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Attempt to Increasing Freshness in Gluten-free Tin Bread by Adding Distilled Monoglycerides, α-Amylase and Glucose Oxidase

Försök att Öka Färskhet i Glutenfritt Formbröd Genom Tillsats av Destillerade Monoglycerider, α-Amylas och Glukosoxidas

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Abstract

The market for gluten-free products is still developing and producers seek to offer consumers products as close to standard market products as possible. Therefore, product development of textural and sensory issues in gluten-free bread is crucial for the industry. This experimental explores effects of additives improving freshness of gluten-free bread. From screening of thirteen additives, one emulsifier and two enzymes where selected, combined and tested at different levels: distilled monoglycerides (DMG) from hydrogenated rapeseed oil; a maltogenic α-amylase (AA) and a glucose oxidase (GO). Gluten-free tin bread was used as model recipe. Height, volume and specific volume were negatively affected by DMG. All three additives increased the fibre content (resistant starch). Maximum softness was obtained when adding high amount of AA and low amount of DMG. No significant effect on springiness was detected. Cohesiveness decreased with raised amounts of AA and DMG. Chewiness correlated with hardness. Sensory evaluation showed divergence regarding which attributes mediated 'freshness'. A harder bread with 0.5% DMG, 125 ppm GO and no AA, was perceived as freshest. This same combination did not get any votes in a 'squeeze of the loaf' test. In the latter test, a bread without DMG but 350 ppm AA and 125 ppm GO was most preferred. Despite deviating sensory results, statistically significant results could be obtained: α-amylase increased softness, hence consumer preference, while the emulsifier increase perceived freshness when eating the bread. Consumers are a valuable resource in product development.

Keywords: emulsifier, α-amylase, glucose oxidase, gluten-free bread, freshness

Sammanfattning

Marknaden för glutenfria produkter fortsätter utvecklas i takt med att livsmedelsproducenter försöker erbjuda konsumenterna en upplevelse så nära standardprodukt som möjligt. Produktutveckling av texturmässiga och sensoriska brister i glutenfritt bröd är därför avgörande för industrin. I detta arbete undersöks effekten av tillsatser som förbättrar färskhållning av glutenfritt bröd. Efter screening av tretton tillsatser valdes ett emulgeringsmedel och två enzymer ut, kombinerades och tillsattes i olika mängder: destillerade mono-glycerider (DMG) från hydrogenerad rapsolja; ett maltogent α-amylas (AA); och ett glukosoxidas (GO). Som modellrecept användes ett glutenfritt formbröd. Höjd, volym och specifik volym påverkades negativt av DMG. Samtliga tre tillsatser ökade fiberhalten (resistent stärkelse). Maximal mjukhet erhölls vid tillsats av hög mängd AA och låg mängd DMG. Ingen signifikant effekt på elasticitet upptäcktes. Kohesiviteten minskade med ökade mängder AA och DMG. Parametern chewiness (tuggmotstånd) korrelerade med hårdhet. Vilka attribut som medierade 'färskhet' vid sensoriska utvärdering var tvetydigt. I testet uppfattades nämligen ett hårt bröd med 0,5% DMG, 125 ppm GO och ingen AA, som färskast. Samma kombination var minst populär (fick noll röster) i ett "limp-klämmar"-test. I det senare testet föredrogs ett bröd utan DMG men med 350 ppm AA och 125 ppm GO. Trots tvetydigt sensoriktest kunde statiskt säkerställda resultat erhållas: α-amylas ökar mjukheten, följaktligen konsumenternas önskemål när man klämmer på brödet, medan emulgeringsmedlet ökar den upplevda färskheten när man äter brödet. Konsumenter utgör en värdefull resurs inom produktutveckling.

Keywords: emulgeringsmedel, α-amylas, glukosoxidas, glutenfritt bröd, färskhet

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1 Introduction

The gluten-free market has grown tremendously the latest years and is still increasing (Haman, 2017). This, although literature and industry indicate unsatisfactory texture. It commonly crumbles, gets dryer, harder and stales more easily than wheat bread. This sensory issue is also revealed in consumer surveys, indicating dissatisfaction with textural and sensory qualities (Olsson, 2017). Consumers expect the gluten-free products to be as good as their wheat containing counterparts, why texture development is a major challenge for bakers and food technicians in this industry (Hager et al., 2012). This market gap is desirable for gluten-free product manufacturers to fill. Improvement of freshness in gluten-free bread is manageable, according to literature. However, could the additives suggested in the literature provide better bread, if added in combination?

Gluten has a unique role in the traditional making of bread, why it has been so hard to replace (Arendt & Dal Bello, 2008). Research show that a mix of different flours, hydrocolloids and proteins are needed to replace gluten and create gluten-free products of good quality. Commercial products also include other ingredients to increase shelf life and provide a softer texture. Emulsifiers and enzymes are commonly suggested as bread improvers (Levenstam, 2010; Arendt & Dal Bello, 2008). These have shown successful inhibition of aging in gluten-free bread. One of the mechanisms these additives interfere with is retrogradation, a phenomenon of starch aggregation during storage of starchy food. The rapid decline of freshness in gluten-free bread is partly due to the high starch content and the absence of gluten. Indirectly, it is due to a not yet satisfied area of product development.

Despite several solutions on the textural issue, apparently it is not enough. Consumers are demanding, yet unspecific. One of the remaining knowledge gaps is the combined effect of additives on gluten-free bread, and what correlations may exist. Another is the freshness concept, which is subjective, influenced by the context.

1.1 Aim

The aim of this study is to investigate if freshness could be improved by adding emulsifier together with enzymes, and what their combined effect may be, in what amounts. Ideally this report contributes to a process of developing a future, healthy gluten-free tin bread with good volume and which age slower than today's gluten-free bread. This project aims to provide increased knowledge in using emulsifier and enzymes in combination, hence development of gluten-free bread recipes.

1.2 Objective

Screening, by systematically test baking of five emulsifiers and eight enzymes, results in three additives for further investigation. Selected emulsifier and enzymes applies to a starch-based model recipe according to an experimental design. Seven days after baking, they are analysed instrumentally and sensorially. Whether the additives contribute to increased freshness is revealed by the ranking of the sensory panel and by comparison to the reference bread (no additive).

Food producers seek to offer consumers an experience as close to standard market products as possible. Therefore, product development of textural and sensory issues in gluten-free bread, such as in this study, is crucial for the industry. For an optimal result, more comprehensive changes of the recipe are presumably needed. Yet, minor findings regarding specific additive interaction contribute to the main knowledge in the long run. If successful, this study could result in an updated recipe for a commercial bread. Realistically, this process contributes to knowledge and potentially better future product development processes.

1.3 Hypothesis

Addition of emulsifier and enzyme is expected to improves textural freshness in gluten-free tin bread, where at least one of the bread samples will be perceived as fresher than the reference.

1.4 Delimitation

In the scope of this project, the aim has been limited to target the texture issue only, with especial emphasis on improved freshness. The study focus on additives, and not how these could be combined with other ingredients.

2 Background

2.1 Wheat and Gluten Sensitivities and Diseases

Individuals with coeliac disease (CD), wheat allergy (WA) or non-coeliac gluten sensitivity (NCGS) react negatively to wheat products in a way that healthy people do not (Hager, 2012). CD is an autoimmune disorder, originating from the gastro intestinal tract, where the immune system is triggered by dietary gluten in genetically susceptible individuals (Catassi & Yachha, 2009; Arendt & Dal Bello, 2008). Characteristics for the disease are specific antibodies against gluten, genetic haplotype and intestinal inflammation (EFSA NDA Panel, 2014).

This chronic disease can develop at any age throughout life (Svenska Celiakiförbundet, 2019) and the only treatment of today is lifelong gluten-free diet (GFD). Approximately 1% of the western population is diagnosed with coeliac disease (Catassi and Yachha, 2009; EFSA NDA, 2014). However, in Sweden as many as 3% are estimated to suffer from CD, though the majority being undiagnosed (Svenska Celiakiförbundet, 2019). From no longer being an unusual childhood disease, the prevalence today is increasing (Ivarsson, 2013). A wide variety of symptoms of different severity is associated with the disease. Some even live with the disease unnoticed. Ultimately, gluten-free products are needed.

2.2 Gluten-Free Bread

2.2.1 Gluten

Cereal proteins are grouped into categories. In wheat, 80-85% of the total protein content fall under the category of storage proteins. These are what develop into gluten upon dough making. Storage proteins are commonly divided in two: Gliadins making the dough viscous; and glutenins providing elasticity to the dough. Together these proteins provide viscoelasticity and a strong gluten network characteristic for wheat bread. People with CD are intolerant to fractions in the gliadin. Since these protein sequences is not only found in wheat but also in barley and rye, coeliac patients are advised not to consume those species.

2.2.2 Gluten-free Bread

Gluten-free bread is commonly based on starch. It usually also includes protein and hydrocolloids (Gallagher, 2009b). Without gluten, the dough does not need long time mixing and usually result in a pourable, liquid batter rather than a dough system (Arendt & Dal Bello, 2008). In EU a product could be called "gluten-free" if the gluten content is not higher than 20 ppm (20 mg/kg). Normal wheat bread could contain approximately 40 000 to 60 000 mg gluten/kg (Livsmedelsverket, 2019).

Due to high starch content, many commercial gluten-free baked goods exhibit low textural quality (Arendt *et al*., 2002; Gallagher, 2009b). These quality defects likely result in a dry and sandy mouth-feel. High amount of starch along with the absence of gluten contribute to rapid staling and crumbling bread texture (Gallagher *et al*., 2009a). It is a technological challenge to replace gluten, which cannot be substituted by one single ingredient (Arendt & Dal Bello, 2008). A variety of polymeric substances mimicking the viscoelastic, gas-holding properties and good crumb structure of gluten is therefore needed (Toufeili *et al*., 1994). Examples of ingredients replacing gluten include enzymes, sourdough and hydrocolloids. Houben *et al*. (2012) means that a multi-composition of these replacers is crucial in order to obtain satisfying taste, structure and a high volume.

2.2.3 Starch & Aging

Starch is a very abundant constituent organised in granules within the plant cells (Witczak, 2015). Granules are dense, insoluble structures 0.1-200 µm in size and differ in shape. They possess a semi-crystalline organisation made up of two different types of (phase separated) glucose polymers: amylose and amylopectin (Hug-Iten *et al*., 2001). Amylose is a linear molecule forming tough gels and strong films. Amylopectin is a much larger and highly branched molecule forming soft gels and weak films (Witczak, 2015). Amylose easily crystallize while amylopectin retrogrades in a slower manner. The distribution of amylose and amylopectin is commonly 25-28% and 72-75% respectively, but differs depending on botanical source and variety (Arendt & Dal Bello, 2008). The diversity among starches, such as gelatinization temperature and granule size and shape, affects the quality of the bread (Hager *et al*., 2012)

Rice is the most commonly used flour for gluten-free baking, on the market and in the literature (Hager *et al*., 2012). It is good for gluten-free baking for its bland taste and lack of colour (Hager *et al*., 2012; Gallagher, 2009). Its proteins are hypo-allergenic (Moore *et al*., 2004). However, they possess a hydrophobicity making them insoluble and incapable of contributing to a viscoelastic dough necessary for gas retention. Thus, making the bread compact with a low specific volume (Gallagher, 2009). The choice of rice cultivar is of further importance as they differ in amylose content (Hager *et al*., 2012). This affect gelatinization temperature, general pasting behaviour and viscoelastic properties, hence baking behaviour. Another widely used starch source is corn (Hager *et al*., 2012). Unlike rice it has a more distinctive flavour and more colour (Arendt and Dal Bello, 2008). Potato starch, usually added in smaller amounts, improves fresh keeping of bread (Hager *et al*., 2012). Its big granules, susceptible for breakage, possess swelling power and increase water-binding capacity of the system. The different functions makes it common to combine starches from several botanical sources.

Starch granules within the plant cell as well as the native granules itself are inaccessible to most amylolytic enzymes – namely resistant starch. Starch is extracted from the seed by milling, which cause some damage to the granule. This greatly affect starch properties (Hoseney, 1994). Damaged starch has higher water absorbing capacity and is more susceptible to enzymatic hydrolysis.

Starch is not soluble in water in its native form. Untreated, starch granules could absorb up to 50% of its dry weigh and regain its original size upon drying (Arendt & Dal Bello, 2008). Upon heating, the starch water slurry undergoes a fairly extensive transition, grouped into phases. Heating of starch suspension over a certain temperature cause an irreversible swelling of the granule and a rearrangement of the molecular order within the granule (Arendt & Dal Bello, 2008). Secondly, amylose leak from the granule out in the inter-granular phase. Lastly, granules visibly ruptures, causing a temporary decrease in paste viscosity and raised susceptibility to enzymatic treatment. The initial stages of starch thermal transition in water is referred as gelatinization, whereas the latter as pasting.

Gelatinization is a central process in breadmaking, happening in the oven. The extent is determined by the type of starch (crystalline structure, amylose content, granule size and shape) and water availability. Other ingredients might affect water availability, such as highly water absorbing fibres etc. Reduced water availability retards gelatinization and vice versa. As a consequence, in later stages retrogradation is also affected. Pasting begins above gelatinization temperature and means continuing swelling and leakage of amylose (Arendt & Dal Bello, 2008). Upon cooling, amylose molecules gradually form a continuous network of double helices in which swollen and deformed starch granules are distributed. This three-dimensional network (starch paste) is connected via hydrogen bonds and holds large amounts of water. Gradually this network moves tighter. This mechanism enables that a stable crystalline gel structure could be formed within the bread a few hours after baking. Starch act as glue for flour particles within the dough (Ward & Andon, 2002).

Continuously during storage, amylose and amylopectin re-associate, a phenomenon called retrogradation. Amylose has a rapid retrogradation, making it an essential structural element of bread and determining factor for initial volume (Eliasson, 2010). The short side chains of amylopectin re-crystallize considerably slower than amylose and could take several days or up to weeks. This mechanism occurs within the gelatinized granule. Hence, amylopectin regulates the long-term development of the gel structure and the amylose retrogradation determining the initial hardness of the starch gel - the baked product. Retrogradation is affected by pH, salt, sugar and lipids (Eliasson & Gudmundsson, 2006).

During storage bread loses freshness (Hoseney, 1994). It stales, crust toughens, crumb gets firmer and less elastic, loss of flavour and moisture. Staling is quick in gluten-free bread due to the high starch content. Staling is a process when water migrate from crumb to crust. The crust become soft and leathery (Eliasson and Larsson 1993). Aging is typically associated with increased firmness and crumbliness of the crumb. Both amylose and amylopectin retrogradation plays an important role in that process. The firming of the crumb is caused partly due to hardening of the granule (molecular reorganization), inter molecular hydrogen bonds, amylose network formation (retrogradation) as well as amylose and amylopectin interacting with each other.

2.2.4 Hydrocolloids

Hydrocolloids are long chained compounds, usually polysaccharides gelating in water-based systems (Hager *et al*., 2012). They are used in gluten-free formulations to mimic the function of gluten (Hager *et al*., 2012; Toufeili *et al*., 1994; Gurkin, 2002). Although added in small amounts (<1%) their effect on the textural properties are significant (Arendt & Dal Bello, 2008). Their wide range of effects in bread include increased loaf volume (Arendt Dal Bello), moisture retention, retardation of starch retrogradation and improved sensory properties such as cell size distribution and crumb hardness (Hager *et al*., 2012). No general effect could be attributed to the group of hydrocolloids (Anton & Artfield, 2008). Usually more than one hydrocolloid is used in formulations in order to obtain the full range of the desired properties. Too high dosage might cause negative effects, such as excessive rise of dough (Hager *et al*., 2012; Arendt & Dal Bello, 2008)

One common hydrocolloid used in gluten-free breads are hydroxypropylmethylcellulose (HPMC) (Hager *et al*., 2012). HPMC is a synthetic cellulose derivative and thickening agent. Addition of methyl and hydroxypropyl groups to the cellulose chain leads to a polymer with high surface activity, and a hydrophobic-hydrophilic balance making it emulsifying. Through its hydrophilic groups, HPMC has high water retention properties. These groups of different polarity provide interfacial activity within the system during proofing and forms gel networks during the bread-making process. The network structure of HPMC formed during baking increases viscosity and strengthen the walls of the expanding cells in the dough. Consequently, the gas retention during baking, hence the final loaf volume is increased (Arendt & Dal Bello, 2008). Textural studies have also shown positive effect of HPMC on crumb structure, reduced firmness of bread crumb, reduced moisture loss during storage and anti-staling effect (Guarda *et al*., 2004). HPMC preferably binds to starch (Collar *et al*., 2001) and retard amylopectin retrogradation (Arendt & Dal Bello, 2008). Gallagher (2009) reports positive effects of inclusion of HPMC in gluten-free rice bread and Haque and Morris (1994) further highlights the positive effects of combining HPMC with psyllium husk.

2.3 Market & Trends

Gluten-free is no longer a trend (Arendt & Dal Bello, 2008). It has now reached the mass market as not only individuals with clinical reasons consume gluten-free products (Haman, 2017). The increasing numbers could partly be explained by: In Sweden, 67% more bread is consumed today compared to in the 1990s (Jordbruksverket, 2018) along with an increasing prevalence of coeliac disease diagnoses (Ludvigsson & Murray, 2019). Food industry analyst at Mintel®, Amanda Topper (2014), explains the gluten-free market growth is driven by people that are not diagnosed with coeliac disease. Common motifs for exclusion of gluten is to test if one is gluten intolerant or sensitive. Also, undiagnosed individuals which are in the risk group might consume GFD preventively (Arendt and Dal Bello, 2008). Consumer studies indicate gluten-free options are perceived as healthier by many consumers (Hamann, 2017) and that healthy living is a primary driver of the gluten-free sales (Topper, 2014). On top of the health aspects, influences from friends and family are what drives the initial purchase of gluten-free products. The gluten-free dieters are predominantly young millennials (Hamann, 2017).

Although the gluten-free market is approaching a degree of maturity, it remains strong with double-digit growth rates in most countries (Hamann, 2017). Gluten-free sales is increasing in Sweden. Product ranges are expanding at several retailers (Herou, 2018). Sales figures from one of the biggest grocery retailers on the Swedish market show that sales of fresh gluten-free bread increased by 50% from April 2018 to April 2019[1]. Even colonial atmospheric packed gluten-free bread (+7%) and

1. Frösell, Paula. Senior Brand Manager Health, Food & Meals. ICA Sverige AB. 2019. Email 7 May.

frozen gluten-free bread (+5%) have increased. However, the sale of frozen gluten-free bread is the biggest category, probably for conveniences. The gluten-free trend is large also in US where 30% of the citizens tries to cut down on gluten (Schierhorn, 2018). Those who need to cut down on gluten for medical reasons in US, including CD, WA and NCGS, are 7% in total.

Gluten-free products are more expensive than their traditional counterparts, partially due to in many cases a limited range of products (Arendt & Dal Bello, 2008). This in combination with comparably lower product quality is experienced as poor value for money. However, a positive transformation in product quality have developed. Where improvement in taste, texture and ingredients of gluten-free products, driven by a growing interest for gluten-free food, have led to greater satisfaction among consumers. Consequently, variety and retail availability increases. Hence, the gluten-free market attracts a broader group, eg. family members to coeliac patients. Further product development is yet central in the gluten-free sector. A member survey among young gluten-free consumers reveal a majority (53,3%) consider quality and taste of gluten-free alternatives is not as good as traditional ones (Olsson, 2017).

2.3.1 Product Development

Product development is an essential, integrated part of the business strategy (Arendt and Dal Bello, 2008). Effective communication between marketing, science and technology within a company is critical. Marketing ideas need to be successfully translated into products and technically expertise need to make reliable decisions, and vice versa (Arendt & Dal Bello, 2008). Screening is crucial for rationalizing the process, assessing the product viability. Only promising products shall be invested in, to avoid economical loss. Wennström and Mellentin (2003) point out the importance of incorporating the consumer early in the process to identify consumer need. Consumers might not know what new products they desire. However, by interpreting consumer behaviour and purchase motifs, failure rates could be reduced in the innovation process (Arendt and Dal Bello, 2008).

Consumers are becoming more demanding and expecting gluten-free options to match the taste and texture of their traditional counterpart (Haman, 2017). Unfortunately, even transformation of familiar food products to gluten-free formulations is a technological challenge for food processors (Arendt & Dal Bello, 2008). Texture as the main issue, yet provides opportunities in being innovative. Functional needs, such as the ability to successfully spread something on the bread, still need to be satisfied.

The segment perceiving gluten-free as healthy is also looking for improved nutritional value and simpler ingredient list (Hamann, 2017). To succeed with new products, consumer acceptance of the ingredients is important (Wennström & Mellentin, 2003). High sugar content and a large number of additives are examples of issues to overcome. Further on, there is no “one size fits all” in this diverse consumer segment. Hence, designing new gluten-free products shall be done with the consumer in focus (Arendt & Dal Bello, 2008).

2.4 Sensory Aspects

Sensory properties relate directly to consumer acceptance and product quality (Arendt and Dal Bello, 2008). Sensory awareness of texture is generally subconscious until expectations of textural features are not met. When it occurs, awareness and criticism towards the deficiencies arouse (Bourne, 2002).

The sensory character of bread is experienced through a complex interaction of several aspects, all from appearance of the bakery to mouth-feel when eating. It includes colour, aroma, texture and taste. Their combined effect thereby contributes to the consumer experience, acceptance and potentially awareness. Sensory evaluation of gluten-free bread basically assesses these involving factors. Texture attributes during chewing up until swallowing could include hardness, crumbliness and cohesiveness. Springiness is perceived by observing the crumb ability to spring back to its original shape after pressing on it (Matos *et al*., 2012). When the objective is to compare several samples according to one attribute, a simple ranking test with randomized (complete) block design, is suitable.

Analysing sensory properties provides a link between consumer and the product, and could be used to ensure consumer acceptance (Meilgaard *et al*., 2007). Expectance of which food attributes will be accepted versus rejected could be obtained by studying consumer reaction in combination with well-defined sensory attributes (Kilcast, 2010). Sensory evaluation help designing better products that gain consumer acceptance.

2.4.1 Texture

Food texture stems from its structure and refers to the sensory experience of interacting with a food (Chen and Rosenthal, 2015). It accompanies taste in the experience of food, primarily the way food feels in the mouth, although other senses are involved too. Without its texture, food lose its identity to us (Schiffman, 1977; Schiffman *et al*., 1978). It plays such major part of our recognition of foods, that with only the taste to base our experience on, many products are hard to identify.

Definitions of food texture have been expressed in many different ways. One of the founders of texture analysis describes texture as such: "Texture is the sensory and functional manifestation of the structural, mechanical and surface properties of food detected through the senses of vision, hearing, touch and kinaesthetic" (Szczesniak, 2002). Hearing could be appropriate to include when associating texture with crisp or crunchy textures, and vision for instance observing the rate of flow (Bourne, 2002). Sense of movement and position is what is meant by the sense of kinesthetics. Any influencing factor on the structure, such as ingredient interaction, storage and packaging, will affect the texture of the food (Chen & Rosenthal, 2015).

Consumers are conscious about food texture, in bread especially (Bourne, 2002). Products must fulfil their purposes, e.g. hamburger bread must not crumble and sandwiches must be able to be spread without breaking apart. Textural quality is therefor of economic importance (Bourne, 2002). It is known that consumers prefer soft bread crumb, and relate softness to freshness.

The primary objective of food texture analysis is exploration of how food material feels, behaves and performs (Food Technology Corporation, 2019). Food texture could be approached in two ways; sensory or instrumentally. Sensory being the more subjective form as the other could be monitored. Consumers evaluate food primarily through touch and mouth feel, and use a wide variety of sensorially oriented terms to describe food quality. Hard, crisp, juicy, soft, creamy and crunchy are among the most used words to describe food texture, according to studies in US, Japan and Australia (Bourne, 2002). Sensory evaluation is time-consuming and subjective, why sometimes instrumental methods are prioritised (Bourne, 1978; Stable Micro Systems Ltd, 1997). Although instrumental texture analysis aims to correlate with sensory evaluations, distinction between terminology shall be made due to these not always being highly correlated (Lawless & Heymann, 2010). A representatively method for measuring bread is a compression test. It reflects a consumer evaluating the freshness of a sliced bread by pressing on it and experiencing initial mouthfeel (Peleg, 1983; Szczesniak, 1966; 1987).

2.4.2 Texture Profile Analysis

Texture profile analysis (TPA) is a compression test known as the “two bite test”, quantifying multiple textural parameters in one experiment. These parameters aim to correlate with sensory parameters and accounts for the diversity of textural identity of different foods (Szczesniak *et al.*, 1963). The method has been widely used for quality control and characterisation of food since it was invented by the General Foods Corporation Technical Centre in 1963. (Trinh & Glasgow, 2012).

In this method, a bread sample is compressed by a pre-set distance. The force required is determined quantitatively and displayed as a two-peak diagram. Peak height, length and area is used to calculate TPA parameters. The peak force is known as the hardness and is a fundamental measure for calculating further parameters. As the sample is compressed a second cycle, the two compressions could be compared and produce information corresponding to what chewing the food sample would be like.

Parameters of TPA group into primary and secondary parameters (Bourne, 2002; Stable Micro Systems Ltd, 1997). Hardness, or firmness, may be taken as a measure of freshness and quality. Cohesiveness (ratio of peak area 2 by 1) indicate the strength of the internal bonds within the matrix of the sample (Szczesniak, 1963). Practically, it is showing to what extent a sample could be deformed before it ruptures. Springiness is a parameter defining the samples ability to recover between the cycles, where a low value indicates a bad recovery. Chewiness is the product of springiness, hardness and cohesiveness, reflecting the energy needed to chew a sample before it is ready to swallow. Originally, chewiness is applied to solids which bread is considered as (Food Technology Corporation, 2019), whereas the parameter gumminess is applied to semisolids. Calculating them both for the same sample is wrong yet a common mistake (Szczesniak).

Common sense and proper operational settings are important to avoid misleading results (Trinh & Glasgow, 2012). The instrumental measurements should mimic the sensory experience of a person (Lawless & Heymann, 2010). The TPA method have developed over the years for these reasons (Trinh & Glasgow, 2012). Lastly, combining texture analysis and sensory evaluation provide a more complete picture of the benefit of additives.

2.5 Improvement of Gluten-free Bread

Consumers expect gluten-free products to be as good as their wheat containing counterparts, why texture improvement is a major challenge for bakers and food technicians (Haman, 2017). Consumers interprets any deviation from the standard as a loss in quality, affecting purchasing behaviour (Food Technology Corporation, 2019). The goal of developing products is to meet these expectations as well as maintaining them throughout the shelf-life.

Selecting bread improver is challenging. What the different functional additives do to the basic ingredients (different starches and flours) is complex (Houben *et al*., 2012).

2.5.1 Emulsifiers

Emulsifiers are compounds possessing both polar and non-polar moieties. These are needed in the gluten-free system to stabilize gas bubbles (Casper & Atwell, 2014). They are coating the air cells, interacting with both the hydrophobic inside of the cell and the hydrophilic batter. Emulsifiers are widely used to improve crumb structure and retard staling (Houben *et al*., 2012; Levenstam, 2010). Polar lipids complex with amylose, wedges in the central cavity of a single helix. Along with inhibition of migration of water, the reduced aging could be explained (Gudmunsson & Eliasson, 1990). Studies have shown that emulsifiers could increase softness and retard aging of gluten-free bread (Levenstam, 2010). The result was also that the bread with emulsifier had more uniform crumb.

2.5.2 Enzymes

Enzymes are widely used in the baking industry as a functional ingredient (Hager *et al*., 2012; Arendt & Dal Bello, 2008; Gallagher, 2009). Enzymes in baking could be naturally derived from the raw material or added with intention (Arendt & Dal Bello, 2008). Gallagher (2009) suggests three categories of enzymes in breadmaking; dough structuring agents, fresh bread quality improvers and shelf life extension. Similar is mentioned by Hager *et al*. (2012). Arendt & Dal Bello (2008) mentions amylases and oxidases among others as functional ingredients in gluten-free bread. Another enzyme used is transglutaminase, cross-linking proteins of different sources, e.g. soy proteins, casein, gelatine or pea legumin.

There are several types of enzymes with activity on starch (endo- and exo-acting amylases, debranching enzymes and transferases) (Arendt & Dal Bello, 2008). The often-encountered problem of accelerated staling and crumby structure in gluten-free bread could possibly be inhibited by starch hydrolysing enzymes such as α-amylase.

α-Amylase

Several aspects are included in the function of an amylolytic enzyme; function on native starch granules, specificity and degradation products formed (Gallagher, 2009). The family of α-amylases (GH13) include a variety of enzymes, hydrolysing α-(1,4)- and/or α-(1,6)-linkages between glucose residues of the starch backbone.

α-Amylases (EC 3.2.1.1) are typical endo-enzymes, acting on starch, glycogen and related polysaccharides. They generate low molecular weight α-dextrins after random hydrolysis of the α-(1,4)-linkages in the starch polymers (Hoseney, 1994; Bowles, 1996).

The resistant starch fraction mainly consists of crystallised amylose (Kettlitz *et al*., 2000). Hydrolysis with α-amylase post resistant starch formation increase the relative amount of resistant starch. This, because the amount of non-resistant material is decreased (Thompson, 2000b). If amylolytic hydrolysis is carried out prior to retrogradation, the mobility of the polymers is increased, inducing molecular reassociation.

Starch fractions differ in their resistance to enzymatic degradation (Witczak, 2015). Debranching enzymes have shown to induce production of resistant starch (type III). Amylopectin sterically hinders crystallisation of amylose. If amylopectin is debranched, it loses this function, hence amylose crystallisation increases. Gujral *et al*. (2003a, 2003b) found when investigating rice bread that amylolytic enzymes might be helpful in preventing gluten-free bread staling. Rosell (2009) states that α-amylase inhibit retrogradation of amylopectin during storage whereupon it could provide prolonged shelf life of the bread.

Glucose Oxidase

The enzyme family oxidases catalyse redox reaction with oxygen as electron acceptor. Glucose Oxidase (GO; EC 1.1.3.4) is a flavoprotein and act specifically on the CH-OH group of glucose, together with oxygen, to create hydrogen peroxide and glucono-lactone (which spontaneously transforms to gluconic acid) (Webb, 1992).

$$\beta\text{-D-Glucose} + O_2 = \text{D-Glucono-1,5-Lactone} + H_2O_2$$

Studies of gluten-free bread improvement have found GO to have good potential in textural quality. Gujral and Rosell (2004a; 2004b) tested addition of GO in rice bread finding improved rice bread quality. They suggest the effect was due to promoted formation of rice protein network by GO catalysing inter- and intra-molecular cross-links between rice proteins. However, the need of hydrocolloid still remain since this process alone cannot replace gluten, they mention. Other authors suggest that the functionality of GO probably is due to the generated hydrogen peroxide (Goesaert *et al*., 2006), since it promotes oxidative cross-linking between proteins and/or other components.

3 Experimental Procedure

3.1 Literature Review & Screening

Literature study on product development of gluten-free bread was performed to support screening of bread improvers; emulsifiers and enzymes. The screening was initiated by contact with different firms, which the screening samples were derived from. A total of five emulsifiers and eight enzymes were test baked by applying them one by one to a model recipe. Before screening, the model recipe used in this study was baked multiple times to adjust and fine-tune water and HPMC levels. Selection of additives to study further was done by experience-based evaluation.

3.2 Raw Material

Commercial Swedish baking ingredients were used for a model recipe: rice flour (22.2%); maize starch (18.5%); potato starch (3.7%); psyllium husk (0.7%); hydroxypropyl methylcellulose (HPMC) (0.4%); bakers yeast (2.8%); rapeseed oil (2.8%); table sugar (1.9%); iodinated salt (0.8%); sorbic acid (0.21%). Tap water (46.1%) in the lab of Semper in Sundbyberg was used. The dry matter/water content ratio was 0.98.

Gluten-free bread improvers (one emulsifying and two enzymatic; referred to as additives) were studied at five different levels of addition, in different combinations with each other (referred to as samples). The levels of addition (w/w dry ingredients) were based on common levels used in the gluten-free baking industry but were adjusted after preliminary test baking. The three additives were: distilled monoglycerides, DMG, from hydrogenated rapeseed oil (0.0, 0.2, 0.5, 0.8 and 1.0 %; Dimodan® HR, Danisco DuPont, Denmark); a maltogenic α-amylase, AA (0, 142, 350, 558 and 700 ppm; Novamyl®, Novozymes, Denmark); a glucose oxidase, GO (0, 51, 125, 199 and 250 ppm; BakeZyme® Go Classic GF 10.000, DSM, the Netherlands).

3.3 Bread Making

3.3.1 Experimental Design

A response surface experimental design (combinations of additives and execution order of samples) was produced as a central composite design using Minitab version 18.1 (2017). The letters indicate which order the recipes were baked (A-T) and the numbers (1-20) represents the order of the recipes if they would to be structured in the order of the central composite design. Conversion of the table to numerical order could be found in the result section, for more convenient comparison regarding correlations.

Table 1. *Experimental Design – levels of additives and execution order*

Run order abbrev.	Sample	DMG (%)	AA (ppm)	GO (ppm)
A	10	1.0	350	125
B	13	0.5	350	0
C	8	0.8	558	199
D	2	0.8	142	51
E	19	0.5	350	125
F	9	0.0	350	125
G	5	0.2	142	199
H	6	0.8	142	199
I	17	0.5	350	125
J	12	0.5	700	125
K	11	0.5	0	125
L	18	0.5	350	125
M	16	0.5	350	125
N	14	0.5	350	250
O	3	0.2	558	51
P	20	0.5	350	125
Q	1	0.2	142	51
R	7	0.2	558	199
S	4	0.8	558	51
T	15	0.5	350	125

3.3.2 Dough Preparation

Twenty-two types of dough were made: two references (ref) made according to the model recipe; and twenty formulations made in based on the model recipe, but with the further addition of additives according to Table 1. Each formulation was produced once which generated four loaves. The centre value combination was repeated, hence produced in six replicates.

Doughs were prepared based in the procedure described in the AACC method 10-10B (2000). Water for the dough liquid was adjusted to 36±0.2°C and the yeast thoroughly suspended in the water. In formulation including Novamyl®, proper amount was dissolved in the dough liquid. The dough liquid was then added to the dry ingredients and immediately started to be mixed. Dough mixing was carried out in a planetary mixer (Major Titanium KMM020, Kenwood Ltd., UK) started at slow speed and within seconds raised and kept at medium/high speed [speed 2 on the machine]. After one minute of mixing, machine was stopped, bowl scraped and oil according to the recipe added. After additionally two minutes of mixing followed by scraping and one final minute of mixing, the dough was ready. No hand kneading was needed due to the gluten-free dough being rather a liquid batter. Nor was punching or sheeting of dough carried out in this preparation. Hence, the procedure went directly to moulding and proofing after dough mixing.

3.3.3 Baking

500± 0.1 g of the dough was poured into 1.3 litre Teflon bread tins, determinedly spread out with a dough-scraper to remove any air pockets. Tins were then placed in a proofing cabinet for leavening at 32°C (80% moisture) until dough had raised to the edge of the tin (approx. 45 min). Baking was carried out in a ventilated deck oven (Sveba Dahlen, Fristad, Sweden), with steam injection at the beginning of baking, at 240°C for 35 min or longer until minimum of 98°C inner loaf temperature was reached. Loaves were removed from the tins and cooled for 2.5 h at room temperature before packaged in plastic bags and left over-night.

3.3.4 Loaf Measurement

Loaf measurements was carried out the day after baking. Weight and height (middle of loaf) was recorded on all loaves with the plastic bag on. Loaf volume was measured, one loaf for each sample, by rapeseed displacement according to AACC method 10-05 (2000). Specific volume was calculated by dividing volume by weight. Pictures were taken on each sample which are shown in Figure 2. Bread moisture content was determined by drying approximately 1.5 g of sample in 105 °C over-night.

3.4 Fibre

Total fibre content analysis was carried out on seven-day old bread by Eurofins® Food & Feed Testing Sweden (Lidköping, Sweden) according to method AOAC 985.29, on a limited selection of samples and one ref. Expected ability of the additive to affect starch retrogradation, along with experienced based preliminary evaluation of all samples, lead to the selection of bread sample B, O and S. These samples were firstly all low in GO. Secondly, they showed promising effect on improving bread freshness when preliminary evaluated.

3.5 Texture

Seven days old bread loafs, two from each sample, were sliced in a slicing machine and immediately packaged in plastic bags. Slices from the centre of the loaf was selected and punched-out in the middle to obtain cylindrical 29.6 mm in diameter bread samples. The procedure aimed to obtain representative bread samples, therefor big holes were avoided. Texture analysis of the crumb was measured on two punched-out samples stacked together, six times for each sample. TPA was performed with Instron 3343 (Instron Cooperation, USA) equipped with a 35.7 mm aluminium cylindrical probe. Analysis was made in software Bluehill 2 (Instron Cooperative, USA).

3.6 Sensory Evaluation

3.6.1 Simple Ranking Test

A simple ranking test with randomized (complete) block design was performed using a limited selection of samples, to investigate if significant differences in ranking of the bread samples existed. For best utilization of the sensory panel, sample amount was limited to four (excl. ref). Pre-screening evaluated the attributes; appearance (observing of the bread slice), texture (attributes during chewing) and springiness (ability to regain original shape after pressing the crumb). The selection of samples aimed to include a representative range of the experimental: recipes that had indicated improved freshness in preliminary evaluation (sample B and O), as well as samples seen as less successful. Sample K was selected for its low specific volume, and sample F for its rather high specific volume and zero amount of DMG.

The four samples and one ref (k=5) was ranked by n=37 untrained panellists (employees at Semper AB; 70% woman and 30% men). The day of the test, the seven-day old bread loaves were sliced and kept in plastic bags. The room for evaluation was adapted for sensory evaluation according to Lawless and Heyman (2010). The samples were prepared in a separate room and presented as 1.2 cm thick squares of bread slices, excluding crust, on paper dishes assigned random three-digit codes. These were served in randomized order, using a separate randomization for each panellist.

Panellists were asked to feel, smell and taste, and after individual evaluation, rank the bread according to their perception of freshness. Panellists choose time for testing individually during normal working hours. With the bread samples, water was served as a neutralizer and participants were asked not to conversate with each other.

3.6.2 Consumer Preference 'Squeeze' Test

The five bread samples used in sensory ranking (k=5) were evaluated by same sensory panel (n=37) in a consumer preference test. The panellist got to pinch and feel the five unsliced loaves, covered in coloured plastic bags. They then voted for which single one they would have chosen to buy if they were in the shop.

3.7 Statistical Analysis

3.7.1 Loaf Measurement & Texture

Loaf measurements and TPA data were statistically evaluated by regression analysis in Minitab version 18.1 (2017). Data of the ref breads were excluded in these analyses. Response surface regression models were made for all responses (measurements and textural parameters), excluding non-significant (p>0.05) interactions and quadratic factors. The regressions were used to produce surface plots and contour plots showing the interactions between the additives.

3.7.2 Sensory Evaluation

Data from the Simple Ranking Test was tabulated and rank sums for each sample was calculated. The null hypothesis (H_0) stated that mean rating for all samples are equal, and the alternative hypothesis (H_a) stated that mean rating of at least two of the samples are different. Minitab version 18.1 (2017) was used to perform Friedman analysis (significance level p=0.05); Tukey Pairwise Comparison determining which samples was different; and General Linear Model to extract the adjusted R-squared value (the coefficient of determination).

A schematic diagram of how the method was proceeded is shown in Figure 1.

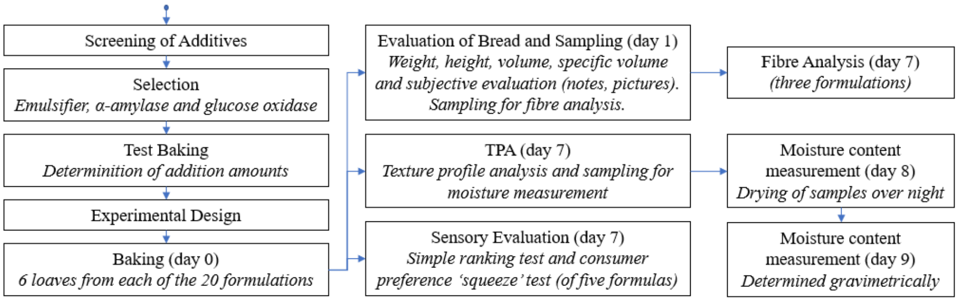

Figure 1. Schematic diagram of the method procedure.

4 Results

4.1 Screening

Screening of additives, initiated by a literature review and contact with different firms, was done by applying the thirteen additives one by one to the adjusted model recipe. When adding HPMC in too high quantities, the bread rose inappropriately fast and to a large extent, why adjustment was crucial before screening. Additives screened were tested within quantities recommended by each manufacturer.

From the screening, a limited number of additives with potential on prolonging and increasing freshness attributes in gluten-free bread, could be extracted. Unsuccessful additives were directly excluded. Borderlines were evaluated based on experience and subjective impressions (finger-feel, taste and mouth-feel). The final three additives selected for further testing were: Dimodan® HR (DMG); Novamyl®(AA); and BakeZyme® Go Classic GF 10.000 (GO). These were test baked in order to optimise the range of addition. The lower limit was decided to be zero (0 ppm), hence it was the upper limit that needed to be established. Levels of addition could be viewed in Table 2, now presented in sample numerical order.

4.2 Baking

Having determined the span of addition amount, Minitab (2017) was used to produce the test design shown in Table 2. The table is showing what amounts of DMG, AA and GO were added in which bread formula (sample). Each formula was based on the model recipe described in the method section. The baking was carried out in the randomised execution order according to the test design, presented in Table 1.

Table 2. *Test design of additive evaluation*

Sample	Run order abbrev.	DMG (%)	AA (ppm)	GO (ppm)
1	Q	0.2	142	51
2	D	0.8	142	51
3	O	0.2	558	51
4	S	0.8	558	51
5	G	0.2	142	199
6	H	0.8	142	199
7	R	0.2	558	199
8	C	0.8	558	199
9	F	0	350	125
10	A	1.0	350	125
11	K	0.5	0	125
12	J	0.5	700	125
13	B	0.5	350	0
14	N	0.5	350	250
15	T	0.5	350	125
16	M	0.5	350	125
17	I	0.5	350	125
18	L	0.5	350	125
19	E	0.5	350	125
20	P	0.5	350	125
Ref		0	0	0
Ref		0	0	0

4.2.1 Photo Documentation

Figure 2 is showing photo documentation of the one-day old bread samples. The ref obtained the highest volume and clearly have among the smallest, fine-walled cells. Appearance most alike the ref was sample B, O, Q and S, which all had minimum addition of GO (51 ppm). Sample C, J and S, whit a big hole in the top, all have high levels of DMG and AA in common. T, M, I, L, E and P are the six central replicates which in general had medium size crumb cells and medium to poor perceived springback. Samples with big holes were generally harder and appeared greasier.

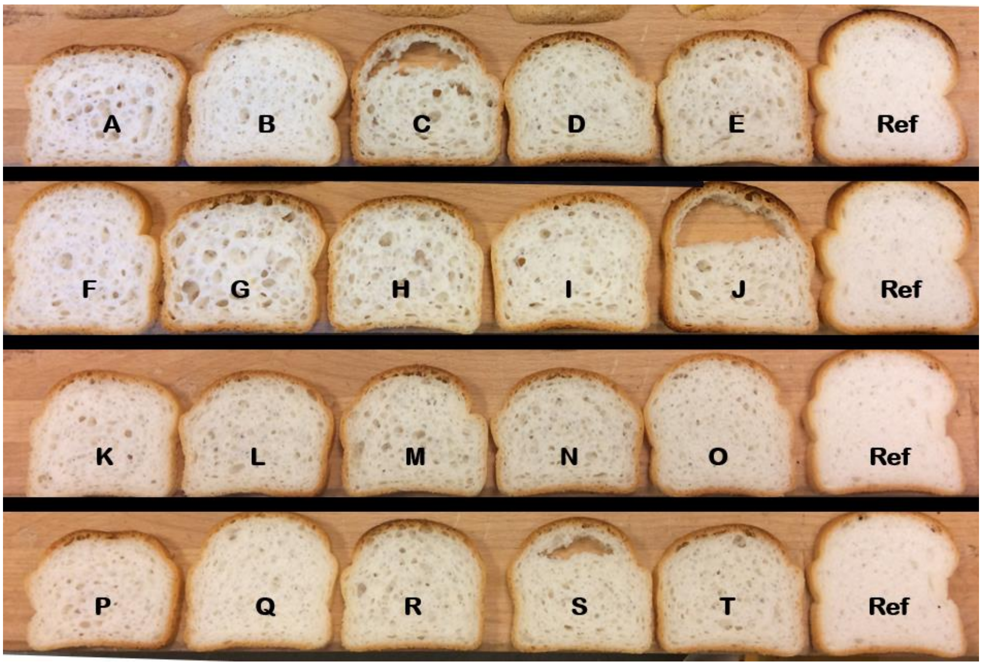

Figure 2. Photo documentation of bread samples: all twenty formulas and references (included in each photo for proper comparison).

4.2.2 Measurements

Measurement of weight, height, volume and calculation of specific volume took place day 1. Moisture content was determined after 7 days of storage in room temperature. Results are compiled in Table 3. Samples with the highest weight (sample S, G and T) where not the samples measuring the highest moisture content, which was sample Q and the two ref. Hence, weight and moisture content did not correlate. Height varied between 6.7 and 8.3 cm (excl. ref) and the lowest bread was 81% of the highest bread height. The centrum samples had generally low specific volume, as well as high DMG samples. The ref bread had medium-low weight, but reached high values of the other parameters (height, volume, specific volume and moisture content).

Table 3. *Results from loaf measurements weight, height, volume, specific volume and moisture content of gluten-free bread samples with added DMG, AA and GO at different amounts*

Sample	Run order abbrev.	Weight (g)	Height (cm)	Volume (ml)	Specific volume (cm^3/g)	Moisture content (%)
1	Q	404.0	8.1	1180	2.9	51.2
2	D	401.6	7.2	1055	2.6	49.9
3	O	408.8	8.0	1190	2.9	50.4
4	S	414.7	7.0	1070	2.6	49.3
5	G	418.3	7.4	1210	2.9	49.3
6	H	406.6	7.3	1100	2.7	50.5
7	R	405.9	7.5	1120	2.8	50.7
8	C	401.2	7.9	1160	2.9	49.9
9	F	406.9	8.3	1240	3.0	50.1
10	A	404.7	7.0	1060	2.6	49.4
11	K	411.0	7.1	1080	2.6	50.2
12	J	393.4	7.5	1200	3.1	50.1
13	B	399.7	8.1	1205	3.0	49.8
14	N	405.9	7.1	1080	2.7	49.5
15	T	414.6	7.2	1070	2.6	50.4
16	M	409.1	7.4	1080	2.6	50.4
17	I	406.8	7.6	1160	2.9	50.3
18	L	412.4	7.0	1060	2.6	50.5
19	E	411.1	7.5	1135	2.8	50.5
20	P	405.8	6.7	1020	2.5	50.5
Ref 1	Ref 1	388.2	8.6	1300	3.3	50.8
Ref 2	Ref 2	406.0	8.4	1280	3.2	51.2

Fibre analysis was done to a smaller selection of formulas that showed high potential in increasing freshness over time at an early stage. These were sample O, S and B and results are shown in Table 4. Sample S had approximately 42% more fibre than was detected in the ref.

Table 4. *Dietary fibre content in gluten-free bread formulas: O, S, B and ref*

Sample	Run order abbrev.	Dietary fibre content (g/100g)
3	O	3.2
4	S	3.4
13	B	3.3
ref	Ref	2.4

4.2.3 TPA

TPA was carried out on the one-week old breads. The results from TPA is compiled in Table 5, showing median values of six replicates. Standard deviation (SD) ranged between 0.07 to 1.59, except for chewiness which ranged from 0.60 to 8.00. The textural profile of the ref bread in this study is medium hardness, medium-low springiness, highest cohesiveness and medium-high chewiness. This profile is what is baseline for comparison.

Table 5. *TPA results showing texture profile of gluten-free bread (n=6)*

Sample	Run order abbrev.	Primary TPA parameters			Secondary TPA parameters
		Hardness (N)	Springiness (mm)	Cohesiveness	Chewiness (N*mm)
1	Q	5.2	8.5	0.36	15.9
2	D	5.9	9.0	0.31	16.3
3	O	4.6	8.7	0.29	11.7
4	S	6.1	8.6	0.32	16.9
5	G	6.1	8.8	0.36	19.4
6	H	5.6	8.8	0.31	15.1
7	R	4.8	8.6	0.32	13.2
8	C	7.7	9.0	0.28	19.7
9	F	4.8	9.1	0.32	13.8
10	A	5.9	9.1	0.32	16.9
11	K	7.2	9.0	0.33	21.3
12	J	6.0	8.4	0.31	15.7
13	B	4.5	9.1	0.34	13.8
14	N	5.6	8.8	0.29	13.9
15	T	5.3	8.6	0.31	14.2
16	M	5.4	8.7	0.32	15.0
17	I	5.0	8.6	0.32	13.4
18	L	6.4	8.6	0.33	18.0
19	E	5.1	9.0	0.32	14.4
20	P	7.1	8.8	0.29	17.9
Ref 1		6.0	8.7	0.38	19.9
Ref 2		5.4	8.5	0.38	17.6

4.2.4 Statistical Analysis of Instrumentally Measured Bread Attributes

Raw data was statistically analysed in Minitab, in which a response surface experimental was carried out and level of significance of the contribution of each additive was derived. Factors significant for the studied bread attributes are shown in Table 6 below.

Table 6. *P-values for the effect of DMG, AA and GO on textural parameters. All p-values are shown for the model factors (non-significant response italicized). Non-significant quadratic factors and interactions are not included in the table*

	Weight	Height	Volume	Specific volume	Moisture content	Hardness	Springiness	Cohsiveness	Chewiness
DMG	*0.385*	0.005	0.007	0.025	0.011	0.020	*0.343*	*0.075*	0.044
AA	*0.133*	*0.336*	*0.344*	*0.231*	*0.361*	*0.552*	*0.109*	0.031	0.028
GO	*0.479*	*0.171*	*0.576*	*0.505*	*0.362*	*0.101*	*0.868*	*0.085*	*0.274*
DMG×DMG^2					0.046		*0.057*		
AA×AA^2						0.056			0.017
GO×GO^2					0.017				
DMG×AA					0.047	0.041		0.047	0.004
DMG×GO		0.029			0.007				
AA×GO	0.024				0.018				

Bread weight was affected by a significant interplay between the α-amylase (AA) and the glucose oxidase (GO). Both increase bread weight, however when added in high dosages, they maintained a low weight. Bread height was negatively affected by addition of the distilled monoglyceride (DMG). This negative effect was increased in high addition of GO. Volume was significantly affected only by DMG, which decreased the volume. Although not significant, studying the contour plots in Figure 3 revealed the enzymes also had an impact on volume. AA had an increasing effect and GO a decreasing effect. The specific volume (volume/weight) showed similar results as the parameter volume, with correlating contour plots and same profile in Table 6. Bread samples having large volume also possessed a high specific volume (cm^2/g), as can be seen in Table 3. Highest specific volume in this study was obtained by excluding DMG or GO, or by adding maximum dosage of AA. Exclusion of AA or adding medium amount of all three additives in combination produced loaves with low specific volume.

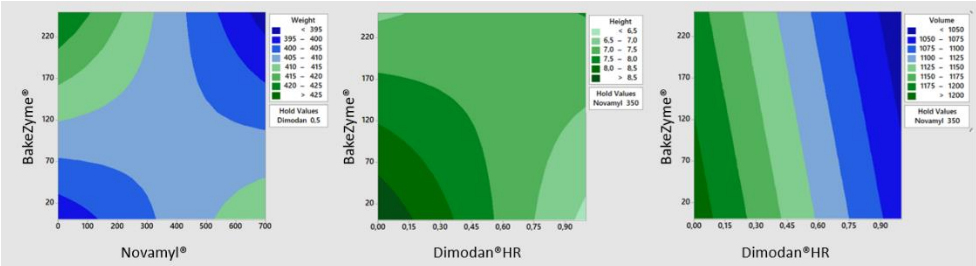

Figure 3. Contour plots showing interactional effects of additives on: weight; height; and volume.

Moisture content is evidently affected by the tested additives (Figure 4). DMG and GO both decrease the moisture content. However, applied together their effect subside. The correlation between DMG and AA is more complex.

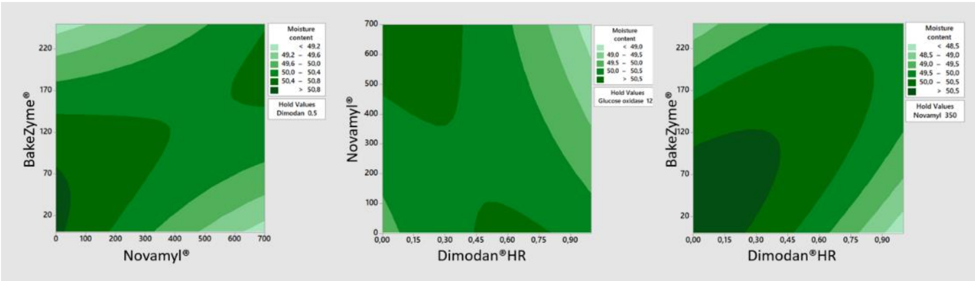

Figure 4. Contour plots showing interactional effects of additives in moisture content.

Hardness is affected by DMG and AA and their action are influenced by each other (Figure 5). AA seem to lower hardness at an optimum around 400 ppm. However, together with high dosage of DMG, increasing amount of AA makes the bread harder. DMG increase hardness. Maximum softness is obtained when adding high amount of AA and low amount of DMG. No significant effect on springiness was detected. However, DMG (Dim×Dim^2 p=0.057) is lowering springiness at an addition optimum around 0,45%. Highest values of springiness were derived at either zero or high amounts of DMG. Lowest springiness was derived by maximum dosage of AA and medium dosage DMG. AA has a significant effect on cohesiveness along with DMG. Cohesiveness decreases with raised amounts of AA and DMG. Chewiness (hardness x cohesiveness x springiness) correlate with hardness, showing similar contour plots. Alike hardness, chewiness is significantly affected by DMG and AA. DMG increase chewiness and AA seem to lower chewiness with an addition optimum at around 450 ppm. The interaction of additives is shown in Figure 5.

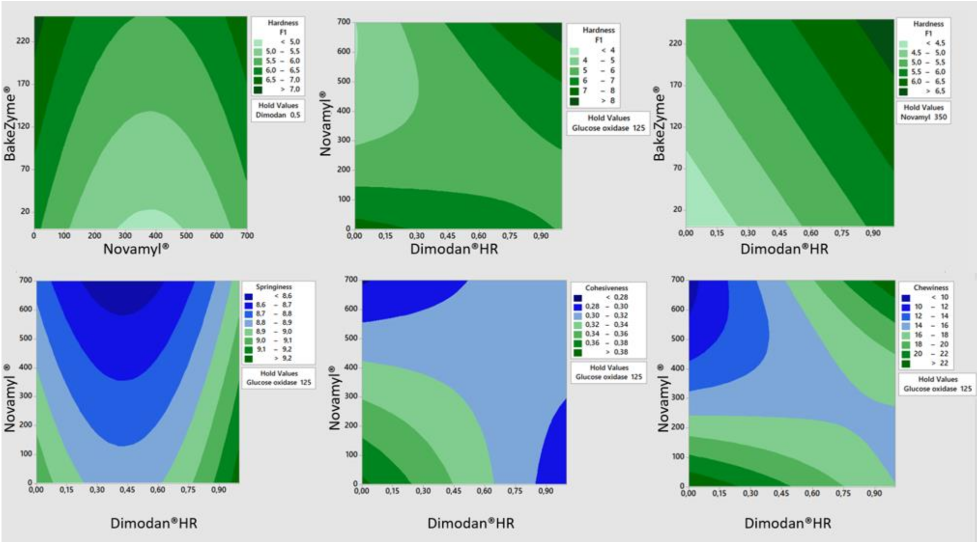

Figure 5. Contour plots showing interactional effects of additives in: hardness; springiness; cohesiveness; and chewiness.

4.3 Sensory Evaluation

The sensory panel, consisting of untrained individuals, was presumed to be best utilized by being provided a scaled down selection of formulas. The selection of bread formulas to include in the sensory test went as follows: all 20 samples from the design were preliminary evaluated subjectively and documented. Perceived successful combinations of the additives were noted. Of those, the two best were picked along with two other samples covering different parts of the spectrum. The design of this study is presented in a diagram of Figure 6. It illustrates how the different formulas relate to each other. The four selected samples: B; F; K; O, and one ref were baked seven days before the sensory test.

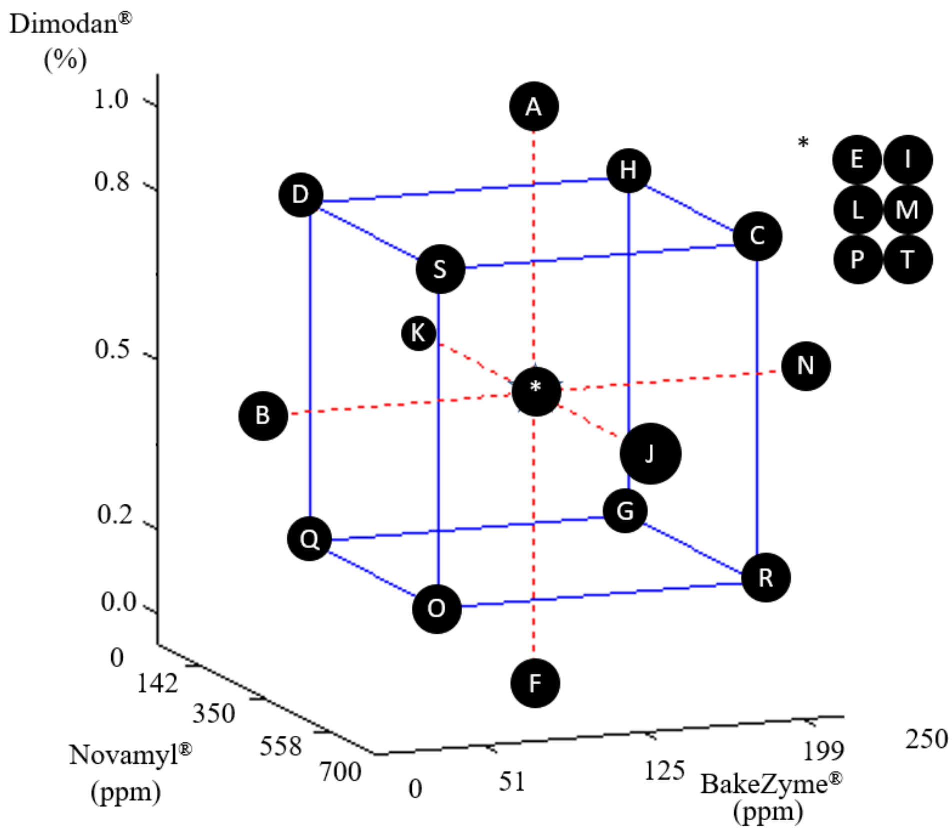

Figure 6. Overview of the experimental design, indicating run order abbreviations and their recipes composition of additives.

4.3.1 Simple Ranking Test

The consumer ranking test was carried out evaluating one attribute; freshness. The sensory panel (n=37) was given the task of ranking five gluten-free bread according to what they perceived as freshest. The result of the ranking test is shown in Table 7, where the highest number is what the panel perceived as freshest. Sample K was ranked as being freshest, which contained medium DMG, medium GO and no AA.

Table 7. *Results from simple ranking test: Rank of gluten-free bread samples B, F, K, O and a ref on freshness*

	Sample Ref	Sample B	Sample F	Sample K	Sample O
Rank sum, R	128.0[b]	87.5[c]	94.5[c]	159.0[a]	86.0[c]
R^2	16 384	7 656	8 930	25 281	7 396

Values with different superscript are significantly different at p<0.05

The result from the Friedman analysis confirmed rejection of H_0 at significance level 0.05, meaning at least two samples are ranked different. The Tukey pairwise comparison of the ranking result determined sample K was ranked higher than sample Ref, which both were ranked higher than sample B, F and O. There was no statistically significant difference in ranking between sample B, F and O. These had roughly similar parametrical profile. Sample K was different from these on several aspects. The General Linear Model indicated an adjusted R-square value of 28%. This percentage is the coefficient of determination of the variance, meaning that this ranking test explains 28% of the panellist's choice of rank order.

Figure 7. The sensory evaluation room and panellist ranking coded samples for degree of freshness.

4.3.2 Consumer Preference 'Squeeze' Test

The sensory panel was, after consumer ranking test, asked to vote for which bread they would buy if they were to buy a gluten-free tin bread. The same selection of bread formulas was coded and presented as un-sliced loaves in coloured plastic bags to not reveal any loaf colour. Only one vote was made possible. The result of the voting is presented in Table 8, showing sample F was the most popular loaf, followed by sample O. Sample K, winner of the consumer ranking test, did not receive any votes in this test.

Table 8. *Rank sum from the consumer preference 'squeeze' test evaluating sample B, F, K, O and a ref*

	Sample Ref	Sample B	Sample F	Sample K	Sample O
Rank sum	3	6	16	0	12

5 Discussion

Freshness is divergent, perceived differently depending on context, yet affectable. Additive interplay is complex and need to be fine-tuned. Clear goals are therefor of major importance. Specify goals by observing consumer reaction, along with keeping track of parameters by TPA.

Attributes scoring better freshness in this sensory test was firmer, more dense and chewy bread. This bread includes medium amount of Dimodan®HR and BakeZyme®, and no Novamyl®. The panel was informed the bread they were testing was a tin loaf, although the bread least alike this category of bread won the consumer ranking test. However, the consumer preference ‘squeeze’ test show more correlating results with the literature: that consumers associate softness to freshness. They were not advised to pick the freshest bread but rather to choose the one they would buy. Although consumers aim for the freshest bread. Two of the softest bread of the entire study was the once ranked no. 1 and 2 in the ‘squeeze’ test. Interestingly the winner of the consumer ranking test did not receive any votes in the ‘squeeze’ test. The ‘squeeze’ test results have the reversed order of the sensory ranking test order. The ref bread was not top ranked in either of the ranking test. Freshness is hence possible to affect, even if mode of improvement might differ. These findings support that improving freshness in bread should be preceded by a thorough pre-study determining as detailed as possible what the goals are and exactly what defects need to be improved in the product which is under development.

Knowing consumer liking is a corner stone in setting the guidelines for product development. Consumers might not know what they want, they know what they like (Arendt & Dal Bello, 2008). Divided sensory results is once again a proof of this. Further on, these results could be useful in developing bread that the consumers actually desire. The results indicate this is not classical toast bread, but rather levain-like bread. However, in the case of the particular product category of CO_2 packaged loaves stored on the shelf in room temperature, softness while feeling the loaf is much more critical than the experienced freshness of the bread in the sensory test.

What is aimed to be improved, the *freshness,* is sometimes hard to detect instrumentally. Parameters such as crumbliness, taste, mouth-feel influenced by water distribution, cell distribution and crumb homogeneity are only perceived sensorially.

The gluten-free bread baked in this study differ significantly, from the ref and among each other. The effect was clearly observed already after baking, see Figure 2 and is confirmed in the statistics. Successful results have previously been obtained when using emulsifier in gluten-free bread (Levenstam, 2010). However, the results in this study cannot be considered as successful, since the emulsifier decreased the volume and increased hardness, although this emulsifier performed well in the sensory test. Perhaps, the amount or technique of addition was wrong or simply just the emulsifier of selection not perfectly right. Improved crumb softness can be expected by the addition of protein-connecting and also starch-crashing enzymes in the right dosage (Houben *et al.*, 2012). Accordingly, the addition of AA increased softness, even in this study. However, the effect of GO was not as satisfactory in this experimental. Presumably due to the recipe being too low in protein, since the suggested mode of action of GO involves protein cross-linking. Fibre content was elevated in all three samples tested. This is probably derived from resistant starch formation, induced by the additives. To obtain a higher moisture content, AA shall be used, and combining DMG together with GO avoided. For DMG and AA together, nor high or low quantities of them both should be added simultaneously.

Instrumental- combined with sensory texture analysis is a suitable concept of measuring bakery goods (Food Technology Corporation, 2019), also shown in this study. By running texture analysis, statistically significant textural profiles could be assigned the different samples, and coupled to their level of sensorially perceived freshness.

5.1 Limitations

The selection of additives was derived by screening and evaluation based on our best knowledge. There might exist more interesting, better interacting enzymes, to study. However, literature supports the selection of enzymes and emulsifiers. The model recipe was adjusted once, before the experiment. For deeper knowledge of the full potential of the additives, levels of HPMC should be adjusted after finding a promising combination of additives. When adding HPMC in too high quantities, the bread rose too fast, however with high level of emulsifier the volume was low. Further on, additives might have optimum levels which not have been spotted.

Baking is a handicraft full of potential human errors. Pouring the dough into the tin is a critical step. Dough liquid temperatures, thorough dissolution of yeast, different leavening time until dough had raised to the edge of the tin and fluctuating oven temperatures is further examples of errors that might affect the results. Variation in biological material used is naturally occurring. There was only one dough sample prepared of each combination, however this was made into six bread which increased the reliability of the method.

TPA was made six times for each sample, which is standard. Main potential errors are wrongly programmed test settings, producing faulty values. If that would be the case, the bread samples are internally compared, eliminating the impact of that potential error. The sensory test evaluated four out of twenty samples. Ultimately all would be included. However, the four selected samples are believed to be representative as well as the best way to utilize an untrained panel is by providing a limited number of samples. The number of panellists is large enough to provide reliable results. Other potential errors are, although the sensory room and instructions followed guidelines, there might always arouse misunderstandings or exchange of words between test persons. Freshness is a broad term, definitely affecting the sensory results. Yet, there was a point in using a wide and fundamental term for this untrained panel.

The focus of this study, including three additives in the design, is a limitation in itself. The results show how these additives interact and what their combined effect are on the bread. The effect of one single improver will thus be omitted. However, these effects are well documented in the literature.

5.2 Future research

There are many enzymes to investigate further, especially their action on specific raw material. Houben *et al*. (2012) suggests future research to be more specific for the activity of different functional additives on the different starches and flours, in order to give better directions. The recipe of the sensory top ranked sample “K” could be developed using optimal addition of HPMC and adding a protein source. Further on, although this study provided divided results, it might prevent a sensory analytic to ask about “freshness” in this broad kind of sense done here.

6 Conclusion

From the chosen bread improvers, significant improvement of the perceived, as well as measured, freshness was detected. The α-amylase Novamyl® produces a bread that consumers would have preferred as a soft bread in the shop. The hydrogenated monoglyceride Dimodan®HR make the bread appear fresher in a consumer ranking test. This additive is more suitable for a more compact-type bread rather that toast bread. The glucose oxidase BakeZyme® did not present any clear desirable effects for improving freshness in this test. Their optimal amount of addition is not established by this study. However, this study might contribute to further knowledge about the potential of these additives. The fact that the ref bread did not score highest in the sensory ranking, nor the preference 'squeeze' test, further supports a positive effect of the additives on the freshness.

With ambiguous results, this study explored another dimension to the freshness concept - the consumer. Freshness is to some extent subjective hence consumers are a valuable resource in product development.

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Appendix 1 – Popular Scientific Summary

Fresher Gluten-free Bread

The additives doing magic

Gluten-free is no longer a trend. This market has grown tremendously the latest years and is still on the rise. People eat gluten-free for various reasons, nowadays not only because of celiac disease. However, the number of people getting celiac disease is actually rising, too.

Are you one who thinks gluten-free is a healthy option? Market surveys indicate many consumers would agree on that. In the US, as many as 30% of the population tries to cut down on gluten. Where in Sweden, one of the biggest grocery retailers (ICA) just increased their sales of fresh gluten-free bread by 50% the latest year.

Consumers are demanding and not specific in their wishes at the same time. Most expect gluten-free products to be as good as their wheat containing counterpart. It is learned that consumers might not know what they want, they know what they like. This means, consumers are not really aware of, for example, texture until it deviates from the standard. That is when suspicion arise and the consumer might disregard the product. Since this would be very unluckily for a bread manufacturer, they prevent this basically through product development of texture.

Gluten-free bread has major textural problems. It crumbles and gets dryer and harder than normal wheat bread. Gluten has a unique role in the making of ordinary bread, why it is so hard to replace. Research show that a mix of different gluten-free flours, proteins and fibre that absorbs water (hydrocolloids) is the best recipe to make gluten-free bread. Psyllium husk is one hydrocolloid, for example. Commercial products also include other ingredients to increase shelf life and provide a softer texture - emulsifiers and enzymes.

Emulsifiers act as adding oil in the machinery and lubricate the cogs. Enzymes works as if some cogs would be modified, tweaking their function slightly.

These could potentially do magic in the bread, as they are added in small amounts yet could cause major textural changes. The phenomenon occurring in bread when it goes from fresh to hard and dry is predominantly caused by starch aggregation (retrogradation). It is a spontaneous mechanism happening in starchy food during storage, from the moment of baking until the bread does not exist anymore. In gluten-free bread which mainly consist of starch, this mechanism is a little too dominant. At least if you are unhappy with hamburger bread dry cracking or sandwich crumbs crowding up in your spreadable butter. Luckily, there are emulsifiers and enzymes that have shown successful inhibition of gluten-free bread turning hard and dry so fast. These additives have been investigated one by one, not so much how they correlate when added together. For this is substances you cannot just add in approximate quantities. You need to know the optimal amount, which might be different if you add two, or even three, at the same time. Potentially they affect each other.

Why not add a selection of additives, and see what happens?

We believed addition of emulsifier and enzyme would improve textural freshness in gluten-free tin bread. We wanted to see if any sample with additive would be recognized as fresher than the untreated bread, when testing them on a sensory panel

The process started with reviewing the literature and eventually by screening several additives. The three most successful additives (emulsifier (E471), α-amylase and glucose oxidase) were selected for further investigation by incorporating them in a recipe for a plain gluten-free bread. Seven days after baking, they were analysed instrumentally and sensorially.

Results

The additives tested showed more or less an effect on all parameters tested, except for one. This was springiness, a measure for the ability of the bread to regain its original shape after pressing down on it. This was unexpected since this is a common bread measure. All three additives highly influence moisture content in different ways and also raise the fibre content in the bread. The bread got softest from adding α-amylase and cutting down on the emulsifier. The level of hardness correlated with another parameter measuring the energy needed to chew the sample before it is ready to swallow (chewiness). The emulsifier made the bread lower in volume. When applying additives, the cohesiveness, indicating the strength of the internal bonds within the structure, decreased.

In the sensory test, different additives made different favoured effects. To explain this further, when adding higher amount of the α-amylase and less of the others, a softer bread was created. This bread was favoured in an experiment where the sensory panel was asked which out of five bread loaves they would buy in the store, only by touching and squeezing the bread. On the other hand, when adding much more emulsifier, medium glucose oxidase and excluding the α-amylase totally, a bread with increased perceived freshness was produced. This combination was ranked highest in a tasting test where the sensory panel was asked to organize five samples in order from freshest to least fresh. This same combination did not get any votes at all in the 'squeeze of the loaf test'.

Why even bother?

For an optimal result, more comprehensive changes of the recipe are presumably needed. Yet, minor findings regarding specific additive interaction contribute to the main knowledge in the long run. Ideally this work contributes to a process of developing a healthy gluten-free bread in the future. This project provide knowledge in using emulsifier and enzymes in combination, hence development of gluten-free bread recipes.

If successful, this study could result in an updated recipe for a commercial bread. Realistically, this process contributes to knowledge and potentially better future product development processes.

Conclusion

The ambiguous result highlight that freshness is perceived differently depending on context.Yet, it is something you CAN affect. How the additive interplay is way complex and need to be fine-tuned. It helps if you know exactly what you are aiming for, since different additives contribute to different freshness parameters. You figure this out by taking advantage of observing the consumer, which is an important resource.

Appendix 2 – Instructions Sensory Evaluation

Sensory evaluation was carried out during one day. Below are the instructions for the consumer preference ‘squeeze’ test shown in Figure 8, and the simple ranking test shown in Figure 9.

Du är på affären och ska handla glutenfritt formbröd

Det finns ingen innehållsförteckning att läsa på och du ser heller inte brödet i förpackningen. Du kan bara känna på bröden.

Vilket bröd väljer du?

(Ringa för det alternativ du väljer)

A B C D E

Figur 8. Instructions for sensory consumer preference ‘squeeze’ test.

RANKING TEST

Date: 2019-03-27 Prov # : ____________

Type of sample: ***Gluten-free tin loaf***.

Characteristic studied: ***Degree of freshness***.

Instructions

1. Note each sample code below according to its position in front of you.
2. Taste the samples from **left to right** and take notice to the ***degree of freshness***.

   Wait 30 seconds between samples and rinse palate as required.

3. Write "1" in the box of the sample which you find ***freshest***.

   Write "2" for the next, … , and "5" for the ***least fresh***.

   Tip: first arrange the samples in a provisional order, and then resolve the positions of the samples by more careful tasting.

4. If two samples appear the same, make a "best guess" as to rank their order.

Code	_____	_____	_____	_____	_____
Rank	☐	☐	☐	☐	☐

Comments: ____

____

____

Figur 9. Instructions for the sensory simple ranking test.