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Trials for replace industrial phosphate salts with milk calcium and guar gum powders as a natural product to improve the quality of minced beef

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Abstract
There are many consumer requirements in processed foods which include minced form with priority to the product safety and quality. The accepted texture for the consumers should be not either excessive soft or very firm. Also, cooking loss which be accompanied by loss of extractives and shrinkage of the product are also interested by consumers. Guar gum (GG) can lead to improve texture of the final product, increase production yield and prolong the product shelf life. In this research, addition of either 0.5% GG or 0.3% Di-sodium phosphate (Na_2HPO_4)to the beef minced meat resulted in retain high amount of moisture content (64.09±0.05 & 64.32±0.07), high pH (6.07±0.25 & 6.00±0.20), Aerobic plate count (APC) and mold and yeast count (log_{10}cfu/g±SD) were less by using GG 0.5% (4.50±0.02and3.61±0.01) as compared with control(4.91±0.01and4.12±0.11) and with Na_2HPO_4 recorded (4.70±.0.02 and 3.70±0.65),respectively. Also, there were no significance differences (P>0.05) between Milk calcium (MC) (0.3 & 0.5%) and GG (0.3%) as they revealed moisture content (63.44±0.30,63.55±0.04and 63.50±0.05), pH values of (5.75±0.05, 5.85±0.05 and 5.90±0.20), APC (4.49±0.04,4.48±0.04 and 4.54±0.02), respectively. Cooking loss was less by using 0.5% of GG & 0.3% Na_2HPO_4 (70g / 28% for both) followed by 0.5% MC (71g / 28.4%), then 0.3% each of MC & GG (72g / 28.8% for both). The obtained results proved that 0.5% of either GG or MC could be used as a nature preservatives replacers for the synthetic Na_2HPO_4 which have the same properties either as antimicrobials or protect the product quality as they retain high amount of moisture resulted in decreasing of cooking loss and subsequently increase the product palatability and protect the consumer health from the side effect of synthetic or chemical preservatives which will be discussed.

Keywords: *Milk calcium (MC), Guar gum (GG), Di-Sodium phosphate (Na_2HPO_4), APC, Mold count, Cooking loss, Moisture %, pH.*

Introduction

Color, odor, texture and overall acceptability of meat products are important factors in consumer choice and ranks the first prerequisite among consumer requirements and satisfaction. Furthermore, the frying yield and size reduction of processed meat products are important factors for manufacturers, they arise as a result of the degradation of meat protein. Also, they affect the economic purpose and the packaging which also looks uncomfortable along with the product size. Gelatinization and high-water holding capacity of gums can overcome such processing problems. Otherwise, GG has highwater-binding ability which is considered the major functional property of food product that positively affect the texture of meat products (**Ulu, 2006 and Kilincceker *et al.*, 2009).**

Guar gum considered a versatile polymer for the food industry. It is also considered a polygalactomannan derived from the endosperm of the legume plant seeds *Cyamopsis* tetragonolobus . It provides extremely high viscosities even at a very low concentrations (≤1% w/v) in aqueous solutions. Due to these properties, it is widely used as thickening agent, foam stabilizer and emulsifier in several food industries. Furthermore, it has a prebiotic effect results in the lowering of blood glucose and blood cholesterol levels. Guar gum is also used in preparation of low glycemic food products due to its high content of dietary fibers. Guar gum or guar bean is a natural polysaccharide with a high molecular weight that named E412 in European additives which is used in a wide range of applications in food, medical, pharmaceutical, textile, paper, agriculture, cosmetics and bioremediation (**Patel *et al.,* 2014 and Gupta and Variyar, 2018).**

Guar gum has its unique property of collecting a large quantity of water, resulting in high viscosity which is responsible for its adhesion to the hydrophilic surfaces. Guar gum products showed a pronounced temperature effect when the solutions are heated. This is caused by loss of water of hydration around the polymer molecule which makes the GG most applicable as natural polymer (**Reddy and Tammishetti, 2006**). Because of these properties, GG is used in a large number of industries including food processing **(Prabhanjan *et al.,* 1989).**

Guar gum is used to deliver drug to colon due to its drug release retarding property and susceptibility to microbial degradation in the large intestine, its gelling property retards the drug release as well as its susceptibility for degradation in the colonic environment (**Macfarlane *et al.,* 1990).**

The guar plant measured about 0.6m high and pods of 5-12.5cm long, it contains an average 5 -6 light brown seeds. Guar gum found to be insoluble in fats, hydrocarbons, alcohols, esters and ketones. Water is the only important solvent for GG.

Minced meat usually has a shorter refrigerated storage life when compared to meat that had not been minced, and particularly affected by microbiological changes. Generally, bacterial and mold spoilage of meat are of high growth rate during refrigeration storage (**Jones, 2004).**

The growth of yeasts and molds within food products is always seen on outer products surface, during their growth, they metabolize some food components and produce metabolic end products, this causes the physical, chemical, and sensible change of food leading to food spoilage. Such food intoxication when consumed will results in a group of diseases ranging from mild to severe lung infections, or even whole-body infections (**Fleet and Praphailong, 2001; Karabagias, 2018 and Pronadisa, 2018**).

Phosphates are widely used as food additives as they support the processing properties and functional characteristics of meat, poultry and their products. They are used in order to improve the water holding capacity through elevation the products pH, antimicrobial, inhibit lipid oxidation, reduce cooking loss, maintain meat products color stability and keeping its textural properties (**Xiong, 2005; Long *et al.*, 2011 and Sebranek, 2015**). Furthermore, phosphates in combination with salts can improve protein functionality and subsequently, increase the ionic strength, resulted in reducing product weight loss during cooking (**McKee and Alvarado, 2004; Long *et al.*, 2011; Petracci *et al.,* 2013; Sebranek, 2015 and Sindelar, 2015).** Despite the multifunctional benefits, phosphates takes its way to the end of its use in the incoming decade because of poor consumer perception associated with health risks (**Petracci *et al.*, 2013; Watanabe *et al.,* 2016** and **Kim *et al.*, 2017;**). Therefore, recent years has observed an increase in efforts to find phosphate substitutes from natural sources (**Ruusunen *et al.*, 2003; Jarvis *et al.,* 2012; Casco *et al.*, 2013 and Cho *et al.*, 2017).**

Natural calcium powders, which are widely used and distributed in the food industry, include oyster shell calcium (OSC), egg shell calcium (ESC), marine algae calcium (MAC), and milk calcium (MC). Each of these natural calcium powders has their own unique physico-chemical properties and sensory characteristics, as they differ in their basic sources from raw materials and manufacturing methods. They are used with successful replacement of synthetical chemical preservatives (**Bae *et al.,* 2017**). Therefore, this paper sheds light on the ability of GG and MC as natural preservatives with concentrations of 0.3% and 0.5% to be safe replacers to Di-sodium phosphates (Na_2PHO_4).

Material and Methods

The preservatives used in the experiment.

Milk Calcium (MC) AR, AVI-CHEM LAB., India, CAS: 7440-70-2

FW: 40.08, Min. Assay (99.5%).

Guar gum (GG) AR., AVI CHEM. LAB., India, CAS: 9000-30-0.

Dibasic Anhydrous Purified Sodium Phosphate (**Na_2HPO_4**), India.www.Labachemia.com

All preservatives used in this study were of analytical grade and the doses of the preservatives used in the present study (0.3% and 0.5%) were recommended through several investigators who have used the same concentrations or through international references that have approved the use of such concentrations **(SCF, 2001 & 2003, Yoko Kawamura, 2008 andUS-FDA, 2019)**

Preparation a solution of preservatives according to Srichamroen (2007)

Aqueous solutions (0.3% and 0.5%) were prepared on a weight-to-volume (w/v) basis using clean distilled water in a boiling water bath for 30 min, with gentle stirring to ensure homogeneity. The solutions were cooled and held at 4°C for 2 h, with gentle stirring to ensure homogeneity.

Sample preparation

One sample weighted one and half kg of fresh beef meat was purchased from butcher shop in Cairo, transferred under strict hygienic measures to the laboratory as soon as possible, minced with addition of ice water and 1.5% of NaCl. Minced meat sample was divided into six sub-samples (250 g for each) and treated as follows:

The 1st sample considered control without any treatment,

The 2nd sample was treated with addition of 0.3% Na_2HPO_4,

The 3rd and 4th samples were treated with 0.3% and 0.5% of MC powder, respectively and

The 5th and 6th samples were treated with 0.3% and 0.5% of GG powder, respectively. The samples then subjected to the following examinations as well as the experiment was repeated in triplicate to carryout statistical analysis.

Chemical analysis

Determination of moisture content: -

Moisture contents of raw minced meat samples were determined by hot air oven method at 105±2°C according to **AOAC (2002).**

Determination of pH: -

Ten grams of raw minced meat were homogenized and mixed thoroughly with 100 mL of distilled water for measuring of pH using a digital pH meter (Suntex TS-1, Taiwan) equipped with a probe-type combined electrode (Ingold) through direct immersion of electrode into the mixture at room temperature according to the method recommended by **AOAC (2002).**

Bacteriological examination

Preparation of sample homogenate (APHA, 2001):

Twenty-five grams of the examined samples were aseptically transferred to a sterile stomacher bag and homogenized with 225 ml sterile buffered peptone water (0.1%) for 30-60 seconds to give an initial dilution of 1/10.One ml of the initial dilution was transferred by means of sterile pipette to another sterile tube containing 9 ml of sterile buffered peptone water (0.1%) then mixed thoroughly by using vortex for 5-10 seconds to obtain the next dilution (1:100). Repeat this operation to obtain further decimal serial dilutions up to 10^6.

Aerobic plate count according to APHA-(2001):

From the previously prepared decimal serial dilutions, 0.1 ml of each dilution was inoculated by means of surface spread method onto the surface of duplicate plates of Standard Plate Count Agar(Oxoid,CM0463)and incubated at 35°C for 48±2h.,count plates with 25-250 colonies and recorded as $\log_{10}$cfu/g sample

Enumeration of mold and yeast according to ISO 21527/1(2008) ;

About 0.2mL.from each dilution was transferred to DG18 dechlorane rose Bengal agar plates, distributed by sterile glass spreader.

Plates were incubated at 25°C±1°C for 5 to 7 days.Counts were recorded as $\log_{10}$cfu/g sample.

Determination of cooking loss according to (Bae *et al.*, 2017)

Weight of each sample prior to cooking and then again after cooking and cooling to calculate the cooking loss according toas the following calculation:

Cooking loss (%) =

$$\frac{\text{Weight before cooking} - \text{Weight after cooking}}{\text{Weight before cooking}} \times 100$$

Statistical analysis

Statistical analyses were run in triplicate and results were reported as mean values and standard deviation (Mean±SD). using of Statistical Packaging for the Social Science (SPSS) Ver. 20. A p-value less than 0.05 ($p \leq 0.05$) was considered statistically significant.

Results and Discussion

Guar gum (GG) or MC able to be used as a natural preservatives replacers for the synthetic Na_2HPO_4 in meat products as minced meat which have the same antimicrobial characteristics as well as protect the product quality as they retain high amount of moisture content and a subsequently, resulted in decreasing of cooking loss, increase the product palatability and protect the consumer health from the side effect of synthetic or chemical preservatives.

As regards to chemical analysis, the obtained data in **Table (1)** concerning that mean±SD of pH values showed significance difference ($P<0.05$) between 0.5% GG (6.07±0.25) and each of control samples (5.70±0.20) and 0.3% MC (5.75±0.05).While, no significance differences ($P>0.05$) of recorded pH between all other treatments. By analyzing the data in Table (1), it was clear that 0.5% GG was the pest preservative which have the nearly same characteristic pH as 0.3% Na_2HPO_4 (6.00±0.20), followed by 0.3% GG (5.90±0.20) then milk calcium 0.5% (5.85±0.05). The alkaline nature of phosphate used as a preservative in meat products is a means to improve water retention by elevating the pH and ionic strength of meat mixtures **(Sebranek, 2009 and Choi *et al.*, 2014).** Thus, the pH is an important factor that determines the best replacer of synthetic phosphate in meat products depending on the ability of these alternatives to retain water and subsequently increase the product water holding capacity thus improving the product yield as shown in **Fig. (1)** in which the data cleared that when the pH increased, the moisture content also increased. The same results were obtained in the present study by using 0.5% GG as phosphate alternative and it succeeded to elevate the pH of the product to6.07and the moisture content reached to64.09%.The maximum permissible level of phosphates in meat and poultry products was 0.5% as determined by **USDA-FSIS (2015),** while it is used by 0.3-0.4% in meat product industry **(Sebranek, 2009).** Furthermore, the results agreed with **Jeong (2016)** who stated that there was no significance difference ($P>0.05$) between pH of control samples and samples treated with MC

Table (1). Chemical and microbial finding of control and treated minced meat samples by using different preservatives

Treatments		Chemical finding		Microbial finding	
Preservatives	Concentration	pH	Moisture	APC	Mold & Yeast count
Control	------	5.70^{A}±0.20	64.49^{A}±0.04	4.91^{A}±0.01	4.12^{A}±0.11
Di sod.Phosphate (Na_2HPO_4)	0.3%	6.00±0.20	64.32^{B}±0.07	4.70^{aB}±.0.02	3.70±0.65
Milk calcium (MC)	0.3%	5.75^{B}±0.05	63.44^{abC}±0.30	4.49^{abE}±0.04	3.50^{a}±0.20
Milk calcium (MC)	0.5%	5.85±0.05	63.55^{abD}±0.04	4.48^{abcD}±0.04	3.48^{a}±0.01
Guar gum (GG)	0.3%	5.90±0.20	63.50^{abE}±0.05	4.54^{abd}±0.02	3.76±0.02
Guar gum (GG)	0.5%	6.07^{ab}±0.25	64.09^{abcde}±0.05	4.50^{ab}±0.02	3.61^{a}±0.01

The mean difference was significant at the 0.05 level between small and capital superscripted letters within the same column.

The results of moisture contents (Mean±SD) assured that the samples treated with GG (0.5%) had a high significant value (P<0.05) of moisture content (64.09±0.05), which considered as indication of the water holding capacity, even when compared with GG 0.3% (63.50±0.05) (**Table 1 and Fig. 1**). In the same subject, the higher moisture content was recorded in treated samples with Na_2Hpo_4 (64.32±0.07), while there was no significance difference (P>0.05) in moisture content between treated samples with both MC, 0.3% and 0.5% as they recorded nearly the same moisture content (63.44±0.30 and 63.55±0.04), respectively. The obtained results in the current research were complied with **Bae *et al.* (2017)** as they reported that samples treated with MC showed the lowest moisture content (66.17%) as compared with other treated samples (P<0.05) and subsequently has lower water retention power than inorganic phosphate. The variation in moisture content of meat products may be due to the type and amount of ingredients of preservatives used, as well as the type of meat products (**Cofrades *et al.*, 2008; Lee *et al.*, 2011 and Casco *et al.*, 2013**).

The obtained data in Table (1) also proved that both MC (0.5%), MC (0.3%) and GG (0.5%), were the pest preservatives as they achieved the lowest APC levels (4.48±0.04, 4.49±0.04 and 4.50±0.02 log_{10}cfu/g), followed by 0.3% GG (4.54±0.02log_{10}cfu/g) and finally 0.3% disodium phosphate (4.70±.0.02 log_{10}cfu/g). All treatments using MC and GG were better than Na_2HPO_4 as they were able to reduce APC significantly as compared with control one.

Moreover, the results in Table (1) indicated that MC of both 0.5% and 0.3% followed by GG 0.5% had a significant reduction of mold and yeast counts (3.48±0.01, 3.50±0.20 and 3.61±0.01 log_{10}cfu/g) as compared with other treatments and also the control one, while treated samples with Na_2HPO_4were recorded 3.97±0.65 log_{10}cfu/g of mold and yeast count. The obtained results agreed with **Suarez *et al.* (2005)** who concluded that Na_2HPO_4 had a little inhibitory activity and had no significant effect (P>0.05) on molds isolated from foods. Moreover, GG was recommended by the microbiological specifications in the **EC regulation No. 1333/2008**, European Food safety Authority (**EFSA ANS Panel, 2012**) and **EU Regulation No. 257/2010** [mentioned into the EU specifications of guar gum (E 412)] as polysaccharidic thickening agents that help in clearance of *Salmonella* spp. and *E. coli* and reduce total aerobic microbial count (APC) as well as for total combined yeasts and molds count (TYMC) from food products. In this regard, **Hamdani *et al.* (2017)** mentioned that GG showed strong antibacterial activity against gram negative bacteria.

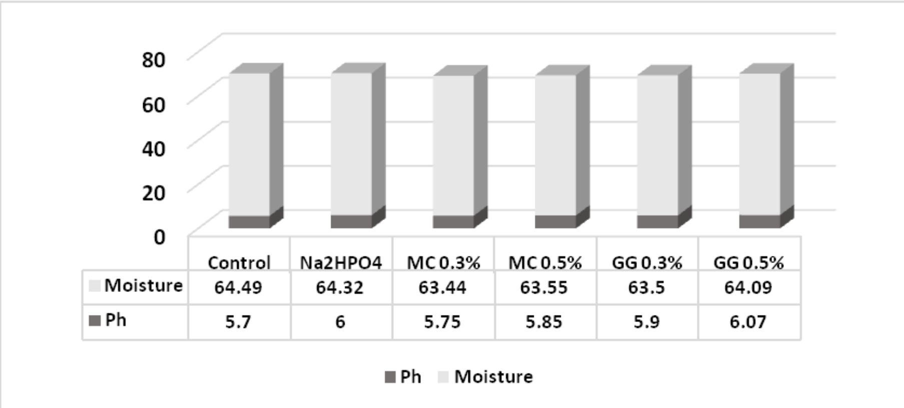

Fig. (1): Correlation between moisture content and pH of minced meat using different preservatives

Guar gum hydrolysate (GGH) increases glucose intolerance and lower the level of hypertriglyceridemia **Suzuki and Hara (2004)**. Furthermore**,** GG containing methotrexate for treatment of colon cancer (**Chuurasia *et al.*, 2004**). Also**,** GG and its derivatives used in treatment of various diseases as cholera, constipation and diarrhea. Moreover, GG and its solution also used in eye-drop formulations (**Lampe *et al.,* 1992).**

The obtained results in the currents study were also compatible with **Park *et al.* (2008)**, **Lee *et al.* (2011)** and **Jarvis *et al*. (2012 & 2015)** as they reported that GG and alginic acid were good and suitable replacers for inorganic phosphate in meat products. Furthermore, **Choi *et al.* (2014)** added that a combination of calcium and whey protein found to have a high-quality parameter and consequently can be used as Na_2HPO_4replacers.

Table (2) and Fig. (2) revealed non significance difference (P>0.05) between mean pH value (6.25±0.10) of 0.3% Na_2HPO_4 and 0.5% Guar gum(6.35±0.05). In turn, less cooking loss was recorded in both treatments (70g / 28%) as it considered the least value recorded among the preservatives used in this study, followed by 0.5% MC (6.20±0.05) and 28.4% cooking loss, then 0.3% of both MC and GG were recorded (6.10±0.01and 6.14±0.03) and cooking loss was 28.8% for both, with absence of significance difference (P>0.05). While, all preservatives were recorded significance difference (P<0.05) as compared with control samples. The correlation between pH and cooking loss proved that more increase in pH resulted in less cooking loss. Also, the obtained data proved that phosphates can be successfully replaced by using either GG (0.5%) or MC (0.5%). Furthermore, there were a significance difference (P<0.05) between cooking loss of control samples and all other preservatives, meaning that all preservatives used had the same level of water retention.

Table (2). Cooking loss and pH (mean log_{10}cfu/g ± SD) of tested samples with different preservatives (250 g each)

Treatments		**Meat characteristics**		
		pH	**Cooking loss**	
Preservative	**Concentration**		**Amount (g)**	**%**
Control (1)	**------**	**5.80^{A}±0.20**	**78^{A}±1**	**31.2**
Na_2HPO_4	**0.3%**	**6.25^{a}±0.10**	**70^{a}±1**	**28.0**
Milk calcium	**0.3%**	**6.10^{aB}±0.01**	**72^{a}±2**	**28.8**
Milk calcium	**0.5%**	**6.20^{a}±0.05**	**71^{a}±1**	**28.4**
Guar gum	**0.3%**	**6.14^{aC}±0.03**	**72^{a}±1.5**	**28.8**
Guar gum	**0.5%**	**6.35^{abc}±0.05**	**70^{a}±1.5**	**28.0**

Control (1): minced meat without preservatives

The obtained results agreed with those reported by **Chubzikowski (1971) and Wang and Murphy (2003)** as they stated that GG has the ability to produce highly viscous solutions even at low concentrations due to (i) its high molecular weight (up to 200,000 to 300,000 Daltons) and (ii) the presence of the extended repeating unit formed by hydrogen bonding. This feature allows guar gum to be soluble and gel forming even in cold water as well as retain moisture content and decrease cooking loss**.** In this respect**, Demirci *et al.* (2014)** concluded that the moisture content increased in cooked meatballs formulated with GG. This because GG form a strong template that prevent the migration of water from cooked or fried foods to the surrounding media**.** These results conformed with that of the present study.

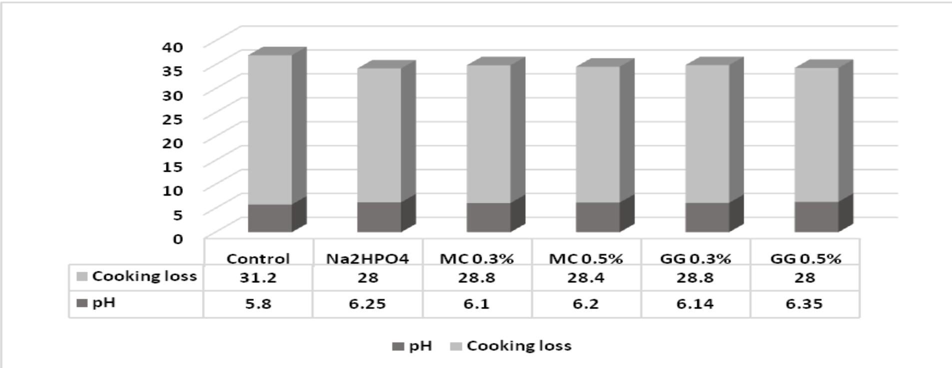

Fig. (2): Correlation between cooking loss and pH of minced meat using different preservatives

Kilincceker and Yilmaz (2016) found that GG in a concentration of 0.5, 1 and 1.5% showed better effect through increased both of frying yield and moisture retention**.** These results comply with that obtained in the present study meaning with increasing the concentration of GG (0.5% as compared with 0.3%), the quality properties of minced beef concerning cocking loss**,** shrinkage and moisture content were improved**.** Also agreed with **Ibrahim *et al.* (2011)** and **Tabarestani and Tehrani (2014)** who concluded that GG as a hydrocolloidal substance is useful for increasing the binding properties of minced beef as well as decreases the water activity of meat products. Thus, enhancing the desirable structure, texture and avoid moisture loss during cooking. In the same subject, various investigators **(Caprioli *et al.*, 2009; Özen *et al.*, 2011** and **Lopes *et al.*, 2015**) had discussed the effect of addition of different gums at various concentrations in meat products which assisted in enhancement of its quality during manufacturing and helped in reducing the level of lipid oxidation. Moreover**, Srichamroen (2007)** stated that Up to 0.5% concentration of GG solution was giving a higher significant viscosity at low temperature. In addition, it was stable over a wide pH range of about 1.0–10.5. This is due to its nonionic and uncharged behavior. Final viscosity of GG is not affected by the pH, but the hydration rate showed variation with any change in pH **(Mudgil *et al.*, 2014).** In food industry, GG is used as a novel food additive in various food products for food stabilization and as fiber source **(Morris, 2010).** It is liked by both manufacturer and consumer because it is economic as well as a natural additive. It is used in variety of foods because it changes the behavior of water present as a common component in various foods. Guar's "generally recognized as safe" (GRAS) status. In this regard**, Long *et al.* (2011)** and **Sindelar (2015)** found that 0.5% MC showed the highest cooking loss (p<0.05) in examined minced meat samples.

Conclusion and Recommendation

From the previous data, it could be concluded that all the nature preservatives used in the current study has the ability to be substitutes for synthetic Na_2HPO_4, in terms of their safety and quality characters including their ability to retain water and subsequently, minimize cooking loss by increasing the pH of the product. But the preference among these additives to GG as it is characterized by many other features represented by:

- It has a strong hydrogen bond forming tendency in water which makes it a novel thickener and stabilizer.
- It is characterized by its popularity in the industry because its low cost and economical . In food industry, it has wide applications in ice cream, sauce, beverages, bakery and meat industry. It is also used in food products for supplementation as dietary fiber. Its consumption reduces the risk of heart diseases by reducing the cholesterol level in body, control diabetes and maintains the bowel movement in human beings.
- These features makes GG used in wide applications in the industries like food, pharmaceutical, textile, oil, paint, paper and cosmetics.

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