# STUDIES ON FORMULATION AND PROCESSING ASPECTS OF BREAD USING NATURAL SOURCES

# THESIS SUBMITTED FOR PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF

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# THIS PROJECT IS DEDICATED TO MY BELOVED PARENTS AND MY SENIOR MS.DEBASMITA PATHAK

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## DECLARATION OF ORIGINALITY AND COMPLIANCE OF ACADEMIC ETHICS

I hereby declare that thesis contains literature survey and original research work by the undersigned candidate, as a part of Master of Technology in Food Technology and Biochemical Engineering.

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I also declare that, as required by these rules and conduct, I have fully cited and referenced all materials and results that are not original to this work.

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#### CERTIFICATE OF RECOMMENDATION

This is to certify that Payel Dasgupta (Class Roll No: 001410902001, Registration No: 129079) has carried out the research work entitled "Studies on formulation and processing aspects of bread using natural sources" under my supervision, at the Department of Food Technology and Biochemical Engineering, Jadavpur University, Kolkata. I am satisfied that she has carried out this work independently with proper care and confidence. I hereby recommend that this dissertation be accepted in partial fulfillment of the requirement for awarding the degree of **Master of Technology in Food technology and Biochemical Engineering**.

We are very much pleased to forward this thesis for evaluation.

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#### CERTIFICATE OF APPROVAL

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#### ABSTRACT

Raw and ripe Mango peel is now of great importance and is seeking attention by the food sectors to a great extent due to its high phenolic content and antioxidant properties. High dietary fibre and other bioactive compounds like Vitamin E ,Vitamin C and carotenoids also adds nutritive value to the product incorporated with Mango peel powder (MPP). Ripe mango peel was incorporated and optimized at varied levels in wheat bread to figure out the level of fortification under a certain baking condition which was well suited for the physicochemical parameters. The combined effect of raw and ripe mango peel powder (MPP) in equal proportion at different levels in the Gluten free GF) flour formulations were studied. The physical and functional properties of GF flour samples like moisture content, color, water absorption capacity (WAC), oil absorption (OAC) and swelling power (SP) were also studied to correlate well with the final bread properties. The physicochemical and sensory parameters were analyzed which showed a positive impact upon MPP addition to a certain level. The 4% MPP bread showed the best results of the analyzed parameters followed by 2% MPP bread sample. Increasing levels of MPP at 6% showed an inferior bread quality with poor crumb color, appearance and specific volume. The rheological characteristics of the MPP enriched gluten free dough samples were performed and were compared with the wheat dough. The main objective of this study was to observe the effects of MPP on the dough rheology of gluten free samples and its impact on the viscoelasticity. The 2% and 4% MPP dough exhibited viscoelastic properties in a similar trend to wheat dough. Conversely 6% MPP showed the poorest consistency above all. To characterize the wheat breads and gluten free breads enriched with MPP at different levels principal component analysis (PCA) was performed. PCA was done to differentiate the quality of the above mentioned bread samples based on their physical and sensory parameters. The color parameters and sensory parameters found in PCA plot were found to be well enough to explain and segregate the bread samples from one another. Specific volume the most desirable parameter of the bread showed a positive impact on the wheat bread enriched with ripe MPP. A similar impact was shown on the specific volume of the wheat bread samples enriched with both raw and ripe MPP in equal proportions upto level of 4%. On the other hand Gluten free formulated breads enriched with the combination of raw and ripe MPP in equal proportion at varied level exhibited a similar viscoelastic property as that of wheat sample, as well as a positive impact was observed for physicochemical and sensory properties which is close to that of wheat sample at 4% level.

At 6% level both the wheat and gluten free breads showed an inferior quality and poor texture and appearance with darker crumb color and sensory results recorded were unacceptable. Thus the combination of the raw and ripe mango peel powder in equal proportions to the wheat and gluten free bread samples was accomplished successfully.

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# CHAPTER 1:

#### **INTRODUCTION AND REVIEW**

#### **1. 1 GENERAL INTRODUCTION**

Bakery products are an important part of food expenditure. Consumption of bakery products have been falling since the end of world war two in some industrialized countries such as the USA, Canada, the UK and Australia. According to (Hunt and Robbins et al, 2009) bakery products accounted for 9 per cent of the average weekly food consumption. Anon, 2000 estimated the consumption of bread in the UK was still 41.5 kg per person in 1990. Baur, 2001 estimated the western European bread market to be 23.000 million French francs. For several thousands of years, man has used wheat and other cereals to produce bread with an average consumption of a boat 65 kg of bread per capita per year in Europe, it remains and important constituent of a balanced healthy diet. Microbiological spoilage is often the major factors limiting the shelf life of bakery products. Spoilage from microbial growth causes economic loss for both manufacturers and consumer. These losses could be due to many individual cases such as, packaging, sanitary practice in manufacturing, storage conditions and product turnover. (Rachel Needham et al., 2004) convenience, taste and freshness determine to a great extent the appeal of bread products and are expected to remain the driving factors for purchases of bakery products.

India's bakery and cereal market is worth \$1 billion, the 3rd largest in Asia pacific after Japan and Australia. But still per capita per year consumption of bakery product in India is roughly 1 to 2 kg as compared to 10 to 50 kg in advanced countries. The gap is thus wide. There is ample scope for increasing production of bakery products. In our country, out of the 85,000 bakery unit 75,000 operates in the unorganized small-scale sector occupying 65 per cent of the market share (Shraddha et al., 2008). The organised sector in bread industry consists of around 1800 smallscale bread manufacturers, 25 medium scale manufacturers and two large-scale industries. Biscuits and bread which are considered to be the major bakery product and they account for 82 per cent of all bakery productions. The unorganized sector accounts for about half of the total biscuit production estimated at 1.5 million tonnes. It also accounts for 85 per cent of the total bread production and around 90 per cent of the other bakery products estimated at 0.6 million tonnes. The last includes pastries, cakes, buns, rusks and others as suggested by (Saranraj and Geetha, 2012). India belongs to the fastest growing economies in the world. In 2013, India ranked 11th on the list of the largest economies in the world according to the GDP in US dollars. According to the Ministry of Food Processing Industries MOFPI, roughly 35,000 food processing and beverage production companies (2011) are registered in India. Around 50 percent of these are processing units for grain, crops and rice, including mills. The other half is dominated by the dairy sector: 1,493 units are active in the dairy industry; second largest sector is the bakery industry: 1,450 companies produce baked goods. There are 1,200 units active in the beverage sector, around 500 companies are in the cocoa and confectionery business and around 100 companies are in the meat sector.

Bakery products according to Euromonitor, the second strongest segment (after dairy products) within the packaged food sector are baked goods: In 2013, the sales volume of baked goods rose to 4.2 million tons. The per capita consumption at 3.3 kg is, however, very low. An increase in consumption is predicted to happen by 2018.( Source: Euromonitor International 2014, VDMA).

In 1,000 tons	2008	2013	2018	Growth rate 2013-2018
Bakery products	3,251	4,220	5,445	29 %

#### **1.1.1 Different Types of Bakery Products:**

The bakery industry today in India plays an important role within the industrial map of this country. Bakery products are things of mass consumption because of its low pricing and high nutritional value. With very fast growth and moving eating habits of consumers, bakery products have raised in popularity amongst the mass population. This industry, typically, consists of cakes, breads, and biscuits. The bakery industry has been able to gain the third place in creating revenue within the processed food sector. The market size of the overall industry is calculated at US\$ 4.7 bn in 2010 and is predicted to go up to US\$ 7.6 bn in 2015. The highest performing member of the industry still is the biscuit sector, which is expected to over perform the growth of the entire industry.

The bakery industry is the largest among the processed food industries in India with significant growth of 40 per cent. Bread and biscuits are the two major bakery industries account for about 82 per cent of the total bakery products. Bread is the cheapest and ready to eat convenience food taken by large section of population of urban as well as rural. Bread is generally a highly perishable item which has a shelf life of 72 hours in a tropical country like India.

From the production point, these are transported to various cities nearby and even to other states. The time gap between production and actual consumption for bread is estimated to be 18.36 hour or more. By that time the product may have lost its freshness due to staling which is the result of the changes other than those resulting from action of a spoilage organism namely spores of rope mold. These lead to the loss of palatability.

The important changes due to staling are increase in the firmness of the crumb, evaporations of moisture from the crumb; loss of bread flavor; and microbial deterioration. Among these, increase in crumb firmness, which is considered as the most important aspect of staling is recognized by the consumer a long time before the bread products has become unsuitable for consumption. It results in decreased consumer acceptance and great economic losses. Hence much attention has to be focused on this problem, because people never prefer stale products. There is a large scope of technological development in the bread sector of the bakery industry. A brief detail is provided below from the Indian point of view.

#### **1.1.2 Recent advancements**

Discovery of synthetic antioxidants has revolutionized the use of antioxidants in food. The effect of these antioxidants in bakery products were reviewed and found to be effective in enhancing the shelf life. Animal experimental studies have shown that some of the synthetic antioxidants had toxigenic, mutagenic, and carcinogenic effects. Hence there is an increasing demand for the use of natural antioxidants in foods, especially in bakery products. Some of the natural antioxidants such as  $\alpha$ -tocopherol,  $\beta$ -carotene, and ascorbic acid were already used in bakery products. These natural antioxidants are found to be effective in enhancing the shelf life of bakery products but not to the extent of synthetic antioxidants. Baking processing steps may lower the antioxidative activity but techniques such as encapsulation of antioxidants can retain their activity. To enrich the breads with bioactive compounds, antioxidants and fiber content

waste from fruit and vegetable industries like peel, pulp waste, seed etc are often used as an agent for fortification. Fruits and vegetables contain many antioxidant compounds, including phenolic compounds, carotenoids, anthocyanins, and tocopherols (Naczk and Shahidi, 2006). Specially, fruit peels are rich in polyphenolic compounds, flavonoids, ascorbic acid, and many other biologically active components having positive influences on health (Leontowicz et al., 2003). Mango peel was examined and was found to be a good source of phytochemicals, such as polyphenols, carotenoids, vitamin E and vitamin C.(Ajila et al., 2007). Sources of pomegranate waste , apple pomace ,grape peels ,citrus peel are also used as fortifying agent in different types of bread and other bakery products to enhance its functionality.

The gluten content in the flour is mainly responsible for the network formation and helps in retaining the CO<sub>2</sub> gas in dough during the fermentation process with yeast (Gan et al., 1995) resulting in a good crumb structure and texture of the final bread. The gluten breads often resists celiac patients to consume it. Removal of gluten and consequently the lack of a strength protein matrix able to expand and retain gas results in weak batter and high permeability of  $CO_2$  which results in the difficulty of the structure as a result decrease the volume of the bread. Commercially Gluten free breads are mainly starch-based and lacks in fibre, vitamins, and nutrients which drastically affects the nutritive profile of the bread and disturbs the balanced diet of the celiac patients adhering to this GF diet.(Mariani et al., 1998). The increasing number of diagonsed celiac patients and the poor quality of the gluten free breads (GFB) has encouraged the researchers to investigate new ingredients for formulation and new technologies that can function as gluten and improve the quality of the GFB. Starches, gums, gluten free flours (soy, quinoa, teff, buckwheat, rice, sorghum, chickenpea) etc are now being used in the formulations which elevates the physicochemical properties. Gluten free breads enriched with amaranth flour resulted in higher calcium and magnesium and in an increase of the body mass index in rats and gluten free breads enriched with flaxseed flour resulted in higher calcium and magnesium and in an increase of the body mass index and decrease in total cholesterol and LDL fraction in rats (Gambus et al., 2009). This study had proven the use of flax seeds and amaranth flour to formulate in the gluten free diets for human consumption. The Gluten free formulations lack fibre content in them which can be supplemented by utilization of peel wastage from fruits and vegetables rich in fiber content. The inclusion of the bio active compounds from the natural sources (fruits and vegetables) plays a major role in the bread industry. It enhances the

functionality of the bread, increases the antioxidant activity to a higher level .It also increases the volume of the bread renders it to be commercially acceptable and thus enhances the nutritive profile of the bread as a whole.

Various types of fruit peels like pomegranate peels, citrus peels, pineapple peels, grapefruit albedo, mango peel etc are used as a natural source of antioxidants and dietary fiber to enhance the functionality of the bakery products. The inclusion of these peels improves the physical, chemical and sensory properties of the products to a certain level beyond which it renders them unacceptable from consumer point of view. (Okpala and Akpu, 2014) where he studied the effects in physical, chemical and sensory attributes upon incorporation of orange peel flour in bread samples at varying levels. It was found that upon increasing the levels of orange peel flour the chemical parameters shows an increase in trend but the physical and sensory parameters shows a decrease after a certain level of fortification which shows a relationship between the physical and sensory parameters. The overall impact in the parameters upon incorporation of peel can be further analyzed and studied to derive a correlation between the factors using statistical software.

## **1.2 REVIEW OF LITERATURE 1.2.1 PEELS IN FOOD FORTIFICATION**

#### 1. Pomegranate Peel Powder in biscuits

Pomegranate Peel Powder (PPP) is a rich source of tannins, flavonoids and other phenolic compounds. It is incorporated at 3% in wheat and bajra at varying ratios of 60:40, 50:50 and 40:60 respectively in biscuit formulation. The 50% replacement of wheat flour with pearl millet and 3% pomegranate peel powder shows the best sensory properties in biscuits (Vaijapurkar et al., 2013).

#### 2. Banana Peel in Vinegar production

In this study, the matooke peels (banana) are used as an ideal substrate for production of good quality vinegar. It increases the economical and food value of matooke but also provides a way of utilizing banana. The banana wine vinegar production process took 28 days which has physicochemical characteristics of 6.0% (v/v) acetic acid,  $0.5^{\circ}$ Brix, and pH of 2.9 which complied well with the standard ranges of brewed vinegar having an yield of 4.0% acetic acid(v/v) after complete fermentation as per the FDA requirement (Byarugaba-Bazirake et al., 2014).

#### 3. Pomegranate peel powder and pomegranate juice in cookies

In this study peel powder and juice of fresh pomegranate are utilized to fortify cookies as a source of antioxidants. It enhances its nutritional value without any significant changes in its rheological, sensorial and antioxidant properties. The cookies were fortified with( 2.5,5,7.5, 10)% level of fortification with the pomegranate peel powder whereas at 10,20,40 and 50)% with pomegranate juice. The DPPH content is found to increase significantly with the increase in the fortification level as well as a significant increase in the flavonoids and anthocyanins were also reported. It was found that fortification beyond 10% pomegranate peel powder (PPP) or 50% juice is not acceptable since on increase in the level increases the total flavonoid and anthocyanin content which causes bitterness and affects color of the final product beyond a certain limit which thereby affecting the physical quality of the product and finally the affects the commercial

aspect. Pomegranate peel powder as a fortifying agent adds a longer shelf life and nutritive quality of the product and its eating qualities (Paul and Bhattacharyya, 2015).

#### 4. Orange peel fortified biscuits

In this study wheat flour is fortified with orange peels at different levels of 5%,7.5%,10%12.5%,15%..The physico chemical and sensorial properties of the samples were analysed. The overall sensory attributes, color of sample 100:10% has better appearance compared to the other formulated levels. Overall acceptability for 100:7.5% has higher score with a high percentage of protein and other nutrients. The wheat flour incorporated from 5 upto 10% shows the best results without affecting the qualities of the final product (Bugad et al., 2015).

#### 5. Mosambi peel in cookies

Mosambi peel has high antioxidant content which enhances the functionality and improves the storage stability of the food. The main objective of this research is to isolate and quantify antioxidants from mosambi peel. Aqueous extract of mosambi peel was incorporated in cookies at various levels of 1%, 2% and 3%. The physicochemical and sensory attributes of the incorporated cookies are analyzed. The proximate analysis has no significant changes to the control sample but shows a better hedonic response with 2% level of fortification (Imran et al., 2016).

#### 6. Grape pomace in yoghurt

In this study grape pomace is used for fortification in yoghurt at varying levels of 1% to 5% and its effect on the physicochemical and sensorial properties are analyzed .The total phenolic content and the radical scavenging activity increases along with the fortification level but shows a decrease in the sensorial property as the level of fortification increases. A similar trend in physicochemical and sensorial property is observed in 3% level fortified yoghurt when compared to control (Mohamed et al., 2014).

#### 7. Apple pomace in gluten-free cracker

In the present study the two varieties of brown rice flour is used for gluten free cracker formulations with apple pomace as a fortifying agent at levels of 0%,3%,5%,9% respectively. The antioxidant properties and anti scavenging potential shows highest value at 9% level whereas the sensory scores are higher upto 6% level. The sensory scores are desirable and is accepted widely by consumer. Thus the brown rice flour and apple pomace proves to be a good source of nutrition and functional components and can be used as functional ingredient in other bakery products (Mir et al., 2015)

#### 8. Watermelon rinds and Sharyln melon peels in cake

The aim of this present study was to evaluate the physical and chemical properties of watermelon rind (WMR)and sharyln melon peel powders(SMP) and its utilization as partially, substituted of wheat flour at levels of 2.5%, 5% and 7.5% or fat at levels of 5%, 10%, and 15% in cake making. Watermelon rind had high moisture, ash, fat, protein and carbohydrates as compared to SMP. The water absorption capacity, oil absorption capacity and crude fibre content is higher in SMP than WMP. The incorporation of WMR and SMP in cake batters at studied levels enhanced the specific volume of cakes than control. It also retards staling of the cakes and inhibition of lipid oxidation and free fatty acids formation during storage. It is observed that substitution of 5% flour and 10% fat with WMR and SMP produced acceptable cakes which were not significantly different with the control (Hanan et al., 2013).

#### 9. Fruit peels utilization in paneer

Peels from pomegranate, lemon and orange are used as sources of natural antioxidants. Pomegranate peel extracts shows the maximum antioxidant activity compared to the lemon and orange peels. The addition of each peels at (1%, 2% and 3%) level in paneer and the study of its sensory parameters of the samples compared to control. At 2% level the samples are most acceptable and posses the greater ability to prevent the peroxide formation. It also emphasizes the use of fruit peels as a source of natural antioxidants which can successfully replace the synthetic antioxidants (Singh et al., 2014).

#### **1.2.2 PEEL IN BREADS:**

#### 1. Orange peel

The aim of this study is to supplement wheat bread with orange peel at levels of 5%, 7%, 10%. It is observed that the highly nutritious bun was prepared by incorporating 5% orange peel powder without any adverse impact on the overall acceptability of the product. It was found that on 5% level sensory attributes like color, flavor texture and overall acceptability was found to maximum. The texture parameters also shows acceptable results at 5% level incorporation whereas the rheological analysis indicates that addition of orange peels in wheat flour caused an increase in arrived time and a decrease in dough stability in Farinograph and in Extensograph the extensibility of the dough decreased and maximum resistance to extension increased by increasing the levels of nutritional waste in all samples (Raj et al., 2014).

#### 2. Apple pomace in wheat bread

Pomace flour blends were prepared by incorporating 2,5,8 and 11% pomace in wheat flour. Blends were evaluated for their bread making quality. Water absorption increased with the increase in the blends but it did not change significantly upon neutralizing the acidity of apple pomace. Upon increase in the incorporation level a reduction of 42.8% in loaf volume was observed whereas neutralization of pomace acidity in dough resulted only in 26.6% reduction in volume. The loaf weight of the breads prepared from the blends with 11% pomace under neutralized and unneutralized conditions increased by 3.1 and 7% respectively whereas the bread firmness increased from 3N in control to 10N and 12N respectively. Blending of pomace(0-11%) increases the baking time from 20 min for control to 33min for unneutralized and 27min for neutralized blend whereas sensory evaluation upto 5% level was acceptable.( Massodi et al., 1998).

#### 3. Grapefruit albedo

The aim of this research study is to incorporate white grapefruit albedo layer flour in wheat toast bread.It includes the determination of gross chemical composition,the caloric value and mineral content of the bread studied. The physical and sensory characteristics of the studied bread were also assessed.Upon incorporation of the defatted grapefruit albedo layer flour with wheat flour a drastic increase in crude fat,ash and crude fiber (1.57%, 6.41%), (0.59%, 2.20%) and (0.82%, 9.13%) was observed respectively.The mean values of minerals composition of wheat flour and

fortified wheat toast bread with defatted grape albedo flour shows no significant differences in iron and phosphorus between 5 and 10% fortification level but shows an huge increase in the contents of calcium and potassium ranging from 38.00% to 171.00%) and (90.00% to 210.00%)respectively compared to control. The physical and sensory attributes were analyzed for (5 to 20)% deffated grape albedo flour fortification level. It was observed that 5% level of fortification in wheat toast bread shows the best sensory scores. It recommended that utilization of the grape albedo layer flour as a fortifying agent enhances the nutritional value of the toast bread. (Hassan and Ali, 2014).

#### 4. Pomegranate peel

The aim of the present study is the utilization of pomegranate peel powder (PPP) at levels of 2.5%, 5%, and 7.5% to add nutritive value to the bakery product and also to investigate the weight loss activity in rats fed on high fat diet. Pomegranate peel is a good source of fiber and antioxidant bioactive compounds. The chemical composition ,total phenolic content ,total flavonoid content and anti-oxidative activity (DPPH) of PPP and pan breads as well as texture profile analysis, sensory evaluation and physical properties of pan breads were analyzed. Thirty six female albino adult rats divided into six groups of six each. The first group was fed on basal diet followed by second with the positive control. The third group fed with PPP free pan bread followed by feeding of the fortified levels of bread at 2.5%,5%,7.5% in the other three groups respectively. The food intake , food efficiency , body weight, gain% , epididymal adipose tissue and organs along with the serum lipid profile and liver enzymes were analyzed. The results shows that 7.5% fortification of PPP followed by 5% PPP fortified breads reduces the body weight  $(47.61 \pm 1.27\%$  and  $40.65 \pm 1.11\%$ ), The results concluded that pomegranate peel powder fortified bread due to its high fiber content and antioxidative properties is recommended to gain nutritional and healthy benefits to weight loss activity and risk of obesity. (Sayed-Ahmed et al., 2014).

#### 5. Combination of banana peel and pomegranate peel

The pharmaceutical use of the banana peel and pomegranate peel made it useful in the bakery sector t as a fortifying agent and adds value to the final product. The bread samples were prepared by incorporation of banana peel and pomegranate peel powder in equal proportions. The wheat flour is replaced by 10%,,15%,20% and 30% of wheat flour by incorporating wheat flour with banana peel flour and pomegranate peel flour in the ratio of 5:5,10:10,15:15 and 5:10

respectively and the bread samples prepared from this formulations were designated as B1,B2,B3,B4 and is compared to the control sample B0 with respect to its proximate analysis,physic-chemical and sensory parameters. Results show that the B1sample is found to be sensory acceptable. The proximate analysis, moisture content and the antioxidant properties shows a marked increase in the bread sample B1when compared to the bread sample B0. It was also observed that the dietary fibre content increases upto 10 fold to that of control sample. The moisture content of the B1 sample is 33% which renders it free from staling as compared to that of control sample having 27% moisture content. Thus on replacing the wheat flour with 10% banana peel and pomegranate peel flour with in 5:5 ratio each shows the best nutritionally and sensory acceptable breads. (Bandel et al., 2014).

#### 6. Theobroma peel

Cupuassu (*Theobroma grandiflorum*) peel flour (CPF) was used at various percentage as partial replacement in breads. The samples with 9% CPF were major sources of protein, ash ,insoluble fibre.and total fibre with highest moisture with the lowest calorie factor and as well as protein digestibility whereas the 3% samples has the highest of lipids and soluble fibre content. With the increase in the levels of CPF there is a sharp increase in tannins, phytic acid and phenolic compounds. In general it was observed that the addition of CPF did not cause extra browning and the resulted breads has a good appearance. There was a steady reduction in specific volume of breads with added CPF. The acceptance rate including color, texture, flavor and aroma decreases with the increase in addition of CPF (Salgado et al., 2013).

#### **1.2.3 MANGO PEEL FORTIFICATION**

#### 1. Macaroni preparation

Incorporation of mango peel at different levels (2.5,5, 7.5)% in the macaroni preparation and its effect on the cooking properties, firmness, nutraceutical and sensory properties have been studied. The polyphenolic content increases with the increase in the levels of incorporation enhancing the antioxidant properties of the samples when compared to control. The cooking loss and firmness also shows an increasing trend upon incorporation of MPP. Semolina incorporated with 5% level of MPP yields the macaroni of acceptable quality. Results suggested that it is possible to enhance the nutritional value of the macaroni without any adverse changes in the cooking, textural and sensory parameters (Ajila et al., 2010).

#### 2. Yoghurt

Incorporation of ripe mango peel powder at varying levels of 1%,3%,7% and 10% in natural yoghurt not only imparts a nutritive value to the final product but also acts as a preservative by extending the shelf life of the product. Physico chemical and sensory parameters was evaluated and it was observed that yoghurt with 10% formulation shows the best preservative effect and has a texture, flavor and color of acceptable quality (Ruiz et al., 2011).

### 3. Biscuits

In the present study Mango peel powder (5.10.15.20)% and Mango kernel powders are used at different levels (20.30.40.50)% in formulation of biscuits and their effects on rheology, physical, sensory and antioxidant properties are evaluated. There is an increamnet in the antioxidant properties of the Mango peel fortified biscuits as well as mango kernel fortified biscuits. It enhances the nutritive and antioxidant value of the fortified biscuits and offers the most acceptable fortification of biscuits with 10% mango peel powder as well as 40% mango kernel powder respectively (Ashoush et al., 2011).

#### 4. Wine

Studies on the preparation of mango wine by yeast- mango peel immobilized biocatalyst system by repeated batch fermentation was conducted and compared to free cells fermentation at various temperatures. Operational stability of the biocatalyst was good as the ethanol concentrations and with high productivities indicating the suitability of the biocatalyst or even low temperature wine making. The concentration of ethyl acetate was not above 40 mg/l in all cases, and higher alcohols were in wine with immobilized cells indicating an improvement in the product compared to free cells fermentation .Sensory evaluation revealed fruity aroma, fine taste and overall improved quality of wines produced by immobilized system than free yeast cells. The aroma content of the wine using immobilized cells is  $7.9\pm0.73$  compared to that of free yeast cell which is  $6.1\pm0.25$  (Varakumar et al., 2012).

#### 5. Cookies

Mango peels are rich in total dietary fibre content as well as polyphenolic content which makes it suitable to provide functionality and produces nutraceutical products once incorporated in it .In this study (Betsy Ng, 2011) mango peel and gluten free cookie are taken as control sample and is compared with the mango puree and gluten free mango cookie. The oxygen scavenging potential activity shows the least for gluten free cookie after storage for 28 days highlighting the potential of a nutraceutical product.

#### 1.2.4 Fortifications and sensory tests

The main objective of fortification is addition of nutrients to foods and to enhance the functionality of the food in different dimensions. Fortification can be successfully achieved if only the sensory parameters are acceptable by the consumer sector while performing the sensory evaluation.

Sensory evaluation is a scientific discipline used to evoke, measure, analyze and interpret reactions to those characteristics of foods and materials as they are perceived by the senses of sight, smell taste sight, smell, touch and hearing The various sensory tests like discrimination, ranking, scaling tests are usually done to evaluate sensorial parameters of food. Discrimination or difference test is used to determine whether or not a difference in some specific attribute exists between two samples. Triangle test, duo-trio test, multiple comparison test are some of the difference test usually done for evaluation of sensory parameters. Scaling tests refers to the dividing of scale using words for verbal test scale whereas numbers for numerical scaling. Descriptive test refers to flavor profile and QDA (Quantitative Descriptive Analysis) whereas example of preference acceptance test is Hedonic scale rating.

Hedonic scale is one of the most common evaluation techinque of sensory parameters. The scale is divided into 9 divisions ranging from 9 extremely like to 1 dislike extremely. The sensory attributes in the score card of hedonic scale rating are taste, aroma, flavor, color, texture and overall acceptability which is rated within 9 to 1 depending on the final product and finally the consumer acceptance of the product.

Descriptive tests like Quantitative Descriptor Analysis (QDA), Principal Component Analysis (PCA) and Partial Least Square (PLS) are mainly performed to correlate the parameters. A

quantitative descriptive analysis (QDA) approach has gained acceptance for sensory evaluation of various food and dairy products (Stone and Sidel, 1998), including conventionally pasteurized milk (Phillips et al., 1995; Quinones et al., 1998), ice cream (Ohmes et al., 1998; Roland et al., 1999), and cheese (Ordonez et al., 1998). The principle of QDA is based on the ability to train panelists to measure specific attributes of a product in a reproducible manner to yield a comprehensive quantitative product description amenable to statistical analyses. In a QDA approach, panelists recruited from the general public work together in a focus group to identify key product attributes and appropriate intensity scales specific to a product. This group of panelists is then trained to reliably identify and score product attributes. As panelists generate the attribute terms, the resulting descriptions are meaningful to consumers, and thus, analyses provide information amenable to modeling predictions of consumer acceptability. QDA results can be analyzed statistically and then represented graphically. Principal component analysis (PCA) is a widely used multivariate analytical statistical technique that can be applied to QDA data to reduce the set of dependent variables (i.e., attributes) to a smaller set of underlying variables (called factors) based on patterns of correlation among the original variables (Lawless and Heymann, 1998).

PCA is used to correlate the physical/chemical and sensorial parameters of the bread samples. The experimental results were analyzed using Principal Component Analysis (PCA) with full cross-validation. PCA constitutes the most basic statistical method of all multivariate data analysis and involves decomposing one "data matrix" into a structure part (model) and a "noise" part (error). The main propose of all multivariate data analysis is to decompose the data in order to detect and model the "hidden phenomena". Visualization of the results of PCA is usually achieved by plotting pairs of the first few principal components (PC). Iordăchescu et al, 2013 has studied the two types of gluten free bread and their physico chemical and sensory properties were assessed and then correlated with each other using PCA. The physico-chemical properties like specific volume, porosity, moisture content and acidity were correlated with the sensory parameters with the operational kneading process. Crust cohesiveness and roughness is inversely proportional to porosity, elasticity, roughness of mass, crust firmness and denseness of crumb. It can be observed that the astringent, sour stickiness and tacky film are directly correlated. PC2 is given by samples moisture.

## **1.3 OBJECTIVES**

- The main objective is to enhance the functionality of the bakery product (bread) using natural sources.
- To utilize Mango peel waste as a fortifying agent due to its high fibre content and antioxidant property as well as its cost effectiveness.
- Utilization of ripe mango peel powder as a natural source of antioxidants in wheat bread.
- Formulation of Gluten free breads and the effect on its physicochemical, textural and sensory parameters on combination with both raw and ripe mango peel powder.
- To study the effect of the viscoelastic nature of the wheat bread and gluten free bread upon incorporation of raw and ripe mango peel powder in combination.
- To study the instrumental, chemical and sensory profile of the wheat and gluten free bread upon incorporation of raw and ripe mango peel powder in combination and to obtain a relationship between the instrumental and sensory parameters of both using Principal component analysis (PCA).

# CHAPTER 2:

## OPTIMIZATION OF RIPE MANGO PEELS FORTIFICATION LEVEL IN BREAD USING RESPONSE SURFACE METHODOLOGY

#### **2.1 INTRODUCTION**

Ripe mangoes are eaten fresh often as a dessert fruit, and are also cooked, dried, and canned. They are used in chutneys, jellies, and jams. Mango is processed to a maximum extent, thereby producing high quality of solid and liquid wastes. Solid wastes, constituting mango kernels, peels & pulp residue contributes about 40 to 50% of total fruit waste out of which, 5 to 10% is pulp waste and 7 to 24% as peel waste (Larrauri et al, 1996). Approximately, 0.6 to 0.8 million tons of mango peel is generated annually in India. These wastes are either used as cattle feed or dumped in open areas, where it adds to environmental pollution.

Although enriching white wheat bread with peel brings many beneficial health regarding metabolic benefits. But it also adversely affects the physical and textural parameters along with sensory attributes (Wang et al, 2002). This implies that the enrichment of bread with ripe mango peel powder must be optimized. Such optimization can be performed using response surface methodology (RSM) (Collar et al, 2007).

The mathematical and statistical approach of response surface methodology (RSM) has been used to optimize formulation and process parameters for the manufacture of "healthy" breads such as wholemeal oat bread (Mc Carthy et al., 2005), gluten-free breads (Angioloni et al., 2012) and wheat-legume composite flour breads (Villarino et al., 2015 and Zhang and Dutta, 2006). However, it is not evident in studies that ripe mango peel powder is being used for formulation of bread using RSM to optimize the formulation and process parameters. The control of parameters like baking temperature and baking time combination is basically an engineering problem that is critical to the successful implementation in the context of fortification of bread. The aim of this study was to use RSM to assess the effects of formulation that is percentage of incorporation of ripe mango peel. Along with the process parameters which compiled parameters like baking temperature on the physical and sensory qualities of ripe mango peel fortified wheat bread and to optimize the levels of these parameters to produce acceptable quality bread with maximum level of ripe mango peel powder incorporation.

### 2.2 MATERIALS AND METHODS

#### 2.2.1 Raw Materials

Ripe mangoes were procured from an orchard in Kolkata. White refined flour, granulated sugar, salt, refined oil as shortening agent were purchased from the local grocery stores of Jadavpur, Kolkata, India. Compressed Baker's yeast (Safe Yeast Company Pvt. Ltd., Mumbai, India) used as the leavening agent in bread preparation.

#### 2.2.2 Chemicals

The Folin-Ciocalteu reagent, sodium carbonate, ethanol (Merck Specialities Pvt. Ltd., Mumbai, India), gallic acid (sd fine-chem Ltd., Mumbai, India) sodium nitrate, aluminium chloride, sodium hydroxide and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, St. Louis, MO, USA) were used in the investigation.

#### 2.2.3 Preparation of the raw material

Ripe mango peels were prepared by peeling freshly bought and ripe mangoes which were followed by washing with tap water to remove adhering dust and dirt particles. The peels were placed in a Oven drier (Reliance Enterprise, Kolkata, India) at 60 °C for 8 hr. For ease in utilization the dried peel was powdered using a Grinder ( $GX_7$ , Bajaj Electricals Ltd, India). The powdered peel for subsequent use was stored in air-tight packets in a refrigerator.

#### 2.2.4 Bread Preparation

The ingredients and their proportions required for the preparation of wheat bread samples were refined wheat flour 100g, sugar 6.0%, salt 2 %, instant dry yeast 3%, refined oil 4.0% and water 60.0%. The dry mix was prepared by mixing flour, sugar, salt. Baker's compressed yeast was dissolved in water (20 ml) and kept at the 37°C for the activation of the yeast cells. Small amount of sugar (2gm) were added to the warm water for activation of the yeast cells. Mixing is an important step for achieving homogenous as well as soft dough. Here, mixing was carried out manually according to the straight dough method. The dry ingredients, shortening and the activated yeast were added in a bowl and water added and then kneaded until the dough was elastic and the required consistency was reached. After this, the dough was rounded and kept in a

bowl for the first proofing at the room temperature (30°C) for about 40 min. The bowl was covered with a wet cloth to maintain a relative humidity of 80-90%. After the first proofing, the dough was punched and worked lightly so that the excess gas could escape and the gas cells are redistributed. The dough was then shaped to fit lightly in greased bread molds. The dough was again kept for the final proofing for about 1 hour at 40°C. Finally, after second proofing, the breads in molds were baked in rotary oven (CM HS108, Chanmag Bakery Machine Co. Ltd., Taiwan) at different experimental temperatures and time. After baking, the prepared bread samples were cooled for about 1 hour at room temperature and then analyses carried out. The formulated flours with 2%, 4% and 6% ( ripe mango peel powder) follow the same bread making procedure as that of control bread sample.

#### 2.2.3. Experimental design and statistical analysis

In this work, a central composite design was set up to look for the best experimental conditions. In this study three levels of each predictor/ independent variable were incorporated into the design, where the three independent variables (factors) used here were: baking time  $(X_1)$  baking temperature  $(X_2)$  and percentage of fortification  $(X_3)$  in Table.1 and the dependent variables (response) studied was loaf specific volume (Y). To study three factors (predictor/ independent variables) at three levels would require, whereas use of the central composite design required 20 runs or experiments. For each of the response variables, model summaries and lack of fit tests were analysed for linear or quadratic models. The cubic model was aliased because there were not enough points for this type of model.

The responses can be described by the following second order polynomial function for predicting the responses in the experimental region:

$$Y = b_0 + b_1 X_1 + b_2 X_2 + b_3 X_3 + b_{11} X_1^2 + b_{22} X_2^2 + b_{33} X_3^2 + b_{12} X_1 X_2 + b_{13} X_1 X_3 + b_{23} X_2 X_3$$

Where Y is the predicted response;  $X_1$ ,  $X_2$  and  $X_3$  are the independent variables;  $b_0$  is the offset term;  $b_1$ ,  $b_2$ ,  $b_3$  are the linear effects;  $b_{11}$ ,  $b_{22}$ ,  $b_{33}$  are the squared effects;  $b_{12}$ ,  $b_{13}$ ,  $b_{23}$  are the interaction terms. The regression coefficients were then used to generate contour maps from the regression models. Keeping one variable constant at the centre point and varying the other variables within the experimental range, the 3D plots were generated.
# 2.2.4 Physico Analysis

# 2.2.4.1 Loaf specific volume

Bread samples were weighed after cooling and the volumes of the loaves were measured by the seed displacement method (Steffolani et al., 2015). From these, the specific volume of the loaf was calculated as: Specific volume (ml/g).The specific volume (cm3/g) was calculated as described in

Specific volume  $(cm^3/gm) = \frac{Losfvolume of bread}{Weight of bread}$ 

### 2.2.5 Statistical analysis

Statistical analysis of variance (ANOVA) and multiple regressions were performed using the software, Design Expert® Version 7.1.6 (Stat-Ease, Inc., Minneapolis, USA) to fit the equation. Significant terms in the model (linear, interactive and quadratic) for the response were found by ANOVA. The sequential F-tests value decides the significance of the model. Various descriptive statistical analysis such as p value, F value, degrees of freedom (DF), sum of squares (SS), coefficient of variation (CV), determination coefficient ( $R^2$ ), adjusted determination of coefficient ( $R^2$ a), predicted determination of coefficient ( $R^2$ p) evaluates the experimental data and shows the statistical significance of the developed quadratic mathematical model. Three-dimensional response surface plots were generated after fitting the data into the model to predict the relationship between the factors and response.

Independent	Unit	Symbol			
variables (Factors)					
				1	1
			-1	0	+1
Baking temperature	°C	$X_1$	10	20	30
Baking time	min	$X_2$	200	220	240
Amt of incorporation	%	X <sub>3</sub>	1	5	9

Table 1: Independent variables and their levels in the central composite design

#### 2.3 RESULTS AND DISCUSSION

#### 2.3.1 Experiment design analysis

The results of total 20 runs statistically designed batch experiments were performed for different combinations of the process variables in order to optimize and study the combined effects of independent variables (baking temperature, baking time and % of fortification) on the specific volume(response) of the final product are tabulated in Table1 along with the experimental and predicted values of the response. The experimental data was fitted into the various models (linear, interactive (2F1), quadratic and cubic) to obtain regression equation. In this study three different tests sequential model summary statistics, lack of fit tests and model summary statistics are carried out to decide about the adequacy of the models among various models and to represent the maximum specific volume. The results are tabulated in Table 2.

From Table 2, linear and interactive (2F1) models exhibited lower  $R^2$ , adjusted  $R^2$  and predicted  $R^2$  compared to the quadratic model whereas the p-value is higher of the former. The cubic model is aliased. Therefore the quadratic model incorporating linear, interactive and quadratic terms was chosen to describe the effects of the process variables on the response (specific volume). Furthermore, analysis of variance was also used to check the adequacy of the model.

Run	Temperature	Time	Amount of	Specific volume
	٥C	Min	Incorporation (%)	Cm <sup>3</sup> /g
1	220.00	20.00	5.00	3.6
2	220.00	20.00	5.00	3.86
3	220.00	20.00	1.00	3.40
4	240.00	20.00	5.00	3.00
5	220.00	20.00	9.00	3.03
6	220.00	20.00	5.00	3.86
7	240.00	10.00	9.00	2.03
8	200.00	10.00	1.00	2.69

9	200.00	30.00	1.00	2.99
10	200.00	10.00	9.00	2.43
11	200.00	20.00	5.00	3.01
12	240.00	30.00	1.00.	2.95
13	220.00	30.00	5.00	3.60
14	220.00	10.00	5.00	2.94
15	200.00	30.00	9.00	3.22
16	220.00	20.00	5.00	3.86
17	220.00	20.00	5.00	3.80
18	220.00	20.00	5.00	3.50
19	240.00	30.00	9.00	2.60
20	240.00	10.00	1.00	3.35

Table 2: Results of regression analysis of optimization of Specific volume using second-order polynomial method.

SOURCE	Sum of	DF	Mean	F-value	p-value	Remarks
	squares		square		Prob >F	
Mean	203.01	1	203.01			
Linear	0.81	3	0.27	1.04	0.4025	
2F1	0.71	3	0.24	0.88	0.4751	
Quadratic	3.00	3	1.00	21.10	0.0001	Suggested
Cubic	0.081	4	0.020	0.31	0.8626	aliased
Residual	0.39	6	0.066			
Total	208.01	20	10.40			
		·				
			Lack of fit tes	st		
Linear	4.06	11	0.37	14.91	0.0040	
2F1	3.35	8	0.42	16.93	0.0032	
Quadratic	0.35	5	0.070	2.83	0.1390	Suggested
Cubic	0.27	1	0.27	10.89	0.0215	aliased
Pure Error	0.12	5	0.025			

Model summary statistics								
Source	ce Std.dev. $R^2$ Adjusted $R^2$ Predicted Press Remark							
				$R^2$				
Linear	0.51	0.1629	0.0059	-0.4508	7.25			
2F1	0.52	0.3047	-0.0163	-3.0303	20.14			
Quadratic	0.22	0.9051	0.8197	0.5274	2.36	Suggested		
Cubic	0.26	0.9213	0.7508	-65.2930	331.26	aliased		

Table 3: Sequential model fitting for the response (specific volume)

# 2.3.2 Fitting of the second order polynomial equation

Design –Expert software generated a second order polynomial equation by applying multiple regression analysis on the experimental data to express a relationship between the process variables and response. The final equation obtained is in the terms of forward regression of the reduced quadratic model and is given below in the coded factors.

# $Y = 3.61 - 0.041X_1 + 0.19X_2 - 0.21X_3 - 0.20X_1X_3 + 0.18X_2X_3 - 0.53X_1^2 - 0.32X_3^2$

Where Y is the specific volume X1, X2, X3 are the coded values of the baking temperature, time and % of fortification respectively.

#### 2.3.3 Statistical Analysis

The statistical significance of the regression equation was calculated by the corresponding F and p values as tabulated in Table 3. The model F-value of 10.88 implies that the model is significant. There is only 0.02% chance that a Model F- value this large could occur due to noise. Values of Prob >F less than 0.0500 indicate that model terms are significant. The lack of Fit F-value 0f 3.21 implies the Lack of Fit is not significant relative to the pure error. There is a 10.87% chance that a Lack of Fit F-Value this large could occur due to noise. The R<sup>2</sup> of the predicted model was 0.8639. Adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable. In this study the ratio was found to be > 12 which indicates an adequate signal and confirm that the model is significant. The predicted sum of squares (PRESS) value of 2.11 shows how each

Source	Coefficient	Sum of	Degree	Standard	Mean	F-	p -
	estimate	squares	of	error	square	value	value
			freedom				
Model	3.61	4.32	7	0.064	0.62	10.88	0.0002
А	-0.041	0.017	1	0.040	0.017	0.30	0.5960
В	0.19	0.37	1	0.040	0.37	6.50	0.0254
С	-0.21	0.43	1	0.057	0.43	7.56	0.0176
AC	-0.20	0.34	1	0.057	0.34	5.93	0.0314
BC	0.18	0.27	1	0.057	0.27	4.70	0.0510
$A^2$	-0.53	0.89	1	0.032	0.89	15.71	0.0019
$C^2$	-0.32	0.32	1	0.032	0.32	5.69	0.0344
Residual		0.68	12		0.057		
Lack of		0.56	7		0.079	3.21	0.1087
fit							
Pure		0.12	5		0.025		
error							
Cor total		5.00	19				
Std.dev.	0.24			R <sup>2</sup>		0.8639	
Mean	3.19			Adjusted R <sup>2</sup>		0.7845	
C.V.%	7.47			Predicted R <sup>2</sup>		0.5785	
Press	2.11			Adeq.precision		12.383	

point in the model fits the design. A high value of R  $^2$  and a low PRESS value indicate a good model.

Table 4: Analysis of variance for Response surface reduced Quadratic Model for the specific volume as response.

# 2.3.4 Influence of process variables:

Three factors and three levels RSM analysis was used in this study to investigate the influence of process variables such as temperature, time and % of fortification on the specific volume of bread. To understand the interaction between the independent variables and estimate the

response of specific volume over the independent variables, three dimensional response surface plots (3D) were plotted from the developed model. In this study, the model has 3 factors. So, the 3D plots have drawn by maintaining one factor at constant level, whereas two factors varied in their range to understand their main and interactive effects on the dependent variable.

# 2.3.5 Interactive Effects of the independent variables

The interactive effects of time temperature is less pronounced in Fig 1.The temperature of 219.30°C and time 23.87 min are the optimum conditions for the achieving the highest specific volume of bread. The curve flattens and increases insignificantly after reaching its optimum conditions. Exposure of the bread sample at higher temperatures and longer duration often decreases the bread volume and affects the quality of the bread. The interaction between time and level of MPP addition is shown to be optimum in Fig 2.at 3.95% level in duration of 23.88 min. At 219.30°C and 3.95% level it shows the optimum interactive effects of temperature and fortification level. The optimization has reached its maximum which can be evident with the corresponding eigen analysis.



Design-Expert® Software

Sp Vol





FIG 1: Response surface plots representing the effects of process conditions on specific volume.

## **2.4 CONCLUSION**

Three factors at three levels Centre Composite Design (CCD) was successfully employed to optimize and study the individual and interactive effects of process variables such as temperature, time and % of fortification on the specific volume of the bread (response). Results indicated that the factors selected in this study had a significant effect on the specific volume. From the ANOVA results a high correlation second order polynomial regression equation was developed which could be employed to optimize the time, temperature and level of fortification of ripe mango peel powder in bread and its impact on the response (specific volume). The optimized baking temperature obtained from the 3D plotted graphs are 219.30°C baking temperature, 23.88 min time and 3.5% level of MPP formulation.

# CHAPTER 3:

# EFFECT OF MANGO PEEL POWDER TO IMPROVE RHEOLOGICAL CHARACTERISTICS OF GLUTEN FREE DOUGH

#### **3.1 INTRODUCTION**

Importance of dough rheology is well recognized in predicting and controlling the quality of baked products. Variation in the elastic and viscous behavior of different dough formulations are considered as key quality factors in bakery applications. Gluten in wheat is responsible for the structure forming ability and visco-elastic properties required to produce good quality breads. The interactions of the gliadin and glutenin through covalent and non-covalent bonds forming the gluten complexes resulting in the visco-elasticity of the dough offering the ability to withstand the stresses during mixing and retention of the gas during fermentation and baking producing a light weight baked product with good textural properties (Lindsay and Skeritti, 1999).

Gluten rich products are not recommended for the celiac patients as they are intolerant to gluten and are strictly advised to adhere on the gluten free diet. The absence of gluten in the gluten free (GF) formulations is a major technological challenge, as it is the essential structure building protein. The absence of gluten impairs the capacity of the dough development during mixing, fermentation and baking. It results in a liquid like batter unlike dough formation in wheat and results in breads with a poor crumb structure and texture and other baking defects (Gallaghar et al, 2004). Recently there has been an increasing trend in the GFB formulations by incorporation of different varieties of flour and starch combination like sorghum flour and cassava starch (Onyango et al, 2009) and enzymes like protease (Hamada et al., 2012) dairy proteins, gums like hydrocolloids and xanthum gums into a GF flour base to improve their final baking quality by stimulating a visco-elastic behavior of the batter. Enrichment of gluten free bread (GFB) formulations with fibres like psyllium with HPMC gums (Haque and Morris et al, 1994) showed an improved rheological properties and final quality of the product due to the presence of natural soluble fibre content.

Similarly, natural sources like fruits peels are used as a fortifying agent in GFB formulations as it imitates the role of gluten and improves the visco-elastic behavior due to its high fibre content such as orange pomace in gluten free bread and apple pomace in extruded snack (Shea et al, 2015) and black carrot dietary fibre concentrate and xanthan gum in rice based eggless gluten free muffin (Singh and Kaur et al, 2015). The fruit peels not only increase the visco elasticity of the batter but also improves its nutritive and antioxidant property.

Thus, the aim of this study is to incorporate both raw and ripe mango peel powder (MPP) in equal proportions at different levels in the GFB dough formulations to improve and achieve the visco-elastic behavior as that of the wheat dough.

#### **3.2 MATERIALS & METHODS**

#### 3.2.1 Raw Materials

Raw and Ripe mangoes were procured from an orchard in Kolkata. White refined flour, sorghum flour, rice flour, granulated sugar, salt, refined oil (as shortening agent) were purchased from the local grocery stores of Jadavpur, Kolkata, India. Instant dry yeast (Best Products, Kolkata) used as the leavening agent in bread preparation.

#### **3.2.2 Preparation of Mango peel powder (MPP)**

Raw and ripe mango peels were prepared by peeling freshly bought raw and ripe mangoes which were followed by washing with tap water to remove adhering dust and dirt particles. The peels were placed in a freeze drier (International Commercial Trade, Kolkata) at -40 °C for 15 hr. For ease in utilization the dried peel was powdered using a Grinder ( $GX_7$ , Bajaj Electricals Ltd, India). The powdered peel for subsequent use was stored in air-tight packets in a refrigerator.

#### 3.2.3 Preparation of dough samples

The dough sample of wheat bread consists of wheat flour with water and gluten free dough samples consists of rice: sorghum (1:1) ratio, guar –gum, varying levels of raw and ripe MPP in equal proportions and water.

Type of dough	Ingredients
Wheat dough	Refined wheat flour (10g) and 7.5ml water
Control GFB	Sorghum : rice (5:5),10 ml water, 0.1% guar gum
2%GFB	Sorghum: rice (5:5), 10 ml water, 0.1%guar gum, 2%MPP (1:1raw
	and ripe respectively)
4% GFB	Sorghum: rice (5:5), 10 ml water, 0.1%guar gum, 4%MPP (2:2 raw
	and ripe respectively)
6%GFB	Sorghum: rice (5:5), 10 ml water, 0.1% guar gum, 6%MPP (3:3 raw
	and ripe respectively)

Table 1: Composition of dough samples

#### 3.2.4 Rheological method

Dyanamic oscillatory tests were performed in a controlled stress rheometer (Physica MCR 51; Anton Paar, Graz, Austria). Parallel plates of 49.986mm and 3mm gap were used and the measurements were monitored with RheoPlus software package (version 2.65). A temperature of 25°C was maintained during the measurements with a water circular device (Neslab RTE 7;Thermo Electron Corporation, Bremen, Germany). The samples were rested for 300 seconds before measuring and excess of samples were removed off. A strain sweep test was carried out at a shear stress of 0.1-100 Pa keeping the frequency constant at 1 Hz to obtain the linear viscoelastic region. Based on the results a stress value included in the linear viscoelastic region was used in frequency sweep test with the frequency range varying from (0.1-100) Hz (Mario et al., 2014). The values of elastic modulus G'(Pa), viscous modulus G'' (Pa) are obtained and then is plotted in the graph against the frequency (Hz). Experimental data were described by power-law equations (Mariotti et al., 2009).

 $G' = K' \cdot x^{n_1}$ 

 $G'' = K''.x^{n''}$  Where G' is the storage modulus, G'' is the loss modulus, x is the frequency (Hz) and K', K'' are the consistency index, n' and n'' are flow behavior index.

#### **3.2.5 Statistical analysis**

The experimental design was completely randomized, with three replicates. All data were expressed as mean values  $\pm$  SD. All the experimental data were analyzed statistically for analysis of variance (ANOVA) with Microsoft Excel 2007.The comparisons between the mean values were tested using Duncan's new multiple-range test at a level of p≤0.05.

## **3.3 RESULTS AND DISCUSSION**

A strain sweep test was performed from which a stress value obtained (5 Pa) in the Linear viscoelastic region (LVR) and the frequency sweep test was performed using that stress value to study the effects of MPP on the rheological characteristics of the dough samples. The consistency of the dough samples were studied and being compared with the visco-elastic nature of the wheat dough sample. The MPP enriched gluten free dough sample at 2 % and 4% level shows almost similar consistency index and is higher than the control gluten free dough sample whereas the consistency of the 6% MPP dough sample is lowest due to high content of insoluble fibre (Ahmed et al., 2013). The 2% and 4% dough samples showed a visco-elastic property similar to wheat dough sample. The wheat dough sample exhibits viscoelastic nature where G' is greater than G" and log-log plot of storage modulus (G') versus frequency was well fitted in the power law model with a value of R<sup>2</sup> 0.99. The 2% and 4% dough samples showed similar trend in its visco-elastic property with values of  $R^2 0.96$  and 0.97 respectively in which both are in close agreement with the R<sup>2</sup> value of wheat dough sample according to power law equation suggested by Mariotti et al., 2009. The 6% level MPP dough sample shows a higher G" to that of G' indicating a viscous nature of the dough. The flow index behavior n' and n" respectively for G' and G" showed all the dough samples shows a shear-thinning (pseudoplastic behavior). Dough samples of wheat, 2% and 4% GF showed a similar flow behavior index with low viscosity. The flow behavior index (n' and n") of 6%MPP (G6) showed lower values indicating the behavior of the sample to be less viscous but the consistency index (K' and K") also shows lower values and the G" exceeds G' which dominates the viscous nature.

Types of dough	G'			G''		
sample	K'	n'	$\mathbf{R}^2$	K''	n''	$\mathbf{R}^2$
Wheat dough	5.16±0.25 <sup>a</sup>	0.09±0.01 <sup>b</sup>	0.99	4.36±0.21 <sup>a</sup>	$0.08 \pm 0.004^{d}$	0.54
GFB control	4.98±0.25 <sup>a</sup>	$0.08 \pm 0.01^{bc}$	0.66	3.93±0.19 <sup>b</sup>	0.25±0.01 <sup>a</sup>	0.86
GFB-2%	5.06±0.25 <sup>a</sup>	0.15±0.007 <sup>a</sup>	0.96	4.18±0.21 <sup>ab</sup>	$0.12 \pm 0.006^{\circ}$	0.64
GFB-4%	$5.11 \pm 0.25^{a}$	$0.075 \pm 0.05^{bc}$	0.97	4.33±0.21 <sup>a</sup>	$0.08{\pm}0.004^{d}$	0.54
GFB-6%	$4.77 \pm 0.24^{a}$	$0.042 \pm 0.002^{\circ}$	0.32	$3.47 \pm 0.17^{\circ}$	0.19±0.01 <sup>b</sup>	0.84

Table 2: Application of the power law equation to the frequency sweep test of wheat and gluten free bread dough.

For each parameter values followed by the same letter are not significantly different at  $p \le 0.05$ 

# **3.4 CONCLUSION**

The rheological characteristics of the GF samples incorporated with MPP (raw and ripe) in equal proportions at levels (2,4and 6) % were studied and compared with the wheat dough sample. Addition of 2% and 4% MPP in GF formulations showed a similar value of elastic modulus, consistency index and flow index behavior and fitted the best in the power law model. 2% and 4% dough samples showed the highest values of storage modulus and exhibit a similar nature of viscoelasticity as in wheat dough .The 6% GF dough showed the least viscoelastic nature and gives a negative impact to the final product with poor textural and sensorial properties. Thus the utilization of natural sources proves to be one of the best alternative to improve the rheological characteristics of gluten free dough samples and achieve the viscoelastic region similar to the wheat sample.

# CHAPTER 4:

# EFFECT OF RAW AND RIPE MANGO PEEL ON THE QUALITY OF GLUTEN FREE BREAD

**4.1 Introduction:** The Gluten Free Bread (GFB) usually has a lower profile of physical and sensory parameters when compared with the wheat breads. The addition of hydrocolloids (xanthan and CMC) is used for alleviating the quality parameter of GFB (Mohammadi et al., 2014). Partial replacement of starch content with maltodextrins in GFB formulations often improves the bread volume, crumb hardness and enhances the product quality (Witczak et al., 2010). However application of enzymes (transglutaminase, papain) in GFB formulation helps to improve its physical parameters to a greater extent.

Nowadays fruit peels are also used as fortifying agent in bakery industry due to its high dietary fibre content and antioxidant properties (Mir et al., 2015). Both raw and ripe mango processing industries incurs a huge amount of peel as bio-waste. Recently raw and ripe mango peels have attracted considerable attention in the scientific community due to their high content of valuable compounds, such as phytochemicals, polyphenols, carotenoids, enzymes, vitamin E and vitamin C, which have predominant functional and antioxidant properties which makes it suitable to use as a functional ingredient and to add value to the final product. From the findings of the literature survey it is seen that ripe Mango Peel Powder (MPP) is used as a fortifying agent in various food sectors. Enrichment of wine (Varakumar et al., 2012), incorporation of MPP in wheat based biscuits (Aslam et al., 2014) also enhancement of the antioxidant properties and fibre content in gluten free cookies enriched with MPP (Betsy 2011) are some of the research studies being investigated.

Ripe mango peel has higher antioxidant scavenging potential than the raw mango peels. On the contrary, the total phenolic content is higher in the raw mango peels to that of ripe mango peels. This can be well supported by the data in the literature of (Kim et al., 2010). The higher values of TPC in raw peels may be responsible for the astringency and bitter flavor with low antioxidant property as compared to ripe peels when used for fortification. The combined effect of the raw and ripe mango peel in equal proportion may compensate the negative impacts of the individual peels and brings forth a good quality product with high nutritional and nutraceutical value.

Thus the aim of this research work is to incorporate the mixture of raw and ripe Mango peel powder in equal proportion at 2%, 4% and 6% level in the GFB formulations and to assess the influence of fibres on flour and dough characteristics also to evaluate the physical and chemical (antioxidant) parameters of the bread samples along with the sensory properties of the final product.

#### **4.2 MATERIALS AND METHODS**

**4.2.1 Materials:** Gluten free flours sorghum and rice, sugar, salt, refined oil, egg and instant dry yeast (Best Products, Kolkata) were purchased from the local market of Jadavpur, Kolkata, India. Guar gum (HiMedia Laboratories Pvt. Ltd., Mumbai, India) is used as a thickening agent.

**4.2.2 Chemicals:** The Folin-Ciocalteu reagent, sodium carbonate, ethanol (Merck Specialities Pvt. Ltd., Mumbai, India), gallic acid (sd fine-chem Ltd., Mumbai, India) sodium nitrate, aluminium chloride, sodium hydroxide and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, St. Louis, MO, USA) were used in the investigation.

**4.2.3 Preparation of Mango peel powder (MPP):** Raw and ripe mango peels were prepared by peeling freshly bought raw and ripe mangoes which were followed by washing with tap water to remove adhering dust and dirt particles. The peels were placed in a freeze drier (International Commercial Trade, Kolkata) at -40 °C for 15 hr. For ease in utilization the dried peel was powdered using a Grinder (GX<sub>7</sub>, Bajaj Electricals Ltd, India). The powdered peel for subsequent use was stored in air-tight packets in a refrigerator.

**4.2.4 Bread preparation:** The control bread was prepared by the combination of sorghum flour (SF) and rice flour (RF) in equal ratio (1:1). Straight dough method is employed for the dough preparation. The SF+RF along with other dry ingredients is kneaded with water followed by the other ingredients like egg white and oil. The prepared dough is then put in the mould pan and is then covered with at a wet muslin cloth for proofing for 1hr at 35°C. The proofed dough is then baked in a rotary oven (CM HS108; Chanmag Bakery Machine Co. Ltd., Taipei, Taiwan) at 220±2°C for 20 mins. The baked loaf is further cooled for 1h prior to further physical and chemical analysis. The formulated flours with 2%, 4% and 6% (raw and ripe mango peel powder in equal proportion) follow the same bread making procedure as that of control bread sample.

**4.2.5 Moisture content:** The moisture content of the flours and bread samples were analyzed by (AACC International 2000). 3g of the sample is taken and placed in hot air oven at 105°C for 3 hrs. It is kept to cool and weights are taken.

**4.2.6 pH:** The pH of the different flour samples were measured using a pH meter (Thermo Orion Basic pH Meter, Model 420A pH/mV/ORP/temperature meter).

**4.2.7 Color:** The whiteness index of the flour and bread samples were measured by Hunter Lab color measurement system (Color Flex 45/0, D 65, 100 observer; Hunter Associates Laboratory Inc. Reston, VA, USA). The CIE L\*a\*b\*system was considered. The obtained results were expressed in L\*lightness (0; black to 100; white), a\* redness (–; green to +; red), and b\* yellowness (–; blue to +; yellow) values. The whiteness index of the flour and bread samples was calculated based on the following equation (Ulziijargal et al. 2013). Whiteness index (WI) = 100 -  $([100 - L^* + a^* + b^*))$ .

**4.2.8 Water absorption capacity:** The water absorption capacity of the flours was analyzed from Tsai's method 1998 with slight modifications in it. Water absorption capacity (WAC) is calculated by the following equation:

**4.2.9 Specific volume:** Volumes of the loaves were measured by the seed displacement method. From these, the specific volume (Steffolani et al., 2015) of the loaf was calculated as described in Eq.

Specific volume  $(cm^3/gm) = \frac{loafvolume of bread}{weight of bread}$ 

**4.2.10 Texture Analysis:** The textural profile analysis (TPA) of the crumb of bread samples was determined by the texture analyzer (TA-XT Plus Texture Analyzer, USA.). The analysis was done by a 35mm diameter probe compressing the sliced bread (40 mm  $\times$  40 mm  $\times$ 30 mm) at a test speed of 2mm/s and a trigger force of 5g.Hardness of the crumb was measured based on the peak height and is expressed in terms of g- force.

**4.2.11 Preparation of the extract:** Samples (1 g) in 80% aqueous ethanol was sonicated in a sonicator (Trans-o-sonic/D150-IM, Mumbai) at room temperature (approx.  $25^{\circ}$ C) for proper mixing of particle and centrifuged in cold (4°C) at 8944×g for 15 min and the supernatant extracted by using a filter paper. The extracts were transferred into plastic tubes, and kept in the refrigerator until analysis.

**4.2.12 Total phenolic content:** The total phenolic content (TPC) was determined according to Singleton et al., 1997. After incubation at room temperature for 90 min, the absorbance of the mixture was observed at 750 nm. The results were expressed both as mg of gallic acid equivalents (GAE) per 1 gram of sample.

**4.2.13 Total flavonoid content:** The total flavonoid content of samples was determined with slight modifications of Xu and Chang, 2007. Total flavonoid contents were expressed as milligrams of catechin equivalents (CAE) per 100 gram of defatted sample.

**4.2.14 DPPH radical scavenging assay:** The free radical scavenging capacity of sample extract was determined using the stable 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH). The decrease in absorbance of the resulting solution was measured spectrophotometrically at 517 nm (Yu et al., 2003). The percentage of inhibition or the percentage of discoloration was calculated as described in Eq.

% Inhibition= (Abs blank-Abs sample) ×100/Abs blank

**4.2.15 Sensory evaluation:** Sensory parameters of the control and bread sample were evaluated by a panel of 10 members including students, staff, and faculty of the Dept. of Food Technology and Biochemical Engineering, Jadavpur University, Kolkata. Sensory parameters evaluated were appearance, color, flavor, taste and overall acceptability for the bread samples based on 9-point hedonic scale ranging from like extremely (9) to dislike extremely (1) for each characteristics. The panelists were trained to become familiar with the Hedonic grading scale as well with these sensory parameters. The panelists were seated in individual sensory booths. Samples were sliced and placed on white plates with given codes. Each panelist was given the control and the formulated samples for evaluation with water for mouth rinsing after each set of evaluation. The evaluation process is repeated thrice.

**4.2.16 Statistical analysis:** All the studies were replicated 3 times and the means were reported. All the experimental data were analyzed statistically for analysis of variance (ANOVA) with Microsoft Excel 2007. Means were compared by Fisher's least significant difference test at a significance level of  $p \le 0.05$ .

#### 4.3 Results and Discussions

#### 4.3.1 Characterizations of flours and its formulations

The physical properties of flours and its formulations are tabulated in Table 1. It is evident that the moisture content (%) of the SF is  $6.61\pm0.33$  and in RF is  $7.93\pm0.4$  both has a markedly significant difference (P $\leq 0.05$ ). The moisture content of the combination of RF and SF in equal proportions is  $7.37\pm0.37$ . There is an increasing trend of moisture content in the flours formulated with 2%, 4%, 6% MPP (equal proportion of both raw and ripe) respectively. However in there is a slight increase in the pH of the flours formulated with MPP compared to that of the SF, RF, SF+RF respectively with no such significant difference. Color of flour has an impact on the crumb color of the final product which is an important parameter to characterize the GFB from the wheat bread .The whiteness index (WI) of the rice flour is reported to be highest in the table whereas the combination of RF and SF flours has a whiteness index (WI) greater than that of SF alone. In the formulated flours of 2%, 4% and 6% there is a decreasing trend in the whiteness index (WI) due to the addition of the MPP. Water absorption capacity of the flour is an important parameter for the dough development and quality of the final product.

Samples	Moisture	рН	WI	WAC
	content (%)			(g/g)
SF	6.61±0.33*	6.5±0.33	79.83±0.83*	3.19±0.09
RF	7.93±0.4*	6.55±0.33	88.67±0.10*	2.91±0.14*
SF+RF	7.37±0.4*	6.48±0.32	82.54±0.05*	2.99±0.15*

SF+RF+2% MPP	8.66±0.43	6.88±0.34	81.23±0.14*	3.09±0.17
SF+RF+4% MPP	8.85±0.44	6.78±0.34	78.58±0.05*	3.33±0.17*
SF+RF+6% MPP	9.02±0.45*	6.62±0.33	77.13±0.05*	3.28±0.16

Table 1: Physical and functional characteristics of different flour samples

Data represent mean value of three samples  $(n=3) \pm SD$ 

Mean values with superscripts (\*) within the same column are significantly different ( $p \le 0.05$ )

#### 4.3.2 Physical properties of bread

The physical parameters of the bread samples are tabulated in Table 2. The height and specific volume is an important quality parameter of the bread loaf. There exists a relationship between height and specific volume of the loaf to that of the water absorption capacity of the dough. The MPP enriched flour samples of 2% and 4% shows a significant increase in the height and specific volume of the loaf compared to that of control. After a significant increase, the height and specific volume tends to decrease on incorporation of MPP at 6% formulation. This is due to the lower consistency and higher water absorption capacity of the dough resulting in unstable air vacuoles within the crumb structure owing to the failure of retention of carbon dioxide gas bubbles resulting in a decrease in the height and specific volume (Shea et al., 2015).

The moisture content of the bread sample reported in Table 2 is agreeable to the values of chestnut flour added GFB (Ilkem et al., 2013). The dietary fibre in MPP has high water binding capacity and allows enhances water absorption capacity of flour as a result increases the moisture content of the formulated bread samples with MPP significantly (P $\leq$ 0.05) to that of control sample (Kamp et al., 2010). The explanation can be further supported with the values of WAC of the flour samples reported in Table 1.

Color of the final bread is another important parameter for consumer acceptability. The color of the crumb is measured by whiteness index (WI). The higher the amount of MPP for fortification the more is the amount of carbohydrates and amino acids present which is responsible for the browning of the crust due to Maillard reaction. The whiteness index (WI) of the bread samples has a significant decrease from ( $64.4633\pm0.015$  to  $49.79\pm0.580$ ). Thus formulated bread samples have a darker crumb color compared to the control sample. The 6% formulated bread sample shows the poorest crumb color of whiteness index compared to the control and other formulated samples rendering it to be unacceptable.

The crumb texture of the bread samples are reported in Table 2. The inverse correlation between the specific volume and hardness of the bread explains the changes of the texture profile of the bread samples (Ziobro et al., 2013).4% level of MPP the volume of the bread is maximum with a softer crumb texture. On addition of 6% MPP there is a significant increase in hardness of the bread compared to the control and other formulated samples due to its high fibre content resulting in dense crumb and more compact cells.

Samples	Height (cm)	Specific	Moisture	Color (WI)	Texture
		volume (cm³/g)	content (%)		(g-force)
SF+RF	5.61±0.28	1.25±0.06	45.00±0.45*	64.46±0.02*	1775.5±28.8 <sup>*</sup>
SF+RF+2% MPP	5.8±0.29	1.21±0.06	47.36±0.86*	54.59±0.58*	2235.33±40
SF+RF+4% MPP	6.0±0.3	1.29±0.06*	48.91±0.85	53.17±0.08*	1921.3±37.5*
SF+RF+6% MPP	5.63±0.29	1.15±0.05*	49.5±0.93*	49.79±0.58*	$2285 \pm 50^{*}$

Data represent mean value of three samples  $(n=3) \pm SD$  Mean values with superscripts (\*) within the same column are significantly different (p $\leq 0.05$ ) Where, WI= Whiteness index

#### 4.3.3 Chemical properties of bread

The total phenolic content of the samples were estimated by Folin- Ciocalteau method. There is a linear significant increase (P $\leq$ 0.05) in the phenolic content of the bread samples enriched with MPP. It ranges from (0.18mgGAE/g to 1.85mgGAE/g) from control to 6% level. Incorporation of MPP results in 9.15 fold increase of the phenolic content in the bread samples. This is due to the presence of the high phenolic content in the raw and ripe mango peel which ranges from 55 to 110 mg/g peel (Ajila et al., 2007). Inspite of loss of polyphenols during baking there is still an increasing trend of the bread samples enriched with MPP.

Total flavonoid components of the bread significantly increases on fortifying with MPP. With the increase in the MPP level the flavonoid component is enhanced from (0.08mgCE/g-0.14mgCE/g) from control bread to 6% MPP level. Mango peel is rich in flavonoids (Marina et al., 2014) which results in the increase of the flavonoid component of the bread samples.

The free radical scavenging potential of the MPP formulated bread samples are determined by DPPH method. It is a stable free radical which has a characteristic absorption at 517nm.The antioxidants present in the MPP react with DPPH radical and convert it to 2, 2-diphenyl-1-picrylhydrazine.The scavenging potential of the antioxidants is due to its hydrogen donating ability and the degree of discoloration of DPPH reflects scavenging potential of the analysed sample. The increase in the scavenging potential of the bread samples formulated with MPP from (0.34% to 34.15%) is due to the higher amount of the polyphenols and flavonoids present in MPP (Ajila et al., 2010). The result implies that no such significant impact on antioxidant compounds during processing of the sample is observed. MPP enhances both the nutritive as well as nutraceutical value of the bread samples due to its high fibre and phenolic content as well as its antioxidant activity.

Samples	Total phenolic content (mg GAE/g)	Flavonoids (mg CE/g)	DPPH (% inhibition)
SF+RF	0.18±0.009*	0.08±0.004*	0.34±0.016*
SF+RF+2% MPP	0.64±0.39*	0.09±0.002*	5.74±0.28*
SF+RF+4% MPP	1.25±0.06*	0.123±0.004*	15.87±0.79*
SF+RF+6% MPP	1.85±0.28*	0.14±0.005*	34.15±0.85*

Table 3: Antioxidant properties of GFB samples on addition of MPP at different levels Data represent mean value of three samples (n=3) ±SD Mean values with superscripts (\*) within the same column are significantly different ( $p\leq 0.05$ )

#### 4.3.4 Sensory evaluation

The bread samples prepared with MPP at different levels were subjected for sensory evaluation in table 4 for appearance, color, flavor, taste and overall acceptability. The sensory parameters like color and appearance upto 4% MPP level have similar scores and are as acceptable to that of the control bread. On increasing level of MPP upto 6% the intensity of whiteness decreases which is supported by the WI values in Table 2. The appearance of the control, 2%, 4% is similar with no much significant difference in it. On increased level of 6% MPP poor crumb appearance is observed due to the fibrous structure making the network dense thereby decreasing the porosity of the loaf. The taste and flavor of the bread samples is enhanced on formulating it with MPP as it has a pleasant characteristic mango flavor. On increasing the level upto 6% there is a slight bitter taste in the sample due to the high polyphenolic content. These data resembles to that of MPP formulated biscuit (Aslam et al., 2014). The overall acceptability of the bread samples shows highest score for 4% formulated sample as compared to control, 2% and 6% respectively The lower score for 6% formulated bread is due to the undesirable crumb appearance, color and taste. Thus it can be concluded that formulating the sample with 4% MPP gives the best sensory attributes.

Samples	Appearance	Color	Flavor	Taste	Overall
					Acceptability
SF+RF	8.4±0.36*	8.03±0.05*	6.67±0.58*	7.33±0.57*	7.63±0.32*
SF+RF+2% MPP	7.6±0.53*	7.0±0.5*	7.77±0.25*	7.93±0.12	7.56±0.25*
SF+RF+4% MPP	8.0±0.5	6.5±0.5*	8.1±0.36*	8.13±0.32*	7.87±0.05*
SF+RF+6% MPP	6.33±0.29*	4.5±0.5*	6.0±0.5*	3.83±0.29*	5.13±0.05*

Table 4: Effects on the sensorial attributes of MPP incorporated GFB samples at different levels

Data represent mean value of three samples  $(n=3) \pm SD$  Mean values with superscripts (\*) within the same column are significantly different (p $\leq 0.05$ )

## 4.4 Conclusion:

The physical, functional and rheological properties of the flour samples fortified with equal proportion of raw and ripe MPP at 2%, 4%, 6% level for gluten free bread preparations were studied to correlate with the physical characteristics of the final bread. The dough sample at 4% level MPP exhibits maximum viscoelasticity compared to that of the control, 2%, 6% sample. Generally incorporation of mango peel powder (MPP) at increased levels increases the antioxidant properties but decreases the physical and sensory parameters simultaneously after reaching a certain level. The fortification of gluten free bread with 4% MPP shows optimum increase in height, specific volume, crumb hardness and sensory properties thus offering it to be the highest acceptable fortified bread. The novelty of this research is to emphasize the combined effects of raw and ripe mango peel powder incorporated at different levels in GFB formulations and its influence and on the final bread quality.

# CHAPTER 5:

# A COMPARATIVE STUDY BETWEEN WHEAT BREAD AND GLUTEN FREE BREAD QUALITY THROUGH PRINCIPAL COMPONENT ANALYSIS

#### **5.1 INTRODUCTION**

Fruit peel waste has received much attention mainly in bakery, dairy and beverage industries as they are great source of antioxidants and dietary fibre. Development of novel foods by adding functional components to the food product provides new opportunities for manufacturers (Krutulyte et al, 2011). From the literature survey it can be cited that use of certain fruit peels in bakery products have enhanced the functionality of the final product like incorporated Mango peel powder in biscuits at varying levels to show the positive effects both in its physical and sensory properties upto certain levels (Ashoush and Gadallah, 2014). Similarly, peels of pomegranate are used for fortification in pan bread (Sayed-Ahmad, 2014). On the other hand there is an increase in gluten free products especially gluten free breads as a result of celiac diseases (Cureton and Fasano, 2009). Gluten free breads is a major challenging issue for the technologists. Due to the absence of gluten, the gas retaining capacity is less resulting in a weaker dough or batter which finally affects the volume and texture of the product. To overcome these, GFB formulations are modified with combination of gluten free flours and starches like tartary and chia flour and fibres like pysillium and sugar beet fibre (Cappa et al, 2013) and are used to enhance the phytochemicals content as well as the physical and sensory properties. Gluten free diets also uses natural sources like apple pomace, mango peel powder in brown rice crackers (Mir et al, 2015) and cookie respectively (Betsy, 2011) to increase the antioxidant activity and shows a positive impact on the physical and sensory properties up to a certain level. Fortification in bread enriches the phenolic and antioxidant activity but simultaneously reduces the sensory quality of the product after a certain level thereby reducing the consumer acceptance (Glanz et al, 1998).

From the literature survey it can be cited that different characteristics of gluten free breads have been evaluated to assess their quality. Despite of the differences in the formulation and characteristics of the GFB to the gluten breads (wheat, whole-wheat, rye and maida) methods applied for evaluation is common. Specific volume, loaf weight, moisture content, color parameters and texture analysis are some of the instrumental parameters to characterize the gluten free breads. Sensory analysis is considered in some of the GFB formulations (Morais et al., 2013). A correlation between physical and sensory parameters to define the quality attributes have not been previously established in wheat and gluten free breads. In addition to that, Principal component analysis (PCA) is employed to identify the best parameters of the quality of the wheat and gluten free bread respectively and draw a comparison between their qualities.

Thus, the aim of the research work is to characterize a range of Wheat bread (WB) and gluten free bread (GFB) samples enriched with raw and ripe mango peel powder in equal proportions and to identify the principal components of both physical and sensory parameters. Four samples of wheat bread enriched raw and ripe MPP in equal proportions at (2, 4, 6) % including control and four samples of GFB enriched raw and ripe MPP in equal proportions at (2, 4, 6) % including control were evaluated with respect to their physical parameters (Specific volume, moisture content, color and texture) and sensory parameters (Traditional bread aroma, fruity aroma, after taste, crumb appearance, crumb color, porosity).

#### **5.2 MATERIALS AND METHODS**

#### 5.2.1 Raw Materials

Raw and Ripe mangoes were procured from an orchard in Kolkata. White refined flour, sorghum flour, rice flour, granulated sugar, salt, refined oil (as shortening agent) were purchased from the local grocery stores of Jadavpur, Kolkata, India. Instant dry yeast (Best Products, Kolkata) used as the leavening agent in bread preparation.

#### 5.2.2 Preparation of Mango peel powder (MPP)

Raw and ripe mango peels were prepared by peeling freshly bought raw and ripe mangoes which were followed by washing with tap water to remove adhering dust and dirt particles. The peels were placed in a freeze drier (International Commercial Trade, Kolkata) at -40 °C for 15 hr. For ease in utilization the dried peel was powdered using a Grinder ( $GX_7$ , Bajaj Electricals Ltd, India). The powdered peel for subsequent use was stored in air-tight packets in a refrigerator.

#### 5.2.3 Bread Composition

The ingredients and their proportions required for the preparation of wheat bread samples were refined wheat flour 100g, sugar 6.0%, salt 2 %, instant dry yeast 3%, refined oil 4.0% and water 60.0%. The dry mix was prepared by mixing flour, sugar and salt. Baker's compressed yeast was dissolved in water (20 ml) and kept at the 37°C for the activation of the yeast cells. Small amount of sugar (2gm) were added to the warm water for activation of the yeast cells. Mixing is an important step for achieving homogenous as well as soft dough. Here, mixing was carried out manually according to the straight dough method. The dry ingredients, shortening and the activated yeast were added in a bowl and water added and then kneaded until the dough was elastic and the required consistency was reached. After this, the dough was rounded and kept in a bowl for the first proofing at the room temperature (30°C) for about 40 min. The bowl was covered with a wet cloth to maintain a relative humidity of 80-90%. After the first proofing, the dough was punched and worked lightly so that the excess gas could escape and the gas cells are redistributed. The dough was then shaped to fit lightly in greased bread molds. The dough was again kept for the final proofing for about 1 hour at 40°C. Finally, after second proofing, the breads in molds were baked in rotary oven (CM HS108, Chanmag Bakery Machine Co. Ltd., Taiwan) at different experimental temperatures and time. After baking, the prepared bread samples were cooled for about 1 hour at room temperature and then analyses carried out. The formulated flours with 2%, 4% and 6% (raw and ripe mango peel powder in equal proportion) follow the same bread making procedure as that of control bread sample.

#### 5.2.4 Gluten free bread

The control bread was prepared by the combination of sorghum flour (SF) and rice flour (RF) in equal ratio (1:1). Straight dough method is employed for the dough preparation. The SF+RF along with the activated yeast are kneaded with water followed by the other ingredients like sugar, egg white, guar gum, oil and salt. Proofing and baking procedures are similar with wheat bread. The formulated flours with 2%, 4% and 6% (raw and ripe mango peel powder in equal proportion) follow the same bread making procedure as that of control bread sample.

#### 5.2.5 Loaf specific volume

Bread samples were weighed after cooling and the volume of the loaves were measured by the seed displacement method (Steffolani et al., 2015). The specific volume  $(cm^3/g)$  was calculated as described in

Specific volume  $(cm^3/gm) = \frac{Loaf volume of bread}{Weight of bread}$ 

**5.2.6 Crumb Moisture** The moisture content of the flours and bread samples were analyzed by (AACC International 2000). 3g of the sample is taken and placed in hot air oven at 105°C for 3 hrs. It is kept to cool and weights are taken.

**5.2.7 Color:** The whiteness index and brownness index of the bread samples were measured using Hunter Lab color measurement system (Color Flex 45/0, D 65, 100 observer; Hunter Associates Laboratory Inc. Reston, VA, USA). The CIE L\*a\*b\*system was considered. The obtained results were expressed in L\*lightness (0; black to 100; white), a\* redness (–; green to +; red), and b\* yellowness (–; blue to +; yellow) values. The whiteness index of the flour and bread samples was calculated based on the following equation (Ulzijargal et al. 2013). Whiteness index (WI) =  $100 - ([100 - L^* + a^* + b^*))$  and of brownness index (BI) (Shittu et al., 2007) of the bread samples were calculated by the equation:

$$BI = \frac{[100(x-0.31)]}{0.17}$$
  
Where x =  $\frac{(a+1.175L)}{5.645L + a - 3.012b}$ 

# 5.2.8 Crumb hardness

The texture profile analysis (TPA) was carried out to evaluate crumb hardness using a texture analyzer. A 35 mm diameter aluminium cylinder probe was used to measure the required compression force. The optimal test conditions were 30-60% deformation, 0.1-2 mm/s cross-head speed and variance coefficient of plunger compressing a sample to 50% of its original height at 0.8 mm/s speed. The dimensions of crumb samples were 40 mm x 40 mm x 30 mm. The data were collected using software, Texture Expert (v 1.22, Texture Technologies, Scarsdale, NY, USA).

**5.2.9 Sensory evaluation:** Sensory parameters of the control and bread sample were evaluated by a panel of 15 members including students, staff, and faculty of the Dept. of Food Technology and Biochemical Engineering, Jadavpur University, Kolkata. Sensory parameters evaluated were Traditional bread aroma (TBA), Fruity aroma (FA), After taste (AT), Crumb appearance (CA), Crumb Color (CC), Porosity (P) for the bread samples based on 9-point hedonic scale ranging from like extremely (9) to dislike extremely (1) for each characteristics. The panelists were trained to become familiar with the Hedonic grading scale as well with these sensory parameters. The panelists were seated in individual sensory booths. Samples were sliced and placed on white plates with given codes. Each panelist was given the control and the formulated samples for evaluation with water for mouth rinsing after each set of evaluation. The evaluation process was repeated thrice.

#### 5.3.0. Statistical Analysis

All the studies were replicated 3 times and the means were reported. All the experimental data were analyzed statistically for analysis of variance (ANOVA) with Microsoft XLSTAT 2007. Means were compared by Duncan test at a significance level of  $p \le 0.05$ .

# **5.3 RESULT AND DISCUSSION**

#### 5.3.1 Physical analysis

The characterization of the wheat and gluten free bread formulations was carried out to identify the most discriminating parameters The instrumental parameters analyzed were moisture content, specific volume, whiteness index, brownness index and texture in terms of hardness. Analysis of data collated using ANOVA showed the instrumental parameters to be significantly different ( $p\leq0.05$ ) among the analyzed bread samples and are tabulated in Table 1. The specific volume of the wheat bread samples upon incorporation of MPP (both raw and ripe in equal proportions) showed significant differences among each other as well with the gluten free bread formulations. The specific volume of the control wheat bread sample showed the highest value of  $3.80\pm0.19$ cm<sup>3</sup>/g. WB2 and WB4 showed a similarity in their specific volume and varied significantly. The trend of decreasing in specific volume of wheat breads upon MPP incorporation is in close agreement with the findings of Ahmad, 2015. The specific volume of GFB formulations shows no such significant differences among them but shows a similarity in the values as in Marco and Rosell, 2008. Generally the moisture content of gluten free breads is comparatively higher than the normal wheat breads. The fibre enriched gluten free breads are usually in the range of 49-53/100g (Sabanis et al., 2009). There is a much variation among all the bread samples and are relatively significant to each other. The color of the crumb has also been an important parameter and is measured in terms of its whiteness index. Great variability was observed in lightness. WBC and GFBC showed the highest value of (66.64±0.07 and 64.46±0.02) respectively compared to the rest of the bread samples. The darkening of the crumb color in GFB2, GFB4 were desirable as gluten free breads usually tend to have lighter color than wheat breads as in agreement with the findings of Gallagher et al., 2003. The bread samples shows a decreasing effect on the BI may be due to the combined effect of raw and ripe mango peel powder (MPP). The greenish color of raw MPP suppresses the effect of yellowish color imparted by ripe MPP and thus had a decreasing impact on the overall BI unlike the effect on BI of Ripe mango peel powder in biscuits by Gadallah et al., 2011. The hardness of the GFB samples showed higher values compared to the wheat bread samples which is mainly due to its complex formation, mainly based on carbohydrate suggested by Matos and Rosell, 2011.

SAMPLE CODES	SP VOL (cm <sup>3</sup> /g)	MC (%)	WI	BI	TEXTURE (g-force)
WBC	3.80±0.19 <sup>a</sup>	37.27±0.95 <sup>e</sup>	66.64±0.07 <sup>a</sup>	50.73±0.47 <sup>b</sup>	1104.67±55.50 <sup>e</sup>
WB2	3.34±0.17 <sup>b</sup>	37.72±0.56 <sup>e</sup>	61.90±0.41 <sup>b</sup>	48.88±0.27 <sup>c</sup>	1248.00±36.43 <sup>d</sup>
WB4	3.5±0.17 <sup>ab</sup>	38.17±0.98 <sup>e</sup>	58.94±0.25 <sup>b</sup>	39.94±0.38 <sup>e</sup>	$1421.00\pm70.50^{\circ}$
WB6	2.8±0.14 <sup>c</sup>	$40.53 \pm 0.92^{d}$	$49.07 \pm 0.62^{\circ}$	38.30±0.46 <sup>f</sup>	1594.00±75.50 <sup>c</sup>
GFBC	$1.25 \pm 0.06^{d}$	$45.00\pm0.45^{\circ}$	64.46±0.02 <sup>d</sup>	52.31±0.74 <sup>a</sup>	1774.66±88.50 <sup>b</sup>
GFB2	$1.21 \pm 0.07^{d}$	47.37±0.87 <sup>b</sup>	54.59±0.59 <sup>d</sup>	41.42±0.41 <sup>d</sup>	1889.00±55.43 <sup>b</sup>
GFB4	$1.24{\pm}0.07^{d}$	48.92±0.85 <sup>a</sup>	53.17±0.08 <sup>d</sup>	38.75±0.28 <sup>f</sup>	2197.00±64.09 <sup>a</sup>
GFB6	1.15±0.06 <sup>d</sup>	49.50±0.93 <sup>a</sup>	49.79±0.58 <sup>d</sup>	34.16±0.21 <sup>g</sup>	2308.00±82.92 <sup>a</sup>

Table 1: Physical parameters of MPP incorporated bread samples

WBC: Control wheat bread, WB2- 2% MPP, WB4- 4% MPP, WB6- 6% MPP. GFBC- Control gluten- free bread, GFB2- 2% MPP, GFB4- 4% MPP, GFB6- 6% MPP.

SP VOL: specific volume, MC: moisture content, WI: whiteness index, BI: Brownness index.

For each parameter values followed by the same letter are not significantly different  $p \le 0.05$ .

#### 5.3.2 Sensory evaluation

Sensory parameters were evaluated by 15 panelist members following the hedonic scale method. The parameters chosen for sensory are interlinked to each other and was well explained with findings of the literature review. The Traditional bread aroma (TBA) and fruity aroma (FA) shared an inverse relationship. WBC and GFBC scores inferred that the control samples had similar bread like aroma as of the commercial bread. Fruity aroma (FA) showed highest value in WB6 due to the intrinsic aroma of the MPP added at 6% level in the WB formulation. On the contrary FA decreases in GFB6 may be due to the dominant nature of the aromatic compounds of gluten free flours. The after taste (AT) scores of WB2 and GFBC had no significant difference and is quite close to the score of WBC whereas WB4 and GFB4 shows no such variation between them but decreases due to the increase in levels of MPP. The samples of WB6 and GFB6 scored the lowest due to the high phenolic content present in MPP and it resembles with the data findings of Aslam et al, 2014. The crumb appearance (CA) and porosity (P) showed a direct relationship where the MPP enriched samples obtained scores in a decreasing order. This may be due to the high fibre content in MPP studied by Larrauri et al, 1997. GFB formulations showed lower values in P and CA due to the complex formation suggested by Matol and Rosell, 2011 and high fibre content (Larrauri et al., 1997). The crumb color was darker as the levels of formulation were higher. This was due to the combined effect of the color imparted by raw and ripe MPP.

SAMPLE	TBA	FA	AT	СА	CC	Р
CODES						
unc		<b>2</b> 0 <b>7</b> 0 1 5 <sup>d</sup>	0.47.0.1.6	0.0010.003		0.05.0048
WBC	$8.20 \pm 0.36^{\circ}$	$2.07\pm0.15^{\circ}$	8.47±0.16°	8.38±0.20*	8.78±0.20*	8.25±0.04*
WB2	7.20±0.20 <sup>b</sup>	3.30±0.09 <sup>c</sup>	7.73±0.07 <sup>b</sup>	7.52±0.34 <sup>ab</sup>	$7.74 \pm 0.08^{b}$	7.57±0.22 <sup>b</sup>
WB4	6.72±0.17 <sup>cd</sup>	4.60±0.1 <sup>b</sup>	6.60±0.08 <sup>d</sup>	7.05±0.24 <sup>b</sup>	6.85±0.05 <sup>cd</sup>	7.14±0.21 <sup>bc</sup>
WB6	5.60±0.18 <sup>e</sup>	6.23±0.38 <sup>b</sup>	5.48±0.32 <sup>e</sup>	6.71±0.23 <sup>b</sup>	5.30±0.04 <sup>e</sup>	5.67±0.24 <sup>d</sup>
GFBC	$7.93 \pm 0.12^{a}$	$1.33 \pm 0.58^{\rm e}$	7.76±0.10 <sup>b</sup>	7.67±0.13 <sup>ab</sup>	8.53±0.11 <sup>a</sup>	7.33±0.58 <sup>b</sup>

GFB2	7.00±0.1 <sup>bc</sup>	1.67±0.58 <sup>de</sup>	7.23±0.08 <sup>c</sup>	7.23±0.35 <sup>b</sup>	$7.00\pm0.50^{c}$	6.77±0.25 <sup>c</sup>
GFB4	6.40±0.36 <sup>d</sup>	3.36±0.19 <sup>c</sup>	$6.84{\pm}0.04^{d}$	7.23±0.35 <sup>b</sup>	6.50±0.09 <sup>d</sup>	6.78±0.11 <sup>c</sup>
GFB6	5.40±0.36 <sup>e</sup>	4.81±0.27 <sup>b</sup>	$5.27 \pm 0.04^{e}$	5.47±0.38 <sup>c</sup>	5.16±0.31 <sup>e</sup>	5.25±0.17 <sup>d</sup>

Table 2: Sensory parameters of MPP incorporated bread samples

WBC: Control wheat bread, WB2- 2% MPP, WB4- 4% MPP, WB6- 6% MPP. GFBC- Control gluten-free bread, GFB2- 2% MPP, GFB4- 4% MPP, GFB6- 6% MPP.

TBA: Traditional bread aroma, FA: Fruity aroma, AT: After taste, CA: Crumb appearance, CC: Crumb Color, P: Porosity.

For each parameter values followed by the same letter are not significantly different at  $p \le 0.05$ 

### **5.3.3 PRINCIPAL COMPONENT ANALYSIS**

PCA is mainly done to identify and combine variables that are not truly independent and also to maximize the variance of the experimental data which can be easily explained with that of grouped variables. In this study, PCA was applied to interpret the results of the experimental data of both instrumental and sensory for the respective bread formulations and to discriminate the best among all the bread samples. The pictorial representation and the plot obtained can be checked and correlated well with the experimental data tabulated in Tables 1 and 2.

The first PCA was performed on the physicochemical parameters analyzed in the bread samples (Fig.1). The first two principle components, F1 and F2, accounted for 96.98 % of total variance (75.11 and 21.86) % respectively. The first component (F1) is characterized by major positive levels of MC and Texture and negative level of SP VOL. For the second principal component the (F2), the variables BI, WI showed higher positive values.

The bread samples were located in two zones of the plot (Fig.1). The GFB2 and GFB4 were located in the positive zones of F1 and characterized by moisture content and texture. The whiteness index (WI) and brownness index (BI) has a positive impact on the GFBC and WBC and was located in the positive zone of F2. Some positive effect on WI and BI was observed for WB2. WB4 and WB2 lie in the negative zone of F2 and are correlated well with the specific volume. Therefore, color parameters could be enough to segregate the control samples from the formulated ones.

FIG 1: (A)

#### FIG 1: (B)

FIG 1: PCA plot of physical variables (A) and Bread samples (B)

WBC: Control wheat bread, WB2- 2% MPP, WB4- 4% MPP, WB6- 6% MPP.GFBC- Control gluten-free bread, GFB2- 2% MPP, GFB4- 4% MPP, GFB6- 6% MPP.

SP VOL: specific volume, MC: moisture content, WI: whiteness index, BI: Brownness index.

The data of six sensory parameters were also analyzed to find the interrelation between the formulations and its acceptability using PCA (FIG.2). The first two principal components F1 and F2, accounted for 93.88% of total variance (85.03 and 8.84) % respectively. The first component (F1) showed positive values with all the sensory parameters mainly with CA and P and a

negative value of FA whereas the second component (F2) showed a highly positive correlation of FA, CA and P but a slight positivity to AT, CC and TBA.

Sensory profiling showed almost all the formulations to have positive values and well correlated. WBC was located in the positive zone of F1 and F2 and highly correlated with CA and P. WB2 which is also located in the positive zone of both the axes had a positive value of CA and P. A correlation between WB6 with FA was clearly depicted in the positive plot of F2. GFB4 and WB4 can be correlated with WB2 with respect to WB2 due to their positioning in between the axes of the positive and negative sides of F1.Both GFBC and WBC was located in the positive zone of F1 and shows a positive value for AT, CC and TBC. It was also observed GFB2 shows a slight positive dependency on AT, CC, and TBA. GFB6 located in the negative zone of F2 shows no correlation with any of the sensory parameters.Variations in the formulations shows positive effects with improvements in certain parameters.

# FIG 2: (A)

FIG 2: PCA plot of sensory variables (A) and Bread samples (B).
WBC: Control wheat bread, WB2- 2% MPP, WB4- 4% MPP, WB6- 6% MPP.GFBC- Control gluten-free bread, GFB2- 2% MPP, GFB4- 4% MPP, GFB6- 6% MPP.

TBA: Traditional bread aroma, FA: Fruity aroma, AT: After taste, CA: Crumb appearance, CC: Crumb Color, P: Porosity.

FIG 2: (B)

## **5.4 CONCLUSION**

The assessments of the instrumental parameters of the wheat bread samples and gluten free bread samples showed a great divergence and the same observations were perceived in the sensory evaluation. Sensory analysis showed a great divergence in crumb appearance (CA), porosity (P), and crumb color (CC). The variations in the formulations showed a positive impact in certain physical and sensory parameters which can be further used for new formulations. Therefore the color and sensory parameters would be the best to characterize the gluten free and wheat bread samples. It can be also used to derive the relationship between the sensory and physical characteristics and their combined effects on the samples in further studies.

## CONCLUSION

In this study, incorporation of Mango peel powder (raw and ripe) in equal proportions in both wheat and gluten free breads showed a positive impact on the physicochemical and sensorial attributes upto certain fortification level. The fortification level of ripe MPP in wheat bread samples were optimized which showed the best impact on the physiochemical parameters. The combination of the raw and ripe MPP in equal proportions showed viscoelastic nature of the gluten free doughs similar to wheat dough upto a certain level. The incorporation of the raw and ripe mango peel increases the antioxidant properties of the gluten free bread samples and enhances the physical and sensorial parameters upto a certain level. The principal component analysis of the MPP enriched wheat and gluten free breads showed a significant variation. The color (Whiteness index and Brownness Index) and sensory parameters were found to be the best to differentiate among the samples.

## FUTURE SCOPE

In the further research work, raw and ripe mango peel can be analyzed based on their individual phytochemicals content, dietary fibre content and other proximate analysis that would be required to enhance the nutraceutical value to a much greater extent for the MPP incorporated breads. Combination of raw and ripe mango peel can also be used in other gluten free bread formulations and other gluten free products to study its effects on the physicochemical and sensory parameters. Due to its high phenolic and fibre content, utilization of the raw and ripe mango peels in a much more effective and broader aspect should be done with a target of increasing its demand in the food sectors and markets.

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