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Phenolic compounds in *Fagopyrum sp*. grains (buckwheat); profile, bioactivity and effect of processing

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Abstract

This study aimed to investigate the health benefits related to chemical compounds found in buckwheat cultivars. Factors influencing the presence of bioactive compounds with assumed health effects were examined. Two species of buckwheat, *Fagopyrum esculentum* (common buckwheat) and *Fagopyrum Tartaricum* (tartary buckwheat) were evaluated. The selection of species was based on the comprehensive documented effects of these as well as their long-raging use as therapeutics in traditional medicine. Buckwheat can be considered an optimal cultivar in sustainable food farming since it has a strong adaptability to harsh environment, making it a versatile crop which can be cultivated worldwide without using pesticides.

Both buckwheat species have in previous studies been shown to contain high amounts of carbohydrates and proteins, rich in vitamins and minerals. Besides, they contain high quantities of phenolic compounds and phytosterols with pharmacological properties and potential to prevent the development of some chronic diseases. Additionally, buckwheat has a unique, balanced macro and micro nutritional composition and is free of gluten and related prolamins, making it prospective as a functional food, suitable for people with coeliac disease. Crude protein has all essential amino acids and are observed to reduce serum cholesterol and cell proliferation. Extracted protein could be used to develop plant-based protein products with low environmental impact.

Phenols, compounds with high antioxidant activity, have been found ubiquitous in buckwheat and were suggested to be responsible for various health modulating effects. Hence, the emphasis in this study was to determine the

plant material that contained the highest amount of phenols and other compounds with biological activity.

The result demonstrated that the highest total phenolic content was found in *F. Tartaricum* (tartary buckwheat). The content was particularly high in the bran followed by the hull and the flour (9.49, 7.71, 3.06, 1.26 GA/g-1 DW, respectively). In *F. esculentum* the total phenolic content ranged from 0,05 ('Novosadska') to 0.3 g (Bosna 1 and 2) GA/g-1 DW. The concentrations obtained varied significantly in the same species depending on location and environment of cultivation. Tartary buckwheat also had a significantly higher content of free phenols compared to common buckwheat, with a co-herently higher antioxidant activity. *P*-hydroxybenzoic, ferulic and proto-catechuic acids were the dominant phenolic acids in tartary buckwheat, and accounted for 83–88% of the total phenolic acid content while ferulic, vanil-lic, *p*-coumaric and syringic acids were estimated as the major individual phenolic acids in common buckwheat.

Among the flavonoids, the most abundant compounds were catechin (monomeric flavan-3-ols), rutin, orientin, vitexin and quercetin. Rutin was the major phenol in common buckwheat seeds (90,5 % of the total phenolic content*)*.

Processing of raw buckwheat somewhat altered the profile of bioactive compounds compared to the raw material. Soaking activates enzymes such as rutin 3-glucosidase which hydrolyses the glycoside rutin to quercetin. Heat treatment increases the fraction of free phenolic compounds but decreases the ester, glycoside, and ester-bound fractions. This may enhance the antioxidant activity.

Regarding absorption and bioavailability, phenols are absorbed differently depending on the chemical composition. Phenolic glycosides, polymers and

esters needs to be hydrolyzed by intestinal enzyme or degraded by microbes in the colon before absorption. Quercetin glycosides, including rutin, can be transported by SGLT1. This proposes querecetin glycosides as inhibitors of glucose uptake. Many flavonoids are however rapidly metabolized in the liver into methoxy derivatives or conjugated derivatives before excretion.

To conclude, buckwheat has a great potential both as a functional food and as a carrier of pharmacological compounds. It can prevent and treat diseases with high mortality rate in both first, second and third world countries. It can also be an option for drugs that is more economically, environmentally and socially sustainable.

Keywords: Buckwheat, *Fagopyrum esculentum*, *Fagopyrum tartaricum*, rutin, quercetin, total phenolic content, gluten-free, functional foods, antioxidants

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1 Introduction

1.1 Background

The Fagopyrum genus belongs to the Polygonaceae family and are composed of 15 species, of which *Fagopyrum esculentum Moench* (common buckwheat) and *Fagopyrum tataricum* Gaertn.(tartary buckwheat) are the most important and abundantly studied species. Due to a long tradition of use as food and in Chinese and Korean medicine, a considerable amount of research has been conducted to investigate health effects of these species.

Traditionally, both seeds and roots have been used to treat chronic diseases such as rheumatic disorders, as well as cancers, hypoglycemia, hyperlipidemia and diabetes. The pharmaceutical and nutraceutical application of both species are currently widely investigated, showing a great potential (Zhang et al., 2018).

F. *esculentum* and *F. tataricum* are both cultivated worldwide and are resilient crops with a low presence of pests and require no or minimal use of plant protection. The need for pollination additionally contributes to recognition of buckwheat as a suitable crop to enhance biodiversity in the agricultural landscape and makes it an optimal constituent in ecological and sustainable food farming (Wieslander et al., 2011).

Buckwheat is a pseudocereal which does not contain gluten. This makes it applicable as dietary component for coeliacs.

For patients with Coeliac disease, a lifelong elimination of gluten-containing grains (including wheat, rye, barley) is currently the only effective treatment. Ingestion of gluten or related prolamins results in mucosal damage causing malabsorption, malnutrition and maldigestion. Consequently, it contributes to gastrointestinal symptoms and symptoms related to nutrient deficiency, such as anemia and osteoporosis (Alvarez-Jubete et al., 2009).

Nevertheless, symptomatic and histological recovery are both generally observed in patients with pathological abnormalities when put on a gluten-free diet, indicating the importance of gluten-free products on the market.

Nevertheless, nutrient deficiencies in both coeliacs and non-diseased following a gluten-free diet have been demonstrated in several studies, indicating an insufficient nutrient supply in gluten-free products (Krupa-Kozak and Drabińska, 2016).

A dietary survey done in the United states which assessed the nutrient intake on a gluten-free diet, reported a lower nutrient quality in gluten-free products and inadequate intake of iron, calcium and fiber in the studied participants (Krupa-Kozak and Drabińska, 2016). In cohesion, a review by Vici et al (2016) assessed a gluten free diet as particularly low in zinc, magnesium, iron, vitamins B and D, calcium, folate and dietary fibers.

The unique chemical composition of buckwheat argues for further development of new buckwheat-based food products. Various products (such as teas, pasta, cookies, breads etc.) are widely consumed in Asian countries, Italy, Slovenia and northern Europe where buckwheat is used in many traditional dishes. Buckwheat is also used as an ingredient in functional foods with therapeutic potential for coeliacs as wells as people with metabolic diseases.

1.2 Nutritional Composition

1.2.1 Starch and dietary fibre

Chemical analysis reported starch to be the major component (>50 % of dry matter, DM), with a high percentage of that being dietary fiber (insoluble and soluble), for example resistant starch (RS). Dietary fiber, including RS, have been associated with positive health effects including a decreased risk of developing diabetes mellitus type 2 and cardiovascular diseases by improving tissue insulin sensitivity, hepatic insulin clearance and fatty acid metabolism (Robertson et al., 2003). Starch also improves postprandial plasma glucose and insulin.

1.2.2 D-chiro inositol

D-chiro inositol (DCI), an inositol phosphoglycan (IPG) is also present in both buckwheat species. It is regarded as an insulin sensitizer since IPG mediators improves glucose metabolism through several cellular mechanisms. DCI deficiency have been demonstrated to cause insulin resistance (IR), partly because of DCI's role as second messenger in the phosphorylation signaling pathways activated by insulin and resulting in the activation of protein phosphatases in the insulin signaling pathway. It was also shown that DCI had a regulatory effect on cytochrome P450 (CYP); specifically on the steroidogenic enzyme genes CYP19A1.The modulation was observed to be beneficial in treating conditions related to IR, for example polycystic ovary syndrome (PCOS) (Sacchi et al., 2016).

1.2.3 Protein

A compliance of published data has demonstrated a high protein content in buckwheat (10.6 and 10.3 g/100 g of DW in common and tartary buckwheat, respectively) with high biological value and a balanced amino acid profile. Buckwheat is particularly rich in leucine and lysine (6.92, 5.84, and 7.11, 6.18g/100g protein in common and tartary buckwheat). Buckwheat is considered as a complete protein source and contain more arginine, aspartic acid and tryptophan than cereal proteins. However, it has relatively low true digestibility (values between 79.9% and 78.8% have been obtained in previous studies) compared to cereal grains, mainly due to crude fiber (e.g. reduced susceptibility for the proteolytic enzymes to the protein fractions) and high tannin content.

Additionally, buckwheat protein products (BWP) have also been associated with preventative nutrition. A prepared buckwheat protein product was in a study able to reduce serum cholesterol levels and the lithogenic index in rats. Both reductions were associated with enhanced excretion of fecal neutral sterols and fecal bile acid, respectively (Tomotake et al., 2007).

BWP significantly reduced cell proliferation in colonic epithelial cells in rats by suppressing induced colon carcinogenesis. Rats fed with BWP showed a 47% reduction in colon adenocarcinoma occurrence compared to those fed with casein (Liu et al., 2001). The effects observed from experimental studies, including anti-obesity, hypocholesterolemic, anti-carcinogenic effects of BWP are estimated to be due to its low digestibility, thus being similar in physiological effect as dietary fiber (Liu et al., 2001).

1.2.4 Micronutrients

Buckwheat possesses a higher level of minerals and trace elements compared to cereals. The levels of Mg, P and K are especially high whereas Zn, Cu and Mn are the most abundant trace elements. The levels of Mg in 100 g of buckwheat (267 mg) are comparable with an RDI for women (280 mg).

However, Mg, Zn, K, P and Co are bound to phytic acid and hydrolysis is required prior absorption so that minerals can pass intestinal walls. Vitamin B3 (niacin), B5 (pantothenic acid), C (ascorbic acid) and E5 (tocopherols) are also present in considerable amounts. Both ascorbic acid and a-tocopherol are potent antioxidants. Germination has been reported to increase the vitamin B1 (thiamin), vitamin B6 (pyridoxine) and ascorbic acid content.

1.2.5 Phenolic compounds

The comprehensive array of health benefits related to buckwheat consumption has specifically been connected to its extensive phenolic profile, specifically flavonoids. Tartary buckwheat has been of special interest since it has previously been shown to contain the highest amount of phenols and possess the highest antioxidant activity when compared to common cereals (Kalinová et al., 2019).

The phenolic profile of buckwheat is complex due to phenolic compounds being ubiquitous in plants.

Dominant phenols in buckwheat are quercetin and its glycosides rutin, isoquercitrin, quercitrin and hyperoside. Other abundant compounds with health effects are chlorogenic acid (administration of 40 mg/kg of CGA decreased the histological severity of pancreatitis (Ohkawara et al., 2017)), catechin, epicatechin, epicatechin gallate (the supplementation of EG reduced the oxidative stress and apoptosis in the brain of flies (Siddique et al., 2014)), orientin, isoorientin, isovitexin, vitexin, procyanidin B2 and procatechuinic acid.

Degradation and modification of these compounds are common during food processing and later at the level of absorption. This makes it difficult to predict bioavailability and bioactivity of the compounds in humans. Nevertheless, rutin has in multiple studies been demonstrated as the most potent antioxidant in buckwheat due to its exceptionally high concentration.

A significant amount of *in vitro* and animal studies has observed protective and inhibitory effects on multiple cellular functions by phenolic compounds related to their antioxidant activity. Antioxidants prevent reactions induced by oxidative stress. Oxidative stress, e. g an excessive production of free radicals and reactive oxygen or nitrogen species (ROS, NOS) from oxidases (for example NADPH, lipoxygenases, cytochrome p450, uncoupling of mitochondrial respiratory chain and endothelial nitric oxide synthase), is the result of toxicity, stress or disruption of homeostasis by either xenobiotics, inflammation or radiation. Reactive radicals readily attack critical biological molecules (lipids, DNA and essential cellular proteins) which alters the "normal" cell and tissue physiology and activates or accelerates pathologies. As quenchers of radicals and radicals chain reactions, phenols can protect cells and tissues from oxidative stress. Among others, a study on protocatechuic (one of the main polyphenol metabolites) by Guan et al (2011) observed it highly potent on reducing oxidative stress-induced neurotoxicity (by for example inhibiting oxygen radical induced caspases) in PC12 cells. The result proposes protocatechuic as a possible drug to promote brain recovery in neurodegenerative diseases (Guan et al., 2011). Flavonoids, the main source of phenols in buckwheat, have been suggested to have therapeutic potential against diabetes, metabolic syndrome and cancer.

There are limited number of epidemiology studies, as well as randomized control trials and cohort studies which makes it uncertain if the observed *in vitro* effects can be translated into *in vivo* situation.

A double blinded randomized crossover trial was done by Wieslander et.al (2011) in Uppsala, Sweden, which assessed the effect of rutin on serum cholesterol and lung capacity. A reduced total serum cholesterol, HDL-cholesterol and improved lung-capacity were observed in 62 healthy female day-care Centre staffs after consumption of 4 buckwheat cookies a day for 4 weeks (Wieslander et al., 2011). The subjects were divided (randomized) into two groups, one group ate cookies containing Tartary buckwheat (360 mg rutin equivalents/day) whilst the other ate cookies containing common buckwheat (17 mg rutin equivalents/day. After two weeks, the two groups switched their type of cookie. The study was done to investigate the hypothesis of rutin having a positive impact on selected biomarkers related to cardiovascular disease (CVD), lower airway inflammation, lung function and dyspnea. Selected biomarkers for CVD were levels of total cholesterol, HDL cholesterol and secretory phospholipase A_2 ($sPLA_2$ group IIA), high sensitivity C reactive protein (HsCRP), eosinophilic cationic protein, myeloperoxidase (MPO), nitric oxide (NO) in exhaled breath for lower airway inflammation. Biomarkers for lung function and dyspnea were FVC (forced vital capacity), FEV1 (forced expiratory volume in 1 second), FEV, % (FEV1/FVC), PEF (peak expiratory flow). FVC increased with 109 %, demonstrating a significant improvement in lung function.

Rutin is a secretory phospholipase A_2 ($sPLA_2$) inhibitor and the suppression of ($sPLA_2$) is seen to attenuate endotoxin induced airway contraction. $sPLA_2$ is involved in the rate-limiting step in eicosanoid biosynthesis by releasing arachiodonic acid from the sn-2 position (the middle position) of membrane phospholipids. AA can generate a wide variety of eicosanoids, including leukotrienes and prostaglandins which play important, complex roles in airway pathogenesis. Leukotrienes generally promotes asthma development. Cysteinyl-leukotrienes for example are broncho constrictors. While no reductions in $sPLA_2$ were obtained in this study, previous studies have assessed both quercetin and rutin as sPLA2 inhibitors(Lindahl and Tagesson, 1997).

Rutin significantly decreased the subjects MPO levels. MPO is released from neutrophil granulocytes as one of the first inflammation responses. Rutin can inhibit LDL-oxidation caused by increased MPO levels and may due to this have an anti-inflammatory effect (Wieslander et al., 2011).
Total serum cholesterol and HDL-cholesterol were significantly reduced but not correlated to type of cookie consumed and thus not related to rutin intake. The cholesterol lowering effect can reduce the risk for cardiovascular disease. The mechanism responsible for this reduction needs more investigation as well as the possible effect on LDL-cholesterol and serum triglycerides.

SGLT1, a sodium coupled co-transporter of glucose, is located in the apical membrane in the proximal part of the small intestine (duodenum and proximal jejunum). Binding of glucose moieties at the c-terminus enables it to transport phenolic glucosides into the epithelial cells. The high affinity characteristic of SGLT1 makes phenolic glucosides potential inhibitors of the glucose transport. Blocking the transport of glucose by SGLT1 can be beneficial in patients with postprandial hypertension caused by DMT2 and IR. The degree of inhibition by phenols varies since the binding affinity is strongly related to the degree and position of the hydroxyl, hydrogen and glyosidic groups (Manach et al., 2004).

More experimental human studies are needed to evaluate the effect buckwheat and its phenolic compounds have on human health. The mechanisms responsible for the observed effects and the pharmacokinetics and bioavailability of phenolic compounds present in buckwheat also needs to be further investigated.

1.3 Objectives

1.3.1 Aim

This study was conducted to further investigate the potential of *Fagopyrum esculentum moench* and *Fagopyrum esculentum tartar* as therapeutic agents and their effect on human health. The emphasis was put on suitability of buckwheat and its components as dietary ingredient for persons diagnosed with coeliac disease, and the nutraceutical and pharmacological properties (i.e. compounds with biological activity and therapeutic potential to treat diabetes, metabolic syndrome and related pathologies). The effect of processing on the bioactive compounds as well as on bioavailability and absorption rate were also investigated.

1.3.2 Research questions

Questions aimed to be answered were:

What is the phenolic profile of *Buckwheat*? Which compounds have been proven to be beneficial to human health?

How does processing affect the chemical composition of *common buckwheat* and *tartary buckwheat*?

What is the therapeutic potential of *common buckwheat* and *tartary buckwheat?*

2 Methods

Relevant data and information was obtained using databases, primarily PubMed, Sciencedirect, Springerlink and AGRIS. Peer reviewed studies were carefully studied and evaluated. Year of publication and other relevant aspects were considered.

2.1 Phenolic profile

For the phenolic profile the studies with a similar analytical technique, including extraction, isolation and quantification of the used plant material were selected. . This was done since the result can vary depending on analytical assay, chromatographic technique and the selected plant material. For instance, a qualitative extraction is crucial to assure that the bioactive components are not lost or degraded during the process. By careful drying, freeze-drying and grinding for a homogenous sample, the kinetics as well as the solvent system-sample surface are intrinsically improved. Solvent system depends on the chemical structure of the extracted compounds. The phenolic compounds were in all chosen studies extracted with methanol, acetone or ethyl acetate.

Terpinc et al., (2016) investigated the phenolic content in malted and raw Darja' and 'Pyra' cultivars of common buckwheat (*F. esculentum*) supplied from Slovenia. Soaking and germination increased the phenolic content. Kilning slightly decreased the phenols in both cultivars which indicate that they are sensitive to heat.

Guo et al. (2012) compared the phenolic content in the hull, bran and flour of Tartary buckwheat, provided by Xichang HangFei Bitter Buckwheat Exploitation Center in China. They identified and quantified the free and bound phenolic compounds in hulls, brans and flours of ten buckwheat cultivars (three varieties *common buckwheat* and seven varieties *tartary buckwheat,).* Tartary buckwheat had the highest content of both total and free phenols. The content was highest in the bran followed by the hull and flour.

Kiprovski et al., (2015) analyzed the phenolic profile in seeds of different *F.esculentum* cultivars from Serbia ('Novosadska'), Slovenia ('Darja', 'Prekmurska' and 'Cebelica'), Bosnia and Herzegovina ('Bosna 1' and 'Bosna 2'), Montenegro ('Godijevo' and 'Lokve'), Austria ('Bamby'), Czech Republic ('Ceska'), France ('La Harpe'), Slovakia ('Spacinska 1'). There was a significant difference in the TPC in the cultivars. Bosna 1 and 2 obtained the highest TPC but it was still significantly lower than the TPC in tartary buckwheat.

Guo et al. (2011) analyzed two *F.tartaricum* samples (Xingku No.2 and Diqing) grown at three locations for free, bound and total phenolic content as well as antioxidant properties. They obtained a noticeable difference in the TPC depending on the location of growth.

The total phenolic content (TPC) in all studies was determined by the Folin-Ciocalteau colourimetric method (expressed as gallic acid equivalents (GAE)) and the antioxidant activity were measured using the same DPPH free radical scavenging assay. Quantification and determination of the individual phenolic compounds were done using a HPLC–MS system with a coupled UV detector.

3 Literature review

3.1 Phenolic profile

3.1.1 Determination of total content of phenolic compounds

As demonstrated in Table. 1, the total phenolic content (TPC) was the highest in the bran and the hulls compared to the flour (FB, fine bran>CB, coarse bran>hull>LF, light flour) in both F. tartaricum and F. esculentum (Guo et al., 2012).

Tartary buckwheat coarse bran (7.71 g gallic acid eq./100 g DW) and fine bran (9.49 g gallic acid eq./100 g DW) had a higher TPC than other studied cereals, pseudo cereals and fruits, including rice (0.25–0.27 g gallic acid eq./100 g DW), wheat (0.48–0.53 g gallic acid eq./100 g DW) and oat (0.05 g gallic acid eq./100 g DW) (Guo et al., 2011).

Total flavonoid content in fine bran (14.07 g rutin eq./100 g DW), coarse bran (9.65 g rutin eq./100 g DW) and hull (4.22 g rutin eq./100 g DW) were also 6-, 4- and 1-fold higher than those of light flour (1.91 g rutin eq./100 g DW), respectively. This is consistent with results from previous studies where the outer layers of Tartary buckwheat have had a higher TPC levels and higher flavonoid levelsthan inner layers.

Terpinc et al. (2016) found that the TPC in *F.esculentum* seeds ranged from 0,05 (‘Novosadska’) to 0.3 g (Bosna 1 and 2) GA/g-1 DW when comparing different varieties (Table.1). ‘Novosadska’ were significantly richer in phenolic acids, proanthocyanidins, flavonols and some of the flavones. In contrast, Bosna 1 and 2 had the highest total flavonoid contents (0.37 and 0.38 m GA/g-1 DW) as well as the highest rutin contents.

In agreement with these studies, Guo et al. (2012) found a higher average TPC in tartary buckwheat hulls (2.38 g GA/g-1 DW) compared to common buckwheat hulls (1.8 g GA/g-1 DW). This was also the case in common and tartary buckwheat bran and flour (Table. 1)

Interestingly, Guo et al. (2011) which studied two Tartary buckwheat cultivars at three locations, observed that Xingku No.2 from Sichuan had the highest phenolic content, followed by Diqing from Gansu. The phenolic content of the Qiqing variety from Gansu was almost two times higher than that of the Xingku No.2 variety from Gansu. As previous studies have indicated, these results may be due to an interaction between the cultivar and the environmental conditions. Phenols also possesses anti-microbial properties and as secondary metabolites they protect plants from pathogens. Vegetables produced by organic or sustainable agriculture have generally a much higher phenolic content since they have been subjected to more stress. (Manach et al., 2004)

Additionally, Terpinc et al (2016) which examined the TPC in two common buckwheat varieties (Pyra’ and ‘Darja’) at different stages in the malting process, demonstrated an 122% and 106% increase in TPC, respectively, after a 64 h germination (Terpinc et al., 2016). This increase was explained by a de novo synthesis of phenolic compounds to promote structural growth and for environmental protection. Since other studies have reported different results, the attitude of increase was suggested to be both specie specific and related to the applied soaking, germination and extraction method.

Table 1. *Total phenolic content (TPC) and flavonoids obtained from three different studies*

Reference	TPC (g of gallic acid eq./100 g DW)	Flavonoid content (g of rutin eq./100 g DW)	Plant material
Guo et al., 2012	9.49	14.07	Tartary buckwheat, fine bran
Guo et al., 2012	7.71	9.65	Tartary buckwheat, coarse bran
Guo et al., 2012	3.06	4.22	Tartary buckwheat, hull
Guo et al., 2012	1.02	1.91	Tartary buckwheat, light flour
Guo et al., 2011	1.80	NA	Common buckwheat, hull
Guo et al., 2011	2.38	NA	Tartary buckwheat, hull
Guo et al., 2011	1.26	NA	Common buckwheat bran
Guo et al., 2011	2.49	NA	Tartary buckwheat, bran
Guo et al., 2011	0.97	NA	Common buckwheat, flour
Guo et al., 2011	1.38	NA	Tartary buckwheat, flour
Kiprovski et al., 2015	0,05	0.05	Common buckwheat 'Novo-sadska'
Kiprovski et al., 2015	0.3	0.38	Common buckwheat Bosna 2

3.1.2 Free and bound phenolic compounds

Phenolic compounds in plants are present in both free and bound form. As bound they are either esterified or etherified and form cross-bridges with carbohydrates or proteins of the cell wall. Phenols and particularly flavonoids often occur as glycosides in plants. The sugar moiety increases the polarity and enables the phenols to be stored in the vacuole. Flavonoids are often in β-glycosylated form, either as O- or C-glycosides, with predominantly glucose or rhamnose as main sugars, but galactose, arabinose, xylose and glucuronic are also common (Martin, 2009). Position of the glyosidic linkage can affect the absorption since it makes flavonoids less lipophilic and decreases the ability for them to passively diffuse across the epithelial membrane (Manach et al., 2004). Free phenols have highest antioxidant and radical scavenging activity (more in section 3.1.4). The antioxidant activity is partly related to the stability of the fenoxyradical and is affected by number and position of the hydroxyl groups on the aromatic ring (the ArOH group).

Tartary buckwheat had a significantly higher content of free phenols compared to common buckwheat; however, the exact content varied widely between species due to effects of environment and location factors. Hence, Guo et al. (2011) demonstrated that free phenolic content ranged from 76% (Diqing variety from Sichuan and Ningxia) to 95% (Xingku No.2 from Ningxia) of the total content.

Moreover, it was suggested that 79.7, 92.9, 94.1%, of the phenolic compounds in the hull, bran and flour respectively were in free form (Guo et al., 2012, 2011). Guo et al (2011) also concluded that the free part accounted for 94% to 99% of the occurring phenols in Tartary buckwheat. The phenolic acid and flavonoid concentrations were also significantly higher in the free phenolic extracts than in the bound, indicating that more bioactive compounds were obtained in the free "fraction". Interestingly, flavonoids in wheat, rice, corn and oat have been shown to be majorly in bound form (Guo et al. 2011). The free phenolic content of Tartary buckwheat was 23-45, 25-50, 2-13, and 3-6-fold higher than that of corn, wheat, cranberry and apple, respectively.

Comparing different milling parts of common buckwheat and tartary buckwheat, tartary had a higher content of free phenols in all parts except the hulls (Table. 2). Free phenols were absolute highest in buckwheat brans (about 92.89%) and flours (about 94.07%). This suggests that the free phenols are predominantly located in the outer layer of the buckwheat seed but can be enhanced by milling and further refinement.

Table 2. *Free and bound phenolic content obtained from two different studies*

Reference	Free phenolic content (g of gallic acid eq./100 g DW)	Bound phenolic content (g of gallic acid eq./100 g DW)	Plant material
Guo et al., 2012	9.31	0.18	Tartary buckwheat, fine bran
Guo et al., 2012	7.49	0.23	Tartary buckwheat, coarse bran
Guo et al., 2012	2.57	0.49	Tartary buckwheat, hull
Guo et al., 2012	0.98	0.04	Tartary buckwheat, light flour
Guo et al., 2011	2.02	0.32	Common buckwheat, hull
Guo et al., 2011	1.38	0.39	Tartary buckwheat, hull
Guo et al., 2011	1.16	0.10	Common buckwheat bran
Guo et al., 2011	2.31	0.17	Tartary buckwheat, bran
Guo et al., 2011	0.90	0.06	Common buckwheat, flour
Guo et al., 2011	1.30	0.07	Tartary buckwheat, flour

3.1.3 Quantification of major phenols

The two main classes of phenols in buckwheat are flavonoids and phenolic acids. Common buckwheat is also rich in tannins, especially condensed tannins (proanthocyanidins, e.g procyanidin B2). Phenolic acids can be divided into groups of hydroxybenzoic acid derivatives and hydroxycinnamic acid derivatives. Flavonoids are the most important group of phenols and include a variety of different classes of compounds- flavonols, flavonoids and flavones. Rutin, isoquercitrin, quercitrin, hyperoside. catechin, epicatechin, epicatechin gallate, orientin, isoorientin, isovitexin, vitexin have been quantified in both common and tartary buckwheat in relevant concentrations in multiple studies.

Guo et al. (2011) demonstrated that *p*-hydroxybenzoic, ferulic and protocatechuic acids were the prominent phenolic acids in tartary buckwheat, and accounted for 83–88% of the total phenolic acid content. Another abundant phenolic acids were gallic, vannilic and syringic acids, *p*-coumaric, caffeic, chlorogenic and ferulic acids (Guo et al., 2011).

P-hydroxybenzoic acid was the most abundant phenolic acid in the fine bran (360.24 ± 9.58 mg/100 g DW), coarse bran (306.93 ± 3.81 mg/100 g DW) and light flour (38.75 ± mg/100 g DW) in tartary buckwheat.

In contrast, ferulic, vanillic, *p*-coumaric and syringic acids were estimated as the main individual phenolic acids in common buckwheat (Kiprovski et al., 2015)

Among the flavonoids, the most abundant compounds were catechin (monomeric flavan-3-ols), rutin, orientin, vitexin and quercetin. Kiprovski et al (2015) found that rutin was the major phenol in *F.esculentum* seeds (90.5 % of the total content) with the highest antioxidant activity due to its high concentration.

However, rutin content in buckwheat varieties may range majorly in seeds depending on the location and growing season. Two varieties of common buckwheat, Bosna 1 and 2, which had the greatest quercetin-3-rutinoside ie. rutin, content had 114.6 and 151.4 mg 100 g^{-1} DW, respectively (Kiprovski et al., 2015)

In comparison, the rutin content in Tartary buckwheat seeds was estimated to range between 1447.87 and 518.54 mg 100 g^{-1} DW. Rutin has been quantified in both the inner and outer layer of buckwheat. The highest rutin levels were found in fine bran (7431.40 ± 2.14 mg/100 g DW) and the lowest in the light flour (287.86 ± 8.16 mg/100 g DW),. 74–99% of rutin also existed in the free form. Rutin is rapidly metabolized into quercetin in contact with hydrolyzing enzymes. Quercetin also forms other glucosides, which makes it difficult to measure its concentration accurately. Guo et al. (2011) found a variation in quercetin from 425.65 to 857.62 mg 100 g^{-1} DW in tartary buckwheat.

3.1.4 Antioxidant activity

In addition to having a significantly higher phenolic content than oats or barley, buckwheat has phenols with a 2-7 higher antioxidant activity in the hulls and bran. Antioxidant activity can be defined as IC_{50} (μg/mL) which is the concentration needed for lowering the DPPH free radical concentration by 50%. Hence, a lower

IC_{50} corresponds to a higher antioxidant activity (less concentration to obtain reduction). The antioxidant activity is often used as a qualitative indicator of the effect and modification of phenols when comparing raw and processed material. Guo et al. (2011) considered free phenols as the major contributors of the total radical scavenging capacity since the levels of free phenols were strongly related to the antioxidant activity. The data in that study demonstrated that the antioxidant activity of free phenols in tartary buckwheat was comparable to, or even higher than certain fruits and grains, including oats, corn, barley, apples and blueberries. Interestingly, the concentration of free phenols in the study was lower than that of fruits and grains (Guo et al., 2011). Phenols in buckwheat probably have a favorable chemical structure that makes them more potent antioxidants.

3.2 Processing

3.3.1 TPC and individual phenols

Costantini et al (2014) performed a study on quantification and comparison of the TPC and flavonoid content in tartary and common buckwheat flour and bread. Tartary and common buckwheat flour had a TPC and flavonoid content of 2.93 and 7.28 g of gallic acid equivalent (GAE)/100 g DW as well as 0.1 and 2.2 g of rutin eq./100 g DW, respectively. This was in agreement with the values in Table.1. Interestingly, the bread samples in the same study only demonstrated a slight decrease in both TPC and flavonoid content, and the final concentrations were still relatively high. Tartary buckwheat bread had a TPC of 5.3 g of GAE100 g DW and a flavonoid content of 1.68 g of rutin eq/100 g DW whereas common buckwheat had 1.65 g of gallic acid eq/100 g DW and 0.06 g of rutin eq/100 g DW (Costantini et al., 2014).

Wieslander et al. (2011) measured the concentrations of rutin and quercetin in cookies made of tartary and common buckwheat flour. Tartary buckwheat cookies had a rutin and quercetin content of 2,530 and 1,620 mg/kg DW, respectively compared to the common buckwheat cookies that had 270 mg/kg DW and 0 (below the limit of quantification), respectively (Wieslander et al., 2011).

The result was supported by Terpinc et al., (2016). In that study, the authors investigated the effect of malting (soaking, germination and kilning) on the TPC and individual phenols in Tartary buckwheat (Terpinc et al., 2016). The rutin levels were observed to be relatively constant throughout the whole malting process (it did however show a 4-fold increase for the ‘Pyra’ variety and a 3-fold increase for ‘Darja’), while for the orientin, vitexin, and isovitexin contents, much greater increases were observed. Soaking (8h at 20 degrees) of the buckwheat had no statistically significant effect on the TPC. Previous studies have reported a negative impact with significant reductions possibly related to leakage into the soaking water or chemical modifications such as formation of insoluble complexes with proteins or polymerization with other LMW phenolic compounds. A similar study (Li et al., 2013) which examined changes in phenols during tea preparation procedures, observed a 50 % decrease in rutin content after soaking for 12-14 at 40 degrees. It is likely that rutin was hydrolyzed to quercetin during processing; quercetin concentrations increased from 0.53 to 15.16 mg/g DW. Hydrolysis of rutin is mediated by the enzyme rutin 3-glucosidase, a flavonol 3-glucosidase which catalyzes the hydrolysis of the 3-glucoside of flavonols such as rutin or isoquercitrin. The concentrations of these compounds were consequently affected by the activity of this enzyme and its location in the seed. Soaking at a higher temperature activates the enzyme in rutin-rich compartments of the grain, resulting in a decrease in rutin and an increase in quercetin (Li et al., 2013).

In the same study, steaming increased the rutin to its initial concentrations, as well as reduced the quercetin and isoquercitin. It was hypothesized to be due to the activation of enzymes converting quercetin and isoquercitin into rutin by 3-glycosylation and rhamnosylation. Roasting (120–150 °C) only slightly decreased the rutin, isoquercitin and quercetin content, indicating that these compounds are relatively heat sensitive (Qin et al., 2013).

Kilning at 80 °C resulted in a lower TPC content compared to heat treatment at 60 °C (Terpinc et al., 2016). Vitexin, and isovitexin slightly decreased during the kilning process, indicating higher heat resistant of these compounds. Rutin decreased significantly during kilning at both 60 degrees and 80 degrees compared to after 88 h germination. However, the total concentration was still higher than in the raw seed. This result is not in agreement with a report that rutin in buckwheat groats is more stable to heat than vitexin, isovitexin, and orientin during roasting at 160 °C for 30 min (Terpinc et al., 2016).

Aglycones are however known to be highly resistant to heat and differences in the obtained results may be due to other factors. The sum of the losses is correlated to the effect the processing method has on vacuoles and apoplasts, i.e. the cellular structures where the phenols are stored. Steaming and frying create less losses than boiling. Boiling may cause leaching of polyphenols from destruction of the cellular matrix (Martin, 2009).

Furthermore, the TPC and the antioxidant activity was the highest in soaked buckwheat seeds. Steaming, drying, roasting and de-hulling (may for example cause oxidative browning) decreased the TPC and antioxidant activity.

3.2.2 Other phytochemicals and nutrients

The chemical composition differs in different parts of the buckwheat kernel. The seed consist of a hull (pericarp), bran (auerlone layer, inner and outer pericarp, testa and hyaline layer), endosperm and germ (including embryo).

The dehulled achene (dried fruit) is called groats. Hulls are high in fiber but are otherwise poor in nutrients. Accordingly, dehulled buckwheat is lower in fiber compared to hulled. Phenolic compounds are mostly present in the bran (i.e the aleuronic and embryonic tissues) which is also richest in macronutrients and dietary fiber.

Bonafaccia et al. (2003) examined the nutritional content in different buckwheat flours and found that the protein content was above 21%, and lipid content approximately 7% in the bran of common buckwheat (Bonafaccia et al., 2003). For tartary buckwheat the protein content of the bran was approximately 25%. In the flour, the values were 10% for protein and 2% for the lipid content. In the same study the samples contained high levels of vitamins B1, B2 and B6. Thiamin (vitamin B1) in tartary buckwheat flour (200 g) satisfy the daily requirement for a diet of 2000 kcal. The highest vitamin content was observed in the tartary buckwheat bran. Dietary analysis also showed excellent content of dietary fiber in both common and tartary flour with most of that being insoluble. This suggests that (especially) the tartary buckwheat bran has a great potential to be used as an ingredient when developing new functional food products.

Bread enriched with husked common buckwheat flour had twice as high protein content compared to regular wheat bread (Lin et al., 2009). Vojtíšková et al. (2014) also noticed that all amino acids could still be quantified in processed buckwheat pasta, therefore making it a complete protein source and a qualitative substitute for wheat, rice or corn pasta (Vojtíšková et al., 2014). An additional study indicated that steaming decreased the protein and crude fat in buckwheat while another saw that cooking increased the protein content in buckwheat-enriched spaghetti as well as increased its bioavailability and digestibility (Nosworthy et al., 2017). The exact effect of processing on the protein content still needs to be evaluated.

Regarding mineral composition, buckwheat was shown to be relatively stable in content during both boiling and steaming. 87% of zinc, 98% of calcium and 100% of iron was retained in steamed buckwheat. All minerals investigated decreased in concentrations by 3 -13% in the boiled samples. The reductions were probably due to minerals leaching to the water during the boiling process. The mineral content increased with longer duration of parboiling. Parboiled buckwheat can thus be recommended to increase mineral content in buckwheat. Both common and tartary buckwheat were similar in mineral content which makes them a good source of iron, zinc, potassium, magnesium, phosphorus, calcium, copper and manganese.

Despite some processing losses, the nutritional properties of buckwheat have still been observed to be superior to wheat and other cereals in processed food products such as bread, pasta, cookies etc. Stokić et al. (2015) reported higher content of dietary fiber and flavonoids in buckwheat-enriched bread which was suggested to decrease the total cholesterol and LDL-cholesterol in the studied participants. Additionally, bread made with 15 % husked buckwheat contained higher amounts of insoluble b-glucan (compared to wheat and unhusked bread) which has an immunostimulating effect (Stokić et al., 2015)

Previous research suggested that high a-glucosidase (a carbohydrate digestive enzyme) and a-amylase inhibitory activity still is obtained after heat treatment (Su-Que et al., 2013). Inhibition of these enzymes was shown to delay carbohydrate digestion and glucose absorption, and consequently, could reduce the risk of developing type II diabetes. Enzyme activity was inhibited by phenols; hence, a high phenolic content increases the anti-diabetic effect of buckwheat. Particularly proanthocyanidins were shown to be responsible of the inhibitory effects. A lowering of the glycemic and insulin indexes was also noted as effect of buckwheat-enriched bread consumption, possibly caused by the formation of indigestible starch after heating the buckwheat flour and by the amelioration of insulin resistance through D-chiro-inositol (Su-Que et al., 2013).

3.3 Bioavailability, absorption and bioactivity

As discussed in the section 3.2, buckwheat has both unique nutritional composition in terms of micro and macronutrients as well as is abundant in phytochemicals with health effects.

Its high fibre content, optimal amino acid composition and mineral and vitamin content makes it applicable as potentially beneficial dietary components to prevent malnutrition, obesity, diabetes, hyperlipidaemia and hypertension.

Minor phytochemicals in buckwheat, particularly phenolic compounds have been demonstrated to both have a quenching effect on oxidative stress as well as specific interactions with cellular components which makes them applicable as potential therapeutics.

Multiple *in vivo* studies have confirmed the effects of these compounds but the potential is however related to their bioavailability and pharmacokinetics which determines the level of absorption and hence, the concentration and activity in blood plasma and tissues.

3.3.1 Bioavailability of phenolic compounds

Despite the acknowledged health effects, flavonoids are known to have low bioavailability because of chemical instability in the gastrointestinal tract, poor absorption in the intestine and degradation by endogenous enzymes. Furthermore, flavonoids are fermented into various metabolites in the large intestine by the gut microbiota (Rocchetti et al., 2018). Free phenolic compounds are probably mainly absorbed in the upper gastrointestinal tract whereas bound fraction needs to be further metabolized in the colon where it forms phenolic acids such as hydroxybenzoic and hydroxycinnamic acids (Qin et al., 2017). The polarity and the high molecular weight of the bound compounds inhibit their absorption. Aglycones and phenolic acids are smaller and more lipophilic which enables them to diffuse passively through the epithelial membrane. However, phenols are generally present as glycosides or esters and needs glycosidases or esterases to carry out the hydrolysis. Some intestinal enzymes have been suggested to preform phenol deglycosylation but the main degradation is carried out by colonic microbes(Martin, 2009).

Microbes preform diverse deglycosylation reactions which releases aglycons that are degraded into simpler phenolic acids. Rutin and quercetin are often metabolized into 3,4-dihydroxyphenylacetic acid (3,4- DHPAA), 3-hydroxyphenylacetic acid (3-HPAA), and 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid, HVA). Quercetin is known to have the highest reducing and antioxidant activity, followed by rutin. 3,4- DHPAA has an equal activity as rutin while -HPAA and HVA had a significantly lower activity. Evidence show that the antioxidant properties of these compounds are closely related to the free hydroxyl group in the carbon ring (Giménez-Bastida et al., 2017).

Similarly, the bioactivity is also determined by the affinity to serum albumin in plasma, which differs between flavonoids and is related to position and degree of hydroxylation, glycosylation and methoxylation. A higher affinity decreases the flavonoid distribution to the cells. Hence, the structure-affinity relationship greatly affects the cellular response to dietary flavonoids (Pal and Saha, 2014)

Since studies have demonstrated a noteworthy difference in rate of absorption and distribution of quercetin in blood plasma when provided as dietary rutin or quercetin, it was suggested that the absorption of quercetin in humans primarily depends on the sugar moiety.

Quercetin can be absorbed both in the small and large intestine but as glycoside it must be de-glycosylated to be absorbed (Manach et al., 1997).

It’s not yet completely understood how quercetin glycosides are transported through the small intestinal mucosa, but several carrier mediated processes have been proposed. The site of absorption seems to be different for different quercetin-glucosides (differences have been demonstrated between quercetin-4′-O-glucoside and rutin (quercetin-3-O-rutinoside). An involvement of the glucose carrier SGLT1 in the transportation of quercetin glucosides have been suggested. After transportation, the glycosides are possibly hydrolyzed inside the cells by a cytosolic β-glucosidase. The involvement of the lactase phloridzine hydrolase (LPH), a glucosidase at the border of the apical membrane of the small intestine, is another suggested pathway for absorption. LPH catalyzes extracellular hydrolysis of some glucosides, which allows the diffusion of the aglycone across the membrane (Manach et al., 2004).

In the case of buckwheat tea compared with the isolated compound (quercetin), the plant matrix did neither effect the rate or extent of absorption in a major extent (Qin et al., 2013).

During absorption, phenols are conjugated in both the small intestine and in the liver by CYP450 enzymes. This is phase I in the metabolic detoxification process.

Most of the absorbed phenols are then further processed to conjugated derivatives by methylation, sulfation, and glucuronidation during Phase II metabolism. They are then biliary or urinary eliminated.

It is necessary to take into consideration both phenolic acids arising from microbial breakdown as well as conjugated metabolites formed in the liver, and not only consider free quercetin form, when investigating the biological effect and activity of polyphenolic compounds.

Even though free quercetin is one of the most potent antioxidants it is probably present in minor concentrations, mainly due to conjugation and interaction with plasma proteins (Manach et al., 1997).

4 Discussion

Comparison of several varieties of Fagopyrum esculentum Moench (common buckwheat) and Fagopyrum tataricum (tartary buckwheat) demonstrated that the chemical composition of buckwheat is highly dependent on specie, variety (i.e genotype) as well as location and environment parameters (phenotype). Tartary buckwheat is the specie with the most documented health benefits, mostly due to its high content of phenolic compounds. The adaption to harsh environmental conditions (since traditionally being cultivated in extreme climates) has facilitated the development of protection mechanisms, including production of secondary metabolites for protection against ultraviolet radiation, pathogens, and herbivores. Low temperature can increase the production of phenolic compounds by enhancing synthesis of phenylalanine ammonia lyase (PAL) in plants. Moreover, high altitude and long sunlight hours with higher UV radiation can positively affect the activity of phenolics synthase. This knowledge can be used to increase the levels of phenolic compounds in buckwheat cultivars. The results from this study indicated that antioxidants including flavonoids and phenolic acids are concentrated in the bran of common and tartary buckwheat. Phenols are absolute highest in tartary brans. The bran had the greatest antioxidant properties, total phenolic and flavonoid contents and phenolic acid concentration followed by the hull and flour. Most nutritional and functional components are concentrated in the embryo and aleurone of grain, making the bran richest in all nutrients.

Buckwheat flour is the most common fraction of buckwheat and consists mainly of the starchy endosperm. The hull, which acts as the seed's first-line defense system, contain higher phenolic concentrations.

The result in this study promotes the use of particularly milled Tartary buckwheat bran as a dietary ingredient to develop functional foods rich in antioxidants (Guo et. al 2011). It has been reported that buckwheat-enhanced wheat bread has higher antioxidant properties than wheat bread. This study also suggests that hulled buckwheat flour could be used to further increase the dietary fibre in buckwheat products. Both buckwheat bran and flour can be used to enrich food products with vitamins and minerals, especially gluten-free products which usually are low in micronutrients. Soaking and germinating the buckwheat may also increase the amounts of minerals and vitamins present. Germination has also been observed to increase the level of phenolic compounds in buckwheat. Since buckwheat have high levels of phytase, cooking or quickly soaking it may be enough to degrade the phytic acid.

Buckwheat flour is reported especially valuable for diabetics and the obese due to its high protein and insoluble fibre contents. Hence, the use of buckwheat flour in bread may contribute to the lowering of the glycaemic and insulin indexes as well as the dietary fibre content and the protein (with an equal physiological effect) may increase post-meal satiety, decreasing proceeding hunger and ameliorate constipation.

Processing does not tremendously affect the chemical composition of buckwheat but the exact impact of different processing methods and techniques needs to be further investigated. Methods used to increase the solubility of phenolic compounds may increase the absorption of these and possibly increase the effect they have on pathological abnormalities.

Research suggests that phenolic compounds in their free form may be absorbed in the upper gastrointestinal tract, while compounds in bound form are fermented in the colon. Thus, phenols in buckwheat are probably mainly absorbed in the small intestine tract compared to wheat, corn, rice and oat, and are easier available for organisms. This may exert a more preventative effect in the upper GI tract compared to the other grains (Qin et al., 2017).

Putative targets of buckwheat are remarkably abundant. Orona-Tamayo et al used a network analysis approach to investigate the relationship between the gene and protein targets of tartary buckwheat and its pathways. The analysis suggested 20 compounds and 28 key-targets with effects against Diabetes mellitus type 2, hypertension, hyperlipoidemia (Manach et al., 1997). Further research in this area can discover the molecular targets of rutin based drugs.

Flavonoids are important regulators of certain nuclear receptors related to the progression of metabolic disorder (Avior et al., 2013). These nuclear receptors include peroxisome proliferator-activated receptor (PPAR), which are involved in lipid metabolism; estrogen receptor (ER), which regulate estrogenic activity; farnesoid X receptor (FXR), which is important in cholesterol metabolism; and pregnant X receptor (PXR), which is involved in steroid and xenobiotic metabolism. If dietary flavonoids can selectively affect these receptors, they can possibly be used as pharmaceutical compounds. However, more knowledge is needed on the mechanism of interactions between flavonoids and nuclear receptors before flavonoids can be applied in pharmaceutical industry.

Rutin can attenuate pro-inflammatory gene expression by supressing the mRNA levels of IL-1β, IL-6, IL-10, IL-13 and TNF-α(Nosratabadi et al., 2009). The suppression of cytokine patterns by rutin can for instance suppress colitis and colorectal carcinogenesis. This, in addition to the suppression of MPO and secretory phospholipase A2, shows rutins potential in the prevention and treatment of many inflammatory diseases. Developing pharmaceuticals with rutin should be looked more into, it may enhance the absorption and the therapeutic effect. Since flavonoids have not demonstrated any toxicity when administrated in large quantity, they can be used without concern of major side-effects.

I would advise to look further into the interaction between rutin and other quercetin-glycosides and the SGLT1 transporter. It may provide more knowledge about the absorption of quercetin glycosides as well as elucidate the possibility of using them to inhibit SGLT1. Inhibition of SGLT1 has been promoted as a target for reducing postprandial hyperglycemia.

5. Conclusion

Buckwheat as dietary component is ideal due to it being a sustainable, environmental friendly source of both macro and micronutrients. As a resilient crop, it can be cultivated worldwide and be used to combat malnutrition in low-income countries. As a dietary source of bioactive compounds with therapeutic effect, it can be used to prevent or treat conditions in people with a low socioeconomic status. It is for example a great option for treating hypercholesterolemia in patients which cannot use statins. Flavonoids as pharmaceuticals should be designed to target receptors such as PPAR, SGLT1 or proteins involved in the inflammatory response, for example phospholipase A. With a suitable method for drug delivery (for example a liposome or a biological nanoparticle layer) they can be very useful in clinical medicine.

Tartary buckwheat bran and flour should be used together with other pseudo cereals to increase the nutritional quality in gluten-free products.

Buckwheat is recommended to be included in diets of both healthy and unhealthy individuals. As part of a plant-based diet it provides a good amount of natural phenols and antioxidants that may reduce the risk for developing CVD, DMT2, cancer and increase longevity. The role of nutrition and lifestyle in disease occurrence is still a rather unsolved mystery. Difficulties includes conducting and designing scientific studies which are ethical, inexpensive and have well-designed research methologies. With more interdisciplinary human observational and experimental research on nutrition in disease aetiology, we can come closer to knowing how nutrition can be used as and during treatment and for prognosis.

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