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Production of Nutraceutical Chocolate Brownies by Incorporation of *Magnifera Indica* Leaves Extract

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Abstract

Product development is a key to success for any food industry; hence a lot of new products are launched in the market on regular intervals. Chocolate brownies were prepared by the incorporation of 5%, 10%, 15% and 20% aqueous extract of Magnifera Indica leaves with partial substitution (50%) of wheat flour by gram flour. The finished products were evaluated for shelf life, antioxidant, organoleptic and physico-chemical characteristics. The brownie sample with 10% aqueous extract of M. Indica leaves was found to be acceptable according to the sensory panel. Both of the control and samples with M. Indica have shown better organoleptic scores, making them acceptable. The moisture(%), ash(%), fat(%), titrable acidity(%), proteins(%), carbohydrates(%), fiber(%) and antioxidant activity as DPPH inhibition (IC50 ppm) was found to be 5.6 ± 0.1, 1.44 ± 0.01, 37.8 ± 0.05, 2.2 ± 0.001, 12.4 ± 0.04, 15.65 ± 0.04, 11.2 ± 0.1 and 92.23 ± 0.9 respectively. The calorific values indicate that the brownie sample with M. Indica contains only 7.6% of the total daily calorie requirements of an adult, and may not cause weight gain. The shelf life, investigated at ambient (27°C) and refrigerated (5°C) temperatures, of control sample was found to be 6 and 15 days and for the samples with M. Indica was 8 and 25 days respectively. The constituents of the product produced in the current study are natural, no artificial colour and flavour was added to prepare the brownies. The research concludes that M. Indica extract and gram flour impart the antioxidant, anticancer, anti-inflammatory, anti-diabetic and other medicinal properties in the chocolate brownie. It also has the market competent price therefore the product is likely to gain the attraction of consumer and improves the market trends.

Keywords: New food product development, Brownie cake, Nutraceutical product, Magnifera Indica

Introduction

Mango (*Mangifera Indica Lam*), one of the most popular tropical fruit, belongs to the family of *Anacardiaceae*. Mango tree flourishes in tropical and sub-tropical climate, particularly in those places where there is a good rainfall (Pay 2005). The antioxidants present in mango plant have captured the attention of a lot of researchers. In biomedical applications, mango leaves, stem and pulp have successfully been used for anticancer activities (Percival, Talcott *et al.* 2006); for free radical and antioxidative scavenging (GARRIDO, DEYARINA *et al.* 2008).

Mango leaves are reported to have anti-inflammatory, anti-diabetic, anti-cancer properties. It is known to contain antioxidants, vitamin E, tannins, flavanoids, Polyphenols. Mango leaves have been used to cure diabetes, high blood pressure and as a source of dietary supplements (Duke and Allen 2006). Different in vitro studies have been carried to use the mango leaves for lowering blood glucose level, cholesterol, improving immune system and cure for neurological disorders (Okwu and Ezenagu 2008). Mangiferin is the most dominant compound of mango leaves that has been found suitable to cure diabetes (Ganogpichayagrai, Palanuvej *et al.* 2017), show antiviral effects (Shah, Patel *et al.* 2010), to promote guts and gastrointestinal protection (Imran, Arshad *et al.* 2017), prevent from

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cancer (Ganogpichayagrai, Palanuvej *et al.* 2017, Rajendran, Rengarajan *et al.* 2015), to reduce cholesterol level, triglycerides and free fatty acids in cardiac tissue and blood tissues (Nair and Devi 2006, Arozal, Suyatna *et al.* 2015). Researches indicated that the mango leaves contain no harmful component and do not cause any acute and long-term toxicity (Zhang, Li *et al.* 2014).

Gram flour has several health benefits reported earlier such as curing diabetes, decreasing the risk of colon cancer (Wallace, Murray *et al.* 2016), role in weight control (Nestel, Cehun *et al.* 2004), to improve digestion (Wallace, Murray *et al.* 2016, Murty, Pittaway *et al.* 2010) and to reduce cholesterol level and boost heart health (Pittaway, Robertson *et al.* 2008).

Due to antioxidant activity of mango leaves, its extract is used as a natural preservative in pharmaceutical and food industry. In food industry, mango leaves extract also used as natural antioxidant in replacement of synthetic antioxidant (Ponce, Casas *et al.* 2013). In java and Philippines Tender mango leaves are consumed as vegetable because of good source of many compounds (Rai, Basak *et al.* 2007). A beverage formulated with 20% mango leaf extract presented the highest percentage of antioxidant activity (38.63%) and inhibition of enzymes α-amylase (41.9%) and α-glucosidase (37.53%). Results showed that the biological properties of beverages could be an alternative for the control of free radicals and glucose levels (Medina-Saavedra, Herrera-Corredor *et al.* 2020).

The effect of half and full replacement of wheat flour by chickpea on the cake quality was determined. It is concluded that it's possible to use chickpea flour with wheat flour in combination but when the concentration of gram flour increases the volume of cake decreases, the texture become firmer gummier like (Gularte, Gómez *et al.* 2012). The impact of substituting the wheat flour by 100% of chickpea flour on quality, nourishing and tactile properties of breads was investigated. The bread with toasted chickpea flour had a highest specific volume and the soft texture whereas the bread obtained from the sprouted chickpea shows the lowest overall acceptability about the tactile properties (Ouazib, Garzon *et al.* 2016). Wheat flour was partially substituted with chickpea flour on different concentrations (10%, 20% and 30%) and the dough rheology and baking performance was observed. Chickpea flour provides good quality characteristics in terms of volume texture, internal structure and pores (Mohammed, Ahmed *et al.* 2012). Biscuits were prepared with the chickpea flour and plantain flour. Results show that the final product contains about 19.3% protein content and 3.6% crude fiber. By the addition of chickpea flour and plantain flour sensorial properties of biscuits also increase (Yadav, Yadav *et al.* 2012).

Brownies are a kind of solid chocolate cake, originally a flour and hard dough made from wheat flour, eggs, fat, granulated sugar and chocolate by baking (Ligarnasari, Anam *et al.* 2018). Brownies usually contain less balanced nutrients; therefore, it is regarded as food that contains a less nutritional value that can be utilized by the body (Winarti and Nurdjanah 2005). Impact on the physical attributes, substance qualities and sensory attributes of brownies prepared with dark cumin oil and substituted sweet potato flour were explored. The result demonstrated that the best equation of substituted brownies which was

included 0.05% of nigella sativa oil had accurate water content, fat content, protein content, sugar contain, fiery debris content, IC50 cancer prevention agent and E complete phenol (Ligarnasari, Anam *et al.* 2018). A study to investigate the optimal percentage of brownies substituted with rice bran dietary fiber (3%, 6%, 9%, and 12%) was carried out. The results indicate that 3% to 6% rice bran dietary fiber is appropriate for production of brownies (Yeom, Kim *et al.* 2016).

In the current study, nutraceutical brownies are prepared where *Magnifera Indica* leaves and gram flour are added to impart medicinal properties. There are several brownie products sold in the market have medicinal properties, but no brownies with mango leaves extract has been reported in the literature earlier. Therefore, the introduction of this first ever prepared brownie will help in improving the health of customers as well as the product cycle of the industry. The pharmacological screening and then market research may further be carried out expending the area of study.

Methodology

Chemicals and Glassware

Analytical grade chemicals were used for analysis. All glassware was pre-rinsed with 10% HCl followed by deionized water.

Raw Materials

All ingredients such as flour, gram flour, sugar and butter were purchased from open market located at Nazimabad and Newtown, Karachi. Branded milk powder (Nestlé Nido), cocoa powder (Rossmoor) and cooking chocolate (Rossmoor) were used. Fresh leaves of *M. Indica* were collected directly from locally grown tree at Orangi Town, Karachi. Identification of the *M. Indica* leaves was confirmed by the Botany Department of the University.

Preparation of extracts

The dirt and other possible impurities were removed by washing the sample with distilled de-ionized water. Drying of *M. Indica* leaves was carried out at room temperature for fifteen days. The dried plant was grinded into powder and10gm of powdered leaves were extracted using water:ethanol (50:50 v/v) as a solvent by soxhlet extraction method (De Castro and Priego-Capote 2010).

Preparation of Brownies

Table 1: Composition of Brownie

S.No	Ingredients	Sample1	Sample2	Sample3	Sample4
1.	Wheat flour	100gm	100gm	100gm	100gm
2.	Gram flour	100gm	100gm	100gm	100gm
3.	Sugar (Sucrose)	180gm	180gm	180gm	180gm
4.	Butter	150gm	150gm	150gm	150gm
5.	Unsweetened cocoa powder	80gm	80gm	80gm	80gm
6.	Unsweetened cooking chocolate	150gm	150gm	150gm	150gm
7.	Eggs	6	6	6	6
8.	Mango leaves powder extract	0gm	54.5gm	109gm	163.5gm
9.	Milk	60gm	60gm	60gm	60gm

5, 10, 15, and 20% aqueous extract of *M. Indica* were incorporated in chocolate brownies to produce four different proportions (Table-1). Other ingredients for all formulations were fixed. Chocolate and butter were melted in a stainless steel pan, and were added to whisked egg yolk. Then cocoa butter, flour and *M. Indica* leaves extract were added to the mixture. Egg white was whisked with sugar and then added to the mixture. The mixture was stirred until a well combined batter was obtained. Thereafter the batter was poured in mould and was baked in an oven at 175^{o}C for 20-30min (Foophow, Phoohinkong *et al.* 2019).

Organoleptic Evaluation

Standard 9-point hedonic scale procedure was used to carry out the sensory (organoleptic) evaluation of the finally produced brownies (Amerine, Pangborn *et al.* 1965). The organoleptic score each parameter such as overall acceptability, colour, texture, flavor was analyzed by 20 trained/and semi trained panelist. Similar sized brownies were presented to the panel with coding, with water to rinse their mouth between samples.

Packaging Material and Storage

The metallic coated polythene wrapper was used to wrap the final product, 50-micron polythene bags were used to pack and were kept for storage at room temperature at 27^{o}C and were refrigerated at 5^{o}C.

Physical and Chemical Analysis

Physical tests of the final product such as moisture, ash, pH, colour, bulk density and chemical analysis such as sucrose, reducing sugar and fat contents, titrable acidity, peroxide value, crude protein, crude fiber and carbohydrates were carried out according to the standard methods of AOAC (Helrich 1990). The texture was analyzed by texture analyzer (CT3 Texture Analyzer, Brookfield AMETEK, MA), and the calorific value was estimated by Bomb Caloriemeter. Antioxidants were analyzed by DPPH method (Amin and Lee 2005).

Microbial Analysis

Standard Plate Count (SPC) method was used to record microbial counts. Petri dishes with Potato Dextrose Agar (PDA) as the cultivation medium were kept for incubation at $37 \pm 5°$ C for 2 days. The colonies were counted by colony counter (Munsch-Alatossava, Rita *et al.* 2007).

Qualitative Phytochemical Screening

Alkaloids were detected by Mayer's reagent (Potassium Mercuric Iodide) and Wagner's reagent (Iodine in Potassium Iodide), glycosides by Borntrager's test, saponins by forth and foam test, triterpine by Salkowski's test, steroids by Libermann Burchard's test, phenol by Ferric chloride test, tenins by gelatin test, resin by acetone water test and flavanoids by alkaline reagent test and lead acetate test as prescribed earlier (Somkuwar and Kamble 2013, Jhaumeer Laulloo, Bhowon *et al.* 2018, Aiyelaagbe and Osamudiamen 2009).

Shelf life of brownies cake

During storage of control and sample with *M. Indica* at room temperature 27^{o}C and refrigerated temperature 5^{o}C, organoleptic analysis and Free Fatty Acid (FFA) was recorded everyday (till the sample is deteriorated) to determine the shelf life of brownies. Standard 9-point hedonic scale procedure was used to carry out the sensory (organoleptic) evaluation (Amerine, Pangborn *et al.* 1965).

Texture Profile Analysis

Texture Profile Analysis was carried out by a method reported earlier (Flores 2007). Brownies were cooled and analyzed after 24 h of baking. Brownies were cut into 3cmx3cmx3cm piece and analyzed using texture analyzer (CT3 Texture Analyzer, Brookfield AMETEK, MA). The probe used was 75 mm compression plate (P/75) and the settings used for this analysis were strain 50% at distance of 10mm with force of 5.0g. The texture parameters that were obtained include hardness, adhesiveness, cohesiveness, springiness and chewiness. The texture parameters of each brownie were averaged from 3 replicates.

Result and Discussion

The presence of various active secondary plant metabolites as revealed by the phytochemical screening (Table 2) supports the resourcefulness of the plant and can justify medicinal properties of mango plant; flavonoids are known to be synthesized by plants in response to microbial infection. Tannins have antibacterial and antiseptic properties whereas triterpenes and steroids have analgesic and anti-inflammatory effects (Duke and Allen 2006).

Table 2: Phytochemical screening of *MangiferaIndica*leaves

Phytochemical substances	Tests	Results
Alkaloids Test	Mayer's	+
	Wagner's	+
Cardiac Glycosides Test	Modified Borntrager's	+++
Saponins Test	Froth test	-
	Foam test	-
Triterpenes Test	Salkowski'sTest	++
Steroids Test	LibermannBurchard's test	-
Resins Test	Acetone-water Test	+++
Phenols Test	Ferric Chloride Test	++
Tannins Test	Gelatin Test	++
Flavonoids Test	Alkaline Reagent Test	++
	Lead acetate Test	++

+++: appreciable amount, ++: moderate amount, +: trace amount, - : absence

5, 10, 15, and 20%*M. Indica*leaves extract were incorporated in chocolate brownies to produce four different compositions. All other ingredientswere kept constant. The brownie samples with 10% *M. Indica* was found to be acceptable according to the sensory panel (Table-3).

Table 3: Standardization of Chocolate Brownies Recipe

Parameters	Sample1	Sample2	Sample3	Sample4
***M. Indica* extract (%)**	**5**	**10**	**15**	**20**
Organoleptic acceptability score	7.10±0.6	7.65±0.5	7.15±0.6	6.9±0.6
Remarks	Not Selected	**Selected**	Not Selected	Not Selected

The sensory score of selected fresh brownies has been summarized in Table-4. The colour of final product was observed to be 8.1 and 7.6, the texture 8.0 and 7.7, flavor 8.1and 7.5 and the taste was8.7 and 8.8for control and sample prepared with *M. Indica*. Both the control and sample with *M. Indica* were found to be acceptable. The better organoleptic scores have made both of the products acceptable.

Table 4: Sensory score of fresh chocolate brownie

Parameters	Control	Sample with MI
Colour	8.1±0.4	7.6±0.5
Texture	8.0±0.4	7.7±0.5
Flavour	8.1±0.4	7.5±0.5
Taste	8.0±0.4	7.8±0.5
Organoleptic acceptability score	8.05±0.4	7.65±0.5

The physical analysis of control and the sample containing *M. Indica* is summarized in Table-5, whereas textural properties of brownies containing 10%, *M. Indica* are presented in Table 6. The colour of control brownie was light brown, extract of *M. Indica* cause the colour of finished product to one tone darker. The colour of brownie might also be influenced by the thermal process and flavonoid content of *M. Indica.* It has been reported in literature that solution containing flavonoid will appear darker i.e. red, orange or yellow when thermally processed. The food product containing sugar appears to be darker when getting caramelized (Priecina and Karlina 2013). The calorific values (152.14cal) indicate that the brownie sample with *M. Indica* contains only 7.6% of the total daily calorie requirement (2,000 calories per day) of an adult, and may not cause weight gain.

Table 5: Physical Characteristics of the Fresh Brownies

Parameters	Control sample	Sample with *M. Indica*
Texture Analysis (N)	12 ± 0.01	11 ± 0.1
Crust Colour	Shiny Light Brown	Light Brown
Crumb Colour	Brown	Dark Brown
Bulk density (gm/ml)	0.378 ± 0.01	0.34 ± 0.01
Calories (cal)	154.6±3.4	152.14±3.5

Mean± SD results are the average of 3 replicas for each analysis

Hardness, adhesiveness, springiness, cohesiveness and chewiness of brownies were significantly affected with incorporation *M. Indica* (Table-6). Additionally, springiness of brownies with 10%, *M. Indica* increased 3.4% from the control brownies. Increased moisture content (Table-7) in brownies after incorporation of *M. Indica* could be the major reason affecting the textural changes. Additionally, higher specific volume (Table 5) in *M. Indica* enriched brownies contributed to lower resilience due to lack of air inside the matrix. Hence, the ability for brownies to return to its original form was significantly lower than control. The fat soluble flavonoids and milk protein might also have interacted to reduce the hardness of the sample with *M. Indica.*

Table 6: Texture profile analysis of brownies

Parameters	Control sample	Sample with *M. Indica*
Hardness (g)	3262±326.0	2536±199.6
Adhesiveness (g.sec)	-1.5±1.3	-5.5±4.0
Springiness	0.56±0.03	0.58±0.02
Cohesiveness	0.35±0.03	0.36±0.02
Chewiness	642.65±60.0	604±58.6

Mean± SD results are the average of 3 replicas for each analysis

The results of chemical analysis of the fresh final product are summarized in Table-7. Although fibre is always associated with increased hardness, the increased moisture content with addition of *M. Indica* may overcome the effect of fibre in *M. Indica* brownies, resulting in reduced hardness with cake-like texture. The fat content is found to be within the range of 38% in both controlled and the sample with *M. Indica.* It is reported to be in the limit which is imparting texture to the brownie and also playing a part in the mouth feel of the product. The titerable acidity (TA) was found to be 2.0% in the control and 2.2% in the final product. The milk protein positively affects the value of titerable acidity.

Peroxide value is found to be less than the detection limit. This indicates that the product will stay stable for a longer time, and will not get rancid or lose its characteristics. Protein contents were observed to be higher in 12.6% in control sample than 12.4% in sample with *M. Indica*. Milk proteins are functional compounds therefore; they can interact with fat-soluble materials and reduce the loss of their functionality during high temperature and oxidation process.

Antioxidant activity as IC50 was observed to be higher in 128.3ppm in control sample than 92.23ppm in sample with *M. Indica. B*etacarotine, anthocyanin, tocopherl and phenol present in *M. Indica* leaves affect the amount of antioxidant IC50. Theobromine present in dark chocolate added to the brownies also influences the antioxidant activity. It has been reported in the literature that the total phenolic content is an indicator to determine the hydrophilic activity of antioxidants. The more the phenols present in the product, the lower IC50 antioxidant activity will be (Priecina and Karlina 2013). The lower IC50 values show better antioxidant activity of the brownie produced in the current study.

Moisture content of control sample was found lesser (4.8%) than the sample with *M. Indica* (5.6%). Due to difference in water activity migration of moisture in both of the products is observed. Higher the water activity; more rapid the migration of moisture can be observed.

Table 7: Chemical Analysis of the Fresh Brownies

Parameters	**Controlled sample**	**Sample with *M. Indica***
Fat Content (%)	37.6 ± 0.05	37.8 ± 0.05
Titrable Acidity (%)	2.0%± 0.002	2.2%±0.001
Peroxide Value	< the Detection Limit	< the Detection Limit
Protein (%)	12.6 ± 0.1	12.4 ± 0.04.
Fiber (%)	10.8 ± 0.1	11.2 ± 0.1
Carbohydrate (%)	16.76 ± 0.05	15.65 ± 0.04
Antioxidants IC50 (ppm)	128.3 ± 1.0	92.23 ± 0.9
Moisture Content (%)	4.8±0.1	5.6±0.1
Ash (%)	1.2±0.01	1.44±0.01
Microbial Analysis		
Standard Plate Count (CFU/ml)	$3.2x10^7$	$2.8x10^7$

Mean± SD results are the average of 3 replicas for each analysis

The ash content in the control sample is found to be lesser (1.2%) than the ash content in the sample with *M. Indica.* It is mainly because *M. Indica* extract is

obtained from a plant source which could be the reason of higher value in the later product. The ash content in both of the samples was found to be in acceptable limits, making both of the chocolate brownies acceptable.

Plate count value was found to be within the limit i.e. $3.2x10^7$CFU/ml for control and $2.8x10^7$CFU/ml for the sample with *M. Indica.* Confections are generally resistant to bacterial growth, but pathogens (if present) survival in the product for a long time is possible.

The shelf life of brownies determination is shown in Table-8. Organoleptic score of brownies stored at various temperatures are altered within 25 days of storage time. After 6 days of storage at room temperature and at 5°C mold were grown over the control samples of brownies and after 8 days on samples with *M. Indica.* Aroma, colour and texture changes occur due to oxidation reaction, causing damage to food characteristics. Damage in food quality is also caused by the large numbers of microbe grown over the brownies and by poor physical handling. Product degradation is accelerated at extreme temperature; the testing temperature of shelf life. The shelf life is determined by temperature and distribution condition (Dewi 2010).

The shelf life of chocolate brownies can be extended by using carefully selected specific packaging material and process. The packaging material is able to extend the shelf life of product by manifesting the flavor and texture changes. The knowledge of shelf life is the most important aspect in new food product development. Proper application of chemical kinetic principles to food quality loss allows for efficiently designing appropriate shelf-life tests and maximizing the useful information that can be obtained from the resulting data.

Table 8: Shelf life determination of chocolate brownie

Parameters	**Control**	**Control**	**Sample**	**Sample**
Temperature (°C)	**5**	**27**	**5**	**27**
Shelf life (Days)	15	6	25	8

Free Fatty Acid (FFA) was another parameter used to determine the rate of decline in the quality of brownies. FFA shows the amount of free fatty acids in a product. Fig-1 shows that the FFA value of brownies increases with the length of storage time at all two storage temperatures. The increase in FFA percentage is due to oxidation of the product. The oxidation reaction of brownies is caused by the contact of oxygen with fat containing in the brownies.

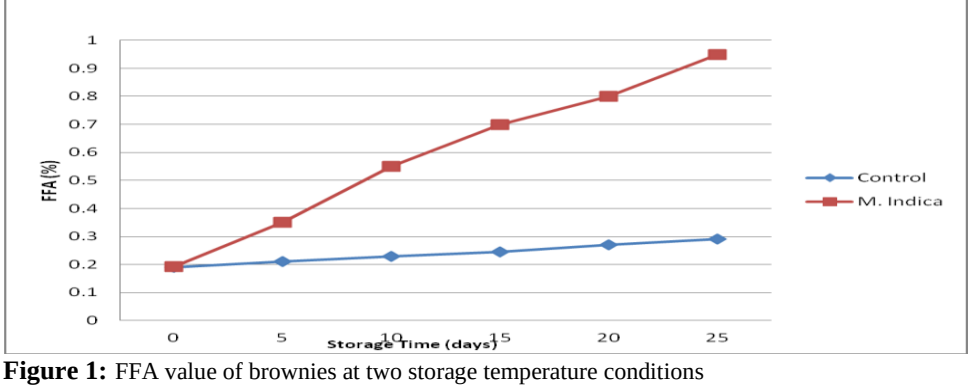

Figure 1: FFA value of brownies at two storage temperature conditions

The constituents of the product produced in the current study are natural, no artificial colour and flavour is added to prepare the brownies. The cost of toffee was calculated as per existing prices at the time of the study. It also has the market competent price therefore the product is likely to gain the attraction of consumer and improves the market trends.

Conclusion

Chocolate brownies were prepared by the incorporation of 5%, 10%, 15% and 20% aqueous extract of *Magnifera Indica* leaves with partial substitution (50%) of wheat flour with gram flour. The finished products were evaluated for shelf life determination, antioxidant, organoleptic and physico-chemical characteristics. The brownie samples with 10% *M. Indica* was found to be acceptable according to the sensory panel. Both of the control and brownie samples with *M. Indica* have shown better organoleptic scores, making them acceptable. The moisture(%), ash(%), fat(%), titrable acidity(%), proteins(%), carbohydrates(%), fiber(%) and antioxidant activity as DPPH inhibition (IC50 ppm) was found to be 5.6 ± 0.1, 1.44 ± 0.01, 37.8 ± 0.05, 2.2 ± 0.001, 12.4 ± 0.04, 15.65 ± 0.04, 11.2 ± 0.1 and 92.23 ± 0.9 respectively. The calorific values indicate that the brownie sample with *M. Indica* contains only 7.6% of the total daily calorie requirement of an adult, and may not cause weight gain. The shelf life investigated at ambient and refrigerated temperatures, of control were found to be 6 and 15 days and brownie samples with 10% *M. Indica* 8 and 25 days respectively. The research concludes that chocolate brownie with 10% *M. Indica* extract was produced with the antioxidant, anticancer, anti-inflammatory, anti-diabetic and other medicinal properties.

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