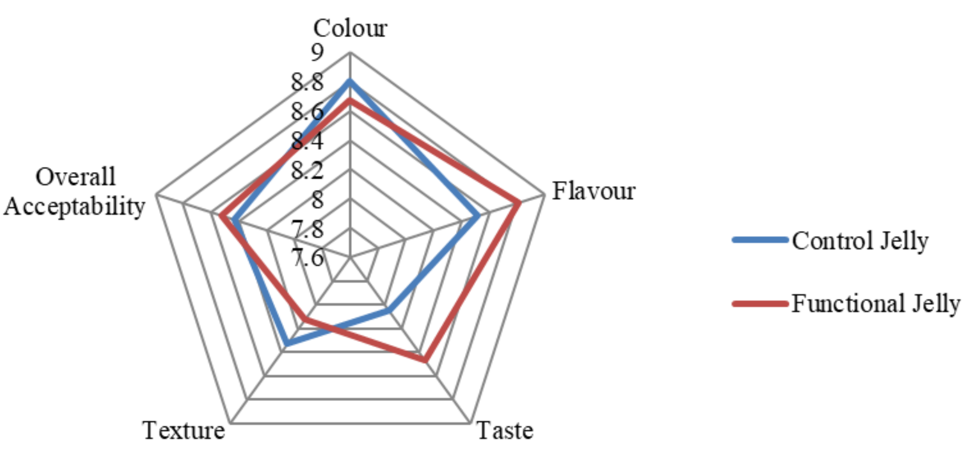

Fig 4: Sensory evaluation of control and functional jelly

Conclusion

This study was aimed to develop functional jelly with herbal extracts (lemongrass, ginger and mint) that could lead to improvement in the polyphenols and masking bitterness of lemon juice. Although physico-chemical and phytochemical properties of jelly did not differ much, still jelly developed with incorporation of herbal extracts was found to have fairly high amount of total phenols, total flavonoids and antioxidants which highly beneficial for human health. It can be considered healthier than other jellys. Rheological dynamics revealed that the functional jelly had non-newtonian, shear thinning properties. The functional jelly showed lower and more stable modulus despite it had weaker gel network and lower viscosity. The functional jelly was found to have higher overall acceptability in sensory evaluation. The functional jelly prepared from underutilized Assam lemon enriched with herbal extracts offers potential health benefits by helping in the prevention of chronic lifestyle diseases. Additionally, it highlights the scope for value addition to Assam lemon enhancing its utility and market potential.

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