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Swedish University of Agricultural Sciences

Faculty of Natural Resources and  
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# Evaluation of acute appetite effects of crisp bread of rye

– Results from two cross-over studies

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– Results from two cross-over studies

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## Abstract

Fibre rich rye products have been shown to have superior effects on appetite compared to white wheat bread through mediation of prolonged feelings of fullness, less hunger and less desire to eat. Moreover, consumption of rye products compared to white wheat bread has resulted in lower energy intake after a subsequent meal.

The aim of this thesis was to evaluate the acute effects of a commercial crisp rye bread on appetite in terms of hunger, fullness and desire to eat in a four hour perspective, compared to a white wheat bread reference as part of a breakfast. The energy intake after an *ad libitum* lunch was also evaluated.

The analysis is based on results from two randomised cross-over studies. Appetite was rated by visual analogue scales. In total, 20 and 21 subjects completed the studies, respectively. The participants were 25-53 years old and had BMI 20-26. The studies differed in terms of total energy content of the breakfasts and proportion of energy coming from the treatment product. Differences between breakfasts within the two studies were evaluated using mixed models with repeated measures appropriate for cross-over designs.

Study 1 showed a lower perceived hunger and desire to eat after eating the rye breakfast, while effects on fullness were less pronounced. This is likely due to the high energy content of the breakfast or the lower proportion of test product masking the difference in effect on appetite.

In Study 2 the energy content of the breakfast was lower than in Study 1 and the test product constituted a larger proportion of the breakfast. Results showed significantly higher perceived fullness, less hunger and less desire to eat as well as a lower energy intake at lunch after eating the rye breakfast compared to the control.

Rye crisp bread affects satiety through contributing to lower perceived hunger, higher perceived fullness and less desire to eat in a four hour perspective compared to a wheat reference bread. It also led to a lower energy intake after an *ad libitum* lunch, which may be the result of positive effects on appetite.

Keywords: rye, appetite, VAS, crisp bread, dietary fibre, whole grain

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# 1 Introduction

## 1.1 Background

Nutrition and health are important factors in the prevention of chronic disease related to lifestyle, such as obesity, diabetes type II, cardiovascular disease and certain forms of cancer (Smith & Tucker, 2011). A diet rich in dietary fibre and bran products has been associated with a decreased risk for several of these diseases (Landberg, 2012), as well as with a lowered risk of weight gain and obesity partly mediated by lowering effects on appetite that prevents excess energy intake (Williams *et al.*, 2008). The satiating effect of different foods can thus play a role in weight management, for example through the effect on meal size (Blundell *et al.*, 2010).

Rye products, including porridge and bread, are interesting in this context as they have been linked to increased and prolonged feelings of satiety (Isaksson, 2011; Isaksson *et al.*, 2011; Isaksson *et al.*, 2009; Isaksson *et al.*, 2008; Leinonen *et al.*, 1999; Rosén *et al.*, 2011a,b,c; Rosén *et al.*, 2009) and a lower energy intake at subsequent meals (Rosén *et al.*, 2011c) as well as to induce favourable glycaemic profiles (Bondia-Pons *et al.*, 2011; Leinonen *et al.*, 1999; Rosén *et al.*, 2011a,b,c; Rosén *et al.*, 2009) compared to white wheat reference products.

Rye products are traditionally consumed as whole grain and they are unique amongst commonly grown cereals in having the highest content of dietary fibre, ranging from 18% to 22% when including fructans (Andersson *et al.*, 2009). The Western diet is generally low in dietary fibre, and average consumption is below 25-35 g/day, which is the daily recommended intake for the adult population (Nordic Council of Ministers, 2004). This was recently demonstrated by the dietary survey Riksmaten 2010-11 (National Food Agency, 2012). The most commonly consumed rye product is bread, including sour dough breads, crisp breads and sifted rye flour breads (Isaksson, 2011). Bread is also the main source of die-

tary fibre for Swedish adults (National Food Agency, 2012), suggesting a high potential for the production of added value products with health benefits in this product segment. However, no study has so far investigated the effects of crisp bread on appetite and energy intake in a subsequent meal.

## 1.2 Objectives

The objective of this thesis was to evaluate the effect on appetite by a commercial rye crisp bread compared to a commercial control bread made of wheat as part of a normal breakfast in two different cross-over trials, where the energy content as well as the proportion of energy originating from the test products differed.

The hypothesis was that consumption of rye crisp bread affects appetite through contributing to lower perceived hunger, higher perceived fullness and less desire to eat in a four hours perspective compared to a wheat reference bread, as well as leading to a lower energy intake after an *ad libitum* lunch.

## 1.3 Delineation

This thesis has focused on the effects of a commercial rye crisp bread on appetite and not other rye products, and for this reason different processing methods that might affect appetite have not been evaluated. Only acute or short-term effects on appetite and energy intake are investigated, thus long-term effects which may be relevant for weight maintenance were not evaluated. Furthermore, the mechanisms underlying any differences in effects on appetite are not investigated in this thesis.

## 2 Theoretical background

### 2.1 Appetite and its definitions

Appetite is commonly used as a collective term, covering all aspects of motivation or inhibition of eating (Isaksson, 2011). Hunger is the biological drive to eat and is perceived through physical sensations from the stomach, limbs or head and cognitive sensations of light headedness or weakness (Blundell *et al.*, 2010). When eating, a feeling of fullness arises resulting from neural, endocrinal and environmental changes. This process, called satiation, consequently leads to the cessation of eating. It describes the intra-meal, or between-meals, satiation and influences the size of the consumed meal (Blundell *et al.*, 2010). After eating, inter-meal or post-ingestive satiety arises leading to the inhibition of further eating, a decline in hunger and an increase in perceived fullness after the meal (Blundell *et al.*, 2010). Based on this definition, a food that has high satiety produces a longer period of inter-meal satiety, *id est* the period of time between meals when the subject does not perceive feelings of hunger (Gerstein *et al.*, 2004). All appetite parameters, hunger, fullness and desire to eat, have been found to contribute to explaining the subsequent energy intake (Flint *et al.*, 2000).

### 2.2 Regulation of appetite

The regulation of appetite is a complex process perceived as subjective feelings of hunger, fullness and desire to eat (Flint *et al.*, 2000). These sensations are mediated through physiological as well as psychological factors that vary throughout the course of a meal (Flint *et al.*, 2000). Biologically, the regulation of appetite involves afferent neural and endocrine signalling that arises in the gastrointestinal tract in response to the mechanical and chemical properties of the ingested food

(Cummings & Overduin, 2007). Satiating signals arise from the stomach, the proximal and distal small intestine, colon and pancreas, and are mainly evoked in two ways; through gastric distension and release of peptides from endocrinal cells which have either a suppressive (anorectic) or stimulating (orexigenic) effect on appetite (Cummings & Overduin, 2007). Appetite regulating peptides that have been shown to have an anorectic effect on appetite include cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1) and peptide YY (PYY). Ghrelin is an extensively studied orexigenic peptide, which has a powerful effect to increase food intake in humans (Cummings & Overduin, 2007).

### 2.3 Factors that influence satiety

The satiating effects of food are a result of several factors including sensory, cognitive, post-ingestive and post-absorptive processes that lead to satiety and satiety (Blundell *et al.*, 2010). These processes are influenced by the characteristics of the food or meal in terms of macronutrient composition, fibre content, energy density, volume, physical structure and sensory qualities such as texture or palatability as well as environmental cues including the emotional state of the subjects (Blundell *et al.*, 2010).

Different macronutrients have been found to have different satiating effect, with protein being the most satiating and fat the least satiating and these proportions should thus be kept constant in appetite studies to minimise a confounding effect (Blundell *et al.*, 2010). The effect of satiety can also vary within a group of macronutrients as is the case for carbohydrates, where fibre consistently has been shown to have a higher satiating effect than digestible, complex carbohydrates and mono-saccharides (Howarth *et al.*, 2001).

While metabolic factors influence how much we eat, sensory factors have been suggested to be more involved in what we eat through associating food with environmental and social cues through learning, although these factors affect each other through learning. For example palatability has been shown to strongly affect *ad libitum* intake of food (Blundell *et al.*, 2010).

## 2.4 Rye

### 2.4.1 The composition of rye

Rye (*Secale cereale* L.) is a cereal widely grown mainly in the Northern, Central and Eastern European countries due to its cold resistant properties. It is traditionally consumed as whole grain (Andersson *et al.*, 2009). Rye stands out amongst commonly grown cereals through having the highest content of dietary fibre, ranging from 18% to 22% when including fructans (Andersson *et al.*, 2009). The most commonly consumed rye product is bread, including sour dough breads, crisp breads and sifted rye flour breads (Isaksson, 2011).

Rye has gained interest from the food industry and researchers due to its health promoting properties, including increased and prolonged feelings of satiety and a lower energy intake at subsequent meals (Isaksson, 2011). Rye products have also been found to induce favourable glycaemic profiles with a lower increase in serum insulin after a meal without the corresponding effect in blood glucose levels (Juntunen *et al.*, 2003; Rosén *et al.*, 2011a,b,c; Rosén *et al.*, 2009).

Due to its high content of dietary fibre, including arabinoxylan, fructan and  $\beta$ -glucan as well as the bioactive components cinnamic acids, alkylresorcinols, sterols, vitamins and minerals, rye is considered a healthy cereal (Andersson *et al.*, 2009). Arabinoxylan and  $\beta$ -glucan have been shown to lower levels of serum cholesterol, which is an independent risk factor for cardiovascular disease (Leinonen *et al.*, 2000).

### 2.4.2 Rye and appetite

High fibre rye products including bread, porridge and flakes have been found to have a superior satiating effect compared to white wheat bread (WWB) when consumed as part of an iso-caloric breakfast (Isaksson, 2011). Rosén *et al.* (2011a) found that whole grain rye bread as part of a breakfast contributed to increased fullness compared to WWB, during three hours after intake. Similarly, Isaksson *et al.* (2009) found that rye bread baked on sifted rye flour, intermediate rye fraction and rye bran all suppressed appetite until lunch time (8.30-12.00) compared with WWB, but rye bran bread had the strongest effect on satiety. Rye bread breakfasts also resulted in decreased hunger and desire to eat in the afternoon compared to WWB (Isaksson *et al.*, 2009). Rosén *et al.* (2011c) found that rye bread decreased appetite and was associated with a 16% lower energy intake at lunch compared to WWB. In accordance, Pasma *et al.* (2003) concluded that a breakfast with complex carbohydrates is to prefer compared to a breakfast with simple carbohydrates due to higher satiety.

Similarly, rye porridge has been found to affect appetite through increased satiety, lower hunger and lower desire to eat up to eight hours after breakfast, compared with WWB (Isaksson *et al.*, 2008; Isaksson *et al.*, 2011). Porridge differs from bread in having a low energy density due to its high water content. Consequently, it needs to be served in larger volumes to provide the same amount of energy as the reference WWB, which in itself can increase within-meal satiety and decrease food intake (Isaksson *et al.*, 2011).

Food structure is known to affect metabolism, glycaemic index and satiety (Isaksson, 2011). Rye porridge with whole rye kernels increased satiety, decreased hunger and desire to eat before lunch and decreased appetite in the afternoon compared to WWB (Isaksson *et al.*, 2011). However, Isaksson *et al.* (2011) found no effect of structure between rye breads with whole or milled rye kernels, which both increased satiety and lowered desire to eat compared to WWB, but did not affect hunger. Furthermore, Isaksson (2011) found that the amount of rye may be important for the effect on appetite, as bread breakfasts with lower amounts of rye resulted in weak effects, particularly in the afternoon.

## 2.5 Measuring appetite

### 2.5.1 Cross-over design

As feelings of appetite are both physiological and influenced by previous experience and expectations, there is large inter-individual variation in how feelings of hunger, fullness and desire to eat are perceived (Stubbs *et al.*, 2000). For this reason, within-subject design is recommended for assessing appetite. Cross-over designs have been shown to be more accurate than between-subject comparisons, thus requiring less subjects for reaching sufficient statistical power to detect meaningful differences (Flint *et al.*, 2000). In cross-over design, each subject receives all treatments in random order. Thus, all treatments are given to an equal number of subjects in a balanced and randomised design (Blundell *et al.*, 2010).

### 2.5.2 Visual analogue scales

Subjective appetite is commonly measured through the use of visual analogue scales (VAS), a unipolar 100 to 150 mm long line scale anchored with terms such as 'not at all' to 'extremely', used for self-reporting (Blundell *et al.*, 2010; Flint *et al.*, 2000). The study participants assess their appetite using VAS before and after

the consumption of a food or meal and in regular time intervals, often every 30 minutes, during three to five hours (Blundell *et al.*, 2010). The original questionnaire for appetite measurement contained six questions and was developed by Hill and Blundell (1992). Based on this, three questions are now commonly used together with VAS to measure all aspects of appetite; hunger ('How hungry are you right now?'), fullness ('How full are you right now?'), and desire to eat ('How strong is your desire eat right now?') (Blundell *et al.*, 2010; Isaksson, 2011; Rosén *et al.*, 2011a,b,c). These questions have been accepted and used consistently in several countries and with different test groups and stimuli (Blundell *et al.*, 2010). Measures of appetite using VAS have been shown to represent high within-subject reliability and validity when used under controlled conditions, in within-subject designs with repeat measurements and when used together with objective measures such as volume or amount of food eaten at an *ad libitum* meal (Stubbs *et al.*, 2000). VAS scores are quantified through measuring the distance from the left end point of the scale to the mark made by the subject, and results can then be analysed statistically (Flint *et al.*, 2000). VAS has traditionally been presented to the study subjects on paper together with a pen, but it is now often used as a portable electronic appetite rating system (EARS) which has been shown to be reliable to use in appetite research (Stratton *et al.*, 1998; Stubbs *et al.*, 2001).

### 2.5.3 Study participants and study instructions

When good experimental conditions and a within-subject design are employed, 20-25 participants are considered sufficient to capture a difference of 10% in ratings of appetite (Flint *et al.*, 2000). This number of study participants in appetite studies is thus considered to result in sufficiently high statistical power and minimise the risk for type II errors (Flint *et al.*, 2000).

Although the subjects should reflect the intended use of the tested product (Blundell *et al.*, 2010), certain subject-related factors need to be considered in appetite studies as the perception of appetite is highly subjective and has been shown to be affected by physiological, psychological, social and cultural factors (Gregersen *et al.*, 2011). According to Gregersen *et al.* (2011), appetite ratings differ among subjects depending on age, gender and level of physical activity, and to some extent also with nicotine use and menstruation cycle. High age has been associated with a decreased food intake and earlier perceived fullness, and children and young people can have difficulties with compliance to instructions (Gregersen *et al.*, 2011). Furthermore, moderate to high levels of physical activity lead to higher ratings of hunger and prospective consumption compared to light or no exercise (Gregersen *et al.*, 2011). Nicotine suppresses hunger, increases satiety

and reduces energy intake and is thus a potential confounder in appetite studies (Gregersen *et al.*, 2011). It has also been debated whether a very high or low body mass index (BMI) and concerns about diet or weight influence appetite ratings, with yet no clear conclusions made (Blundell *et al.*, 2010; Gregersen *et al.*, 2011).

Possible confounders in satiety studies include antecedent levels of physical activity, energy depletion and food intake (Blundell *et al.*, 2010). It is therefore important that subjects are given pre-study instructions in order to isolate the factor under study (Blundell *et al.*, 2010; Flint *et al.*, 2000; Stubbs *et al.*, 2000).

#### 2.5.4 Test meal and intervention products

The test meal should be suitable for the time of the day when it is served (Blundell *et al.*, 2010), and should not differ in palatability (Flint *et al.*, 2000). The study conditions should be controlled using a non-preload or placebo treatment which, in appetite studies on cereal products, commonly is a white wheat product (Blundell *et al.*, 2010). In order to ensure that the shown effect is due to the factor of interest, the control products should be standardised in terms of energy content, macronutrient composition, physical state (solid or liquid), weight or volume, and sensory and cognitive characteristics (Blundell *et al.*, 2010). When the test product is served as part of a breakfast, the total energy content should not exceed that of a normal breakfast as this could affect the subjective feelings of appetite (Isaksson *et al.*, 2009). For the adult population, breakfast should contribute with 20-25 E%, for the sedentary population 1900-2488 kJ (Nordic Council of Ministers, 2004). Isaksson *et al.* used in their studies breakfasts with a total energy content of 1658 kJ (Isaksson *et al.*, 2008), 1960 kJ (Isaksson *et al.*, 2009), 1850 kJ for the porridge breakfasts and 2550 kJ for the bread breakfasts (Isaksson *et al.*, 2011).

#### 2.5.5 Statistical analysis

Data from cross-over studies in which appetite has been measured using VAS, is preferably analysed statistically using a model that analyses appetite as a function of multiple dependent time points (Blundell *et al.*, 2010). This also minimises risk for type I errors, compared to analysing appetite as a function of independent time points. For this reason, it is recommended to use a repeated measure analysis or the total area under the curve (tAUC), with repeated measure analysis having the benefit of better handling missing values than the tAUC (Blundell *et al.*, 2010). For tAUC, a difference in appetite of 8-10% between treatment and control is considered to be of practical relevance (Flint *et al.*, 2000).

## 3 Materials and methods

### 3.1 Study design

In both studies, assessments of subjective appetite ratings were made through the use of electronic VAS. The intervention product was evaluated in the same subjects in each study following a randomised, balanced and single-blinded cross-over design, in which all participants had both test breakfasts, as shown in Figure 1. Study days were separated by a wash-out period of six days to ensure that any effect of previous treatment was eliminated.

Study participants were instructed by e-mail to abstain from consuming any food or beverage after 20.00 the day preceding the study, and that their last meal should not contain any products rich in fibre such as whole grain foods, fruit and vegetables. They were also instructed to not perform any vigorous physical exercise during the 24 hours before the study occasion. If these criteria were slightly

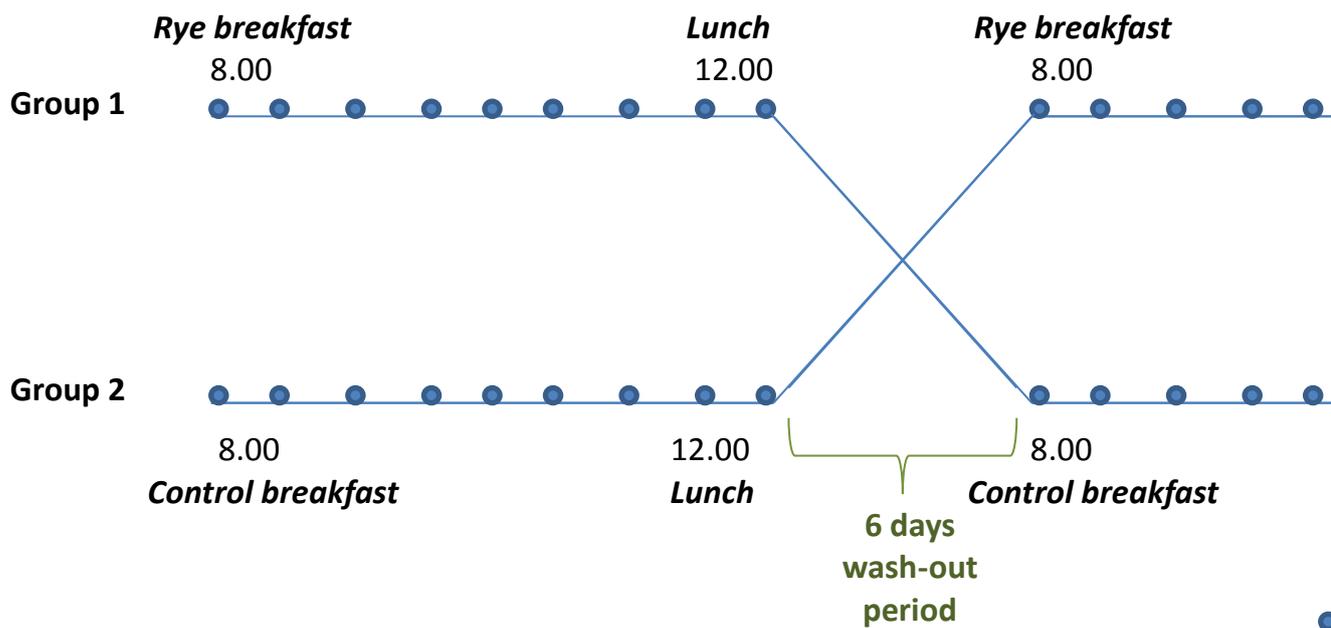


Figure 1. Study design showing the cross-over design and sequence of treatment and control, with breakfast being served at 8.00 and lunch at 12.00 in between which appetite was registered every 30 minutes, indicated in the figure with blue dots.

violated, participants were instructed to repeat the same activity before the next study occasion to minimise any confounding effects.

In the morning of the test day, participants were instructed to come fasted to the study premises at Ultuna Campus, Swedish University of Agricultural Sciences (SLU), to consume the test breakfast. Upon their arrival, participants were weighted, asked about their age and provided with a hand-held computer, model z22 (Palm Inc, Sunnyvale, USA) and instructed on how to use the electronic VAS to score their feelings of hunger, fullness and desire to eat (Figure 2). Additionally, a fourth question, "How tired do you feel right now?", was rated on VAS. This question was not analysed in this thesis. In Study 2, a few of the computers did not work accordingly and thus scales on paper were used on which appetite was scored with a pen. These values were entered manually into the Excel-datasheet. While eating their breakfast, participants were asked to fill out a form with questions on their date of birth, height, weight, when their last meal and drink were and what they ate/drank, what time they started their breakfast and when their test lunch would be, if they had coffee/tea with their breakfast and if they used milk and/or sugar/sweetener and how much, in order to ensure compliance.

1. *How hungry do you feel right now?*  
Not at all hungry |-----| Extremely hungry
  
2. *How full do you feel right now?*  
Not at all full |-----| Extremely full
  
3. *How strong is your desire to eat right now?*  
Not at all strong |-----| Extremely strong

Figure 2. Questions used to assess subjective appetite together with VAS.

Two small conference rooms and a nearby kitchen at the Department of Food Science at SLU were used for the study. Study participants were seated together at round tables, with one room for participants eating the intervention breakfast and one room for the control breakfast. Conversation was allowed with the exception of discussing the study or comparing ratings. Participants were not allowed to eat or drink anything between breakfast and lunch, but were allowed to perform their usual occupation.

### 3.2 Study participants

Healthy men and women aged 18-65 with a BMI of 18.5-28.0 (normal weight to slightly obese) were recruited through advertising in local newspapers and at Ultuna Campus and by sending e-mails to employees at the Department of Food Science at SLU, Uppsala, Sweden. 24 participants were recruited for Study 1 and Study 2 respectively. Due to absence on one or both of the study occasions, 21 and 20 subjects completed Study 1 and 2, respectively. For the measurement of energy intake in Study 2, another participant was absent and the analysis was performed on 19 subjects. Information about the study was given both orally and in written form to those who reported interest to participate.

Only those who regularly consumed breakfast, lunch and dinner and who did not follow any diets including vegetarians and vegans were allowed to participate. Additional exclusion criteria included dieting, use of tobacco, physiological or psychological problems with eating, gastrointestinal problems or other medical

conditions that were likely to affect appetite or food intake including food intolerances or allergies, as well as women that were pregnant, lactating or wishing to become pregnant during the study period. Persons aged over 65 were excluded as high age can result in a decreased food intake and more early feelings of fullness, as were children and young due to potential difficulties with compliance.

### 3.3 Test products and meals

#### 3.3.1 Test products and breakfasts

The test products were the same in both studies; the intervention product was a commercially produced rye crisp bread (RCB) and the control product a commercially produced WWB. The breads were served as part of a standardised typical Swedish breakfast together with margarine (Lätta), slices of boiled ham, a small glass of orange juice (2 dl in Study 1 and 1 dl in Study 2) and a choice between a cup of coffee or tea (the same amount and choice of beverage should thereafter be ingested at all test occasions). Additionally in Study 1, Gouda cheese was included in the breakfasts. Margarine was used to make the test and control breakfast isocaloric. The energy and macro nutrient content of the breakfasts are presented per portion below in Table 1 and 2. At macronutrient level, the breakfasts in Study 1

*Table 1. The energy and macro nutrient content of the breakfasts per portion in Study 1*

	Amount (g)	Nutrient					Energy (kJ)
		Fat (g)	Protein (g)	Carbohydrate (g)	Whereof sugar (g)	Dietary Fibre (g)	
<b>Treatment</b>							
Rye crisp bread	80	1.9	7.7	52.0	1.2	12.8	1188
Lätta	30	11.7	0.1	0.9	0.2	0.0	450
Boiled ham	27	0.8	5.9	0.3	0.2	0.0	136
Gouda cheese	25	7.0	5.8	0.0	0.0	0.0	357
Orange juice	200	0.2	1.4	18.0	18.0	1.4	348
<b>Total</b>	<b>362</b>	<b>21.6</b>	<b>20.9</b>	<b>71.2</b>	<b>19.6</b>	<b>14.2</b>	<b>2479</b>
<b>E%</b>		<b>33</b>	<b>14</b>	<b>48</b>	<b>13</b>	<b>5</b>	<b>100</b>
<b>Control</b>							
White wheat bread	108	3.8	9.7	49.7	3.8	3.8	1180
Lätta	30	11.7	0.1	0.9	0.2	0.0	450
Boiled ham	27	0.8	5.9	0.3	0.2	0.0	136
Cheese	25	7.0	5.8	0.0	0.0	0.0	357
Orange juice	200	0.2	1.4	18.0	18.0	1.4	348
<b>Total</b>	<b>390</b>	<b>23.5</b>	<b>23.0</b>	<b>69.0</b>	<b>22.1</b>	<b>5.18</b>	<b>2475</b>
<b>E%</b>		<b>35</b>	<b>16</b>	<b>47</b>	<b>15</b>	<b>2</b>	<b>100</b>

Table 2. The energy and macro nutrient content of the breakfasts per portion in Study 2

	Amount (g)	Nutrient					Energy (kJ)
		Fat (g)	Protein (g)	Carbohydrate (g)	Whereof sugar (g)	Dietary Fibre (g)	
<b>Treatment</b>							
Rye crisp bread	64	1.5	6.1	41.6	1.0	10.2	953
Lätta	20	7.8	0.1	0.6	0.1	0.0	301
Boiled ham	30	0.9	6.6	0.3	0.2	0.0	151
Orange juice	100	0.1	0.7	9.0	9.0	0.7	174
<b>Total</b>	<b>214</b>	<b>10.3</b>	<b>13.5</b>	<b>51.5</b>	<b>10.3</b>	<b>10.9</b>	<b>1573</b>
<b>E%</b>		<b>24</b>	<b>15</b>	<b>56</b>	<b>11</b>	<b>6</b>	<b>100</b>
<b>Control</b>							
White wheat bread	86	3.0	6.9	40.4	3.0	2.6	936
Lätta	20	7.8	0.1	0.6	0.1	0.0	301
Boiled ham	30	0.9	6.6	0.3	0.2	0.0	152
Orange juice	100	0.1	0.7	9.0	9.0	0.7	174
<b>Total</b>	<b>241</b>	<b>11.8</b>	<b>14.3</b>	<b>50.3</b>	<b>12.4</b>	<b>3.3</b>	<b>1561</b>
<b>E%</b>		<b>28</b>	<b>16</b>	<b>55</b>	<b>14</b>	<b>2</b>	<b>100</b>

and 2 differed in total energy content and the part of the total energy content of the breakfasts originating from the test products. In Study 1 the total energy content was 2479 kJ and 2475 kJ for the rye and control breakfasts respectively, which was 40% more than in Study 2 where the rye breakfast was 1573 kJ and the control breakfast 1561 kJ. In Study 1, 48% of the energy content of the treatment breakfast originated from the RCB, whereas in Study 2, 56% of the total energy content originated from the RCB.

### 3.3.2 *Ad libitum* lunch

Four hours after intake of breakfast, participants were served a standardised lunch consisting of the Swedish dish Pyttipanna; a mixture of finely chopped pieces of pork, beef, potato and onion, that had been commercially manufactured and then fried in butter before serving. All participants were served a plate with 600 g of Pyttipanna and a glass with 90 g of pickled beetroot, a glass of water and an empty plate, and were instructed to eat an *ad libitum* amount of food until they felt pleasantly full. Subsequently, the leftovers of each participant were weighted in order to measure the amount of food they had ingested and calculate the energy intake. This procedure was the same in both studies.

## 3.4 Statistical analysis

Data from both studies was transferred from the hand-held computers to Excel (Microsoft Office, version 2007, Microsoft Corporation, USA), in which the figures presented in this thesis were created. Statistical analysis was performed using

SAS (version 9.2, SAS Institute Inc., USA). The level of significance was set at  $P < 0.05$ . Normality of the data was tested graphically by plotting the residual variance when fitted to a linear model, and statistically using Shapiro-Wilk test. When the data showed a non-Gaussian distribution, a logarithmic model was fitted to the data. Criteria for choosing a transformed or a non-transformed model were primarily based on the graphic test for normality and secondarily on Shapiro-Wilk test for normality. Appetite scores were analysed based on VAS scores as well as tAUC, for the three questions respectively, as well as for energy intake. Data from Study 1 and 2 was analysed separately by mixed models appropriate for cross-over designs. For VAS scores the model included the fixed factors occasion, treatment, time point and interaction between time point and treatment as well as between occasion and treatment, and the random factor ID. For tAUC and energy intake, the model did not include the factor time point. Differences between breakfasts measured with tAUC as well as energy intake were compared using least mean squares (LSM) values. When LSM values were log transformed, back transformed values are presented. All mean values presented in these figures are LSM. Values are presented together with the standard error of the mean (SEM), describing the uncertainty of how the sample mean represents the population mean.

## 4 Results and discussion

### 4.1 Results from Study 1

#### 4.1.1 Study participants

The characteristics of the 21 study participants who completed Study 1 are shown below in Table 3.

*Table 3. Characteristics of study participants in Study 1. Mean  $\pm$  standard deviation*

	Men (n = 10)	Women (n = 11)	All (n = 21)
Age (years)	34 $\pm$ 11	44 $\pm$ 15	39 $\pm$ 14
Weight (kg)	78 $\pm$ 12	62 $\pm$ 5	70 $\pm$ 12
Height (m)	1.80 $\pm$ 0.1	1.66 $\pm$ 0.1	1.73 $\pm$ 0.1
BMI (kg/m <sup>2</sup> )	24 $\pm$ 4	23 $\pm$ 2	23 $\pm$ 3

All participants fulfilled the inclusion criteria. Participants were between 18 and 65 years old, did not have a BMI < 18 or > 28, they were not pregnant, did not use tobacco, were not under any form of medication that could affect their appetite and did not suffer from any gastrointestinal problems.

#### 4.1.2 How hungry do you feel right now?

Figures 2 and 3 show the results for the first question, “How hungry do you feel right now?”. Participants felt less hungry after eating the RCB breakfast compared to the WWB breakfast (Figure 2). The difference in hunger appeared to start after 9.00 and was largest at 10.30 and remained until lunch time at 12.00 (Figure 2). Difference in hunger between RCB and WWB breakfasts was statistically significant ( $P < 0.0001$ ). Furthermore, participants felt significantly less hunger ( $P =$

0.0030) depending on occasion, meaning that the size of the effect differed between occasion one and two regardless of breakfast type. This might be an effect of learning. When differences in hunger were evaluated by comparing tAUC, as shown in Figure 3, subjects felt 24% less hunger after RCB breakfast than after the WWB breakfast (P = 0.0189).

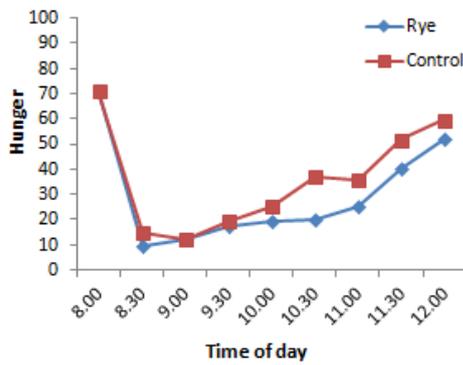


Figure 2. Perceived hunger as reported by the study participants on VAS from before breakfast (8.00) until lunch (12.00).

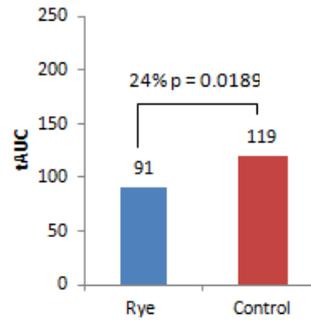


Figure 3. Perceived hunger as reported by the study participants from before breakfast (8.00) until lunch (12.00). Measured as difference between tAUC. Figure is based on LSM values, for rye  $91 \pm 1.2$  and for control  $119 \pm 1.2$ .

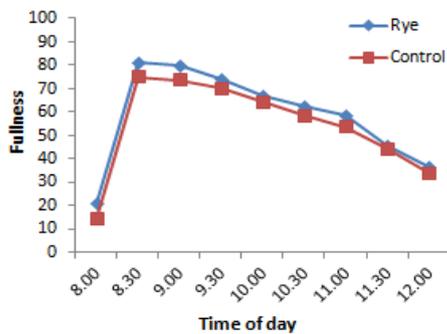


Figure 4. Perceived fullness as reported by the study participants on VAS from before breakfast (8.00) until lunch (12.00).

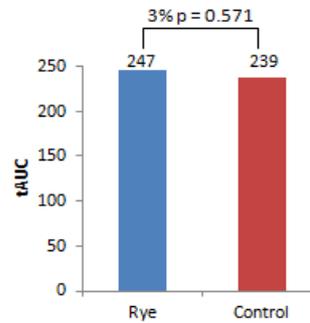


Figure 5. Perceived fullness as reported by the study participants from before breakfast (8.00) until lunch (12.00). Figure is based on LSM, for rye  $239 \pm 17.3$  and for control  $247 \pm 17.3$ .

#### 4.1.3 How full do you feel right now?

Figures 4 and 5 show the results on the question ‘How full do you feel right now?’. Participants felt significantly less full after eating the WWB breakfast compared to the RCB breakfast ( $P = 0.0309$ ). As shown in Figure 4, participants scored higher ratings for fullness after having eaten the RCB breakfast, from directly after breakfast (8.30) until 11.30 where the scores even out. When difference in fullness was analysed as tAUC as shown in Figure 5, no statistically significant difference in perceived fullness was observed ( $P = 0.571$ ). The difference in results from the model with repeated measures and tAUC could be due to the fact that tAUC does not handle missing values as well as repeated measures analysis as there were several missing values in the data set.

#### 4.1.4 How strong is your desire to eat right now?

Figures 6 and 7 show the results on the question “How strong is your desire to eat right now?”. Participants perceived a statistically significant stronger desire to eat with the WWB breakfast compared to the RCB breakfast ( $P < 0.001$ ). The difference in desire to eat was largest between 9.30 and 11.00 and after 11.00 until lunch. However, at 8.30, the desire to eat was slightly lower with the WWB breakfast compared to the RCB breakfast (Figure 6). There was also a statistically significant effect of occasion ( $P = 0.0006$ ). Similarly, when desire to eat was analysed using tAUC, desire to eat was 23% lower ( $P = 0.0208$ ) after eating the RCB breakfast compared to the WWB breakfast (Figure 7).

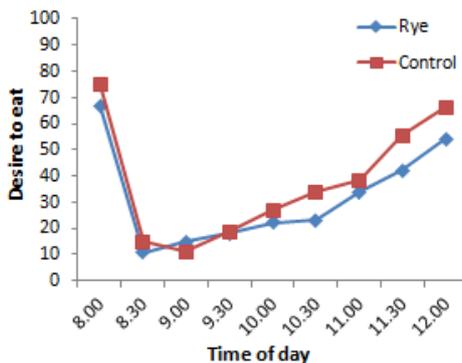


Figure 6. Perceived desire to eat as reported by the study participants on VAS from before breakfast (8.00) until lunch (12.00).

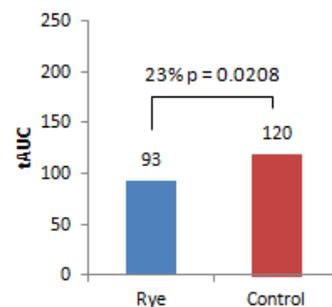


Figure 7. Perceived desire to eat as reported by the study participants from before breakfast (8.00) until lunch (12.00). Measured as difference between tAUC. Figure is based on LSM, for rye  $93 \pm 1.2$  and for control  $120 \pm 1.2$ .

#### 4.1.5 Energy intake

As shown in Figure 8, no significant difference in energy intake after the *ad libitum* lunch was observed in Study 1 ( $P = 0.4130$ ).

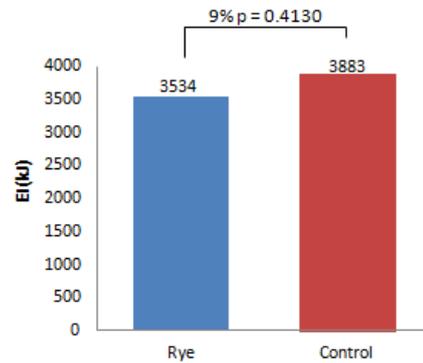


Figure 8. Energy intake at *ad libitum* lunch at 12.00. The figure is based on LSM values, for rye  $3534 \pm 294.9$  and for control  $3883 \pm 294.9$ .

## 4.2 Results from study 2

### 4.2.1 Study participants

The characteristics of the study participants in Study 2 are shown in Table 4. A total of 20 subjects participated in and completed the study.

Table 4. Characteristics of study participants in Study 2. Mean  $\pm$  standard deviation

	Men (n = 6)	Women (n = 14)	All (n = 20)
Age (years)	38 $\pm$ 13	40 $\pm$ 15	39 $\pm$ 14
Weight (kg)	75 $\pm$ 15	62 $\pm$ 6	66 $\pm$ 12
Height (m)	1.79 $\pm$ 0.1	1.66 $\pm$ 0.0	1.70 $\pm$ 0.1
BMI (kg/m <sup>2</sup> )	23 $\pm$ 4	22 $\pm$ 2	23 $\pm$ 3

Participants were aged between 18 and 65 years, did not have a BMI  $< 18$  or  $> 28$ , they were not pregnant, did not use tobacco, were not under any form of medication that could affect their appetite and did not suffer from any gastro intestinal problems, thus fulfilling the inclusion criteria.

#### 4.2.2 How hungry do you feel right now?

Participants were significantly ( $P < 0.0001$ ) less hungry after eating the RCB breakfast compared to the WWB breakfast (Figures 9-13). As shown in Figure 9, this difference appeared at 9.00, thus it was not apparent on the first scoring time point after breakfast. There was also a significant interaction between treatment and occasion, meaning that there was a statistically significant difference ( $P = 0.0116$ ) in hunger scores depending on whether the participant received the RCB or WWB breakfast first (Figures 10 and 11). In both groups, participants were less

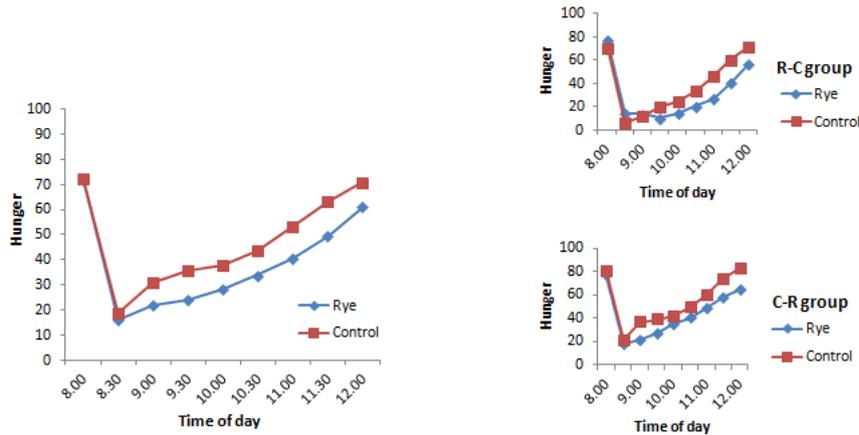


Figure 9. Perceived hunger as reported by the study participants on VAS from before breakfast (8.00) until lunch (12.00).

Figures 10 and 11. Perceived hunger as reported by the study participants on VAS from before breakfast (8.00) until lunch (12.00) Interaction between treatment and occasion divided by sequence.

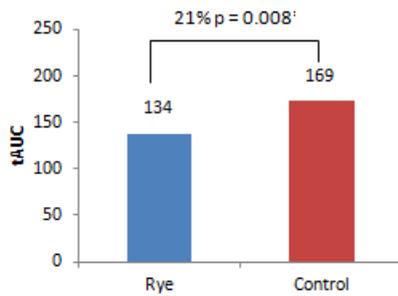


Figure 12. Perceived hunger as reported by the study participants from before breakfast (8.00) until lunch (12.00). Measured as difference in tAUC. Figure is based on LSM, for rye  $134 \pm 13.3$  and for control  $169 \pm 13.3$ .

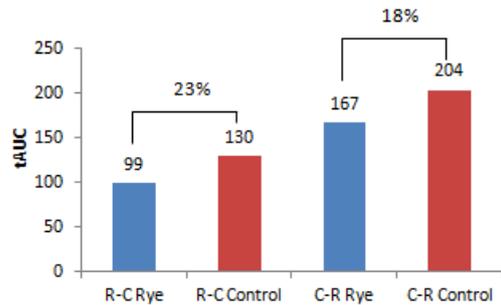


Figure 13. Perceived hunger measured as difference in tAUC, divided by sequence.

hungry after eating the RCB breakfast, although the VAS scores were generally lower in the R-C group than in the C-R group. In the C-R group, the largest difference in hunger was at 9.00 and at 12.00, whereas in the R-C group, participants were initially less hungry with the WWB breakfast. However this change at 9.00 after which the difference remained relatively constant. When hunger was evaluated based on the tAUC, participants felt 21% less hungry after eating the RCB breakfast compared to the WWB breakfast, which is statistically significant ( $P = 0.0080$ ) (Figure 12). Similarly, there was also a statistically significant effect of the interaction between treatment and occasion ( $P = 0.0062$ ), with a larger difference in hunger after eating the breakfasts within the R-C group (23%) than in the C-R group (18%). Additionally, participants generally felt more hunger in the C-R group than in the R-C group (Figure 13).

#### 4.2.3 How full do you feel right now?

Participants felt significantly more full ( $P < 0.0001$ ) after eating the RCB breakfast compared to the WWB breakfast. The difference in perceived fullness was apparent from 9.00 and increased slightly towards the end of the measured time period (Figure 14).

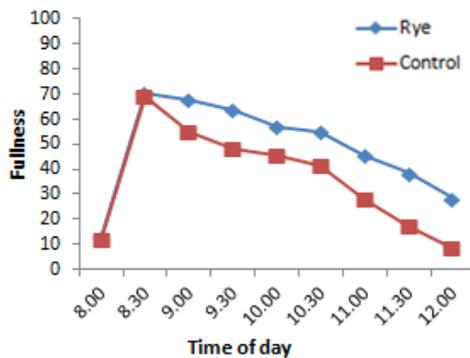


Figure 14. Perceived fullness as reported by the study participants on VAS from before breakfast (8.00) until lunch (12.00).

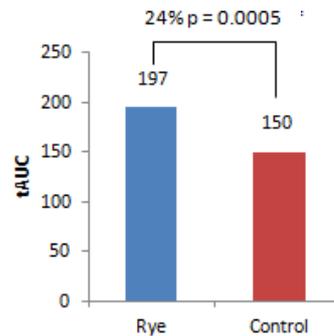


Figure 15. Perceived fullness as reported by the study participants from before breakfast (8.00) until lunch (12.00). Measured as difference in tAUC. Figure based on LSM, for rye  $197 \pm 14.9$  and for control  $150 \pm 14.9$ .

A statistically significant difference in perceived fullness between RCB and WWB breakfast was observed for tAUC ( $P = 0.0005$ ). Participants felt 24% more full after eating the RCB breakfast compared to the WWB breakfast (Figure 15).

#### 4.2.4 How strong is your desire to eat right now?

Figures 16-20 show the results for the question “How strong is your desire to eat right now?”. Desire to eat was significantly lower after eating the RCB breakfast compared to the WWB breakfast ( $P < 0.0001$ ). At the first measurement after breakfast, participants had an equally low desire to eat independent of breakfast type. However, after 9.00, participants reported a lower desire to eat with the RCB breakfast compared to the WWB breakfast (Figure 16). There was also a statistically significant effect of occasion ( $P = 0.0173$ ), showing that desire to eat differed based on occasion independent of treatment. Furthermore, there was a statistically significant interaction ( $P = 0.0280$ ) between treatment and occasion (Figure 17 and 18), hence desire to eat differed based on the order in which RCB or WWB breakfast was served to the participants. In the R-C group, the difference between the breakfasts in desire to eat was less clear than for the C-R group, as participants initially felt a lower desire to eat with the WWB breakfast and first after 9.30 there was a shift towards feeling lower desire to eat with the RCB breakfast. This difference appears to diminish towards the end of the study time period (Figure 17). In the C-R group where participants first received the WWB breakfast, the RCB breakfast led to a lower desire to eat from 8.30 until lunch at 12.00 (Figure 18).

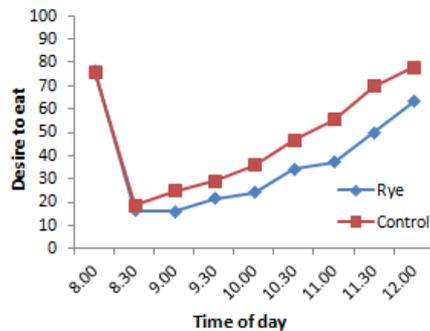
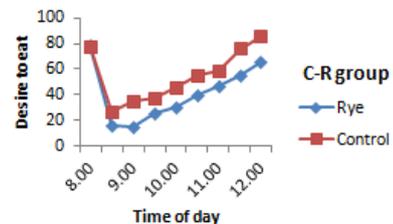
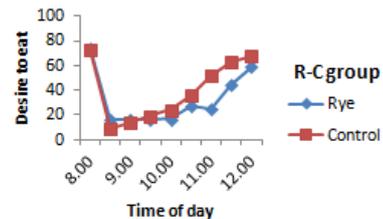


Figure 16. Perceived desire to eat as reported by the study participants on VAS from before breakfast (8.00) until lunch (12.00).



Figures 17 and 18. Perceived desire to eat as reported by the study participants on VAS from before breakfast (8.00) until lunch (12.00). Interaction between treatment and occasion divided by sequence.

A statistically significant difference in desire to eat was observed when using tAUC ( $P = 0.0198$ ), with participants reporting a 21% lower desire to eat after eating the RCB breakfast compared to the WWB breakfast (Figure 19). However, there was also a statistically significant effect of the interaction between treatment

and occasion ( $P = 0.0439$ ), meaning that the difference in desire to eat was statistically different between the group that received the RCB breakfast first (the R-C group) and the group that received the WWB breakfast first (the C-R group) (Figure 20). In both treatment sequences, participants felt a lower desire to eat after eating the RCB breakfast compared to the WWB breakfast. However, this difference was largest (28%) in the C-R group compared to 21% in the R-C group. Desire to eat was overall higher in the C-R group than in the R-C group (Figure 20).

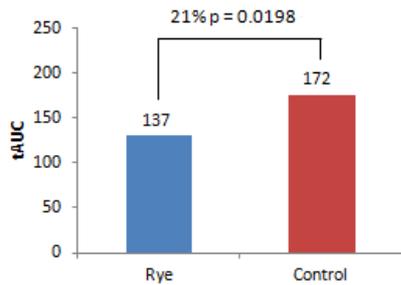


Figure 19. Perceived hunger as reported by the study participants from before breakfast (8.00) until lunch (12.00). Measured as difference in tAUC. Figure based on LSM, for rye  $137 \pm 13.1$  and for control  $172 \pm 13.1$ .

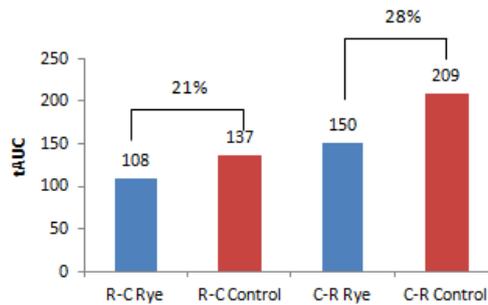


Figure 20. Perceived hunger is measured as difference in tAUC, divided by occasion.

#### 4.2.5 Energy intake

The difference in energy intake at the *ad libitum* lunch is shown in Figure 21. The energy intake of the study participants was significantly lower with 8% after having eaten the RCB breakfast compared to the WWB breakfast ( $P = 0.0237$ ). However, there was also a statistically significant effect of occasion ( $P = 0.0103$ ).

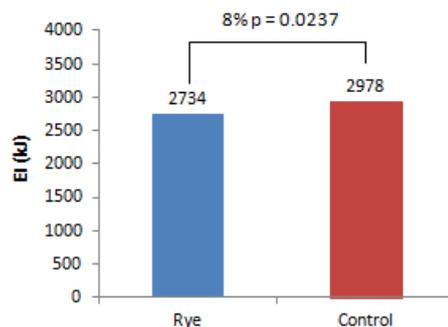


Figure 21. Energy intake at *ad libitum* lunch at 12.00. The figure is based on LSM values, for rye  $2734 \pm 131.2$  and for control  $2978 \pm 131.2$ .

### 4.3 Comparison between Study 1 and Study 2

#### 4.3.1 Difference in breakfast composition between the studies

The breakfasts in Study 1 and Study 2 differed in energy content as well as in the proportion of the total energy content of the breakfasts originating from the RCB. In Study 1, the energy content of both RCB and WWB breakfasts was higher than in Study 2, and the proportion of the energy content originating from the RCB was larger in Study 2 than in Study 1 (Figure 22).

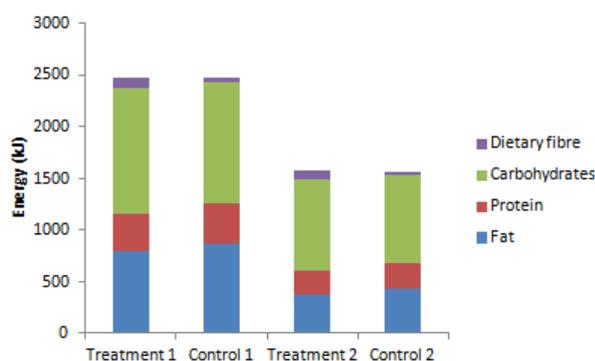


Figure 22. Energy content and proportion of the energy content coming from the different macronutrients. Study 1 is indicated as 1 and Study 2 is indicated as 2.

In Study 1, no difference in energy intake after the *ad libitum* lunch was found between the RCB and WWB breakfasts. A reason for this might be that differences were minimised due to the higher energy content of the breakfasts in Study 1, or that the effect of the RCB was smaller due to the fact that it was a smaller proportion of the total breakfast. In comparison, in Study 2, where the RCB constituted a larger part of the breakfast and the breakfasts had lower energy content, all effects on appetite and energy intake were statistically significant. This masking of effects in Study 1 compared to Study 2 is likely the result of the high energy content more than a result from differences in proportion of test product. This is based on the comparison between control curves in Study 1 and 2, where participants feel more hunger, less fullness and more desire to eat with the WWB breakfast in Study 2 than with the WWB breakfast in Study 1 (Figures 23-25). The total energy

content of the breakfasts in Study 1 lay close to the upper recommended limit, while the energy content of the breakfasts in Study 2 lay slightly below the lower recommended limit (Nordic Council of Ministers, 2004), but should still be considered to be in the range of a normal breakfast.

#### 4.3.2 Hunger

Subjects in both studies felt significantly less hunger ( $P < 0.0001$ ) after eating the RCB breakfast compared to the WWB breakfast (Figure 23). In Study 2 the difference in perceived hunger between RCB and WWB breakfasts remained relatively parallel from 9.00 until lunch at 12.00, whereas in Study 1 the lines fluctuated more and a clear difference between RCB and WWB breakfasts is observed first from 10.00. For both studies, control and test breakfasts appear to have equal effect on hunger at the first time point after breakfast. Participants in Study 2 scored approximately 10 VAS units higher on hunger for both breakfasts compared to Study 1, indicating that participants in study 2 generally felt hungrier throughout the study time regardless of breakfast type than participants in Study 1 (Figure 23). This is probably due to the larger amount of breakfast that was served in Study 1 compared to in Study 2. When comparing hunger based on tAUC, both studies showed statistically significant treatment effects, with 24% and 20% less hunger after eating the RCB breakfast compared to the WWB breakfast in Study 1 and 2, respectively. In Study 1 there was an effect of occasion, and in Study 2 an effect of the interaction treatment and occasion, suggesting that the order of treatments appeared to play a role. This could be the result of a learning effect. In both studies, there was a significant effect of time point ( $P < 0.0001$ ), as expected.

#### 4.3.3 Fullness

In both studies participants felt fuller after eating the RCB breakfast compared to the control breakfast (Figure 24). These differences were significant in both studies, although the difference was larger in Study 2. This is likely caused by the larger amount of breakfast in Study 1. In Study 2, fullness measured as difference between tAUC was statistically significant. Thus although results from Study 1 were slightly unclear, Study 2 showed that there was a statistically significant increased feeling of fullness after eating RCB for breakfast than WWB. In both studies, there was a significant effect of time point ( $P < 0.001$  in Study 1 and in Study 2  $P < 0.0001$ ), as expected. In Study 2 there was also a statistically significant effect of the interaction between time point and treatment.

#### 4.3.4 Desire to eat

Results from both studies showed that subjects had a statistically significant lower desire to eat throughout the study period after eating RCB breakfast compared to WWB breakfast (Figure 25). In Study 2, however, the effects differed slightly depending on the sequence of treatment and control. For both hunger and desire to eat, the effect of treatment appears to be more pronounced in the subject group that received the control breakfast first. This could be the result of a learning effect. In both studies, there was a significant effect of time point ( $P < 0.0001$ ), as expected.

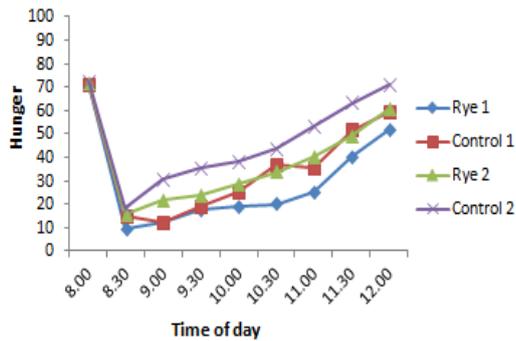


Figure 23. Comparison between perceived hunger as reported by the study participants on VAS from before breakfast (8.00) until lunch (12.00) in Study 1 (indicated as 1) and Study 2 (indicated as 2).

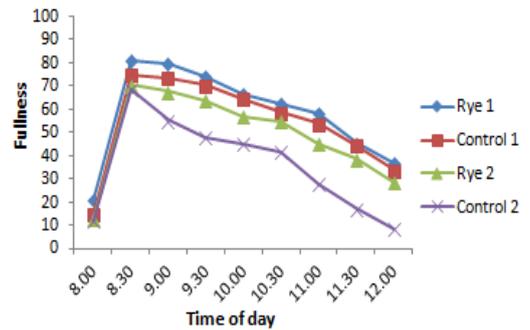


Figure 24. Comparison between perceived fullness as reported by the study participants on VAS from before breakfast (8.00) until lunch (12.00) in Study 1 (indicated as 1) and Study 2 (indicated as 2).

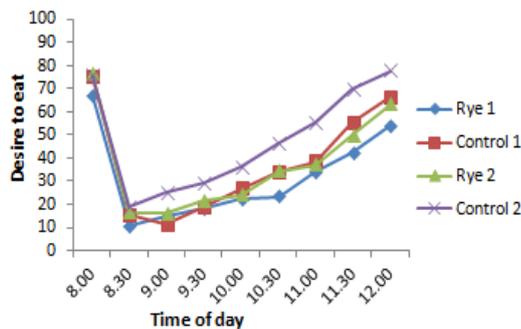


Figure 25. Comparison between perceived desire to eat as reported by the study participants on VAS from before breakfast (8.00) until lunch (12.00) in Study 1 (indicated as 1) and Study 2 (indicated as 2).

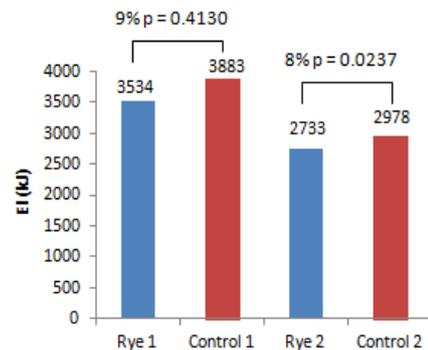


Figure 26. Comparison between energy intake at ad libitum lunch served at 12.00 in Study 1 (indicated as 1) and Study 2 (indicated as 2). The figure is based on LSM values.

#### 4.3.5 Energy intake

Participants in both studies had a lower energy intake at the *ad libitum* lunch, with a 9% and 8% lower energy intake respectively after eating the RCB breakfast compared to the WWB breakfast (Figure 26). This difference in energy intake was, however, only statistically significant in Study 2. In Study 1, a difference could have been masked by the high energy content of the breakfast.

#### 4.4 Comparison to other studies

The strength of using cross-over design in appetite studies is the within-subject comparison, which reduces the risk of confounding effect caused by subjects being different (Blundell *et al.*, 2010). For this reason, comparison with results from other studies should be performed with caution. Furthermore, there are differences in methodology that might affect the results such as different types of rye products, different composition of meals, different study designs and different statistical treatments of the data. However, the superior effect of rye products compared with wheat reference products on appetite has been shown in several studies (Isaksson, 2011; Isaksson *et al.*, 2009; Leinonen *et al.*, 1999; Rosén *et al.*, 2009; Rosén *et al.*, 2011a,b,c) of which the studies of Isaksson *et al.* (Isaksson, 2011; Isaksson *et al.*, 2009) are most similar to the two studies discussed in this thesis. Isaksson *et al.* (2009) found that rye bread and porridge compared to WWB led to reduced perceived hunger, more perceived fullness and less desire to eat in the time span 8.30-12.00. The curves based on VAS were similar to those in Study 1 and 2, in that the curves for hunger and desire to eat followed the same pattern while the curve for fullness was inverted (Isaksson *et al.*, 2009), indicating that study participants tend to rate fullness as the opposite of hunger and desire to eat. Unlike in Study 1 and 2, Isaksson *et al.* (2009) found a difference between appetite responses for rye bread compared to WWB starting at the first time point after breakfast, whilst this difference appeared first around the second time point after breakfast in Study 1 and 2. Furthermore, Rosén *et al.* (2011c) found that consumption of a rye bread breakfast compared to a WWB breakfast was associated with a 16% lower energy intake at lunch, which complements the findings of Study 2.

Isaksson *et al.* had an energy content of the bread breakfasts of 1960 kJ (Isaksson *et al.*, 2009) and 2550 kJ (Isaksson *et al.*, 2011), where effects on appetite of the rye bread breakfast compared to the WWB breakfast were clear at the lower energy content but less clear with the higher energy content where an effect on hunger could not be shown. This might support the theory that higher energy content of the breakfast masks differences in appetite ratings. Furthermore, factors such as structure and large volume of the test food have been shown to decrease

appetite and might thus have a confounding effect (Isaksson, 2011). The RCB evaluated in Study 1 and 2 is made from milled whole grain rye flour and has a small volume compared to porridge. Despite this, clear appetite suppressing effects are shown compared to the WWB breakfast, indicating that the shown effects are not due structure or volume.

## 4.5 General discussion

### 4.5.1 Methodological considerations

The quality of data differed to some extent between the studies, with Study 1 having more missing values and responses fluctuated heavily for some individuals. This raises the question whether the participants understood the questions and how to use the scales to assess their subjective appetite. The result will be loss in precision. As this appears to be random, it will probably not introduce any bias in the result. The overall results of Study 1 still show a more satiating effect of RCB breakfast compared to WWB breakfast, although differences are small and less consistent than in Study 2. Missing values did not appear to influence the results in the study. In Study 1, the normality tests showed that the data was normally distributed for some questions and for some not, which indicates that the sample size was slightly too small to provide robust estimation of the distributions. In Study 2, only a few values were missing and the individual plots were more consistent. However, the results of three participants had to be excluded due to not participating at both test occasions. Results from 20 participants were analysed in Study 2, which still is enough to obtain a sufficiently high statistical power and minimise the risk for type II errors according to Flint *et al.* (2000).

A few participants from Study 1 also participated in Study 2. However, a possible learning effect from this is not expected to have a confounding effect in Study 2 due to more than six months between the studies. If such a learning effect existed, it would probably be that the participants learned how to use the VAS to assess their appetite, and thus were more likely to use it accurately in Study 2.

In the statistical analysis of Study 1 and 2, both repeated measure analysis and tAUC was performed. Both these methods are recommended methods for appetite studies, since they consider appetite responses as the result of multiple, dependent variables (Blundell *et al.*, 2010). These analyses complement each other in the way that tAUC provides estimation of the treatments for the entire test period, and thus facilitates comparison, while plots of VAS scores better show how the dynamics in appetite ratings over the measured time period.

The statistical analyses on tAUC and energy intake were performed using LSM, which is preferable since LSM values only show the actual effect of treatment adjusted for the other factors in the model. It is therefore expected to be the best estimate of the true treatment effect. However, the plots of appetite scores against time shown in the present thesis are based on unadjusted values. It needs to be recognised that the statistical analyses have fitted the data to a statistic model hence adjusting it for several factors, which can make the values slightly different from those initially observed. Furthermore, back transformation of logarithmic values tends to make them somewhat lower than the original observational data.

#### 4.5.2 Relevance of the results

These are the first studies to investigate the effects of RCB on appetite and energy intake in a subsequent meal, at two different energy intakes. The results of Study 1 and 2 both show that a breakfast including RCB compared to a breakfast with WWB has superior effects of appetite, although results were clearer with a lower energy content of the breakfast. Study participants were significant less hungry, felt fuller and had a lower desire to eat until lunch at 12.00 after eating the RCB breakfast compared to the WWB breakfast, although there was some variation depending on sequence. Participants in Study 2 also had a significantly lower energy intake after an *ad libitum* lunch after eating the RCB breakfasts compared to the WWB breakfast, suggesting that link to the effects of RCB on appetite. Based on these findings, substitution of WWB with RCB in the breakfast could likely contribute in weight management, although long-term studies are needed to confirm this. Thus from an application perspective, a point of consideration is whether the effects on appetite are large enough to mediate weight loss or facilitate weight management. Additionally, rye products have a high content of dietary fibre and biologically active components which have been linked to health benefits (Andersson *et al.*, 2009), which further contributes to RCB being a value added product.

## 5 Conclusions

Results from both studies show that RCB when served as part of a breakfast compared to a breakfast with WWB, had superior effects of appetite. The evaluated studies differed in terms of total energy content and proportion of the energy content that originated from the test product, with Study 2 having lower energy content and the test product constituting a larger proportion of the breakfast meal than in Study 1.

Study 1 showed a lower perceived hunger and desire to eat after eating the RCB breakfast compared to the WWB breakfast, while effects on fullness were less pronounced. This might be due to the high energy content of the breakfast or the lower proportion of test product masking the difference in effect on appetite.

In Study 2 results showed more perceived fullness, less hunger and less desire to eat as well as a lower energy intake at lunch after eating the RCB breakfast compared to the WWB breakfast.

RCB affects satiety through contributing to lower perceived hunger, higher perceived fullness and less desire to eat in a four hours perspective compared to WWB. In Study 2 but not in Study 1, the findings were verified by a lower energy intake at an *ad libitum* lunch.

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## References

- Andersson, R., Fransson, G., Tietjen, M., & Aman, P. (2009). Content and molecular-weight distribution of dietary fiber components in whole-grain rye flour and bread. *Journal of Agricultural and Food Chemistry*, 57(5), 2004–2008.
- Blundell, J., De Graaf, C., Hulshof, T., Jebb, S., Livingstone, B., Luch, A., Mela, D., *et al.* (2010). Appetite control: methodological aspects of the evaluation of foods. *Obesity reviews: an official journal of the International Association for the Study of Obesity*, 11(3), 251–70.
- Bondia-Pons, I., Nordlund, E., Mattila, I., Katina, K., Aura, A.M., Kolehmainen, M., Orešič, M., *et al.* (2011). Postprandial differences in the plasma metabolome of healthy Finnish subjects after intake of a sourdough fermented endosperm rye bread versus white wheat bread. *Nutrition journal*, 10(116), 1-10.
- Cummings, D.E. & Overduin, J. (2007). Review series Gastrointestinal regulation of food intake. *Health Care*, 117(1), 13–23.
- Flint, A., Raben, A., Blundell, J.E., & Astrup, A. (2000). Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *International journal of obesity and related metabolic disorders: journal of the International Association for the Study of Obesity*, 24(1), 38–48.
- Gerstein, D.E., Woodward-Lopez, G., Evans, A.E., Kelsey, K. & Drewnowski, A. (2004). Clarifying concepts about macronutrients' effects on satiation and satiety. *Journal of the American Dietetic Association*, 104(7), 1151–1153.
- Gregersen, N.T., Møller, B.K., Raben, A., Kristensen, S.T., Holm, L., Flint, A. & Astrup, A. (2011). Determinants of appetite ratings: the role of age, gender, BMI, physical activity, smoking habits, and diet/weight concern. *Food & nutrition research*, 55, 1–10.
- Hill, A.J. & Blundell, J.E. (1992). Nutrients and behaviour: research strategies for the investigation of taste characteristics, food preferences, hunger sensations and eating patterns in man. *Journal of Psychological Research*, 17, 203-212.
- Howarth, N.C., Saltzman, E., & Roberts, S.B. Lead Review. *Nutrition Reviews*, 59(5), 129-139.
- Isaksson, H. (2011). *Satiating Effects of Rye Foods*. PhD Thesis, Swedish University of Agricultural Sciences, *Acta Universitatis Agriculturae Sueciae*.
- Isaksson, H., Rakha, A., Andersson, R., Fredriksson, H., Olsson, J., & Åman, P. (2011). Rye kernel breakfast increases satiety in the afternoon - an effect of food structure. *Nutrition journal*, 10(31), 1-10.

- Isaksson, H., Fredriksson, H., Andersson, R., Olsson, J. & Åman, P. (2009). Effect of rye bread breakfasts on subjective hunger and satiety: a randomized controlled trial. *Nutrition Journal*, 8(39), 1-8.
- Isaksson, H., Sundberg, B., Åman, P., Fredriksson, H. & Olsson, J. (2008). Whole grain rye porridge breakfast improves satiety compared to refined wheat bread breakfast. *Food and Nutrition Research*, 52, 1-7.
- Juntunen, K.S., Laaksonen, D.E., Poutanen, K.S., & Niskanen, L.K. (2003). High-fiber rye bread and insulin secretion and sensitivity in healthy postmenopausal women. *The American Journal of Clinical Nutrition*, 77, 385-391.
- Landberg, R. (2012). Dietary fibre and mortality: convincing observations that call for mechanistic investigations, editorial. *American Journal of Clinical Nutrition*, 96, 3-4.
- Leinonen, K.S., Poutanen, K.S. & Mykkänen, H.M. (1999). Rye bread decreases serum total and LDL cholesterol. *Journal of Nutrition*, 130, 164-170.
- National Food Agency. (2012). Riksmaten vuxna 2010-11, Livsmedels- och näringsintag bland vuxna i Sverige, Resultat från matvaneundersökning utförd 2010-11, viewed 12 March 2013, <[http://www.slv.se/upload/dokument/rapporter/mat\\_naring/2012/riksmaten\\_2010\\_2011.pdf](http://www.slv.se/upload/dokument/rapporter/mat_naring/2012/riksmaten_2010_2011.pdf)>.
- Nordic Council of Ministers. (2004). *Nordic Nutrition Recommendations, Integrating nutrition and physical activity*. 4<sup>th</sup> ed. Copenhagen: Norden, 14, 13-101.
- Pasman, W.J., Blokdijs, V.M., Bertina, F.M., Hopman, W.P.M. & Hendriks, H.F.J. (2003). Effect of two breakfasts, different in carbohydrate composition, on hunger and satiety and mood in healthy men. *International journal of obesity and related metabolic disorders journal of the International Association for the Study of Obesity*, 27(6), 663-668.
- Rosén, L., Silva, L.O.B., Andersson, U.K., Holm, C., Ostman, E.M. & Björck, I.M.E. (2009). Endosperm and whole grain rye breads are characterized by low post-prandial insulin response and a beneficial blood glucose profile. *Nutrition journal*, 8(42), 1-11.
- Rosén, L., Östman, E.M. & Björck, I.M.E. (2011a). Postprandial glycemia, insulinemia, and satiety responses in healthy subjects after whole grain rye bread made from different rye varieties. 1. *Journal of Agricultural and Food Chemistry*, 59(22), 12139-12148.
- Rosén, L., Östman, E.M. & Björck, I.M.E. (2011b). Postprandial glycemia, insulinemia, and satiety responses in healthy subjects after whole grain rye bread made from different rye varieties. 2. *Journal of Agricultural and Food Chemistry*, 59(22), 12149-12154.
- Rosén, L., Östman, E.M. & Björck, I.M. (2011c). Effects of cereal breakfasts on postprandial glucose, appetite regulation and voluntary energy intake at a subsequent standardized lunch; focusing on rye products. *Nutrition Journal*, 10(7), 1-11.
- Smith, C.E. & Tucker, K.L. (2011). Health benefits of cereal fibre: a review of clinical trials. *Nutrition Research Reviews*, 24(1), 118-131.
- Stratton, R.J., Stubbs, R.J., Hughes, D., King, N., Blundell, J.E. & Elia, M. (1998). Comparison of the traditional paper visual analogue scale questionnaire with an Apple Newton electronic appetite rating system (EARS) in free living subjects feeding ad libitum. *European journal of clinical nutrition*, 52(10), 737-41.
- Stubbs, R.J., Hughes, D., Johnstone, M., Rowley, E., Ferris, S., Elia, M., Stratton, R., *et al.* (2001). Description and evaluation of a Newton-based electronic appetite rating system for temporal tracking of appetite in human subjects. *Physiology & behavior*, 72(4), 615-619.
- Stubbs, R.J., Hughes, D.A., Johnstone, A.M., Rowley, E., Reid, C., Elia, M., Stratton, R., *et al.* (2000). The use of visual analogue scales to assess motivation to eat in human subjects: a review of their reliability and validity with an evaluation of new hand-held computerized systems for temporal tracking of appetite ratings. *British Journal of Nutrition*, 84, 405-415.
- Williams, P.G., Grafenauer, S.J. & O'Shea, J.E. (2008). Cereal grains, legumes, and weight management: a comprehensive review of the scientific evidence. *Nutrition Reviews*, 66, 171-182.



## Rye crisp bread has superior effects on appetite

**A breakfast including crisp rye bread contributes to increased feelings of fullness while lowering feelings of hunger and desire to eat, and thus adds value in terms of health when substituting white wheat bread.**

Choosing high quality food products is an important lifestyle choice when aiming for a long and healthy life. Bread and porridge of rye are traditionally consumed in the Central, Eastern and Northern European countries and are with advantage included in your breakfast meal. Compared to white wheat bread, rye products have a high content of beneficial dietary fibre as well as vitamins, minerals and micronutrients. These nutrients have been linked to decreased risk for several life style related diseases such as diabetes type II and cardiovascular disease. Furthermore, fibre rich products such as crisp rye bread can contribute to weight management through effects on appetite. For this reason, rye products are the healthy choice.

### **Appetite affects our energy intake**

Our biological drive to eat, appetite, is influenced by many factors. These can be both of physiological nature which we notice through a growling stomach or feeling tired, or it can be the result of our mood, our habits and our culture. Appetite is also affected by what we eat, as certain foods make us feel fuller and keep the hunger away for a longer time period than other foods. Appetite can be said to be divided in three perceptions: how hungry we feel, how full we feel and how strong our desire to eat is. All these perceptions together influence how large portions we will eat at subsequent meals and thus our energy intake. Consequently, food products that lead to less hunger, more fullness and lower desire to eat are more likely to make

us wait longer until we eat again and, when we do, eat a smaller amount of food with lower energy content.

### **Rye crisp bread decreases appetite**

Results from two studies that have investigated how a breakfast with crisp rye bread affects appetite compared to the same breakfast including white wheat bread, show that crisp rye bread has superior effects on appetite. Study participants felt fuller as well as less hungry and had a lower desire to eat after eating the breakfast with crisp rye bread, compared to the breakfast with white wheat bread. This difference lasted from breakfast until lunch four hours later.

### **Rye crisp bread and weight management**

Results from the second study also showed that participants ate a smaller amount of food at lunch and thus had a lower energy intake after eating the breakfast with crisp rye bread. This is likely the result of the appetite-lowering effects of eating crisp rye bread compared to white wheat bread. Therefore, substitution of white wheat bread with crisp rye bread could likely contribute to weight management.

Choosing rye crisp bread instead of white wheat bread is a good choice for the health-conscious consumer that aspires a long and healthy life. Rye crisp bread has superior effects on appetite compared to white wheat bread. Consumption of rye bread results in stronger feelings of fullness, less hunger and a lower desire to eat, which can lead to a lower energy intake at a subsequent meal. Rye also contributes with healthy nutrients and dietary fibre, which further contributes to crisp rye bread being bringing you added value in terms of health.