Extraction of Mucilage from Chia Seeds and its Application as Fat Replacer in Biscuits

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Abstract— **Chia seeds (*Salvia hispanica L.*) are considered as one of the "Superfoods" due to its high nutritional value. They are good source of dietary fiber, protein and unsaturated fats. Application of mucilage from chia seeds in food industry has been of interest lately. There are no commercially viable techniques to extract and separate mucilage due to its limited research, low extractability and yield. In the present study, chia mucilage is extracted from white chia seeds by a novel thermal process. The extracted mucilage powder is reconstituted and further incorporated into the biscuits as a gel and investigated for the effect of partial fat substitution (20% to 40 %) on physical properties (spread ratio, color, and texture profile), chemical properties (carbohydrates, protein, fat and ash) and sensory evaluation of biscuits along with the control sample. The yield of mucilage obtained through novel method is 8%. Addition of chia mucilage gel had a positive effect on hardness of biscuits. The color of the biscuits changed from light brown to creamy yellow as the level of mucilage gel increased. The sensory properties of the biscuit were strongly influenced by the mucilage gel level. By considering the overall acceptability of the biscuits, the use of chia mucilage gel can be considered as a potential fat replacer.**

Keywords—Chia seeds. chia mucilage gel, fat replacer, viscosity of mucilage gel.

I. INTRODUCTION

Chia (*Salvia hiapanica*) is an edible seed which is native from southern Mexico. Chia seeds are good source of dietary fiber, protein, antioxidants and poly unsaturated fats such as linoleic and lenolenic acids [1]. Chia seeds are considered as "Super food" and commercially available in India. Due to its nutritional properties and fat binding properties, inclusion of chia flour is considered as value addition in bakery products [2]. When chia seed is immersed in water, a transparent capsule is exuded around the seed known as "mucilage [3]. The high water holding capacity and soluble fiber nature of chia seeds is due to its complex structure which is proposed to be tetrasaccharide with 4-O-methyl-a-Dglucoronopyranosyl units occurring as branches of b-D-xylopyranosyl on the main chain. [4]. The extraction of mucilage from chia seeds is prolonged process and can't be separated easily. Various studies on the extraction of chia seed mucilage has shown yield ranging from 4 to 7 % at temperature 80° C [5]. The typical chia mucilage extraction involves hydration, exudation, drying and sieving process. Microwave assisted extraction (MAE) is recent technique which is gaining interest and has potential to extract efficiently when compared to conventional extraction technique. The principle of MAE depends on the ionic conduction and dipole rotation caused by microwaves followed by heat generation which in turn causes the pressure on the cell wall. This pressure allows the constituents to get ruptured from the cell into the solvent present and thus increasing their yield [6]. Using microwave, mucilage has been successfully extracted with more yield in less time from *Opuntia ficus indica* cladodes when compared to conventional extraction (CE) [7][8].

Health disorders such as obesity, diabetes and heart disease are caused due to excess intake of saturated fats [9]. Currently, consumers' attention is drawn towards low-caloric products, owing to the increasing emphasis on health education. Current major concern in packaged food market is fat reduction and therefore increasing demand for low fat products. According to the reports, in the year 2016, around 13% of the world's adult population (11% of men and 15% of women) were obese [10]. According to recent research reports [11], south Asians are at higher risk for the development of obesity and other non-communicable diseases. Obesity may be related to other noncommunicable diseases [12]. Fat replacement by other substances is a major concern, specifically in bakery products. In biscuits, the addition of fat gives right texture, flavor, mouthfeel and thus contributing to its overall acceptability [13]. Over the years, different carbohydrate-based substances such as maltodextrin [14], polydextrose [15] and inulin [16] that provide similar physical and sensory properties to fats have been used in foods. When used in biscuits, the textural and sensory properties have significantly changed [17][18][19]. Therefore, there is a search for best fat substitute that causes least changes in physico-chemical and sensory properties of food. India is currently the world's largest biscuit consuming nation and is consumed all over the world. Biscuit can contain fat ranging from 16 to 25 gm per 100 g of the product. Indian biscuit market projected to reach $7.25 billion in next four years [20]. Recently, chia mucilage has been studied as a potential fat replacer. Role of chia seed mucilage as a fat replacer has been studied in pound cakes and bread More than 25 % of fat was replaced in pound cakes [21] and 56.6% in breads [22] without effecting their technological properties. However, its function was not studied in biscuits. Role of chia mucilage as stabilizer and emulsifier also has been studied in ice cream [23].

The objective of this study was to extract chia mucilage through MAE and evaluate the effects of its incorporation on the textural, physiochemical and sensory quality of wheat biscuits with the substitution of 20, 30 and 40g/100 g of fat.

II. MATERIAL AND METHODS

A. Material

White chia seeds were donated by M/s. SAMATH, Hyderabad, Telangana. The seeds were commercially grown in the Ananthapur region of Andhra Pradesh. The composition as provided by the supplier: Moisture < 12%, Carbohydrates 40%, Dietary fiber 34 %, Protein 17%, and Fat 32 %.

B. Chia seeeds hydration

Chia seed hydration capacities were determined under room temperature conditions (26°C). Seed to water ratio is maintained at 1: 40. In a 100 ml beaker, 40 ml of distilled water is taken into which sample of 1 gm of chia seeds were placed separately inside a filter paper bag was immersed .The net weight of each bag was weighed every 10 min until it reached a constant value. The experiment was done in triplicate.

C. Mucilage extraction by using microwave

Microwave assisted extraction of mucilage from chia seeds were performed in customized pilot scale microwave oven system with cubical (2.45 GHz, Enerzi Microwave systems pvt ltd, Belgaum, Karnataka). The oven was equipped with digital control system for microwave power which is adjustable from 100 to 2000 W and also modified to record the temperature of the sample placed by fiber optic temperature probes (-50°C to 250°C Reflex Make, Canada). Samples of 10 g of chia seeds were added into 400 ml of distilled water taken in a 500 ml beaker. pH was adjusted and maintained at 8. Then the beaker with aqueous suspension was placed into the oven and ran at 1.3 W/gm until it reached 80 C. At room temperature, the aqueous suspension was stirred continuously with magnetic stirrer for 30 min. The suspension was filtered for removing any extra water and spread on a tray followed by drying in a hot air oven with forced air circulation at 50°C for 12 to 14 hours. The dried seeds were rubbed through a 40 mm mesh screen to obtain mucilage. The weight was recorded, and yield was calculated. Mucilage gel was prepared by mixing 0.1 gm of dried mucilage in 100 ml of distilled water. This mucilage is further used as fat replacer in wheat biscuits.

D. Yield of the mucilage and viscosity of the gel

The yield of the chia mucilage extracted is calculated using following Eq. (1)

$$Y(\%) = \frac{Wt \ of \ mucilage}{Wt \ of \ chia \ seeds} \ X \ 100 \quad \ldots\ldots \quad (1)$$

The viscosity of the hydrated samples (0.125 g/ 100 g) was measured using a Hoppler viscosimeter at 25°C. All analyses were performed in triplicate.

E. Preparation of biscuit

The composition of ingredients in biscuit formulation is mentioned below (TABLE I). The dough preparation was done in a laboratory mixer. Sugar and fat/ mucilage gel were mixed thoroughly and creamed at low level speed. The ammonium bicarbonate, milk powder, sodium chloride and vanilla essence were added into the flour. After pouring appropriate amount of water, cream was added along and dough was prepared by proper mixing. The biscuits were cut with a round cutter of 40 mm diameter and baked on an aluminum tray at 180°C for 10 – 12 min. The biscuits were cooled and stored in aluminum pouches for analysis.

TABLE I. BISCUIT COMPOSITION

Ingredients of biscuits with varying fat levels				
Ingredients	*Control*	*20 % FR*	*30 % FR*	*40 % FR*
Wheat	53.5	53.5	53.5	53.5
Sugar	14.9	14.9	14.9	14.9
Oil	16.6	13.2	11.6	9.9
CMG (0.1%)	0.0	3.3	5.0	6.6
Baking Powder	0.5	0.5	0.5	0.5
Ammonium Carbonate	0.5	0.5	0.5	0.5
Salt	0.6	0.6	0.6	0.6
Milk :Powder	0.8	0.8	0.8	0.8
Essence	0.4	0.4	0.4	0.4
Water	12.1	12.1	12.1	12.1
Total	100	100	100	100

F. Evaluation of biscuit

1) Physico-chemical properties: The physical properties measured were diameter, thickness, average weight, moisture content, and color. Diameter was measured by placing six biscuits edge to edge and thickness by stacking one biscuit above the other. The average of the three such arrangements were taken and considered. The average weight of six biscuits was noted. Moisture was measured using the methods described by [24]. Spread ratio is calculated by the ratio of diameter and thickness. The colour (L∗, a∗, b∗) values of the sample were measured with a Hunter Lab Colorflex colorimeter (Hunter Associates Laboratory, Reston, Va., U.S.A.) and by taking color group as a reference, total color differences (ΔE) were calculated.

The total colour difference (ΔE), in CIE L*a*b* color model was calculated by the Eq (2) [25]

$$\Delta E = \sqrt{[(L*_2 - L*_1) + (a*_2 - a*_1) + (b*_2 - b*_1)]}$$

$$\ldots\ldots\ldots \quad (2)$$

where, and indicate colour parameters of raw cookies (dough), parameter *L** refers to the lightness of the samples, and ranges from black (*L*=0) to white (*L*=100), a negative value of parameter *a** indicates green and a positive one indicates red–purple colour, and positive value of parameter *b** indicates yellow while negative value indicates blue colour. All measurements were repeated in three replicates. Textural analysis was performed by Texture Pro CT V1.3 (Brookfield Engineering Labs, Inc). Four cookies were evaluated from each sample using the three-point break technique. Test type is compression strength and was used under following conditions: Target mode: distance, distance: 2.5 mm, Pre test speed: 2 mm/s, test speed: 1 mm/s, trigger force: 50 g. The hardness (g) and fracturability (mm) were recorded.

The proximate compositions of biscuits were estimated according to [26], on dry basis. The Moisture, total ash, protein, total fat, total carbohydrate content and lipid content were determined. The energy (kcal) of the biscuit was calculated based on the caloric coefficients corresponding to

the protein, carbohydrate and lipid content, according to Eq. (3)

$$Energy\ (k\ cal\ per\ 100\ g) = [Quantity\ of\ protein\ in\ (g) * 4] + [Quantity\ of\ carbohydrate\ (g)] * 4 + [Quantity\ of\ lipids\ (g) * 9]$$

………… (3)

2) Sensory properties: The physical properties measured were diameter, thickness, average weight, moisture content, and color. Diameter was measured by placing six biscuits edge to edge and thickness by stacking one biscuit above the other. The average of the three such arrangements were taken and considered. The average weight of six biscuits was noted. Moisture was measured using the methods described by [25]. Spread ratio is calculated by the ratio of diameter and thickness. The colour (L∗, a∗, b∗) values of the sample were measured with a Hunter Lab Colorflex colorimeter (Hunter Associates Laboratory, Reston, Va., U.S.A.) and by taking color group as a reference, total color differences (ΔE) were calculated.

3) Statistical analysis: All the experiments were done at least in triplicate. The data were compared using analysis of variance (single point ANOVA) and the average values obtained were compared using MS office 2016, with statistical significance (a) set at $p < 0.05$.

III. RESULTS AND DISCUSSION

A. Hydration of chia

Hydration of white chia seeds was studied at a ratio of water to seed ratio of 40: 1. After 30 min, the total weight of the seeds became constant. Unlike the black chia seeds which takes 2 hrs, the hydration time for white chia is less. Fig 1 shows the final water absorption is 83 % indicating that it would require less water for hydration. The test was done at room temperature ($28^{O}C$). Water absorption is faster within first 10 min. similar behavior has been reported in the above study on black chia [5].
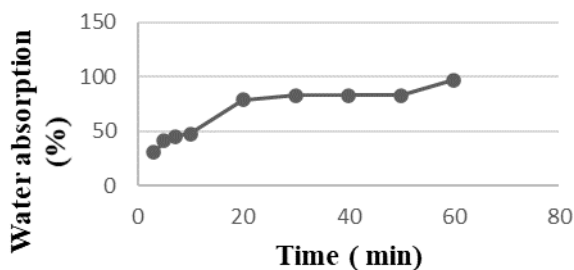

Fig.1. Hydration of Chia seeds

B. Mucilage Extraction

Mucilage was extracted by using microwave at 1.3 W/gm for 15 min. Preliminary studies of time temperature profiles with water in microwave indicated that by application of 1.3 W/gm, water temperature rises to 80ºC. Time and temperature profile correlated with chia seeds and water suspension. As the hydration studies indicated that it would take 30 min to reach maximum water absorption, the seed and water suspension were magnetically stirred for another 15 min after microwave treatment.

C. Yield of the mucilage

The average yield of mucilage obtained was 8% through the microwave extraction. The obtained yield is higher than those reported by [23], [27] and [3] but lesser than reported by [28] through conventional extraction methods at 80ºC with pH of 8 and seed : water ratio of 1:40. Optimization studies are yet to be done with varying power levels, time and water seed ratio. Extraction time was 4 times lesser than the above reported studies. There were no comparative studies for microwave extraction of mucilage from chia seeds. However, a yield of 8.13%, 2% and 4.6% was reported for microwave extracted mucilage from Opuntia ficus-indica Cladodes [8], Trichosanthes Dioica fruit [29] and Trigonella foenum graecum seeds [30] respectively.

D. Viscocity

It can be observed in the rheogram (Fig.2) that the viscosity decreases with increase shear rate or speed. This behavior is called as shear thinning or pseudoplastic. The reason may be due to the change in the structure or orientation of the mucilage. At higher shear rate, the interaction between the polymers are destroyed as they arrange themselves in the flow direction and in turn resulting in low viscosity [31], [32], [33]. The rheological behaviour of the mucilage extracted through microwave correlates with the mucilage extracted through cold extraction [34], and hot extraction methods [24], [35], [36] and Supercritical Fluid extraction methods [37].
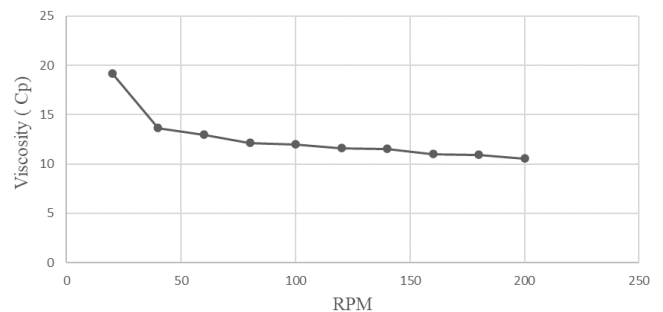

Fig.2. Effect of Shear rate/speed on the viscosity of chia mucilage

E. Physico-chemical properties of biscuit

Table II presents the physical properties of control biscuit and biscuit with fat replaced by mucilage extracted from chia at three different levels. Moisture content in biscuit is one of the important sensory characteristics and in turn responsible for quality [38]. Moisture content increased with the increase of fat substitution. This is due to the water holding property of the chia mucilage. Similar results were reported by [39] when fat was replaced by chia mucilage gel in bread and cakes. Reduction in weight (mass) of biscuit has been considered as baking loss which is caused by the loss of moisture. Although the initial moisture content of the fat replaced biscuits are high when compared to the control, a significant decrease in weight has been observed in fat replaced samples (TABLE II). Similar observation was reported by [40] while studying the effect of different flour composition on qualitative parameters of cookies.

Texture Analysis In biscuit quality, significant differences were observed in spread ratio, breaking strength and fracturability. The desirability of the biscuit quality is indicated by the higher spread ratio [41]. It is used to determine the quality of flour that enables the biscuit to rise [42]. Hence, biscuit prepared with 35 % fat replacement may

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be the most preferred based on spread ratio. Similar findings with respect to the weight, diameter, and spread ratio were reported by another researcher [43]. For a biscuit, textural parameters are very desirable and important quality attribute. Breaking strength, which is the force (g) required to break the biscuit, increased with the increased percentage of mucilage formulation in biscuit. Biscuits which show high values of breaking strength represent less tenderness. The ability of biscuit to regain its original form is measured in terms of fracturability. Fracturability of the biscuit samples significantly reduced as the level of mucilage increased. Relation between breaking strength and distance (mm) at different fat replacements in biscuits is presented in figure. Biscuits which shows high breaking strength values are less tender. Biscuits which shows greater values of fracturability at breaking strength were less brittle. Control biscuits showed the lowest breaking strength and the lower fracturibility. 20 % and 30 % fat replaced biscuits showed higher breaking strength and higher fracturibility than control samples. 40 % fat replaced biscuits showed high breaking strength but lower fracturability than control samples (Figure 3). Therefore 30 % fat replaced biscuits were considered as highly compressible and less brittle. [44] reported that at formulations with 30 % fat replacement by polydextrose showed high breaking strength than control cookies. It **is** reported that 25 % fat replacement in cookies did not affect penetration force [45].

TABLE II.   PHYSICAL PROPERTIES

Physical properties of control and fat replaced (FR) biscuits					
Sample	Moisture	Spread Ratio	Baking loss (g/100gm)	Breaking Strength (kg)	Fracturatibility (mm)
Control	3.77±0.85[a]	5.9±1.62[b]	10.26±1.33[b]	1.8±1.05[b]	0.49±0.05[a]
20% FR	4.48±1.70[b]	6.1±0.34[a]	13.36±1.76[a]	2.2±0.18[a]	0.59±0.02[a]
30% FR	6.67±1.65[a]	4.5±0.22[b]	17.39±0.25[b]	2.6±0.21[a]	1.09±0.06[b]
40% FR	8.46±0.55[a]	4.4±0.15[b]	20.91±0.88[b]	3.4±0.48[b]	0.49±0.02 [a]

Average of three values with standard deviation, same letter in the line indicates that there is no significant difference between the means (p < 0.05)
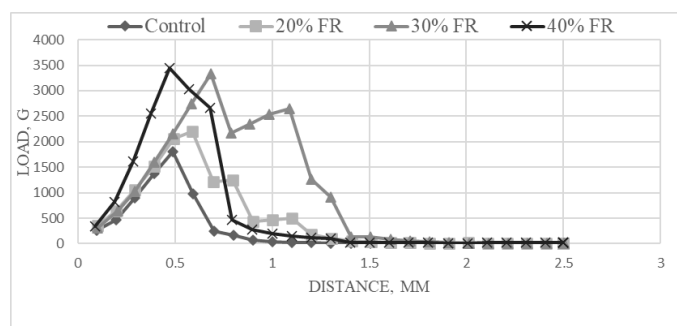

Fig.3. Breaking Strength and fracturibility at different levels of fat replacement(FR)

F. Proximate composition

Table III presents the proximal composition of control biscuits and in fat replaced biscuits. The increase in percentage of fat substitution resulted in a slightly higher protein content. The energy or caloric value for fat replaced samples was lower because of its highly reduced content of lipids. **S**ignificant reduction was reported in fat content of

56.6% in breads and 51.6% in cakes by adding chia mucilage gel and good sensory acceptability [39]. However, results reported by [46] showed a fat reduction of 57% in cakes but does not have good sensory acceptability. There were no significant changes in ash content of fat replaced formulations.

TABLE III.   PROXIMATE COMPOSITION

Mean values of proximate composition of control and fat replaced biscuits (in 100g)					
Sample	Moisture	Spread Ratio	Baking loss (g/100gm)	Breaking Strength (kg)	Fracturatibility (mm)
Control	64.0	10.3	17	3.1	450.2
20% FR	63.8	10.8	13.7	3.1	421.7
30% FR	64.2	10.9	12.2	3	410.2
40% FR	63.9	11.1	10.3	3.2	392.7

G. Color measurement

To observe the undesired color changes, the color of the fat replaces biscuits is measured by comparing with the control sample. The values of color change (ΔE) and lightness (L*) are presented in Fig 4. There are no significant differences in a* and b * values. [44] reported that there was a significant decrease in L* values after adding fat mimetics such as polydextrose and pectin. Similarly, [39] reported decrease of L* values in crust and crumb of breads after adding chia mucilage. In contrary to the above studies L* values were significantly increased with addition of chia mucilage in the present report.
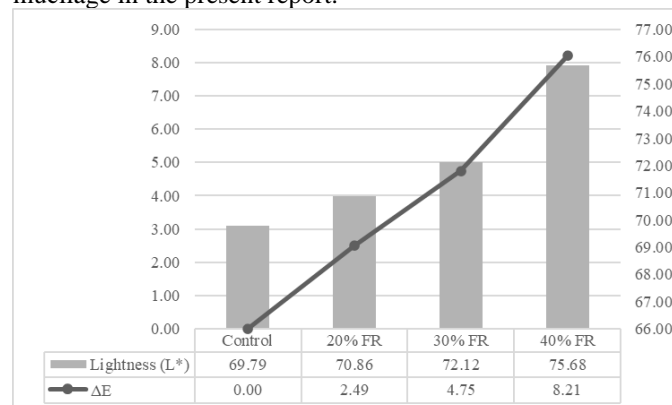

Fig.4. Mean values of color changes in fat replaced (FR) biscuits

H. Sensory Analysis

Figure 5 presents the sensory analysis results of fat replaced biscuits by comparing with control sample. It can be observed that there was no significant difference upto 20 % fat replacement in biscuits in all the evaluated sensory parameters. Control and 20 % FR samples were evaluated a "moderately like" and "like very much" respectively. Therefore, the most acceptable biscuits at 25 % fat replacement which had shown good hardness parameter and acceptable flavor. It was reported that cookies had given good general acceptance at 35 % fat replacement with polydextrose [44].
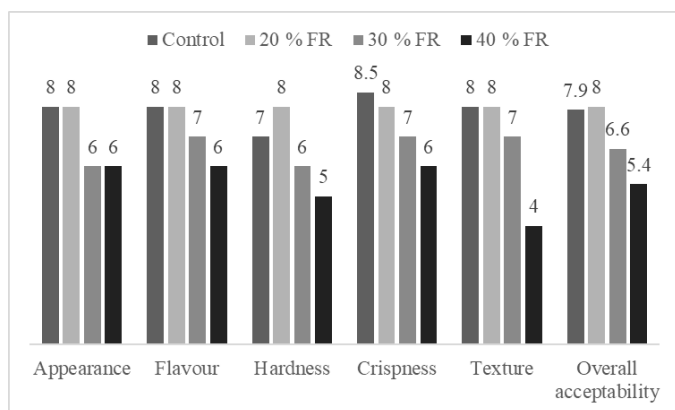
Sensory score of control and fat replaced (FR) biscuits

IV. CONCLUSION

Microwave Assisted Extraction (MAE) of chia seed mucilage gave a better yield than the conventional extraction method. Its rheological properties correlated with the mucilage extracted through other techniques. Further optimization studies are yet to be done at different water seed ratio, time and power levels and seed particle size on mucilage yield and oil yield. The addition of chia mucilage as a fat replacer significantly affected the physico-chemical properties and sensory properties of biscuits. Energy value was reduced as a result of fat substitution. In sensory analysis, biscuits with 25 % of fat replacement with chia mucilage gave overall acceptability similar to control biscuits. A comparative study on shelf life of various bakery products are yet to be done with mucilage extracted through various kind of extraction techniques. Therefore, a complete utilization of chia seed can be achieved thorough MAE for a better yield of oil and mucilage.

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