Sveriges lantbruksuniversitet
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3 in 1 Bread

Functional, Bioavailable and Meeting the Health Claims – How Difficult Can It Be?

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Abstract

Habitual consumption of inappropriate diets has not only been associated with insufficient intake of micronutrients, but also increases risk factors for many chronic diseases such as obesity, diabetes and cardiovascular diseases etc. In the field of food science, new opportunities have evoked where advanced food technology and nutritional science have been combined to develop functional food, which is food products with the purpose to bring the gap between food and health closer. Health features of bread have gained substantial interest in recent years. Given that bread is considered as staple food, enhancing bioavailability of nutrients in bread, in particular nutrients known to be insufficiently consumed may serve as a potential to improve the overall intake.

Linking health claims recently approved by European Commission with bioavailability of iron, iodine and folate and development of flour-based bread became the objectives of the study. Review through literature studies was conducted concerning how bioavailability may be enhanced in bread. Furthermore, a flour-based recipe was developed in collaboration with Fazer where the objective was to estimate whether the requirements of iron, iodine and folate fulfilled the condition to use the health claims and if these nutrients were of bioavailable quantity.

Results showed that bioavailability of iron and folate is increased during the fermentation process involving leavening agents such as yeast and lactic acid bacteria from sourdough. Fermentation causes modification of pH, which contributes to degradation of phytic acid that otherwise are bound to iron as insoluble complex impairing its bioavailability. Furthermore, leavening agents have been reported to compensate for potential losses of folate in the baking process, thereby enhancing overall folate levels. Iodine fortified in salt was shown to be retained after the baking process. However, review of these results is dependent on additional factors ranging from condition of baker's yeast, amount and strains of leavening agents as well as type of grains to baking time. Furthermore, the recipe developed to be tried-out in the test bakery at Fazer Eskilstuna turned out to just partly meet the requirements to use the health claims and partly sufficient in terms of bioavailable quantity.

Using bread product as functional food may potentially improve consumption of those nutrients at risk of insufficient intake. Impact of fermentation methods are one approach to enhanced bioavailability of nutrients in bread. However, interaction among nutrients should not be overlooked as it may affect bioavailability in a negative way. The knowledge of bioavailability may be complex, but in the end, the health claims approved by European Commission only requires that the product is of a certain amount and do not care whether the nutrients are of bioavailable quantity or not.

Keywords: Iron, Iodine, Folate, Bioavailability, Retention, Bread, Yeast, Sourdough, Cereal, Health Claims

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Part One

1 Introduction

For many years, inappropriate diets have been known as one of the risk factors for many chronic diseases (www, World Health Organization, 1, 2012). Incorrect diet can also lead to insufficient intake of important micronutrients and contribute to increase prevalence of deficiencies. Since the 2nd half of the 20th century, major modification in diets have occurred resulting in changes in health and life style (www, Food and Agriculture Organization of United Nations, 1, 2012).

Owing to the combination of advanced food technology and nutritional sciences new kinds of food product categorized as functional food have been evoked (Bizios *et al*., 2011). Although, there are still no official definitions of functional food, it is basically food products that provide benefits to the body, beyond the effects of adequate nutrition, in a way that improves health and wellbeing and/or reduces the risk of disease (Vassallo *et al*., 2008). Attractive food products to be made as a functional food product are found to be muesli bars, yoghurts and breads while the most attractive functional ingredients are suggested to be plant sterols, vitamins, minerals and dietary fibre (Krutulyte *et al*., 2010). In recent years, health benefits of bread and pasta have been the most studied cereal products (Vassallo *et al.,* 2008). As dietary recommendation suggests a significant amount of daily carbohydrate consumption in the diet, it has been of substantial interest to develop functional foods of cereals. Therefore, increasing health benefits of bread products by enhancing nutrients of natural source may serve as a way to further strengthen the relationship between food and health. A possible approach is to link health benefits to health claims. Health claims have been revised by the European Food Safety Authority (EFSA) and recently (25th of May 2012) approved by the European Commission as health promoting information for consumers.

2 Objective

There is an increase interest of food products associated with better health and well-being. The willingness to purchase bread providing a simple and clear health benefit is stronger than bread containing a specific functional ingredient (Bizios *et al.,* 2011). Moreover, consumers are more interested in buying a food item where the functional ingredient is of natural source (Krutulyte *et al.,* 2010). Given that bread is considered as staple food in many countries, it is of relevance to extend the knowledge of "functional bread".

The focus of this thesis was on product development with the added value "health", by developing a bread product taking bioavailability of iron, iodine and folate into account. The purpose of increasing bioavailability of these nutrients may in the long run serve as a potential to improve overall intake of nutrients of which consumption has been known for being insufficient. In the scope of this thesis, two objectives were drawn. The first aim concerns literature study review on how bioavailability of the selected nutrients may be enhanced in different ways in a flour-based bread product. The second part of the thesis aims at analyzing whether it is possible to develop a flour-based bread product where the amount of these nutrients meet the requirement needed in order to make use of health claims about iron, iodine and folate. However, the health claims do not take bioavailability into account. Also, not the entire amount of a nutrient present in an ingredient may be of bioavailable content. Therefore, additional objective would be to analyze whether nutrients meeting the conditions to certain health claims also take into account that the amount is of bioavailable quantity.

3 Method

The idea behind the choice of nutrients to study was partly based on facts from World Health Organization on the most prevalent deficiencies today in the developed countries. In addition, Brödinstitutet published the brochure *Brödfakta* in 2011, which served as inspiration to include nutrient(s) commonly found in bread products. Insufficient intake has been observed for iron, iodine and folate in recent times. Therefore these three nutrients were chosen to be evaluated owing to that they all are presented in bread in a significant amount. As the first aim already stated in the objective involved how selected nutrients might be enhanced in different ways, subsequently bioavailability of these micronutrients became a relevant aspect to study.

Web of Knowledge was the primary database used. In addition, the database Scopus and Pubmed were used as a complement to broaden the survey of studies. Key words used to find research articles related to the objectives were: bioavailability, nutrition, mineral, vitamin, iron, iodine, folate, bread, bread-making, bakery products, cereal, grain, flour, rye, wheat, heat and retention.

Furthermore, the studies selected were all associated with bioavailability/retention of these nutrients. The definition of bioavailability will in this thesis imply nutrient distributed in a way in the product (i.e converted to soluble forms) that it is ready to be utilized. The main hits for iron and folate related to improving bioavailability in bread were focused on impact of choice of leavening agents. As for iodine, research about bioavailability in iodized salt in bread was focused to be reviewed.

The chosen studies were critically reviewed according to the following questions as template:

- To what extent has the objective been achieved?
- What knowledge has the study contributed to the field?
- What additional research questions have evoked by the results?
- What is not stated/mentioned in the study, yet relevant to take into consideration?

Part of the results from the review served as foundation for the next part of the thesis which was to develop a flour-based bread recipe to be tried out in the test bakery at Fazer Eskilstuna.

3.1 Delineation

Among the research articles found through the databases, only articles published from year 2000 onwards were selected to be reviewed. Even though some of the selected scientific papers aimed at evaluate bioavailability of different cereal-based food items, only results concerning bread were reviewed. For iron and folate, the bioavailability as a result of leavening agents was limited to yeast and sourdough (and to some extent lactic acid). The bioavailability of iron and folate was limited to cereals (mainly wheat and rye), thus availability of other commonly used food items in bread such as other types of grains, as well as seeds, dried fruits and vegetables were neglected. Only research concerning bioavailability of endogenous iron and folate in leavening agents were included, thereby excluding exogenous fortifications. However, exception was made for iodine as iodized salt was part of the aim. There are certainly additional nutrients of which intake has been observed as insufficient and may be suitable to be enhanced in bread products as well, yet the review were restricted to iron, iodine and folate. Furthermore, the health claims desired to be fulfilled did not cover other nutrients but iron, iodine and folate. Finally, the review was conducted as evaluating the nutrient's bioavailability separately and not an outcome of interactions as it may complicate the interpretation of the overall bioavailability.

4 Iron

Iron is an important mineral responsible for many functions in the body (www, Food and Agriculture Organization of United Nations, 2, 2012). Most of the iron in the body is presented in red blood cells as haemoglobin. Iron is needed as oxygen carrier from the lungs to the different tissues. Furthermore, iron is represented in several enzyme systems of various tissues including enzymes synthesizing steroid hormones and bile acids. Iron also acts as signal controlling in some neurotransmitters. In brief, iron plays an essential role in human metabolic processes.

4.1 Iron Deficiency

Iron deficiency is widespread in the world affecting a large number of people. In the developed countries, iron deficiency is significantly prevalent, in particular among women (www, World Health Organization, 2, 2012). It can sometimes be quite invisible, resulting in stealthy health consequences such as anemia. The prevalence of iron deficiency has led to development of a set of strategies to tackle the problem. Within the nutrition area, dietary diversification is one of the approaches, by which means, increased intake through iron-rich foods, improved iron absorption, fortification and iron supplementation. In 1941, USA started to enrich food products with iron. At that time it was compulsory to add iron salts in wheat flour. Sweden followed the trend with iron fortification as well, at least until 1994. Today iron is no longer enriched in flour in Sweden (Raba *et al.*, 2008). Bread and bakery products represent important part of food products in daily life, thus iron strengthening in bread may offer several health claims possibilities.

4.2 Iron in Cereals

Cereal products are particularly rich in sources of minerals. For instance, iron has shown to make up to 1-5 mg/100 g cereal grains (Frontela *et al.,* 2011). Unfortunately, minerals are formed as insoluble salt complexes with phytic acid. Phytic acid is inositol phosphates (IP) with varying amount of phosphate groups IP6, IP5, IP4, IP3, IP2 and IP1 (Nielsen *et al.,* 2006). It acts as storage compound of phosphorous and is accumulated in the outermost (aleurone) cell layer where it has the ability to chelate the minerals into salt complexes called phytate (Poutanen *et al.*, 2009). As a consequence, the insolubility of the mineral complex not only lowers bioavailability but also impair mineral absorption in humans. In addition, phytate is insoluble at pH higher than 7, hence the mineral complex is hardly degraded during human intake (Nielsen *et al.*, 2006).

4.3 Improving Iron Bioavailability

Subsequently, there are two major ways to create better condition in improving the overall iron intake. The first is selection of iron rich foods, while the second is to overcome the inhibitory barrier of phytic acid (Hurrell, 2002). Cereal fermentation is a process dating back to ancient Egypt, where naturally occurring microorganisms such as yeasts and lactic acid bacteria were activated in the production of beer and bread (Poutanen *et al.*, 2009). Yeast produces carbon dioxide and ethanol, while lactic acid bacteria produce lactic and acetic acids. Presence of these microorganisms is important for liberating iron in the baking process as it results in lowering the pH in the dough. An acidic environment has shown to initiate degradation of phytate (Nuobariene *et al.,* 2012), by activating phytases, a group of enzymes catalyzing the degradation (Nielsen *et al.,* 2006). Phytase hydrolyses the anion phosphates of inositol phosphates, preventing minerals to chelate into phytic acid; hence the amount of the minerals will become more soluble and available (Leenhardt *et al.,* 2005). The reasons behind fermentation in baking were and still are mainly to create extraordinary taste sensation such as new flavors and to improve texture and volume. Lately, knowledge in cereal fermentation has been extended as it also has the ability to overcome the barrier of phytic acid. Interest has gradually evoked to understand how methods can be developed and optimized to increase the bioavailability of mineral complexes (including iron).

4.3.1 Choice of Leavening Agents

Leenhardt *et al* (2005) explained how sourdough fermentation contributed to decrease of pH, thereby reducing phytate content of whole wheat flour. The aim of the study was to analyze disappearance of phytic acid during bread making in the following conditions: no leavening agent, yeast, sourdough and lactic acid. Results showed that a decrease of 35% in phytic acid was detected after 120 minutes in the unfermented dough. Nonetheless, the degradation of phytate was more pronounced when the dough contained either yeast or sourdough. After only 30 minutes the phytic acid degradation in the sourdough fermented dough was the same as the unfermented dough after 120 minutes. The decrease of phytic acid for yeast fermented and lactic acid dough was 20% and 13% respectively after 30 minutes. The degradation for both kinds of dough was stabilized to around 50% after 120 minutes proofing. Furthermore magnesium solubility was evaluated with respect to degradation of phytic acid. Sourdough fermented dough was much more effective in terms of magnesium bioavailability compared to yeast fermented dough and the unfermented dough. After 4h proofing, more than 25% of total magnesium was soluble in the sourdough fermented dough, while yeast fermented dough only yielded about 10%. The solubility of magnesium in the unfermented dough was estimated to 7.5%. The above actions occurred at a decrease of pH to 5.5.

The outcome have contributed to additional understanding in how time is important to changes in pH thereby determining changes in phytate levels. Previous research by Nuobariene *et al* (2012) has shown that yeast for instance exhibit phytase activity already at pH 5.5. The aim of Leenhardt *et al* (2005) was to study how the level of phytate altered by using different leavening agents. However, the pH of flours at initial state did not gain that much attention. It would be an important point to emphasize as wheat varieties were used in the baking. In the study by Nielsen *et al* (2006) phytase activity in the cereals showed to differ due to grinding process. Furthermore, a valuable approach to point out would be to evaluate the effect of different amount of each of the leavening agent. Sanz-Penella *et al* (2012) analyzed the effect of phytate degradation based on four sourdough levels. The results would be broadening insights concerning how the selected quantity of each of the chosen leavening agent contribute to the decrease of phytic acid in relation to time and pH. Thus, the knowledge is not only concentrated to the condition of yeast, sourdough and lactic acid bacteria but also to how the results may be prone to change when different amount of the same leavening agent are added.

Moreover, Leenhardt *et al* (2005) measured the bioavailability of magnesium, which many times tend to be just swiftly mentioned. Results from the study by Frontela *et al* (2011) showed that the iron solubility was estimated to 18.6%±1.3 and 20.4%±2.3 of total iron content for sourdough fermented whole wheat bread and white bread, respectively. The results may indicate that the ability for minerals to become soluble could be similar although different minerals were being studied.

While, Leenhardt *et al* (2005) only analyzed phytic acid activity of one type inositol phosphate (IP6), Nielsen *et al* (2006) evaluated activities of additional type of IPs. Hydrolyses of phosphates of inositol phosphate are key in increasing the bioavailability of minerals. According to Nielsen *et al* (2006) phytic acid requires to be degraded from IP6 to at least IP3 before minerals are released from their complexes.

To conclude, changes in acidifications affect the overall phytic acid content. Currently there is limited research evidence regarding the actual effect of iron in staple foods such as wheat flour (Hurrell, 2002). However, phytase activity has in general been observed to be low in wheat flour dough using strains of baker's yeast (Nielsen *et al.,* 2006). Perhaps, a combination of different fermentations such as of yeast and sourdough fermentation may be an even more optimal in improving bioavailability of minerals? Although more research attention is needed in this field, the results obtained about magnesium solubility may act as indicator for other minerals in terms of bioavailability content. Too what extent is complete reduction of all IPs required for fully access of mineral bioavailability? In other words, will for instance iron and zinc become bioavailable when there is still a pool of IP3 left? The results of Leenhardt *et al* (2005) may not be justified for every case as there are a variety of additional factors affecting what pH the dough finally reaches.

5 Iodine

Iodine is important for the synthesis of thyroid hormones which is important for growth and development of brain and central nervous system (www, Food and Agriculture Organization of United Nations, 3, 2012). Moreover, thyroid hormones are responsible for regulation of energy production and metabolic processes of fat, proteins, vitamins and minerals.

5.1 Iodine Deficiency

Iodine is considered as one of the world's most prevalent micronutrient deficiencies (www, World Health Organization, 3, 2012). It has been a public health concern as deficiency disorders may already start during pregnancy, jeopardizing survival of the child and later on affecting his/her mental health. Even if the most severe type of iodine disorder have become rare, 20% of all people with iodine deficiency live in Europe according to data from 1994 (World Health Organization, 2007a). The strategy developed to prevent further prevalence was through fortification of salt (www, World Health Organization, 3, 2012).

5.2 Iodized Salt in Food

In 1993, universal salt iodization was implemented, resulting in significant progress in decreasing the prevalence of iodine deficiency. Elimination has been quite successful; however efforts are still necessary in order to eliminate the deficiency completely. Despite application of iodized salts, the exposure of iodine is still decreasing in Europe (World Health Organization, 2007a). There have been few iodine deficiency disorder control programme, which partly explain why European Region is the region with the lowest coverage of iodized salt. Only 27% of the households are actually using iodized salt, which according to Winger *et al.* (2008) is explained by an ongoing trend towards using non-iodized salt at home. Globalization has not only increased the assortment of food items, but food items can also be produced in diverse manufacturing processes, which means that iodized salt may not always be used (World Health Organization, 2007a). The majority of food companies are not adding iodized salt for processed food according to Winger *et al.,* (2008) which is also true for Sweden (www, Livsmedelsverket, 2012). In Sweden, use of iodized salt is voluntarily, but the National Food Agency encourages food companies and restaurants to use iodized salt. Bread fortified with iodine has been successfully implemented in many countries of (World Health Organization, 2007a). For instance, the Netherlands has used iodized salt in bread since 1942. Despite the effort, intake of iodine is still inadequate, especially among Dutch women. Furthermore, "Testfakta" conducted a survey investigating the prevalence of using iodized salt among various food companies/restaurants in Sweden (www, Testfakta, 2012). The results published in April 2012 revealed that neither Skogaholm bread nor Pågen bread use iodized salt. Given the prevalence of iodine deficiency, including iodized salt in staple food products may therefore serve as a potential vehicle in improving the intake of iodine (Winger *et al.* 2008).

5.2.1 Choice of Iodized Salt in Bread

Research about iodized salt in food products in terms of bioavailability in bread products has been scarce. Thomson (2009) aimed to analyze stability of iodine from iodized salt when added to selected cereal-based food including bread. Iodized salt content was estimated to 1.10 mg/kg salt and 1.00 mg/kg salt for white bread and grain bread, respectively, of which was maintained after proofing and baking. This study indicated that iodine seemed to be preserved after the baking process.

Even if the author makes clear that the aim has been fulfilled, there are still technological issues to take into account before the stability of iodine can be established for all cases (Winger *et al.,* 2008). One major issue is that iodine, iodate and iodide are all reactive species serving as a strong oxidizing agent. When added into food, in particular in a low pH environment, oxidation or reduction cycles start to accelerate. The conflict that may occur is how iodine may oxidize/reduce vitamins and minerals when placed in acidic environment (Winger *et al.,* (2008), yet the environment is essential to create condition of low pH, thereby increasing the bioavailability (Poutanen *et al.*, 2009).

Thomson (2009) argued that stability of iodine in salts is remained in bread. Yet World Health Organization states that the actual availability varies due to food processing, washing and cooking (World Health Organization, 2007b). Also, Harris *et al* (2003) claims that losses of iodine in commercial baking are estimated to be more than 20%. One possible improvement according to Thomson (2009) would be to conduct a study based on a wider selection of bread types, to estimate whether there are any differences in iodine levels after baking of various type of flour-based bread. Another possibility would be to use the same recipe but perhaps make additional try-outs altering the suppliers of iodized salt. Results achieved would further expand the knowledge of iodine's stability in cereals.

Up to now the limited research results available make it hard to control the validation. General concerns that may not affect the stability or bioavailability of iodine in baking but could affect the condition of overall intake should be taken into account. Examples are iodine losses that may occur already at packaging stage of the salt, such as climatic conditions, texture of the salt and depending on whether the package has been opened before (World Health Organization, 2007b). Also, reasons such as financial issues (Harris *et al.,* 2003) should be overcome. Technological issues needs to be solved and guidelines should be established regarding the existing variability of iodine concentration in iodized salt. Although the manufacturers produce iodized salt, the actual bioavailability remains as a question mark. Furthermore, assuring that consumers at the same time are not at risk for overexposure of iodine is important. Otherwise replacing non-iodized salt with iodized salt in food processing without knowing the actual levels may end up in unfortunate consequences.

6 Folate

Folate is an important vitamin required for DNA synthesis and is contributing to normal metabolic function in humans. As humans are not able to synthesize folates, the vitamin has to be obtained through diet. Women of childbearing age, in particular, are advised to increase their folate intake the first month of pregnancy in order to reduce the risk of neural tube defect (Gujska & Majewska, 2005).

6.1 Folate Insufficiency

In countries where folic acid fortification is not compulsory, there is clearly a difference between the recommended intake and the actual folate intake (Öhrvik *et al.*, 2010). Folate deficiency may not be considered as widespread vitamin deficiency, however people in many countries are exposed to lower folate intake than the daily recommendation (Gujska & Majewska, 2005). A gap of 45% between the recommended folate intake and the actual consumption has been observed (Öhrvik *et al.*, 2010).

6.2 Folate in Cereals

Bread consumption contributes to 13% of the daily folate intake in Sweden and serves as an important source of folate as vegetables also do (Öhrvik *et al.*, 2010). Yet the same author claim that losses of endogenous folate in bread containing flours of wholemeal wheat (13%), wheat (11%), rye (7%), oat (3%) and rye kernels (14%) baked in 200 ^{0}C at 50 min was estimated to 41%.

6.3 Improving Bioavailability

Incorporating natural folates or improving folate content in food items considered as staple foods may improve the overall intake of folate. According to the study by Vahteristo *et al.,* (2002), intake of moderate amounts of rye products and orange juice have shown improvements of the folate status.

6.3.1 Choice of Leavening Agents

In order to get an overview of folate content in bread, Gujska & Majewska (2005) analyzed how levels of folate content changes. The aim was to measure the changes of both endogenous folate and added folic acid in baking process of yeast fermented wheat and rye breads. Results showed that the total content of endogenous folate in wheat flour was 20 µg/100 g on dry basis which after approximately 2.5 hours of proofing increased to 32 µg/100 g and finally decreased to 22 µg/100 g after baking in 230 ^{0}C for 30 minutes. In rye bread, similar pattern were obtained. The total endogenous folate content in start rye flour was measured to 29 µg/100 g which after approximately 1.5 hours of fermentation with baker's yeast rose to 46 µg/100 g and finally decreased to 34 µg/100 g after baking in 235 ^{0}C for 40 minutes. While native folate in both kinds of bread maintained in quite stable level, added folic acid decreased by 12% and 21% for wheat and rye bread, respectively.

According to Gujska & Majewska (2005), it was believed that the stability of endogenous folate was associated with baker's yeast's ability to synthesize folates during proofing. This study also appeared to be in good agreement with previous findings on folate content where fermentation has involved yeast (Kariluoto *et al.,* 2006). Hjortmo *et al* (2008) demonstrated that possible increase of folate also depend on choosing the right strain and of appropriate sources. For instance, the strain *Saccharomyces cerevisiae* has been found in baker's yeast and rainbow trout.

A valid point worth taken into consideration is the time, given that the objective was to study baking process. As Hjortmo *et al* (2008) emphasized, not only the type of cereals is of significance but also leavening time, as these factors influencing the actual folate content.

Kariluito *et al* (2006) examined fermentation of sourdough yeasts and lactic acid bacteria typically found in rye sourdough. The study aimed to analyze yeast's and lactic acid bacteria's ability of producing or consuming folate using a method that models sourdough fermentation steps. The results showed that increase of folate was mainly due to yeasts fermentation which improved the total folate content of the dough. Folate content rose from 6.5 µg/100 g to15-23 µg/100 g after incubation of 19h. Some of the natural lactic acid bacteria on the other hand showed to both produce and deplete folate, although the consumption in this study did not contribute to any significant effect.

The outcomes were in good agreement with previous obtained results regarding the positive effect obtained from growth of yeast (Katina *et al.,* 2007). For instance, the study by Hjortmo *et al* (2008) showed that the level of folates in baker's yeast increased by 1.5 times already after 3h proofing. Nonetheless, to understand every step of the fermentation process has proven to be a complex matter. The catabolic rate of baker's yeast appeared to be due to its ability to gradually adapt to the dough medium depending on its condition (Hjortmo *et al.,* 2008).

Also, a combination of different strains of lactic acid bacteria is often found in sourdough cultivation. Therefore, the knowledge and characteristics identified about each individual lactic acid bacteria may not provide enough information to grasp the whole picture of the folate levels. The same lactic acid bacteria may excrete folates as well as deplete folate thereby affecting the results. Kariluito *et al* (2006) chose to model sourdough fermentation step-by-step in a laboratory environment by using various strains of yeast and lactic acid bacteria. However, in bakeries the sourdough is usually kept active by back-slopping (adding flour and water at regular intervals) which will change and affect the quality of the sourdough (Rosenquist & Hansen, 2000). In the end, the reliability of the results remains rather vague.

Given that the objective of Kariluito *et al* (2006) was to examine the ability to synthesize and deplete folate, attention should have been placed on the influence of lactic acid bacteria to pH as it most likely will play an important role in terms of nutritional quality. Sanz-Penella *et al* (2012) monitored pH and lactic acid bacteria during the fermentation process. Results showed that the pH was slightly higher in control dough without lactic acid bacteria than with dough with these bacteria.

Finally, folate changes during rye bread baking have been scarcely studied thus knowledge about folate levels have increased especially in rye sourdoughs. It is hard to provide coherent results and arguments since production of folate may occur in other stages in the baking process. The folate content seems to be increased by yeast's ability to synthesized folates, while understanding natural occurring lactic acid bacteria and their influence on folate levels in the dough should gain much more attention. The source of leavening agent and its condition is of great importance. The study by Kariluito *et al* (2006) showed that the role of lactic acid bacteria did not seem to be too effective, as total folate depletion was not of significant amount. As some derivatives of folates not found in the raw flour appeared afterwards, yeast and microbiological activity of flour have the ability to compensate for the loss of folates from the proofed dough. The choice of yeast strain is necessary in discovering the most suitable strain to be grown in dough, a conclusion already stated by Nuobariene *et al* (2012). Baker's yeast is one of the food items containing the highest amount of folate. According to *Finnish Food Composition Database* yeast is the third folate-richest food items. Subsequently, this lead to new fields to understand, is there a difference between pelleted baker's yeast and fresh baker's yeast in terms of increase of the total folate content in the dough?

7 Discussion

Even though consumers may be reluctant to change eating habits, they are still interested in buying healthy foods (Vassallo *et al.,* 2008). For food producers, new market opportunities have been encountered. That is to develop food products with the potential to reduce many life style associated diseases (Bizios *et al*., 2011). In the case of incorporating functional ingredients in bakery products (such as bread) consumers' attitudes and preferences rely on the combined experience of perceived healthiness with type of bread, price, texture, taste and aroma. The bioavailability of the three nutrients iron, iodine and folate served as important aspect to evaluate given that intake has been known for being insufficient. Also, they all had in common to be used as fortification in food products in the past. Iodine may still be part of universal salt iodization; however it is not compulsory to use iodized salt in food processing today.

Regarding the results obtained from the reviewed studies, the way bioavailability might be enhanced in flour-based bread were partly dependent on the condition of the choice of leavening agent such as yeast and lactic acid bacteria from sourdough fermentation. However, the outcome of the micronutrient bioavailability has clearly been described to be influenced by additional factors than just optimizing leavening agents. The reviewed studies might on one hand achieve its objective, but on the other hand the results may not be consistent enough to be used as guidelines. Factors such as time, pH and the initial environment etc. may change from one time to another. For instance, Liukkonen *et al.,* (2003) mentioned milling process and types of cereals as factors affecting the concentration of bioactive compounds. Frontela *et al.,* (2011) argued that other compounds (i.e. dietary fiber components) in addition to phytase contribute to iron bioavailability. Iron and folate have shown to be increased during sourdough fermentation, but other vitamins such as thiamin and tocopherol may rather be decreased (Katina *et al*., 2005). On the other hand, yeast has repeatedly shown to increase folate, but thiamin content is increased as well. Furthermore calcium from milk (Frontela *et al.,* 2011) and iodized salt (Winger *et al*., 2008) has been reported to inhibit bioavailability of iron. Therefore, interaction between nutrients should not be overlooked as it may affect the outcomes, which adds to the knowledge of the complexity in trying to improve bioavailability of nutrients. Due to limited research attention justified outcome are hard to obtain. Results are not always comparable since approaches also vary among the studies.

At last, no clarified conclusions can be drawn. Looking at the nutrients separately, leavening agents do have a potential to enhance bioavailability despite that different approaches were used and that some factors taken into account in one study might be neglected in another study. It may be relevant to assure bioavailable quantity as a prerequisite to use the health claims, especially nutrients of which are at risk of insufficient intake. The specific measurement of bioavailable quantity may not be established, but the results may serve as indications. Furthermore, iodized salt may at some point still play an important role; however there may be other staple food more suitable to be iodide fortified. Is distributing iodine in salt still a good vehicle for improving iodine intake given that in the aspect of promoting good health, salt intake are encouraged to be reduced? The development of functional bread product assuring the content of bioavailable vitamins and minerals of natural source may not be a simple task for food producers despite new market opportunities have been evoked.

Part Two

1 Introduction

The fast food trend has contributed to increased bread consumption (www, Brödinstitutet, 1, 2012). Consumers are looking for quick solutions to meals and bread has been fitting into the requirement. Data from Swedish Board of Agriculture shows that consumption of bread reaches up to 55 kg/person and year (www, Jordbruksverket, 2012) and there is no indication that bread consumption will cease (www, Brödinstitutet, 1, 2012). According to Brödinstitutet, the bread trend today is clearly pointing towards healthiness where extraordinary healthy bread is perceived to be baked on sourdough, based on wholemeal and including kernels, seeds and fibers. In Sweden the assortment of bread is estimated to several hundred varieties. The most common varieties range from baguettes, wheat-based bread, flat bread to crisp bread (www, Brödinstitutet, 2, 2012). In addition, Brödinstitutet conducted a survey in September 2011 asking 1200 Swedes whether their eating habits of bread have changed in the past five years (www, Brödinstitutet, 3, 2012). The results showed that one in three Swedes are consuming more sourdough bread than five years ago and just as many claims that they are purchasing unwrapped loaves from in-store bakeries.

2 Objective

There is a trend in Sweden towards consumption of bread presented as unwrapped loaves and of healthy bread products, preferably sourdough fermented. In the Swedish supermarkets, bagels are one of the products sold as unwrapped loaves of bread. It's a versatile bread product in terms of eating moments, suitable for breakfast as well as a snack. Up to now, modest product development has been found about bagels in the Swedish market. Yet, bagels have been popular in USA for ages and the trend has reached Europe, in particular United Kingdom. Given that this thesis is conducted in cooperation with Fazer, the idea was to develop a sourdough fermented bread product of unwrapped loaves to their existing line. Bagels are currently not in Fazer's bread line, thus it is thought to be a good opportunity to incorporate functional properties in a bagel. Not only will the product idea appear to be timely for a potential boom, but also ability to improve health and wellbeing. To be able to market the bread as health promoting, approved health claims reviewed by European Food Safety Authority (EFSA) served as cornerstone in this thesis. The aim was to develop a try-out bagel recipe where following questions were taken into consideration:

- Will the final chosen ingredient(s) added in this test recipe fulfill the European Commission's requirements on making health claim(s) about iron, iodine and folate?
- Will the nutrients meeting the conditions of use the health claim(s) also take into account that the amount is of bioavailable quantity?

3 The European Food Safety Authority

The European Food Safety Authority (EFSA) is an independent agency co-operating closely with partner organizations throughout the EU and beyond (www, The European Food safety Authority, 1, 2012). Part of EFSA's work programme is related to delivering advices in different areas such as food and nutrition, animal health and welfare, plant health and protection. The major role of EFSA is to access and inform all risks associated with the food chain (www, The European Food safety Authority, 2, 2012). EFSA is thereby responsible for delivering advices to risk managers in their policy-making processes. Also, high scientific standards are ensured by carrying out work within feed safety, safety assessment in substances and claims by continually reviewing the scientific work (www, The European Food safety Authority, 3, 2012). Briefly, EFSA's work concern revising legislation, developing new regulatory frameworks and deciding whether or not to approve a certain substances and at what amount (www, The European Food safety Authority, 2, 2012). In that way, EFSA aim at raising awareness and distributing knowledge among various organizations ranging from other national authorities, consumer groups, non-governmental organization to individuals (www, The European Food safety Authority, 4, 2012).

3.1 Health Claims

The numbers of food products sold in EU advertised to contain certain nutritional properties and health benefits after consumption have increased. The European Commission found it necessary to draw up guidelines of permitted health claims (www, The European Food safety Authority, 5, 2012). In December 2006 a regulation was adopted by EU decision-makers where the use of nutrition and health claim should be equally labeled EU-wide. Based on scientific evidence, EFSA were appointed to evaluate and present health claims to be authorized in the EU. The proposal was officially approved by European Commision on the 25th of May, 2012.

The reasons behind setting up guidelines were mainly to protect consumers from misleading labeling and health promotion. Now food has to meet specific requirements to be eligible to make a certain claim. Nowadays, an adequate claim means that the substance subjected of the claim exists in a sufficient amount in the final product to produce a nutritional or physiological effect.

3.1.1 Health Claims about iron, iodine and folate

Health claims about iron, iodine and folate and the conditions to be fulfilled in order to use the approved claims are presented in Table 1. The claims about iron, iodine and folate have been extracted from EUR-LEX (2012).

Table 1. Health claims about iron, iodine and folate and the conditions to be met in order use the claims EUR-LEX (2012)

Nutrient, food or food category	**Claim**	**Conditions of use of the claim**
Iron	Iron contributes to normal formation of red blood cells and haemoglobin, reduction of tiredness and fatigue etc	The claim may be used only for food which is at least a source of iron as referred to in the claim SOURCE OF [NAME OF VITAMIN/S] AND/OR [NAME OF MINERAL/S] as listed in the Annex to Regulation (EC) No 1924/2006.
Iodine	Iodine contributes to normal cognitive function and functioning of the nervous system etc.	The claim may be used only for food which is at least a source of iodine as referred to in the claim SOURCE OF [NAME OF VITAMIN/S] AND/OR [NAME OF MINERAL/S] as listed in the Annex to Regulation (EC) No 1924/2006.
Folate	Folate contributes to normal amino acid synthesis, normal blood formation and maternal tissue growth during pregnancy etc	The claim may be used only for food which is at least a source of folate as referred to in the claim SOURCE OF [NAME OF VITAMIN/S] AND/OR [NAME OF MINERAL/S] as listed in the Annex to Regulation (EC) No 1924/2006.

In accordance to *SOURCE OF [NAME OF VITAMIN/S] AND/OR [NAME OF MINERAL/S] as listed in the Annex to Regulation (EC) No 1924/2006,* claim may only be made where the product contains at least a significant amount (EUR-LEX, 2006). The meaning of significant amount is further referred to the *Council Directive on Nutrition Labelling for Foodstuffs (90/496/EEC)* of which the vitamins and minerals are defined as containing at least 15% of the recommended daily allowances specified in this directive supplied by 100 g or 100 ml (EUR-LEX, 1990).

In the case of iron, iodine and folate the amount required to make the health claim is calculated for a portion of 100 g or 100 ml, is presented in Table 2.

Table 2. The daily recommended allowance for iron, iodate and folate and at what amount 15% of the daily recommended intake corresponds to regarding these three nutrients

Nutrient	**Recommended daily allowance**	**Amount needed (per 100 g or 100 ml) to fulfill conditions of use of the health claim**
Iron	14.0 mg	2.10 mg
Iodine	150 µg	22.5 µg
Folate	200 µg	30.0 µg

4 Materials & Method

The development of the recipe was based on an existing bagel recipe obtained from one of Fazer's flour suppliers as modification of a recipe already customized for large-scale baking would be more convenient. It should be emphasized that this part of the thesis may as well be a "theoretical try-out". However, a hands-on experiment was conducted to show how bread turned out when certain criteria needed to be met. The try-out baking was carried out at Fazer Eskilstuna's test bakery. Iron, iodine and folate are considered as nutrients where insufficient intake has been observed. Review in ways of enhancing intake of these nutrients through literature study served as inspiration to develop a functional food bread product. The results from the theoretical part were therefore taken into account when choosing the ingredients to be included. Information from several food databases such as Swedish National Food Administration, Finnish Food Composition Database, United States Department of Agriculture National Nutrient Database and relevant suppliers served as guidelines in determining what ingredients with high amount of iron, iodine and folate should be selected in this try-out recipe. Finally, theoretical calculations were further performed to show whether the total iron, iodine and folate content of the modified recipe met the conditions to be able to use the health claims. Study by Frontela *et al.,* (2011), Thomson (2009) and Gujska & Majewska (2005) were selected to be used as guidelines to estimate the bioavailable quantity of iron, iodine and folate. The calculation further provided information whether the amount of nutrients needed to make use of that health claim was of bioavailable amount.

A simple subjective evaluation of the final bread was made and the following attributes were studied:

- Volume
- Density
- Visual porosity
- Surface
- Fat spreadability

4.1 Delineation

In the scope of this master thesis, the ingredients tried-out in the recipe were limited to the assortment of Fazer Eskilstuna. Although salt is not iodized in the assortment of Fazer, iodine content was calculated as if it was included in try-out recipe. Furthermore, the actual bioavailability of the nutrients in the bread was not analyzed, neither was the level of nutrients nor pH measured.

4.2 Fazer Eskilstuna's assortment

The raw materials were provided by Fazer Eskilstuna. Only fresh sourdough starter was purchased in a nearby supermarket in Eskilstuna, as it was not in their regular assortment. At Fazer Eskilstuna's assortment, the following products (Table 3) were found and its nutrient content was obtained from various food databases as no nutrition index was available from Fazer's suppliers. The ingredients finally selected to be included in the modified recipe were based on the content of iron, iodine and folate presented in this table.

Table 3. Nutrition content of iron, iodine and folate in the assortment of Fazer Eskilstuna

Ingredient	Iron (mg/100 g)	Iodine (µg/100 g)	Folate (µg/100 g)
Wheat flour	1.08[1]	N/A[5]	23[1]
Wholemeal wheat flour	5.0[1]	N/A	43[1]
Refined spelt flour	2.2[4]	N/A	45*
Rye flour	3.3[1]	N/A	56[1]
Refined rye flour	2.2[2]	N/A	68.4[2]
Wheat bran	11.0[1]	N/A	260[1]
Oat bran	6.3[2]	N/A	46[2]
Oat meal	6.6[2]	N/A	46[2]
Flaxseed (whole)	5.73[1]	N/A	87[1]
Pumpkin seed	15[1]	N/A	60[1]
Sunflower seed	6.8[1]	N/A	60[1]
Millet (whole)	4.8[1]	N/A	27[1]
Millet seed	N/A	N/A	N/A
Melon seed	N/A	N/A	N/A
Poppy seed	9.76[3]	N/A	82
Cardamom	13.97[3]	N/A	N/A
Fennel seed	18.54[3]	N/A	N/A
Yeast (fresh)	4.9[1]	N/A	1000[1]
Spelt flakes	3.6[4]	N/A	45[1]*
Salt	N/A	5000[1]	N/A

[1] Swedish National Food Administration
[2] Finnish Food Composition Database
[3] United States Department of Agriculture National Nutrient Database
[4] Nutrition content from supplier - Saltå Kvarn
[5] N/A = Not available
* Data about spelt (whole) were used

4.3 Recipe

The original recipe is presented in Appendix 1, while the developed recipe is shown below (Table 4). Modification of recipe was based on the stated objective, which was to fulfill the European Commission's requirements on making health claim(s) about iron, iodine and folate. Therefore, the choice of ingredients was with regards to iron and folate. In addition, the selected proportions of ingredients were under supervision of the product developer team of Fazer. The amount of selected ingredients is displayed as % of total dough weight. Only iron and folate content for each ingredient were chosen to be reported. Given that, Fazer is not using iodized salt, iodine was not taken into account in the estimated calculations of the total dough weight.

Table 4. The proportion of the chosen ingredients by total dough weight given in %, and the estimated calculation of iron and folate content per ingredient for recipe 1 and recipe 2

Ingredient	**Recipe 1 (without pumpkin seed)**			**Recipe 2 (with pumpkin seed)**		
	% of total dough weight	Iron (mg) for each ingredient	Folate (µg) for each ingredient	% of total dough weight	Iron (mg) for each ingredient	Folate (µg) for each ingredient
Wheat flour	20.9	4.21	93.6	18.3	4.21	93.6
Refined spelt flour	35.9	14.7	288	31.5	14.7	288
Rye sourdough	7.45	4.59	77.5	6.53	4.59	77.5
Pumpkin seed (including 8g topping/bagel)	-	-	-	12.4	34.3	158
Salt	1.39	0	0	1.22	0	0
Oil	1.02	0	0	0.89	0	0
Yeast	3.10	2.83	576	2.71	2.83	576
Water	26.7	0	0	23.4	0	0
Syrup	3.49	0	0	3.05	0	0
Total amount nutrient of the total dough weight		26.4	1037		60.6	1196

Theoretical calculation of nutrients is further elaborated below:
X = total amount of an ingredient (g) (e.g. wheat flour)
Y = sum of the dough weight when all ingredients were included (g)
Z = content of iron (mg/100 g) in each specific ingredient

To estimate the proportion that each of the ingredient (e.g. wheat flour) accounts for of the total dough weight:
% of total dough weight = X/Y * 100

To estimate the amount of iron (T) for each ingredient (e.g. wheat flour):
T = Z * X

The same procedure was applied for folate.

4.4 Baking process

Two kinds of bagels were made and each variety was made in triplicate according to recipe shown in Table 4. Both recipes are the same except for one single ingredient, recipe 1 is without pumpkin seed while recipe 2 contains pumpkin seed. The first step in the baking process was to combine all dry ingredients (wheat flour, spelt flour, salt). In recipe 2, whole pumpkin seeds were added in the dry ingredients to the three sets of bagels. Then the liquid ingredients (water at temperature 27 ^{0}C, yeast, syrup, oil, liquid sourdough starter) were added into the dry mixture. Mixing time was 1 minute at low speed, followed by 3 minutes at higher speed. The dough turned out to be moderately stiff with smooth surface. After kneading for a short while the dough was cut into 18 pieces à 100 g and shaped into 18 balls. Each ball was pierced with a finger and rotated around the finger to make it look like a donut shape. The bagel was gradually stretched to create a hole about 4-5 cm in diameter. For the three sets containing pumpkin seed additional pumpkin seed à 8 g was added on top of each bagel to make it a nicer appearance and to be able to visually separate the different bagels. The bagels with pumpkin seed added on top weighed 108 g. Bagels where proofed for about 75 minutes at 38 ^{0}C and 80% relative humidity. Thereafter, the bagels were put in the oven. The baking in the oven involved steaming for 45 seconds, baking for 1 minute at 250 ^{0}C, followed by lowering the temperature to 210 ^{0}C where the bagels were baked for additional 9 minutes. During the oven process, steam was released after 4 minutes.

5 Results

5.1 Are criteria to use the health claims met?

The estimated iron, iodine and folate content per 100 g bread for recipe 1 and recipe 2 are presented in Table 5. Iodine is also included in the table even though iodized salt was not used in baking. Therefore, results of iodine are just estimation of if it is to be included in the dough. The table further shows whether the question about the amount estimated met the requirement to use the health claims described in Table 1 and Table 2.

Table 5. Estimated amount of iron, iodine, and folate per 100 g bread for recipe 1 and recipe 2 and whether the nutrients are meeting the health claims

	Recipe 1 (without pumpkin seed)		**Recipe 2 (with pumpkin seed)**	
Nutrient	Amount/100 g bread	Fulfilling condition to use health claim?	Amount/100 g bread	Fulfilling condition to use health claim?
Iron (mg)	1.57	No	3.16	Yes
Iodine* (µg)	77.5	Yes	67.8	Yes
Folate (µg)	61.8	Yes	62.4	Yes

*Iodine is only a hypothetically outcome, as if iodized salt were used in the baking.

Theoretical calculation for recipe 1 regarding the estimated amount of the nutrients is further elaborated. The calculation for recipe 2 followed the similar pattern. Only calculation of iron per 100g is presented below.

The total iron was estimated in Table 4 = 26.4 mg

Y= the total dough weight for recipe 1 when all ingredients were included (g)
Amount of iron (mg) per 100 g = 26.4/Y * 100 = 1.41 mg

During baking in the oven water evaporates thus the final dough weight was 10% less. Therefore, the nutrient content needed to be adjusted to 100 g.

Amount of iron (mg) per 100 g = 1.41 mg/0.9 = 1.57 mg

For recipe 1, iron was the only nutrient not fulfilling the condition to use the health claim. The results further demonstrated that adding pumpkin seed corresponding to 12.4% of the total dough weight turned out to be sufficient to fulfill the health claim for iron. Furthermore, iodine and folate were present in sufficient amount to be able to use the health claims. The estimated results about iodine and folate are applied for both recipes.

5.2 Are requirement about bioavailable quantity met?

The studies by Frontela *et al.,* (2011) and Gjuska & Majewska (2005) were used as guidelines in estimating the bioavailable quantity of iron in flours and folate in baking containing flours and yeast. The study of Thomson (2009) was used for calculation of the bioavailable quantity of iodine in salt, once again as if it was to be included in the dough. However, no relevant literature about bioavailable quantity of pumpkin seed was found thus the outcomes do not provide sufficient information concerning bioavailability of iron and folate for recipe 2.

Data obtained from the study by Frontela *et al* (2011) showed that solubility of iron in sourdough fermented white bread was 20.4%. Both rye sourdough and refined spelt flour was calculated as the results obtained of white bread. In recipe 1 and 2, the same amount of flours were used thus the total amount of iron in flours was estimated to 1.57 mg/100 g, of which the potential bioavailable quantity was 0.32 mg/100 g. On the basis of this selected study, the bioavailable amount of iron in flours was not sufficient enough as the claim states 2.1 mg iron per 100 g or 100 ml. Yet the result is not sufficient for recipe 2 and as no information regarding bioavailability of pumpkin seed was obtained the bioavailability of iron was not possible to judge.

As for iodine, Thomson (2009) showed that iodine content in salt remained the same level after baking. Based on the amount of salt used, the iodine amount was estimated to 72.2 µg/100 g, of which the entire amount is suggested to be remained. This should be enough to be able to use the health claims for iodine, which states 22.5 µg per 100g or 100ml.

Moreover, the study by Gujska & Majewska (2005) showed that folate in bread was retained after baking. The folate levels of flour increased by 10% when yeasts were used as leavening agent. In recipe 1 and 2 the folate content of the flours was estimated to 459 µg, yielding 24.6 µg/100 g and 21.6 µg/100 g, respectively. The bioavailable amount of folate was estimated to 27.1 µg/100 g and 23.7 µg/100 g for recipe 1 and recipe 2, respectively, thereby not sufficient enough to meet the criteria of bioavailable quantity which is 30.0 µg per 100g or 100 ml. The results obtained for wheat flour was used as guideline in this estimation although other types of flour were used in this recipe. Also, the study used baker's yeast as leavening agent while in this baking process a combination of baker's yeasts and sourdough fermentation was used. Similar to estimation of iron bioavailability, the result of folate is not sufficient as no information regarding bioavailability of pumpkin seed was obtained.

5.3 Evaluation of bagel's characteristics

Eighteen bagels from each set of the two varieties were made. Triplicate of each type of bagels were made, thus the sum of all bagels was 108. One bagel of each set was randomly selected to be measured in weight, volume and density. A bucket filled with canola seed were used to estimate the volume of a bagel. Bagel number 1, 2, and 3 represents recipe 1 and bagel number 4, 5, and 6 stands for recipe 2. The bagels, one at a time, were put in the bucket and the excessive seeds was measured. The results are presented in the Table 6.

Table 6. Weight, volume and density of recipe 1(1, 2, 3) and recipe 2 (4, 5, 6)

	Recipe 1 (without pumpkin seed)			**Recipe 2 (with pumpkin seed)**		
Measure	1	2	3	4	5	6
Weight (g)/bagel	96.0	98.0	96.0	103	106	103
Volume (ml)/bagel	200	240	200	200	220	210
Density (g/ml)	0.48	0.41	0.48	0.52	0.48	0.49

T-test for weight, volume and density was made in Excel. The null hypothesis, stating that there are no differences in the weight of the bread from the two recipes $p<0.05$. Therefore, null hypothesis could be rejected, as the weight of the breads from recipe 2 was significantly higher ($p<0.01$). However, the differences were most likely due to the pumpkin seeds added on top of these bagels. Furthermore, neither volume nor density showed significance, thus there are no differences between the two types of bagels. The outer surface for both kinds of bagels appeared to be smooth and the crust color was light brown. Moreover, both of the bagels were hard to the touch when the surface was gently compressed and were slowly recovering its shape when pressed. There were no obvious differences regarding crumb porosity between the two kinds of bagels. Neither was there observed differences between the types of bagels regarding fat spreadability. The type of fat used was Arla's *Bregott® mellan matfettsblandning 60%.* The visual porosity of the breads is further shown as pictures in Appendix 2.

6 Discussion

The first question to find out was if the final chosen ingredient(s) to be included in the test recipe fulfill the European Commission's requirements on making health claim(s) about iron, iodine and folate. The theoretical calculations showed that iron, iodine and folate content in recipe 2 met the requirements to use the health claims. Bagels based on recipe 1 fulfilled the conditions to use the claims in terms of iodine and folate, whereas iron content was missing by 0.53 mg/100 g. The calculation is only true for the chosen combination of ingredients and at the selected amount. As this is a first try-out recipe limited to the assortment of Fazer Eskilstuna, there are certainly many other ingredients suitable to be included in the bread. According to *Finnish Food Composition Database*, basil, oregano and hempseed are other products rich in iron, whereas wheat germ and nettle are folate rich food products. These may be potential ingredients for the next try-out. There are also other types of flours with higher content of iron. In the present experiment the choice of ingredients was mainly a tradeoff between folate and iron, where refined spelt flour was chosen in this try-out. Moreover, information of the content of nutrients was based on the different databases since no nutrient index was obtained from Fazer's suppliers. A food item appeared to contain a certain amount of nutrient according to one database, while another database displayed different amount of the same nutrient in the same food item. Therefore, the actual nutrition content for each ingredient used may not be truly reflected, as the ingredients were not analyzed before baking. The nutrient content clearly depends on which database that has been used. Calculation based on these databases may affect the final results of whether a bagel à 100 g really fulfills the health claims.

Moreover, the second question to be answered was if the nutrients met the conditions of using the health claims. This question also took into account if the amount of the various compounds were of bioavailable quantity? Iodine was the only nutrient fulfilling the condition to use health claim and was in sufficient bioavailable amount. Unfortunately, iodized salt is currently not applied in Fazer's assortment. Neither the estimated iron nor folate levels in flours were of sufficient bioavailable quantity. However, folate is quite abundant in other items such as yeast which was not taken into account in the performed calculations. In addition, information concerning bioavailability of food items besides flours, such as bioavailability of pumpkin seeds, was not analyzed. Pumpkin seed appeared to play an important role as it showed to influence iron to the extent that criteria to use the health claim were met in recipe 2. It would be a valuable aspect to find out whether bioavailability of iron in pumpkin seed contributes to fulfilling the conditions regarding bioavailable quantity for recipe 2. The bioavailable quantity may be higher than theoretically estimated. As mentioned above, information from databases provided ambiguous nutrient content which contributed to uncertainty. Another factor influencing the outcome to the calculation is the chosen studies used as guidelines. These articles are just indicating one of the possible outcomes when bread is baked in a certain way. There are additional methods and factors affecting the final results thereby also influencing the bioavailable quantity of the nutrients. The approach of this baking session was not to imitate a specific method of the reviewed studies, hence the calculations are assumed that present baking process worked out the same as for the reviewed studies. Therefore, the results obtained from the baking served as an indication and not to postulate that the mineral solubility became bioavailable at a definite amount. As it might be an obstacle to reach the requirement in flour-based bread, substituting flours with grains or bran may increase the bioavailability. Nielsen *et al.,* (2006) commented on that the phytate content varied with different type of grains, of which rye in general tended to show higher phytase activity. In this try-out, the purchased sourdough starter was the only ingredient containing rye. The advantage of including sourdough was not only to improve sensory properties, but to increase the health potential further by enhancing the bioavailability of important nutrients.

Another source of error was due inadvertence of not measuring pH during the present bread-making, which made it impossible to guarantee whether reduction of phytate might have occurred. As stated by Nielsen *et al.,* (2006), lowering pH, even at moderate decrease, favors phytate reduction thereby enhancing mineral availability. However, acidic environment may not be beneficial for all nutrients. Winger *et al.,* (2008) suggested that iodine in acidic conditions initiates oxidation and reduction of other nutrients such as Vitamin C and iron affecting the nutritional value. It is important to understand that although focus has mainly concerned ways of decreasing phytic acid, there are other components affecting bioavailability of iron (Frontela *et al.,* 2011).

According to the theoretical calculations, the nutritional value in bread seems to be improved, at least to meet the requirement to utilize health claims approved by European Commission. The present baking session showed that it was possible to make bread with focus on optimizing iron, iodine and folate. In particular, adding the amount of pumpkin seeds to theoretically fulfill the health claim requirement about iron proved in practice to not affect the impression of bread, at least not from the subjective point of view. This is just a first try-out of the product idea, but the results show a potential to enhance the nutritional value in bread. Furthermore, the results indicate that it is possible to include ingredients of natural source in creating functional food, thereby also responding to consumers' preferences. The improvement did only concern the overall nutritional value of iron, iodine and folate and not the bioavailability. In the end, health claims only requires that the product contains a significant amount of a nutrient, which is 15% of daily recommended intake per 100 g or 100 ml and do not care whether the nutrients are of bioavailable quantity or not. However, it ought to be the prerequisite for the claims to even be valid in the first place. Health claims should not only take the significant amount into account, but bioavailability should be of equal importance. Otherwise what would improving health status through functional food be, if bioavailable quantity of the nutrients cannot be guaranteed? Further research would be to evaluate the degree of which a nutrient is absorbed or becomes bioavailable during human intake.

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Appendix 1

Bagels

These bagels are amde from *Bagarens Bästa* wheat flour. Suggested toppings are cheese, sesame seed, poppy seed, wheat bran and parsley etc. The flavor can be further enhanced in the dough with blue berry, raisins or other spices. Just use the imagination.

Ingredients

Art. no.	**Ingredients**	**Weight (g)**
140153	Bagarns Bästa Wheat flour	1 900,0
1	Water	1 000,0
20170	Malt syrup	100,0
21134	Salt	40,0
864221	Olive oil i.e. Virgin	30,0
214061	Baker's Yeast	20,0
Total dough weight:		3 090,0

Appendix 2

Figure 1. Bagel to the left is without pumpkin seed and the one on the right is with pumpkin seed.

Figure 2. A close-up of the bagels without pumpkins seeds showing three replicates batch 1, 2, and 3 (from left to right) to show the porosity.

Figure 3. A close-up of bagels with pumpkins seeds showing three replicates batch 4, 5, and 6 (from left to right) to show the porosity.

Summary

For many years, inappropriate diets have been associated with increased risk factors for many chronic diseases such as obesity, diabetes and cardiovascular diseases etc. It may also lead to insufficient intakes of important micronutrients resulting in long term deficiencies. Since the mid-20th century, changes in diet have occurred, where modifications have influenced on the health and the wellbeing. Also, as food technology has become more advanced and knowledge in nutritional science has increased, a new type of food category has evoked. Functional food is one of the results fitting into this category. The general definition of functional food is food products providing health benefits to the body beyond the effects of adequate nutrition, in a way that improves health and wellbeing. In recent years, health benefits of bread have been the most studied cereal products. As bread is considered as staple food in many countries, a substantial interest in enhancing nutrients of natural source in these food products has developed.

Therefore one of the objectives in this study was through literature study review ways of improving bioavailability of selected nutrients in bread. Bioavailability is in this case defined as nutrients in a state (i.e soluble form or free ions etc) where it is ready to be absorbed. The selected nutrients were iron, iodine and folate as these nutrients are at risk of insufficient consumption or where inadequate intake has been observed. The results from some of the reviewed studies will serve as guidelines in a theoretical estimation of bioavailability of these selected nutrients in a flour-based bread recipe. Thus the aim is also to develop the flour-based recipe. Another part of the thesis is to take health claims into account in this recipe. Health claims are guidelines authorized by European Commission stating the requirements (expressed as amount) a nutrient need to meet to be eligible to make a certain claim. However, health claims do not take bioavailability into account, thus the third objective is to calculate whether the estimated bioavailable amount of a certain nutrient is of the same amount as the quantity needed to be fulfilled to make use a specific health claim. An ingredient can contain a certain amount of nutrient, but not the entire amount is of bioavailable content. In other words, the nutrients will not only be labelled with a certain health claim, but the amount will also be of bioavailable quantity as well. In the long run, assuring that a product contains a certain amount may serve as a potential to improve the overall intake and strengthening the relationship between food and health.

Relevant findings about bioavailability of iron and folate in bread concerned impacts of different fermentation methods in cereals. The mineral happens to exist in cereal products, but unfortunately as insoluble mineral complexes in the cereals. As a consequence the bioavailability is negatively affected impairing absorption in human. Results from studies showed that yeast and lactic acid bacteria from sourdough lower the pH thereby activating a group of enzymes with the ability to prevent these insoluble mineral complexes from being formed. In other words, iron becomes soluble and bioavailability will therefore be enhanced. The bioavailability of folate content in bread was also by influenced by the choice of fermentation methods. Fermentation involving yeast resulted in an overall increase of folate in the dough, suggesting that yeast has the ability to synthesize folate. However, lactic acid bacteria appear to be more complex as the same strain of bacteria may produce or consume folate depending on circumstances. Moreover to review of iodine of which literature studies was limited to iodized salt in bread, showing that io-

dine in salt is remained after proofing and baking. Iodine intake is relevant to improve as exposure of the nutrient has shown to decrease due to globalisation, optional utilization of iodized salt is for food manufacturers and the trend towards using non-iodized salt at home.

While the studies provide some knowledge regarding improvement of bioavailability of these three nutrients, there are additional aspects that could differ from one time to another which most likely will contribute to the uncertainty of the results. Various initial conditions such as milling or grinding process of cereals influence the final outcome. Also, the conditions of fermentation process play an important role as well as the choice of fermentation products. Time, temperature and the amount of a certain ingredient may for instance affect the pH, which in turn change the bioavailability of that nutrient. Interaction between the nutrients has not gained that much attention in this study but should not be overlooked as it adds to the complexity of the actual bioavailability.

The results of iron, iodine and folate served as inspiration for the practical part which was to develop a recipe and theoretically estimate both nutritional content and bioavailable content of these three evaluated nutrients. Moreover, the current bread trend has indicated a preference towards bread presented as unwrapped loaves and baked on sourdough, based on wholemeal with kernels, seeds and fibres included. As this part was in collaboration Fazer bakery, the intention was to develop a recipe for a bread product not existing in their current bread line. The final choice of ingredients was based on the assortment found in Fazer bakery at Eskilstuna. A simple subjective evaluation was conducted of the breads.

Two types of bagels were baked and both of them originated from the same recipe. The only difference between the two bagels was that additional pumpkin seeds were included in the second bagel recipe. Through food databases, data was obtained regarding the nutrients of iron, iodine and folate. Theoretical estimation showed that the amount of a portion of 100g bread partly fulfilled the requirements to make use of health claims about iron, iodine and folate. For instance, the amount of iron met the requirement in the recipe with pumpkin seeds, suggesting that the result was due the pumpkin seeds added. As for the bioavailable content, results from some of studies were used as guidelines in the theoretical estimation. Only iodine content reached the amount needed to make the health claim about iodine and that the amount at the same time was presented as bioavailable for human intake. The same subjective evaluation was applied for both of bagels. The surface appeared to be smooth and the crust colour was light brown. In addition there were no obvious differences concerning crump porosity, nor were deviation in weight, volume and density observed.

Finally, the estimated results are only true for the given proportion of ingredients and the chosen ingredients. No initial nutrient analysis of the chosen ingredients were performed, neither were any nutrition index obtained from the suppliers of the ingredients. The nutrition content for the same ingredient appeared to vary depending on the chosen food database. Also, the baking process did not imitate a specific method from the reviewed studies, thus the results are just serving as indication of one of the possible outcomes assuming that this baking process worked out the same way as the studies. In reality many nutrients are interacting at the same time, which makes actual bioavailability much more complex. However, the results of this study showed that it was possible to bake bread focusing on optimizing iron, iodine and folate. This is just a try-out of a new product idea and the estimated results showed that there is a potential to enhance nutritional value in bread by using natural source. In the end, the health claims do not take bioavailability into account, although it ought to be a prerequisite as the attempt is to improve health status in the long run. Nonetheless, ability to evaluate the degree of which a nutrient is absorbed or becomes bioavailable during human intake is relevant in further research.