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Galali, Y

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Study the influence of waxy wheat flour, inulin and guar gum on quality and microstructure of Pita and Tandoori breads: response surface methodology aids functional food development

Yaseen Galali[1] Gail Rees[2] & Victor Kuri[3]

[1]Food Technology Department, College of Agricultural Engineering Sciences, Salahaddin University-Erbil, Erbil, KRG, Iraq

[2]School of Biomedical Sciences, University of Plymouth-UK, Drake Circus, Plymouth, PL48 AA, UK

[3]School of Biological and Marine Sciences, University of Plymouth, Drake Circus, Plymouth, PL48 AA, UK

Corresponding author:

Yaseen Galali

Email: yaseen.galali@su.edu.krd

Abstract:

A diet high in dietary fibre (DF) is thought to help reduce cholesterol levels, may control blood glucose levels and reduce gut transit time. However, people often fail to consume the recommended quantity of DF. The aim of this study was to supplement two types of bread with some novel functional food ingredients; waxy wheat flour (WF), inulin (IN), guar gum (GG) to develop products rich in DF. Response surface methodology (RSM) was employed to study the effect of DF on two different breads. Breads were assessed using Cryo-SEM for its microstructure pattern, and physical attributes (pita; springiness, chewiness and hardness, Tandoori; hardness and elasticity). The results showed that, for pita bread, the outcome showed that IN and GG alone significantly increased hardness and chewiness and decreased springiness. WF addition only decreased springiness. GG linearly increased pita height and volume, but it quadratically increased weight loss. Moreover, IN increased volume and height in a quadratic way. Regarding Tandoori bread, IN reduced bread toughness, but showed no effect on extensibility. WF alone increased volume and diameter. The microstructure also showed that the

novel ingredients modified starch gelatinisation and gluten-starch matrix in both pita and Tandoori breads differently. Therefore, the influence of the three functional ingredients alone and in combinations influenced quality parameters dissimilarly.

Keywords: Pita/Tandoori bread, Inulin; guar gum; Response surface methodology, Cryo-SEM.

1. Introduction

Diet related diseases are steadily increasing in modern society as a consequence of following an imbalanced diet; high in energy and low in dietary fibre (DF). It is known that a diet rich in DF can have positive effects on health, but still people fail include enough DF in the daily diet (Evans, 2020). The development of functional foods rich in DF that do not alter quality and texture from the usual product might be a useful addition to the consumer's diet. Consequently, imparting more health benefits to the consumers.

DF (including soluble and insoluble) is described as a polysaccharide (complex carbohydrate) that cannot be digested and it arrives in the small intestine intact. . A diet high in DF is associated with reduced risks of some chronic conditions such as cardiovascular disease (Thompkinson et al., 2014), diabetes (Al-Dmoor, 2012), and colorectal diseases (Salvin et al., 2009). These health benefits of DF can be attributed to a number of physiological impacts through modifying macronutrient digestion in the gastrointestinal tract and providing a substrate for fermentation by gut bacteria (Borchani et al., 2011).

Fortification of popular products with DF can be a convenient way to increase fibre intake, and flat bread varieties represent an adequate vehicle for functional fibre ingredients. Bread is a staple food (Al-Dmoor & Galali, 2014) and is consumed regularly everyday with meals throughout the world. Flat bread consumption is steadily increasing at home and on commercial scale in the Middle East, Central Asia and North Africa (Al-Dmoor, 2012). In particular breads like Tandoori and pita breads which are two-layered flat breads are popular. This suits bread to be wrapped and/or folded whilst using with other food products (Borusk et al., 2012).

The addition of DF as a functional ingredient could inevitably influence some aspects of the product properties such as texture, volume, appearance and overall acceptability (Ferrero, 2017). It is therefore necessary to create a product that will carry functional properties and possess a quality and shelf life similar to conventional bread quality. The quality and edibility are indisputable conditions required to meet the need of consumers; otherwise consumer acceptability might be compromised.

The aim of this work therefore was to assess the impact of 20 different combinations of functional ingredients on bread quality and texture (physically and instrumentally assessed). Furthermore, it also aimed to measure the influence of functional ingredients on two types of bread relatively different in dough properties and processing method. The study also intended to observe the microstructure of bread and visualising the influence of functional ingredients in the starch gelatinisation. Finally, the study compared two different techniques of Cryo- scanning electron microscopy (Cryo-SEM) and freeze-dryer method to find out if the freeze-drying technique changed the starch form as a result of drying overnight.

2. Materials and methods:

2.1 Experimental design:

Response surface methodology (RSM) was used to develop two types of breads by adding functional ingredients (FI). Three different novel FI in their physic-chemical properties were selected in order to be used in this study. The amount of the FI utilised in this research was inulin (IN) up to 8%, guar gum (GG) up to 2% and waxy wheat flour (WWF) up to 15%

(HOMECRAFT™ Create 765 National Starch Corn Products Ltd., UK). The ranges are selected according to the data available in the literature about the impact of FI and their doses on the quality and health aspect of products. In the RSM, central composite design (Minitab 16.2.2) was utilised to choose twenty dissimilar mixtures between the FI and then select the best dose (s) that would least compromises bread quality (Table 1).

2.2. Dough and Bread making:

RSM aided designing an experimental to select 20 different substitutions of flour with combinations of inulin (IN) up to 8%, guar gum (GG) (E412) up to 2% and waxy wheat flour (WF) up to 15% (Table 1). Dough was made using the following ingredients (based on 100g flour); 1% active dry yeast, 1.5% salt and 53g of water. Dough samples were made following the previous method used by Borsuk et al. (2012) with some modifications. For pita bread, the dough was sheeted using machines to give 3mm thickness 10cm diameter. The bread was baked at 228°C ± 5 for 3 min in lab oven (Bartlett, Model: E9E, G.E.E Barlett & son Ltd. Hampshire, UK).The bread was then left to cool down to room temperature for further investigation. For Tandoori bread, dough was produced using the same recipe and additives as used for pita, but 69g of water was added to give consistency to the dough. The dough was then transferred to a specialized shop in Plymouth city where Tandoori bread is routinely served. . The baking was conducted in a clay oven (Tandoor) at 300±10 ºC for 1 minute and 10 sec. After cooling, the samples were brought to lab for assessment

2.3. Physical assessment of bread quality:

Physical assessment was carried out using the following parameters.

2.3.1. Weight Loss:

To determine the weight loss, triplicate samples were weighed before and after baking and the weight loss was then quantified using below equation (Borchani et al., 2011).

$$\text{Weight loss} = \frac{\text{weight before baking} - \text{weight after baking}}{\text{weight before baking}} * 100$$

2.3.2. Diameter:

The diameter of the bread samples were assessed twice using a normal student ruler. Three readings per batch were undertaken .

2.3.3. Pocket height (thickness):

Pita pocket height of each sample was measured after cooling the samples using lab Vernier caliper. Three readings per batch were run. For Tandoori bread, same procedure repeated only fifteen readings were taken for each batch of the samples.

2.3.4. Crust colour:

Crust colour was measured using a Minolta colorimeter (Minolta Ltd, Model CM2660d,UK) after calibrating using a white paper assessing 3 dimensions L*, *a**, and *b** which are indications for lightness, redness and yellowness respectively. For pita and Tandoori breads nine and twelve readings were run respectively. The whiteness of the bread was calculated using the mathematical equation below (Borsuk et al., 2012) :

$$\text{Whiteness} = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{0.5}$$

2.3.5. Loaf volume:

Tandoori and pita loaf volume was measured following the standard method of small seed displacement (AACC Method 10-05.01, 2000). Small rapeseeds were poured into a container of known size then the sample was placed and the container was filled to the top with more seed. The extra seed which is not required to fill the container was considered as loaf volume. Three runs per sample batch were conducted.

2.3.6. Instrumental evaluation of bread quality:

A Texture Profile Analyser (TPA) (TA- XT2 – Stable Micro Systems, Gadolming, UK) was utilized to assess pita bread physical quality attributes including hardness, springiness and chewiness. Circles of 25mm diameter were cut out of the bread and then assessed using the following setting; the settings were; pre-test speed: 1.0mm/s; test speed: 1mm/s; post-test speed; 1 mm/s; distance: 40%, with 36mm cylinder probe with (P/36R). Eight runs per every sample batch were tested. On the other hand, to evaluate Tandoori bread quality, penetration test from the TPA was utilized to test extensibility (elasticity) and toughness. The settings used were as following ; pre-test speed: 2mm/s; test speed: 1mm/s; post-test speed; 10 mm/s; distance 60mm with 1inch diameter plastic ball probe with (P1/S). Six runs were performed per batch of every three samples.

2.3.7. Scanning Electron microscopy (Cryo-SEM):

Scanning electron microscopy (Cryo –SEM) (Cryo–SEM - Jeol 6610- Oxford instruments) was used to image microstructure and of the samples with and without novel ingredients. Bread specimens were put on the specimen holder, stuck with colloidal graphite and frozen in liquid nitrogen at a temperature of -190°C. The specimen were then straightaway placed in the Cryo unit and sublimated for 5 min at -90°C and then gold- coated with 90 mA for 1 min and 30 seconds. Later, the specimen were transferred into the SEM chamber and imaged at 5 kV.

2.3.8 Comparing Cryo-SEM and freeze-drying technique in imaging:

Two different techniques, Cryo-SEM and low vacuum-SEM techniques (LVSEM) (LVSEM. Jeol 5600, Oxford instruments) were tested to assess any procedure interference in the imaging process. Two difference samples were imaged with these two different methods. For the Cryo-SEM, the sample does not need to be dried overnight. However, for the LVSEM technique after freezing the sample in nitrogen the samples need to be dried overnight and then stained and imaged.

2.3.9. Statistical analysis:

,RSM central composite designs (CCD) (Minitab 16.2.2) aided to select twenty different mixtures between three novel functional ingredients. Therefore, all the data were analysed using RSM and Minitab software package.

3. Results and Discussion:

3.1. Physical measurement bread quality:

Quality parameters including volume, colour and texture is important to consumers; hence they have a substantial influence on the marketability and desirability of products like bread. Bread quality distinctively relies on the quality and quantity of the flour constituents, in particular biopolymers; gluten and starch and their matrix. Adding, removing and/or substituting these two ingredients therefore could have an influential role on quality and texture of the end-products.

Bread production comes after a series of processes which commences with dough making and ends by baking when a foamy phase of dough transformed to spongy phase of bread. During mixing and then fermentation air cells are produced and these cells are surrounded by walls of starch-protein matrix. Generating more gases then during fermentation and proofing stage causing the cells to stretch and expand. However, the baking stage results in breaking cell gases as a consequence of starch gelatinisation besides cross-linking of proteins hence permits the gas to move towards crust and the cells become disclosed. Adding novel ingredients therefore could change this process and impact on bread quality. For instance, WF could constrain gas moving from one cell to another and then fail to rupture cell walls. Consequently, shrinkage occurs after cooling down to room temperature (Garimella et al., 2011).

The influence of functional ingredients on pita and Tandoori bread physical quality is shown in Table 2. It can be noticed that novel ingredients changed pita bread quality parameters and caused dissimilar alterations. IN caused a noticeable change in bread height but it was dose dependent. At low concentration, IN increased bread height while with increasing IN to 5% reduced pita height. But with increasing to 8%, pita height was improved. This result coincides with earlier findings (Diederick & Peters, 2009; O'Brien er al., 2009), where is found that the addition of %5 of IN caused reduction in the bread volume. The possible explanation for this could be the influence of IN addition on the gluten ability to retain generated gases during fermentation and baking period and compromise gas permeability (Rosell et al., 2001). But, the addition of 7.5% IN into bread formulation increased bread volume and height by 2.3 ml and 1.87 mm respectively. This has been attributed previously to the inclusion of carbohydrates which is thought to help improve bread height and volume via hindering gelatinisation of starch (Roberts et al., 2012). It has been suggested that IN postpones starch gelatinisation which probably stimulates dough expansion during dough fermentation and bread baking (Peressini & Sensidoni, 2009). The microstructure of bread enriched with IN showed the same result, which suggests that IN restricted the starch gelatinisation .

GG considerably ($p<0.05$) increased Pita bread volume and the second largest volume was observed when 2% GG was added. Moreover, GG marginally increased bread height. The outcome of a previous experiment was similar to this result when GG was added into wheat bread (AB., et al., 2012). The authors reported that GG supplementation improved bread volume by almost 100 ml by increasing the amount of GG from 0.25 to 1%. This might be owing to the synergetic impact of GG on the matrix starch-gluten which could impart ability to dough to retain gas, hence increasing final product height. Alternatively, it could be due to modification to gelatinisation and baking properties. Gomez et al (2007) discovered that improving volume of cake supplemented with GG is as a result of the GG increasing of starch pasting properties and viscosity as well as enhancing gas retention ability which restricts gas permeability. Moreover, GG could improve gluten development and improve gas retention capacity. Thus GG led to product volume improvement (Gómez et al., 2007). The volume of bread also depends on the quality and strength of wheat protein, in particular gluten and can be obtained by GG addition (Roberts et al., 2012).

The weight loss as a result of baking the breads was altered significantly (p<0.05) (Fig. 1) with the substitution of flour with novel ingredients. Moreover, substitution of flour with 1% GG the recorded the lowest moisture loss but it was dose dependant. A number studies have stated that hydrocolloids are able to absorb water and retain the moisture content of products (AB et al., 2012; Guarda et al., 2004; Rosell et al., 2001; Tavakolipour & Kalbasi-ashtari, 2007). However, this is mainly driven by the length of storage time. It has also been stated that moisture retention of hydrocolloids might be due to the chemical structure of and active side of hydroxyl group which improves the opportunity to water and hydroxyl group molecules to interact affectively (Guarda et al., 2004). It is also believed that the volume of bread improves as moisture evaporates while baking through pressurizing the cells to expand and thus increasing the volume (AB et al., 2012). Alternatively, it is due to ability of GG in increasing the contact surface and imparts chances to temperature to increase evaporation rate. In addition, pita bread has a flat form with two thin layers and this is totally exposed to heat. Accordingly, it could cause reduction in weight loss after baking.

Colour has a definitive impact on the product acceptability. This implies it has a main sensation role in the decision of rejecting and/or accepting the food product (Angioloni & Collar, 2009). It can be seen that none of the ingredients neither alone nor in combination influenced bread colour. This was also observed for bread diameter.

The novel ingredients supplementation alone compromised some of the quality parameters of the breads and altered dose dependently. However, when it comes to the combination of the novel ingredients, this impact seems to be attenuated and less prevalent. Thus, the qualities of the breads supplemented with mixtures of novel ingredients are close to the control breads.

For Tandoori bread, it is clearly evident that the effect of functional ingredients and their combinations on quality and texture parameters of Tandoori bread is minimal (Table 2). Bread weight loss as a consequence of moisture evaporation during baking showed that there was little change. The data analysis indicated that IN and GG mixtures seemed to enhance moisture loss. This loss can be as a result of the interaction and competition of these two ingredients on the water availability and uptake and their interaction to water. As a result, one may attenuate the effect of another. Hence, it enhanced the moisture evaporation. Another potential reason could be due to the increasing diameter of bread as a result of combinations of GG and IN. This could increase the surface contact of bread to heat and lead moisture to evaporate.

Bread volume was significantly reduced when WF was added to the bread. Substitution of flour with WF up to 10% could improve bread volume while beyond this point the volume seems to decrease. To keep the bread volume, it is crucial to have an ability to maintain the bread volume out of the oven (Rodríguez-García et al., 2012). WF might have the same effect as suggested previously for pita bread. This implies that WF was unable to and breaks cell walls and allows gas to immigrate from the centre to the surface making blisters, hence caused shrinkage after cooling down. Thus substitution of flour with WF caused a considerable collapse in bread magnitude. In addition, it was observed that the bread enriched with WF could not produce blisters and lead to a flat bread with almost no blister some times. Alternatively, it could be associated to the quadratic decrease in diameter with adding WF .So it's obvious that the effect of WF on bread depends on the volume and could be different according to amount added or substituted, type flour and production process.

Regarding the diameter, it is evident that combinations of IN with GG and IN with WF significantly increased ($P<0.05$) the diameter of bread. Once again WF substitution quadratically influenced of bread diameter. In case of WF, it was observed that the baker could not flatten and stretch the dough as it was rather hard and prone to breaking. Whereas, when IN was mixed with GG and WF, it conferred a kind of battery texture to the dough, so the baker could easily flatten and stretch the dough to a larger size. Despite that, it is worth mentioning that increasing volume and diameter of bread volume depends on the quality of flour used mostly biopolymers; starch and gluten and their interaction with supplemented novel ingredients used (Karolini-Skaradzinska., 2007).

3.2. Instrumental assessment of bread quality and texture:

The data showing the influence of novel ingredients on the Pita Tandoori quality parameters instrumentally assessed is presented in Table 3.

Hardness or firmness is described as the amount of energy needed to abolish a given deformation. Springiness is defined as the ability of pressurised- part of a sample to return to their pre-deformed state after the force is eliminated. Whereas, chewiness is considered as the pressure required to masticate a product into a state that could be easy to swallow. But in the TPA is defined as elasticity, cohesiveness and hardness (Balestra, 2009). In fact chewiness is a more complicated procedure as more than one factor is counted including compression, piercing cutting and saliva to lubricate.

Both parameters, chewiness and hardness, were significantly ($p<0.05$) increased when pita flour was partially replaced with IN. Springiness was considerably decreased ($p<0.05$). This all could refer to the firmness impact of IN on bread. This result is similar to previous studies which stated that adding IN into cakes noticeably decreased and springiness increased hardness (Gularte et al., 2012; Morris & Morris, 2012).

It is obvious that that the addition of IN as a source of DF can possesses a negative influence on bread quality through enhancing bread firmness. This is presumably due to the influence of IN addition on the biopolymers in flour. IN could also impact on the process of gas cells creation which could lead to less gas bubbles and impart a less puffy texture. Hence, it could reduce bread springiness. Studying the influence of different DF including IN was undertaken to investigate their influence on bread performance. It has been discovered that IN increased hardness and chewiness (Wang et al., 2002). Zahn et al. (2010) reported that the IN lead to less gas cells and imparted less springy crumbs when they used as a fat replacer in muffins (Zahn et al., 2010). It perhaps requires more force for mastication and chewing the product.

GG supplementation significantly ($p<0.05$) increased bread chewiness and hardness but improved springiness. It has been reported that GG addition increased bread hardness from 1.11g to 2.86g with increasing the percentage of GG from 0.25% to 1% in bread (AB et al., 2012). To assess the influence of GG on the textural and technical properties of pasta, it was discovered that addition of 2.5% GG was enough to decrease softness of pasta (Aravind et al., 2012).

It seems that the inclusion of DF could impart a firmer texture to the product compared to the control. It could be due to the interaction and the behaviour of DF within bread system. The presence DF in the flour is deemed to strengthen cell walls of bread products. Consequently softness of bread is likely to be sacrificed (Skendi et al., 2010). Alternatively, another possible mechanism by which GG influences the pita bread textural attributes could be moisture loss as explained earlier which can reduce springiness of bread and increased chewiness and hardness.

Regarding WF, a decrease in springiness is obvious when WF was incorporated into Pita formulation. It could be due to the fact that WF will not allow gas to immigrate and the cells turned impermeable as a result integrity of WF starch granules. Thus, the generated gases during baking enhances the cells to expand and stretch but unsuccessful to break and rupture, hence maintaining its foamy structure and shrinkages when it is out of the oven (Garimella Purna et al., 2011). These results are in agreement with a previous study where WF was added into muffin and seemed to decrease springiness (Acosta et al., 2011). This implies that WF alters the structure of the crumb in a way that will be less springy. The incorporation of WF into bread results in a keyhole-like crumb, and with increasing the percentage of WF, the crumb cells seemed to larger but numbers were few . Furthermore, it was found that adding more WF will most likely cause shrinkage of the crumb (Garimella Purna et al., 2011). So, it can be concluded that this type of crumb with the early bite (compression) possibly crumb cells walls are broken and the cells might be unable to recover for the following bite as a consequence of break. Consequently, WF causes a bread crumb less springy.
For Tandoori breads, the puncture test is normally used to assess firmness and elasticity of bread which uses a ball probe to compress the bread to a certain distance until the bread ruptures.

It can be seen in Table 3 that IN significantly altered Tandoori bread texture. Substituting 4% flour with IN improved the bread firmness, While adding 8% seemed to drive the product in the opposite direction. The influence of prebiotics on the product texture and quality depends on the dose (Morris & Morris, 2012). These results are similar to earlier studies who stated that adding 3% of IN attenuated bread hardness, whereas increasing the percentage to 8% caused a tougher product texture in comparison with to a control (Koruz et al., 2006). This is possibly be due to the impact of IN on the structure of bread particularly tightening the bread structure and finally promoting firmness (Mujoo & Ng, 2003).

Regarding Tandoori instrumental measurement, only IN showed an influence on bread texture. , None of the other functional ingredients neither alone nor in combination showed any influence on bread the quality parameters (toughness and elasticity).

It is evident that functional ingredients alone altered bread quality. Novel ingredients in combination seem to have less modifying impact on the bread quality and brought about the same quality as the control. This could be a positive point allowing fibre rich bread to be created with a similar quality to the control. Moreover, it can be understood that although the functional ingredients changed some quality parameters significantly statistically, but in reality the changes were very small and did not make any appreciable differences. So the breads seem to be very similar despite these measured changes.

3.3. Scanning Electron Microscopy (Cryo-SEM):

The SEM was used to image the microstructure of the bread samples supplemented with functional ingredients; IN 8%, GG 2%, WF 15%, all the ingredients together (IN 4%, GG 1% and WF 7.5%) and control (without additives). The images of the Cryo-SEM helped to visualise interaction between the novel ingredients and biopolymers within dough bread system. Cryo-SEM images of pita samples are presented in the Figure 1 and the images of the Tandoori bread samples are presented in the Figure 2.

It is obvious from the imaged-structure of the control (1A) that there is a strong connection and – intra-melting between flour ingredients particularly protein and starch. Furthermore, some of the starches seem to be completely gelatinised, but the majority are partly swollen and gelatinised. However, the structure retained its integrity and the starch granules kept their identities. Furthermore, it can also be noticed that the outline of starch granules are also determinable. Finally, granules of starch look embedded in the protein matrix.

The image of bread enriched with all the novel ingredients is presented as the Figure (1B). The microstructure of bread looks to have a similar microstructure to the control. Moreover, it seems that granules of starch are partly gelatinised but identities are maintained. Moreover, starch granules melted in protein and the novel ingredients and the interaction between them can be noticed. But they are less gelatinised and can be recognised more easily than the starch granules of control bread. This could be ascribed to the competition between novel ingredients and granules of starch as the ingredients absorb water more quickly comparing to starch, consequently, starch are less gelatinised and easily detected.

IN bread microstructure (1C) seem to have a sporadic and less consolidated matrix of starch-protein, and the starch granules are clearly seen. Furthermore, the granules are discrete and are not fully embedded by IN. It is also noticeable that IN appears to make intra-strips between the granules which might be attributed to interaction between starch and IN. Furthermore, starch granules seem to be less swollen and slightly gelatinised. This is assumed to be due to the starches and IN competition for moisture, IN has a stronger affinity to water led starch granules less to be hydrolysed. IN is water soluble fibre at ambient temperature and absorbs moisture quicker which deemed to constrains starch swelling and consequently alter its integrity. Tudorica et al (2004) observed that IN addition to bread resulted in starch granules to be less gelatinised and less interacted with its surrounding granules. In a study on the impact of various percentages of IN addition on microstructure of pasta, it was found that IN led to an incoherent form and altered protein-starch matrix integrity. This was attributed to the affinity of IN in which compete with the protein and starches for water it alters the protein-starch matrix. Hence, it causes to an incoherent crumb form (Tudorica, 2004). Additionally, IN competes to interact with protein instead of starch and produces weak matrix of starch-protein (Manno et al., 2009). Similar outcomes have been stated by some authors regarding the influence of IN on pasta microstructure (Aravind et al., 2012).

The image of bread samples substituted with GG (1D) showed that the starch granules are embedded in GG and protein. However some indistinct outlines of starches granules are barely detectable. Furthermore, unlike IN, GG not only does not appear to weaken matrix of protein-starch, but it also seems to build a continuous and strong network starch-protein. This result is congruent with the earlier findings which conclude e that starch in GG enriched bread was covered with galactomannan of the GG and the starch granules were embedded in a continuous matrix (Ribotta et al., 2004). Similarly, some researchers have concluded that GG could form a condense structure when incorporated to bread formulation. A study was conducted on the impact of various hydrocolloids on the characteristics of Puri bread. It has been stated that the addition of GG covered and led starch granules partially hydrolysed(Parimala & Sudha, 2012).

Microstructure image of the pita breads with WF is presented in 3E. It can be noticed that the WF altered Pita microstructure in a dissimilar way to other novel ingredients. Some granules seem to be hydrolysed , but almost all the starches are swollen to some extent. Therefore, some of the starch outlines can be detected. Finally, the microstructure image shows more cavities less smooth structure. It is well-documented that that starch of WF absorbs moisture and swell at a lower temperature which is around 70°C comparing to the wild-species of starch varieties, and the morphology of granules commence to disassociate and change into small particles . Whereas, normal starch granules resist temperature change beyond 90°C and maintain its form). Some researchers studied the influence of various percentage of WF on bread quality and texture properties, they noticed that the addition of WF starch into bread led to microstructure looked disintegrated. Moreover, the image also visualised the crumb protein network which outstretched between granules.

The microstructures images of Tandoori samples incorporated with novel ingredients are shown in Figure 2. It can be seen that majority of control bread starches (2A) are hydrolysed and outlines of the granules are detectable. Whereas, the granules of bread supplemented with all the novel ingredients (2B) seem to be slightly gelatinised and swollen, and rather have maintained their identities in comparison to control. As earlier discussed, the novel ingredients have a greater tenancy to water than starch. As a result of that the gelatinisation of starch is constrained. That could positive since, despite imparting health benefits, but the quality of the product remains uncompromised.

The microstructure of bread enriched with IN (2C) and GG (2D) show the embedment of the starch granules in the gluten and functional ingredients. It is apparent that the starches are less gelatinised and swollen in comparison to the control. However IN shows less integrated and smoothen structure. GG seems to wrap the granules and showed a coherent more smooth structure. The microstructure of bread with WF (2C) seems to be less integrated than control and the granules are more gelatinised than WF in pita bread case of study which gives a relatively smooth structure.

3.4. Interference of Cryo-SEM and freeze-drying technique in imaging:

Cryo-SEM is a relatively new technique that has a number of implementations in the field of food processing. It can be worth testing to compare this technique with a freeze-drying method to see whether the latter technique interferes with the starch microstructure as a consequence of sublimating and drying overnight. The microstructures of the breads supplemented with all ingredients and control bread imaged with Cryo-SEM are presented in the Figure 3A and 3B respectively and same samples imaged with freeze-dryer method are presented in the Figures 3F and 3G.

It can be noticed from the results (Fig. 3F and 3G) that starches imaged with freeze-drying method show shrinkage. Additionally, the starch granules look more tightened and dense, while this is not obviously seen in the Cryo technique (Fig. 3A and 3B). A possible reason might be the consequence of removing moisture in the sample which made starches shrink. It was noticed the physical appearance of the specimens were shrunk and become fragile after freeze-drying step. Therefore, the freeze-drying method might interfere and change the microstructure of the bread particularly if not carefully imaged. However, more investigation is needed to confirm that.

Conclusion:

The influence of different novel ingredients on quality and microstructure of two types of bread varieties was studied. The outcomes of this study indicated that in pita bread, IN and GG increased hardness and chewiness and reduced springiness. In addition to that GG caused an increment in height, volume and weight loss. Whereas, IN only increased bread height. No influence was found on diameter, and colour. The impact was less profound on Tandoori bread since only a few changes were recorded on bread toughness, weight and diameter. Using a combination of functional ingredients could be a convenient way to create fibre rich bread especially Tandoori bread similar to conventional bread quality.

The microstructure of the bread showed that functional ingredients could restrict water absorption by starch due to their greater affinity to water than starch. However, a combination of all the additives seemed to produce a structure relatively similar to control. This study confirmed the importance of choosing a suitable technique to image bread microstructure to eschew any potential effect of preparation procedure on the specimen microstructure. We observed some interference of the freeze-drying technique on starch form, but more studies may be needed to confirm this result.

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Conflict of Interest:

The author declares that there is not conflict of interest

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Table (1) RSM design (CCD) and different selected doses of the FI

	Functional ingredients		
Runs	IN %	GG %	WWF %
1	4	1	7.5
2	4	1	7.5
3	4	1	7.5
4	8	0	0
5	8	0	15
6	8	2	15
7	0	2	0
8	0	2	15
9	4	1	7.5
10	0	0	15
11	0	0	0
12	8	2	0
13	4	1	15
14	4	1	7.5
15	4	1	7.5
16	8	1	7.5
17	4	2	7.5
18	0	1	7.5
19	4	0	7.5
20	4	1	0

IN: inulin, GG: guar gum, WWF: waxy wheat flour

Table (2) The data analysis of the influence of FI on the pita and Tandoori bread qualities (physical assessed)

Factors	**Pita bread**					**Tandoori bread**				
	Volume (ml)	**Diameter (cm)**	**Height (mm)**	**Weight loss (g)**	**Whiteness**	**Volume (ml)**	**Diameter (cm)**	**Thickness (mm)**	**Weight loss (g)**	**Whiteness**
Constant	96.5	9.84	23.14	13.12	66.38	319.9	28.4	30.76	18.07	61.88
IN	2.3	-0.175	1.87	-0.73	0.20	-1.5	-0.2	-0.22	-0.29	0.37
GG	12.2*	0.22	2.29*	1.39	-0.29	-33.8	0.2	1.37	-1.92	3.98
WWF	-1.3	-0.02	-0.09	-0.1	0.65	11.2	0.05	0.58	0.18	-0.53
IN *IN	18.9*	-0.07	5.11*	-3.08	1.79	0.6	0.01	0.09	0.01	-0.08
GG*GG	-4.74	0.03	-1.92	6.29*	0.56	16.3	-0.5	-0.50	-0.11	-1.23
WWF *WWF	-4.41	-0.10	-2.43	-3.91	-1.10	-0.9*	-0.01*	-0.03	-0.02	0.04
IN *GG	6.17	0.07	0.91	0.02	1.13	-1.4	0.10*	-0.12	0.17*	0.01
IN *WWF	5.48	0.01	1.16	0.55	0.45	0.5	0.02*	-0.03	0.01	0.01
GG * WWF	2.98	-0.05	1.70	-0.37	-0.66	-1.0	-0.01	0.02	-0.01	-0.14
**R-SQ	57.73%	53.59%	60.61%	43.17%	28.37%	71.1%	86.0%	49.0 %	80.8%	48.7%

**P value* <0.05

**R-SQ = Regression square coefficient of the fitting model

Table (3) Textural assessment of Pita/Tandoori breads using TPA

	Pita bread			Tandoori bread	
Factors	**Hardness (N)**	**Springiness**	**Chewiness**	**Toughness (g)**	**Extensibility (mm)**
Constant	347.59	0.862	242.88	1404.9	33.8
IN	46.78*	-0.016*	25.12*	-118.8	0.05
GG	48.77*	-0.013*	29.88*	-220.5	-2.5
WWF	7.24	-0.014*	0.47	-9.9	-0.6
IN *IN	21.48	0.016	21.77	13.2**	-0.06
GG*GG	-35.04	0.011	-22.67	60.4	1.3
WWF *WWF	1.15	-0.006	-0.93	0.04	0.03
IN *GG	6.14	-0.001	2.58	14.4	0.03
IN *WWF	6.08	0.001	6.76	-0.7	-0.02
GG * WWF	-17.2	0.012	-5.06	-4.4	-0.02
R-SQ	58.54%	77.37%	54.95%	87.2%	74.3%

**P* value =<0.05

*R-SQ = Regression square coefficient of the fitting model

Chewiness= hardness x cohesiveness x springiness

Values are average of eight replicates

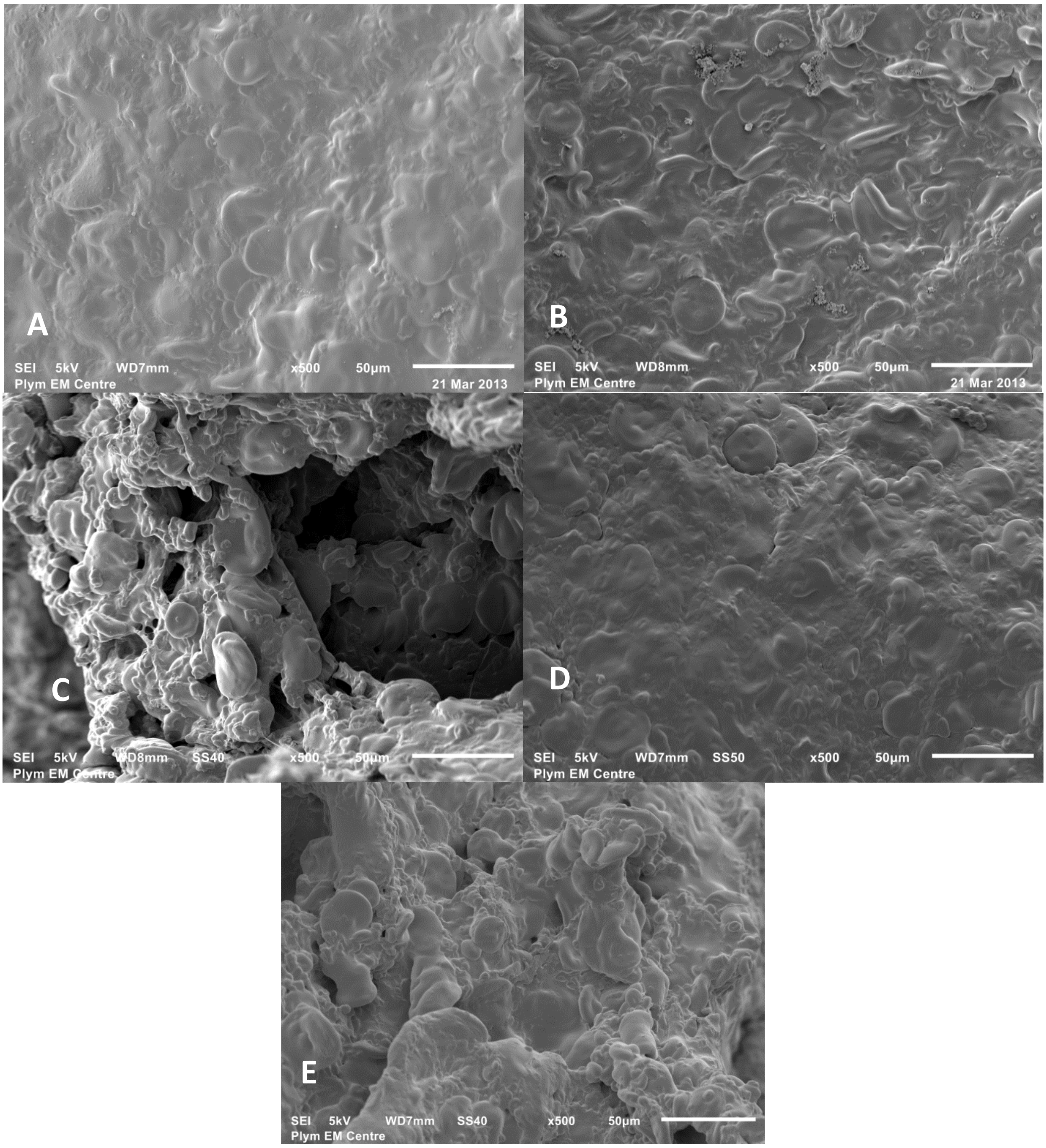

Figure (1) Pita bread, A; control, B; all combinations, C: Bread with IN, D: bread with GG and E; bread with WF.

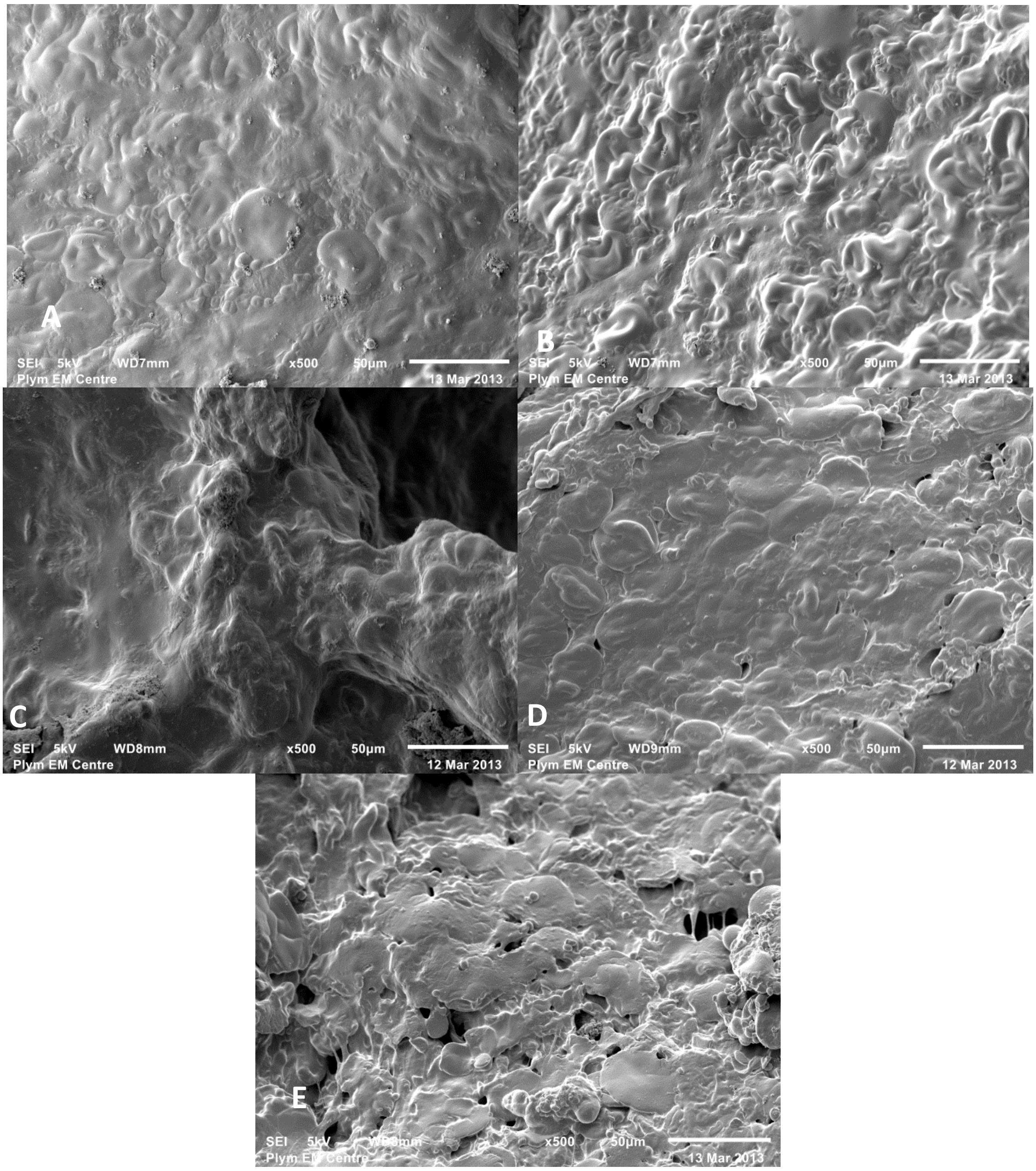

Figure (2) Tandoori bread, A; control, B; all combination, C: Bread with IN, D: bread with GG and E; bread with WF

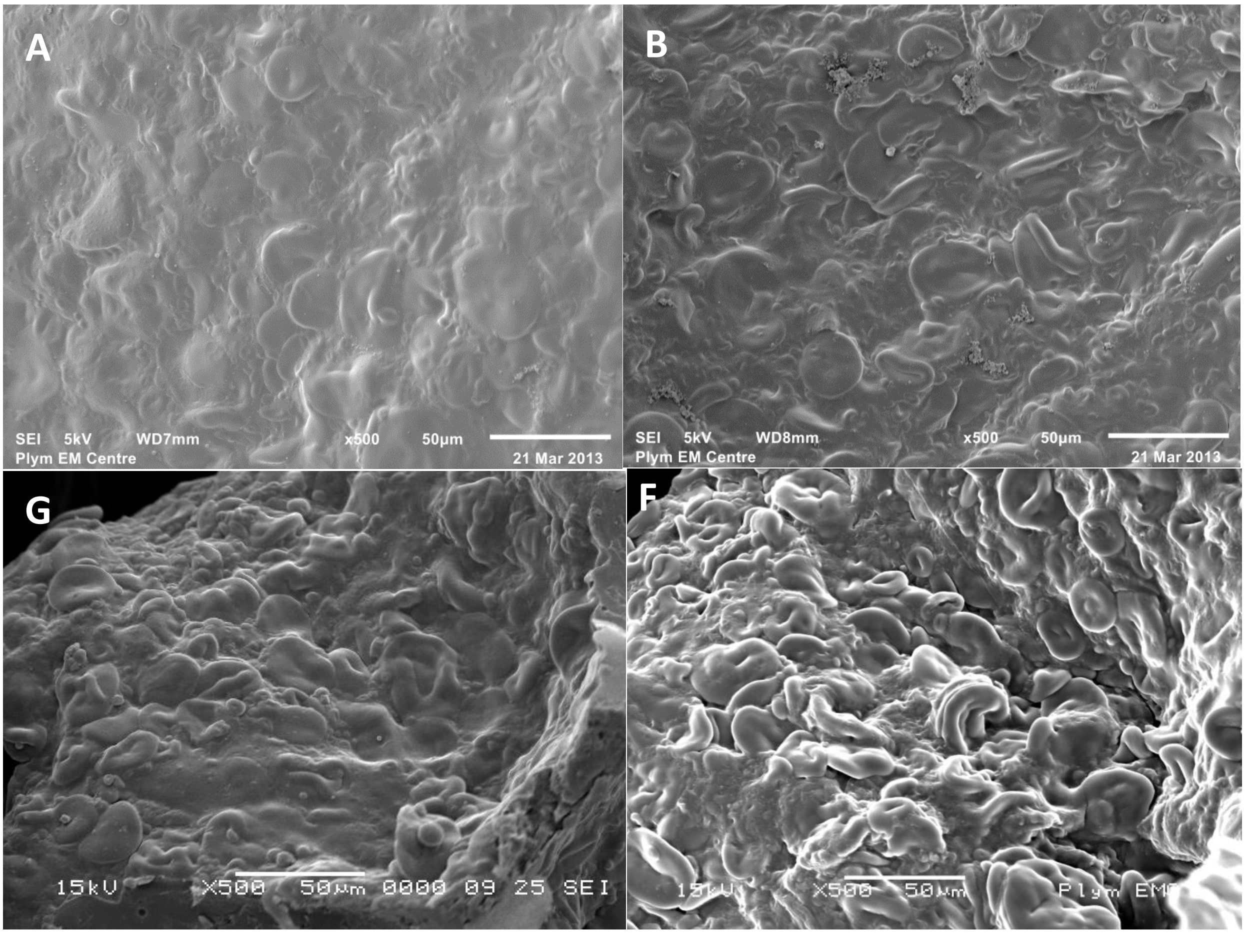

Figure (3) shows different images taken by two different techniques: Cryo-SEM; A) control: B) bread supplemented with the all ingredients. LVSEM; F) control bread, G) bread supplemented with the all ingredients