Sveriges lantbruksuniversitet
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Fakulteten för naturresurser och lantbruksvetenskap

Tocopherols in wheat and rye

– A review of research within the subject

Tocoferoler i vete och råg

– En översikt över forskningen inom ämnet

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Tokoferoler i vete och råg

Tocopherols in wheat and rye

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Abstract

This thesis focuses on tocols or vitamin E found in wheat and rye. Content and structure of tocols, the effects of some types of processing and their health benefits are discussed. The two most common tocols in wheat and rye are tocopherols and tocotrienols. α-Tocopherol has the highest vitamin E activity, while tocotrienols has better antioxidant properties. There are four kinds of tocopherols and tocotrienols, α, β, γ and δ, which have different vitamin activity and antioxidant function. The most abundant tocol in common wheat, einkorn, durum wheat and spelt wheat (*Triticum aestivum*, *Triticum monococcum*, *Triticum turgidum* and *Triticum spelta*) is β-tocotrienol. Almost only α- and β-vitamers is the forms present in wheat. The most abundant tocol in rye (*Secale cereale*) is α-tocotrienol, but it varies among varieties. Only α- and β-vitamers are present also in rye. In general tocotrienols are more abundant than tocopherols in both wheat and rye. Tocopherols are most abundant in the germ and tocotrienols are most abundant in the bran. They are labile in the presence of oxygen and heat, mainly because of their ability to act as antioxidants. Baking has been shown to cause moderate losses of tocols. Malting has no significant effect on tocol content. Milling is a process which separates the bran, germ, hull and endosperm into different fractions. The tocopherols are found mostly in the germ fraction and tocotrienols in the bran fraction. Steaming treatment has been shown to cause a migration of tocotrienols from the bran to the endosperm. Tocols have been shown to be beneficial for the health, for example they can reduce the level of blood cholesterol which is good for the prevention of heart disease. They also have effect in the prevention of neurodegeneration and of some type of cancers.

Keywords: Tocopherols, tocotrienols, wheat, rye, health, processing

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Abbreviations

- HPLC = High performance liquid chromatography
- GC = Gas chromatography
- MS = Mass spectrometry
- α-TE = α-tocopherol equivalents

1 Introduction

Cereals are major components in the human diet and a large topic for research all over the world. They contain several bioactive components (eg. tocopherols). Tocopherols are compounds that exhibit vitamin E activity and are therefore an essential nutrient for humans. If tocopherols are absent in the diet, symptoms like hemolytic anemia or reduced eye motility occurs. Sources of tocopherols are mainly vegetable oils, but cereals have also been shown to contain considerable amounts of tocopherols. Since cereals make up a large part of most people's diet, they are a significant source of people's tocopherol intake. Recent research has shown that several health benefits, such as the reduction of heart disease and cancer, are associated with considerable intake of tocopherols. This is mostly because tocopherols are antioxidants and have a role to protect unsaturated lipids against free radicals and oxidation, which are natural processes that occur in the body.

The purpose of this thesis is to assemble literature about tocopherols in wheat and rye and to explain what influence processing (baking, milling, malting, pasta processing and steaming treatment) and storage have on tocopherol content. Health benefits of tocopherols are also discussed in the last paragraph as well as deficiency and toxicity of tocopherols. Furthermore there is one part about the antioxidant function of tocols, to give a more detailed view on this. Antioxidants are a hot topic in the media.

2 Method

Literature was found in the databases; Primus (the library of Swedish University of Agricultural Sciences) and Web of Knowledge. The following words were used in different combinations; Tocopherols, wheat, *Triticum aestivum*, rye, *Secale cereale*, process effects, baking, stability, health and health benefit. From the original review articles that were found, articles on tocols in wheat and rye, health benefits of tocols and also about processes like, baking, extrusion, steaming treatment and pasta processing on tocopherols, were selected. Also two books were borrowed from the library of the Swedish University of Agricultural Sciences; Fennemas Food Chemistry and Food: The Chemistry of its components.

3 Tocopherols and their derivatives

3.1 Structure and function of tocopherols and their derivatives

Tocopherols are nonpolar, lipid-soluble compounds that are derivatives from a compound called tocol. They have vitamin E activity and are also sometimes called vitamin E (Table 1). They consist of a chromanol ring and a hydro-carbon chain of 16 carbon atoms There are four types of tocopherols; α-, β-, γ- and δ-tocopherol that differ from each other with different side groups (R1 and R2) on the chromanol ring, which can be a methyl group or a hydrogen (Figure 1). α-Tocopherol is methylated at both R1 and R2, β- and γ-tocopherol have one hydrogen and one methyl group each and differ from each other in the position of the methyl group. If the methyl group is on position R1 it is β-Tocopherol and if the methyl group is on position R2 it is γ-Tocopherol. δ-Tocopherol has two hydrogen atoms and have therefore the lowest molecular weight (Gregory, 2007). Their function is to protect biological membranes from oxidation and also to preserve immunologic function (Traber and Stevens, 2011; Meydani *et al*, 1997). The stereochemical configuration of the assymetric carbons at position 2' 4' and 8'have influence on the vitamin E activity (Gregory, 2007). α-Tocopherol has the highest vitamin E activity and is the only form that can be maintained in the body. The other forms (β-, γ- and δ-tocopherol) are responsible for the vitamin E activity in plant tissues (Leenhardt *et al*, 2006). The vitamin E activity is equal in common wheat, einkorn and durum wheat (Leenhardt *et al*, 2006).

Each stereochemical carbon can have R or S configuration which gives rise to eight different stereoisomers (Traber and Stevens, 2011). Synthetic α-tocopherol is called all-rac-α-tocopherol and contains each stereoisomer in equal amounts (Traber and Stevens, 2011). 2R- α-tocopherol is the prominent stereoisomer found in nature (Lampi *et al*, 2008), and this is the only stereoisomer that meets the vitamin E requirements (Institute of medicine, 2000). The stereoisomers which have R configuration on their carbon 2 have high biological activity (Traber and Stevens, 2011).

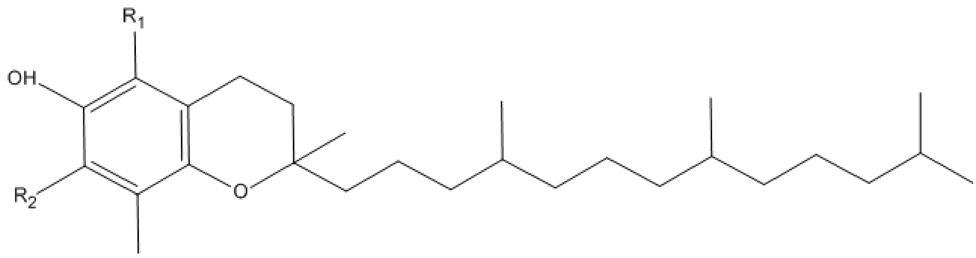

Figure 1. A tocopherol molecule, the side groups (R1 and R2) can be either hydrogen or a methyl group.

The name tocopherol is derived from the greek; τοκκοσ ρερειν which means “off-spring” and “to bear”, respectively (Coultate, 2009). There is also another compound with vitamin E activity which is called tocotrienol who is identical to tocopherols with the exception of double bonds at positions 3’, 7’ and 11’. There are also four types of tocotrienols; α-and β-, γ- and δ-tocotrienol. Tocotrienols have lower vitamin E activity but higher antioxidant activity (Table 1) (Cahoon *et al*, 2003; Gregory, 2007). There is an ester of α-Tocopherol which is called α-tocopheryl acetate, the hydroxyl group on the the chromanol ring is esterified. α-Tocopheryl acetate has vitamin E activity and is also found in plants. Vitamin activity of α-tocopheryl acetate varies according to the particular form present (Gregory, 2007).

Tocopherols and tocotrienols are natural constituents of all biological membranes and contribute to the stability of membranes from oxidation. They are relatively stable compounds in the absence of oxygen, while in the presence of oxygen they act as antioxidants (see antioxidant effects). The water activity dependence of α-tocopherol degradation is similar to that of unsaturated lipids with a rate minimum at the monolayer moisture value and greater rates at either sides of the monolayer. α-Tocopherol can serve as a scavenger to quench nitrogen free radicals (NO. and NO.2) (Gregory, 2007).

The biosynthesis of tocopherols, their chromanol ring, originates from shikimic acid (the shikimate pathway) and their hydrocarbon chain is derived from the compound phytyldiphosphate. The tocotrienol hydrocarbon chain (which consists of three double bonds) is derived from the compound geranyl-geranyldiphosphate (Lampi *et al*, 2008).

3.2 Antioxidant function

Appendix 1 shows a detailed view on the antioxidant mechanism of tocols. Tocopherols have the ability to act as antioxidants by donating a proton and an electron to a peroxyl radical (Gregory, 2007). It is the phenolic hydroxyl group that is donated (Tiwari and Cummins, 2009). This means that it contributes to the oxidative stability of food lipids. Peroxyl radicals react 1000 times faster with tocopherols than with polyunsaturated fatty acids (Traber and Stevens, 2011). α-Tocopheryl acetate exhibit no antioxidant activity in vitro due to the ester bond which blocks the phenolic hydroxyl group, but in vivo it has antioxidant effects because of enzymatic cleavage of the ester bond. δ-Tocopherol has the highest potency to act as an antioxidant followed by γ-, β- and α–tocopherol (Gregory, 2007) and α-tocotrienol has been shown to have a very good antioxidant activity (Cahoon *et al*, 2003).

Tocopherols can quench singlet oxygen and became degraded. The end products which are formed from the reaction have no vitamin or antioxidant activity (Gregory, 2007). α–Tocopherol is most reactive to singlet oxygen, followed by β-, γ- and δ-tocopherol (Gregory, 2007). Furthermore α-tocopheryl radical can react with ascorbic acid or other hydrogen donors and recycle the α-tocopheryl radical to α-tocopherol (Neuzil, Witting, and Stocker, 1997).

Vitamin E stops the chain reaction of oxidation of unsaturated fatty acids, but not the radical formation (Traber and Stevens, 2011).

3.3 Bioavailability

The bioavailability is quite high in individuals with normal fat absorption and the bioavailability of α–tocopherol is the same as to α–tocopheryl acetate (Gregory, 2007). Tocopherols have higher bioacessibility in the gut than carotenoids (Reboul *et al*, 2006). The bioacessibility is dependent on the food matrix and food processing (Reboul *et al*, 2006).

The bioaccessibility of α–tocopherol in white wheat bread is 99 % and 53 % in wheat germ. The corresponding bioaccessibility for γ-tocopherol is 8 % in white wheat bread and 48 % in wheat germ (Reboul *et al*, 2006). The bioavailabilty of α–tocopherol is higher in cereals than encapsulated (supplementation) (Leonard *et al*, 2004). It has been shown that the bioavailability can be improved by vitamin E fortification (Leonard *et al*, 2004).

3.4 Analytical Methods

HPLC (High Performance Liquid Chromatography) is the most common method of analysis of tocopherols because it permits measurement of the specific forms α–tocopherol, β–tocopherol and tocotrienols etc. (Gregory, 2007). Normal phase HPLC with fluorescence detector has been shown to be a good method for analysis of tocopherols or tocotrienols.It provides specificity and sensitivity and can detect all isomeric forms of tocopherols and tocotrienols (Ryynänen *et al*, 2004). Hot saponification has been shown to be a very decent method for the extraction of tocopherols and tocotrienols in rye. Three critical factors were observed; saponification temperature, time and amount of potassium hydroxide (Ryynänen *et al*, 2004). A suitable saponification scheme was formed with 0.5 ml potassium hydroxide for 25 minutes at 100°C. The extraction solvent was n-hexane in 20% ethyl acetate (Ryynänen *et al*, 2004). This method does not degrade α– and β–tocopherol, which is a great advantage (Tiwari and Cummins, 2009). Saponification releases the vitamers by degrading the food matrix and removes the bulk of fat. It improves the tocopherols chromatographic separation. An important thing to consider when analyzing tocopherols or tocotrienols is that they are labile in alkaline solutions and also that β– and γ- tocopherol has very similar structure which makes them hard to separate (Ryynänen *et al*, 2004). Other methods which can be used for analysis of tocopherols are reversed phase HPLC, Gas Chromatography (GC), capillary electro chromatography and Mass Spectrometry (MS) (Ryynänen *et al*, 2004).

4 Content and location of tocopherols in wheat (*Triticum sp.*) and rye (*Secale cereale)*

Cereals (e.g. wheat and rye) contain bioactive compounds, such as tocopherols, and are consumed a lot in the world. They have a major role for most people's vitamin E supply (Hidalgo and Brandolini, 2010). They act as a critical marketing tool because of the increasing consumer awareness of the role of the diet in health promoting disease prevention (Tiwari and Cummins, 2009). In common and einkorn wheat (*Triticum aestivum* and *Triticum monococcum*) tocopherols are located in the germ fraction and the two most abundant tocopherols are α-tocopherol and β-tocopherol (Hidalgo and Brandolini, 2008). The tocotrienols, α-tocotrienol and β-tocotrienol, is found in the bran and germ of wheat and rye, but is most abundant in the bran (Hidalgo and Brandolini, 2008). β-tocotrienol has also been reported to be located in the endosperm (Morrison *et al*, 1982). In einkorn wheat it has been shown that significant quantities of tocotrienols are located in the endosperm (Hidalgo and Brandolini, 2008).

4.1 Content in wheat

Table 2 summarizes the tocol content found in the different wheat species. The tocotrienol/tocopherol ratio is an indication of the proportion of tocopherols and tocotrienols in the kernel (Panfili *et al*, 2003). In common wheat the most abundant tocopherol and tocotrienol were also α-tocopherol (14 µg/g of dry matter (dm)) and β-tocotrienol (35 µg/g of dm), while the tocotrienol/tocopherol ratio was 2 (Hidalgo *et al*, 2006). These results suggest that wheat contain more tocotrienols than tocopherols. However, large variations in amount and composition of tocopherols and tocotrienols were observed in the wheat species (Hidalgo *et al*, 2006).

Einkorn wheat has been reported to contain approximately 50 µg/g dm of β-tocotrienol, 10 µg/g dm of α-tocotrienol, 10 µg/g dm of α-tocopherol and 5 µg/g dm of β-tocopherol. The tocols were present in all four fractions; bran, germ, endosperm and flour (Hidalgo *et al*, 2006). The tocotrienol/tocopherol ratio varied from 2 to 6 in an analysis of 54 einkorn species (Hidalgo *et al*, 2006). A high tocotrienol/tocopherol ratio indicates high antioxidant activity which is good for the nutritional quality (Ryynänen *et al*, 2004). Einkorn wheat is the best cereal source of tocols (Hidalgo *et al*, 2006).

The most abundant tocopherol in durum wheat (*Triticum turgidum*) was α-tocopherol (approximately: 10 µg/g dm), the most abundant tocotrienol was β-tocotrienol (approximately: 30 µg/g dm). The tocotrienol/tocopherol ratio was 3 (Hidalgo *et al*, 2006).

A review on tocopherols in wheat (common, durum, soft, einkorn and dinkel wheat) report a variation between 7 and 20 mg/kg for α-tocopherol, 2 and 10 mg/kg for β-tocopherol, 3 and 25 mg/kg for α-tocotrienol and 15 and 80 mg/kg for β-tocotrienol (Tiwari and Cummins, 2009). They also report that tocopherols and β-tocotrienol is most abundant in the germ and that the tocotrienols are abundant also in the pericarp, aleurone layer, sub-aleurone layer and the endosperm. The level of accumulation of tocopherols is influenced by several factors such as genotype, environment, agronomic inputs and the interaction of these factors (Tiwari and Cummins, 2009). This large variation in amount clearly indicates that the tocopherol and tocotrienol content varies among wheat species.

A study of 175 genotypes of wheat from all over the world (predominantly common wheat) reported an average tocopherol and tocotrienol content of 49 µg/g dm, and observed that einkorn was the best source of tocopherols and tocotrienols (average content: 57 µg/g dm) (Lampi *et al*, 2008). One of the winter wheat varieties contained the least amount of tocopherols and tocotrienols (27 µg/g of dm) and another winter-wheat species contained the highest amount of tocols. No large differences were seen in the content of tocopherols and tocotrienols among winter, spring, durum and spelt wheat (Lampi et al, 2008). The most abundant form was β-tocotrienol (25 µg/g of dm) followed by α-tocopherol (13 µg/g of dm), β-tocopherol (6 µg/g of dm) and α-tocotrienol (5 µg/g of dm). The proportion of tocotrienols in all wheat species were on average 61 % of total tocols. Einkorn wheat had the highest proportion with remarkable 79 %, and the lowest proportion was found in spelt wheat with 63 % (Lampi *et al*, 2008). However, they observed a large variation among the species, and they reported that the tocol content was three times higher in the variety with highest amount of tocols compared to the variety with least tocols. They refer the large differences to environmental factors, genotype and dehulling (Lampi *et al*, 2008). Shewry *et al*. (2010) reported that total contents of tocols were highly heritable in 26 wheat varieties cultivated at four different locations for three years. However, the environment also affected the content, and there was a correlation between total tocol content and mean temperature from heading to harvest.

Other studies have also shown that the environment, such as geographical regions seem to have influence on tocopherol and tocotrienol content in wheat. For example wheat cultivated in the Western Europe (e.g. Italy) has been shown to contain higher amounts of both tocopherols and tocotrienols than wheat cultivated in the Eastern Europe (e.g. Turkey) (Lampi *et al*, 2008; Hidalgo *et al*, 2006). It has also been observed that modern varieties contain more tocopherols and tocotrienols compared to old varieties (Lampi *et al*, 2008). This may be an effect of plant breeding. Furthermore it has been observed that the kernel size have an impact on tocopherol and tocotrienol content in wheat species. Small kernels have a large proportion of outer layer (e.g. bran) and contain higher amount of tocopherols and tocotrienols (Lampi *et al*, 2008).

4.2 Content in rye

Table 2 summarizes the content of the different tocols found in rye. In sifted rye flour it has been reported that the presence of tocopherols and tocotrienols is 6 µg/g dm of β-tocotrienol, 6 µg/g dm of α-tocopherol, 4 µg/g dm of β-tocopherol and 3 µg/g dm of α-tocotrienol (Pironen *et al*, 1986). They also reported the tocopherols and tocotrienols in whole-meal rye flour; 14 µg/g dm of β-tocopherol, 11 µg/g dm of β-tocotrienol, 10 µg/g dm of α-tocopherol and 3 µg/g dm of α-tocotrienol (Pironen *et al*, 1986).

In one study with ten different rye varieties the average tocol content was 49 µg/g dm, with a variation of, 40-54 µg/g dm (Ryynänen *et al*, 2004). The average tocotrienol/tocopherol ratio was 1.7 and varied between 1.3 and 2.1. The abundance of different forms was α-tocotrienol (17 µg/g dm), α-tocopherol (14 µg/g dm), β-tocotrienol (13 µg/g dm), β-tocopherol (4 µg/g dm), γ-tocopherol (1 µg/g dm), γ-tocotrienol (0.3 µg/g dm) and δ-tocotrienol (0.2 µg/g dm) (Ryynänen *et al*, 2004).

A review of tocols in cereals reported that tocotrienols are more abundant than tocopherols in rye, and that α-tocotrienol is the most abundant form (Tiwari and Cummins, 2009). Another report on tocol content in rye flour agreed that α-tocotrienol is the most abundant form in rye followed by α-tocopherol (Nyström *et al*, 2008). They observed a variation between 44 and 67 µg/g dm of tocols in ten rye samples. The average tocol content in rye was 52 µg/g of dm. 60 % of the total tocols were tocotrienols (Nyström *et al*, 2008).

Whole grains of rye has been exhibited to contain between 17 and 28 µg/g dm of tocopherols and tocotrienols, with α-tocotrienol being the most abundant form present followed by α-tocopherol and β-tocotrienol (Zielinski *et al*, 2007).

Rye grains contain high levels of tocopherols and tocotrienols and the tocotrienol/tocopherol ratios observed indicates that rye is a healthy food (Ryynänen *et al*, 2004). It has been reported that high contents of arabinoxylans in rye is associated with high contents of tocopherols and tocotrienols (Nyström *et al*, 2008). The methods for extraction and analysis of tocopherols have great influence on the result. Saponification combined with solvent extraction has been shown to be a decent method for analysis of tocopherols (Nyström *et al*, 2008).

Table 1. *Tocopherol and tocotrienol content in wheat (common wheat, einkorn, durum wheat, and dinkel wheat)* and rye. A (-) indicates that the compound was only found in negligible amounts below the detection limit.

Tocols (μg/g)	Common wheat[a]	Einkorn[a] wheat	Durum[a] wheat	Dinkel[a] wheat	Rye[b]
α-Tocopherol	4-14	8-17	7-13	11-14	8-17
β-Tocopherol	3-8	5	3-5	6-10	4-8
γ-Tocopherol	-	-	-	-	1
δ-Tocopherol	-	-	-	-	-
α-Tocotrienol	6	7-25	3-8	5-6	3-20
β-Tocotrienol	31	29-84	5-40	24-37	9-13
					0.3
γ-Tocotrienol	-	-	-	-	
δ-Tocotrienol	-	-	-	-	

a) Compiled from: Hidalgo *et al*, 2006; Tiwari and Cummins, 2009; Lampi *et al*, 2008.

b) Compiled from: Nyström *et al*, 2008; Tiwari and Cummins, 2009; Ryynänen *et al*, 2004; Zielinski *et al*, 2007.

5 Process effects, influence on stability of tocopherol

The stability of tocopherols is influenced by, light, temperature and substrate (Hidalgo *et al*, 2009), and their amount in cereals is influenced by different processes such as milling, baking, extrusion cooking and malting (Tiwari and Cummins, 2009).

Bleaching of flour can lead to large losses of tocopherols (Gregory, 2007). Drum-drying, extrusion cooking, low temperature storage and when food is exposed to air can also lead to large losses of tocopherols (Coultate, 2006). Tocopherols are however more stable than carotenoids (another antioxidant) against thermal degradation (Hidalgo and Brandolini, 2010).

5.1 Baking (bread and cookies)

The mixing time, pH, temperature and heat have all influence on the content of tocopherols and tocotrienols in baked products (Tiwari and Cummins, 2009). α-Tocopherol, α-tocotrienol and β-tocotrienol content in common wheat, durum and einkorn wheat decreases during baking of bread. The amount of β-tocopherol was low and its evolution during bread could not be measured (Hidalgo and Brandolini, 2010).

β-Tocotrienol have a major role in the change of total tocols during baking and cookie processing (Hidalgo and Brandolini, 2010). 21 % of the tocopherols and tocotrienols were lost during 2-3 minutes of dough kneading in wheat bread, and 28 % was lost during 2-3 minutes of dough kneading for cookie preparation, also with wheat flours (common wheat and einkorn wheat) (Hidalgo and Brandolini, 2010). Another report observed losses between 20 and 60% during kneading of the dough at French bread baking (Håkansson-Wennermark and Jägerstad, 1992). A 30% loss in tocopherol and tocotrienol content has been reported after bread baked with whole-meal flours of common wheat, einkorn and durum wheat (Leenhardt *et al*, 2006). The high losses of tocols during kneading is suggested to be a result of oxidation (Leenhardt *et al*, 2006). Cookies have a higher degradation rate than bread because of the absence of yeasts in cookie dough. The yeast is competing with the lipoxygenase for oxygen and thus limits oxidation of unsaturated fatty acids (Hidalgo and Brandolini, 2010). Cereals with moderate carotenoid contents (e.g. einkorn wheat) exhibit lower loss of tocopherols and tocotrienols during baking. It is suggested that carotenoids have a role in protecting tocopherols from the enzyme (i.e. lipoxygenase) (Leenhardt *et al*, 2006).

Einkorn showed a lower degradation rate of tocopherols than durum and common wheat did. One reason for this could be that einkorn contains more carotenoids which are also antioxidants and will react when peroxyl radicals are formed (Hidalgo and Brandolini, 2010; Hidalgo *et al*, 2006). Another reason may be that the lipoxygenase, which has been shown to be responsible for the loss of tocopherols during baking, is less active in einkorn compared to durum and bread wheat (Leenhardt *et al*, 2006). The absence of linoleic acid in einkorn can also be a possible cause for the lower degradation rate of tocopherols in einkorn. Linoleic acid is a preferable compound for the lipoxygenase (Hidalgo and Brandolini, 2010).

During the leavening phase of wheat bread only small losses of tocopherol or tocotrienol were observed. One reason for this is suggested to be because the yeast had consumed all oxygen and no more was incorporated in the dough (Hidalgo and Brandolini, 2010). In the crust and crumb of bread no significant variation in tocopherol and tocotrienol content were reported (Hidalgo and Brandolini, 2010).

The greatest loss during the baking process is mainly related to oxidative stress, especially when oxygen is incorporated in the dough during the kneading phase. To reduce the loss of tocopherols during baking the following is suggested; shorter kneading time and longer fermentation, and this is mainly to limit the ability for the dough to incorporate oxygen (Leenhardt *et al*, 2006).

5.2 Milling

Milling is a process which separates the bran, germ, hull, and endosperm into different fractions. Because the germ and the bran is removed during the milling process, the tocopherol and tocotrienol content decreases in the flour compared to the whole-grain (Tiwari and Cummins, 2009). It has been reported that the germ does not occur in a specific fraction and is instead recovered in the different by-product fractions and also in the flour (Leenhardt *et al*, 2006).

The level of supplementation and the type of milling fraction have influence on tocopherol and tocotrienol content in wheat and rye flour. The fractions containing bran and germ are enriched with tocopherols and tocotrienols (Tiwari and Cummins, 2009).

5.3 Malting

Malting is a process which includes the following procedures; steeping, germination and kilning. It is tone step in the production of the beverage beer, but malt can also be used in other food products. Malting has very little or no effect on tocopherols or tocotrienols. It may be because tocols are lipid-soluble compounds that are not extracted during the malting process (Tiwari and Cummins, 2009). Malt and beer is mostly made from barley which is not studied in this thesis, but it may also be made from wheat and rye (Schwarz, 2012; Sinclair and Sinclair, 2010).

5.4 Extrusion

The cooking temperature, time and exposure to light (photosensitized oxidation) have influence on the degradation of tocopherols and tocotrienols during extrusion (Tiwari and Cummins, 2009). Extrusion cooking cause a 30 % decrease in tocopherol and tocotrienol content of wheat and rye (Zielinski *et al*, 2001). Same losses of tocols have also been reported by Hidalgo and Brandolini (2010). The loss is related to heat (Tiwari and Cummins, 2009). Temperature seem to have an important role in the degradation of tocopherols, for example, between 110°C and 140°C large tocopherol and tocotrienol losses occur (Shin *et al*, 1997).

5.5 Pasta processing

The loss of tocopherols and tocotrienols during a 7 minutes kneading phase of pasta was reported to be 44 % from white flours of durum wheat semolina, common wheat and einkorn (Hidalgo and Brandolini, 2010). 30% tocopherols and tocotrienols were lost from white flours of durum wheat semolina, common wheat and einkorn wheat during pasta extrusion under no vacuum (Hidalgo and Brandolini, 2010). Under vacuum a tocol loss of 4 % was reported from white wheat flours (Hidalgo and Brandolini, 2010). Another report observed a 35% loss of tocopherols and tocotrienols after kneading and pasta extrusion of durum wheat dough’s (Panfili *et al*, 2005). The loss of tocopherols and tocotrienols during pasta processing is suggested to be due to oxidation, mainly in the preparation of the dough (Hidalgo and Brandolini, 2010).

During drying of pasta no significant amount of tocopherols was lost and this was probably because the temperature (65°C) was sufficient to inactivate the enzymes (i.e. lipoxygenase and lipase) (Hidalgo and Brandolini, 2010).

5.6 Steaming treatment

Steaming is a process that heats up the sample to a specific temperature and certain time. Steaming treatment has shown to have effect on tocopherol and tocotrienol content of wheat, with more loss at higher temperature and longer time. β-Tocopherol was reported to be stable against steaming treatment, i.e. no significant change in concentration after different steaming treatments compared to the control (Hidalgo *et al*, 2008). No other tocopherol or tocotrienol was stable to steaming treatment. A great variation in the degradation of tocopherols and tocotrienols was shown after steaming treatment in five einkorn species and one common wheat species, especially for β-tocotrienol (Hidalgo *et al*, 2008). After steaming treatment α– and β-tocopherol was most abundant in the germ of wheat. α- and β-Tocotrienol were found in both whole flour, endosperm, bran and germ, but were most abundant in the bran (Hidalgo *et al*, 2008). This was suggested to be a migration of tocotrienols.

5.7 Storage

During storage of wheat α-tocopherol and α-tocotrienol are less stable than β-tocopherol and β-tocotrienol (Håkansson and Jägerstad, 1992). Tocopherol and tocotrienol content in einkorn and common wheat decreased during storage. It was dependent on temperature and time, and the degradation in white flour was more rapid than in whole meal flour and as the temperature was increased (Hidalgo *et al*, 2009). The degradation followed first-order kinetics. α-Tocopherol was the least stable tocol (especially in white flour) (Hidalgo *et al*, 2009). One possible explanation for the higher degradation rate in white flour compared to whole meal flour is suggested to be that whole meal flour contains a lot of other substances, such as lignans, phenolic acids and flavonoids which can interfere with the antioxidant effects of tocopherols and tocotrienols during storage (Hidalgo *et al*, 2009; Kamal-Eldin and Appelvist, 1996). One reason that α-tocopherol was least stable can be that it has three methyl groups linked to the chromanol ring compared to the other forms which has only one or two methyl groups (Kamal-Eldin and Appelvist, 1996). Storing temperatures of 20°C or lower is the best way to preserve tocopherols in wheat (Hidalgo *et al*, 2009).

6 Physiological health effects (deficiency, benefits and toxicity)

According to Nordic Nutrient Recommendations (2004) males shall eat 10 mg α-tocopherol equivalents (α-TE) and women 8 mg α-TE every day (Becker *et al*, 2004). One α-TE is 1 mg RRR- α-tocopherol (Becker *et al*, 2004). It has been reported that it is beneficial to eat more tocopherols than the suggested recommendations to reduce the risk of getting chronic disease, such as heart disease (Rimm *et al*, 1993). Another health benefit that has been shown for tocopherols is that they can reduce the risk of colon cancer (Malila *et al*, 1999). The tocotrienols have also been shown to reduce the cholesterol levels in the blood (Quershi *et al*, 1991) and to prevent cardiovascular disease (Raederstorff *et al*, 2002).

Diets which are deficient in tocopherols have showed effects on mitochondrial membranes in humans, i.e. they are more susceptible to oxidation (Coultate, 2009). Specific symptoms that arise from vitamin E deficiency are various degrees of hemolysis which can lead to hemolytic anemia (Traber and Stevens, 2011).

Intake of 40 mg α-TE may give increased bleeding tendency and intakes of more than 300 mg α-TE per day over a long period of time can cause increased mortality (Abrahamsson *et al*, 2006).

The majority of the population in the United States has an insufficient in-take of vitamin E. Only 8 % of the men and 2 % of the women has a sufficient intake according to the estimated average requirements in US (Maras *et al*, 2004).

Supplementation effects of tocopherols has been reported to reduce the coagulation of blood and this is beneficial for individuals with thrombosis (Traber and Stevens, 2011; Glynn *et al*, 2007). Supplementation can however be harmful to healthy people (Traber and Stevens, 2011).

It has been reported that α–tocopherol disappear more rapidly in people that smoke compared to people that don’t smoke, due to the increased oxidative stress (i.e. more free radicals) (Bruno *et al*, 2005). In another study it was shown that α–tocopherol disappearance rates in smokers were equal to that of nonsmokers if 500 mg vitamin C was eaten twice per day (Bruno *et al*, 2006). This suggests that a person that smokes shall eat more vitamin C to prevent losses of vitamin E.

Vitamin E supplements has been reported to be beneficial for the reduction of suffering from cardiovascular diseases for women with an age of 65 years, and this is an example where vitamin E supplements have shown beneficial effects. It has been reported that vitamin E supplements have no beneficial effect on the incidence of cancer or cardiovascular disease for women's (<65 years) and for physicians (Traber and Stevens, 2011).

More health benefits that have been shown for tocotrienols are the prevention of neurodegeneration, induction of immune responses and cancer prevention (Sen *et al*, 2006; Nesaretnam *et al*, 2007).

7 Discussion

The two most common tocols in wheat and rye are tocopherols and tocotrienols. Tocopherols are essential nutrients which are found mostly in the germ of cereals. Tocotrienols are most abundant in the bran. α-Tocopherol has the highest vitamin E activity and is the only tocol that can be maintained in the human body. Tocotrienols are excellent antioxidants, but has less vitamin activity than tocopherols.

The most abundant tocol in wheat is β-tocotrienol followed by α-tocopherol. Einkorn wheat has been shown to contain the largest amount of tocols among different wheat types. In rye, α-tocotrienol is the most abundant tocol followed by α-tocopherol. However, there are large differences in tocol content among both wheat and rye varieties.

The high content of tocotrienols and the presence of tocopherols in wheat and rye flours indicate that these two cereals are good sources of antioxidants and have high nutritional quality. Whole-grain flours contained most tocols due to the presence of bran and germ where tocols are most abundant. It is not surprising that tocopherols and tocotrienols are most abundant in the bran and germ since this is where the fatty acids are located. Tocols are antioxidants and thereby have a role in protecting the fatty acids against oxidation, especially against the highly reactive singlet oxygen.

Baking has been shown to cause moderate losses of tocols and the loss may be due to oxidative stress. The following parameters may influence the loss of tocols during baking of bread and cookies; the presence of yeasts, carotenoid content, lipoxygenase activity, the amount of oxygen and linoleic acid. Shorter kneading time and longer fermentation may reduce the loss of tocols during baking of bread (Leenhardt *et al*, 2006).

The tocols are located in the germ and bran fraction and because these fractions are removed during milling to produce white flour the tocol content will be low in white flour. However, some of the other milling fractions, such as bran, will be rich in tocols. Malting has little effect on tocol content, mainly because tocols are lipid-soluble. Extrusion has been shown to cause moderate losses of tocols and the loss is temperature, time and light dependent. As with baking, the loss of tocols during pasta processing is mainly related to the kneading phase. The extrusion step during pasta making has also been shown to cause moderate losses of tocols.

The stability of tocols is influenced by temperature, oxygen, lipoxygenase activity, the presence of yeasts (in dough's) and linoleic acid and other bioactive compounds (e.g. carotenoids, phenolic acids) which can interfere with the oxygen and free radicals which are formed during oxidation of fatty acids. α-Tocopherol is the most labile form during storage of wheat and rye.

Steaming treatment cause a migration of tocotrienols from the bran to the endosperm and it may be a way to improve the tocotrienol content in, for example white flour.

Tocopherols and tocotrienols have been shown to be beneficial for the prevention and reduction of some diseases which includes; cardiovascular disease, colorectal cancer, reduction of blood cholesterol, neurodegeneration, induction of immune responses and prevention of other types of cancer.

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Appendix

7.1 Appendix 1

Tocopherol react with a peroxyl radical by donating a proton and an electron.The α-tocopheryl radical which is formed is terminated to α-tocopheryl hydroquinone, α-tocopheroxide or α-tocopheryl quinone which has none or very little vitamin activity (Gregory, 2007).

Tocopherols can quench singlet oxygen while forming α–tocopherol hydroper-oxydieneone which then is terminated to α–tocopheryl quinone-2,3-oxide or α–tocopheryl quinone which has negligible vitamin activity.