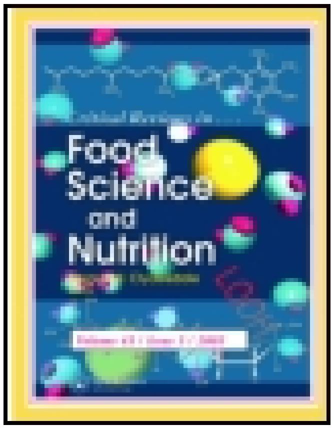

Critical Reviews in Food Science and Nutrition
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/bfsn20

Foods for Special Dietary Needs: Non-Dairy Plant Based Milk Substitutes and Fermented Dairy Type Products

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Accepted author version posted online: 09 Jan 2015.

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Abstract

A growing number of consumers opt for plant based milk substitutes for medical reasons or as a lifestyle choice. Medical reasons include lactose intolerance with a worldwide prevalence of 75% and cow's milk allergy. Also in countries where mammal milk is scarce and expensive, plant milk substitutes serve as a more affordable option. However many of these products have sensory characteristics objectionable to the mainstream Western palate. Technologically, plant milk substitutes are suspensions of dissolved and disintegrated plant material in water, resembling cow's milk in appearance. They are manufactured by extracting the plant material in water, separating the liquid and formulating the final product. Homogenisation and thermal treatments are necessary to improve the suspension and microbial stabilities of commercial products that can be consumed as such or be further processed into fermented dairy type products. The nutritional properties depend on the plant source, processing and fortification. As some products have extremely low protein and calcium contents, consumer awareness is important when plant milk substitutes are used to replace cow's milk in the diet e.g. in the case of dairy intolerances. If formulated into palatable and nutritionally adequate products, plant based substitutes can offer a sustainable alternative to dairy products.

Keywords

Soy milk, oat milk, rice milk, medical nutritional therapy, lactose intolerance, cow's milk allergy

1. Introduction

Plant milk substitutes are water extracts of legumes, oil seeds, cereals or pseudocereals that resemble cow's milk in appearance. There is a wide variety of traditional plant based beverages around the world, for example Horchata, "tigernut milk" in Spain; Sikhye, a beverage made of cooked rice, malt extract and sugar in Korea; Boza, a fermented drink made of wheat, rye, millet and maize consumed in Bulgaria, Albania, Turkey and Romania; Bushera, a fermented sorghum or millet malt based beverage from Uganda, and traditional soy milk originating from China (Cortés et al., 2005; Prado et al., 2008; Kim et al., 2012; Chen, 1989). The most widely consumed plant milk substitute is soy milk, a product that started its journey from Asia to the supermarket shelves in Europe and the US less than hundred years ago. The first commercially successful product was launched in Hong Kong in 1940 and the market grew rapidly during the seventies and early eighties in Asia after the development of technologies for large scale production of mild flavoured soy milk (Chen, 1989). The demand for soy milk in the Western world was initiated by consumers intolerant to cow's milk, but the market expanded in the 1990's and 2000's as a part of a health trend, and grew from USD 300 m to USD 4 bn between 1992-2008 in the U.S. (Organic Monitor, 2005; Patisaul and Jefferson, 2010). After soy received an FDA approved health claim for lowering the risk for coronary heart disease in 1999, more than 2700 new soy products were introduced to the market (Patisaul and Jefferson, 2010).

Soy products are still dominating the market in the Western world, but the emerging of alternative products from other plant sources such as coconut, oat and almond have decreased its share (Mintel, 2011). Overall, the dairy alternative market is still growing: Packaged Facts

(2012) estimated the U.S. market for plant based milk substitutes to have a total value on USD 1.33 bn in 2011, which is expected to increase to USD 1.7 bn by 2016. Also the market for lactose- and dairy free products in general, estimated to be worth USD 3.6 bn in 2010, is growing in the U.S. and Western Europe. The figure includes lactose free dairy products, but much of the growth has been attributed to soy milk like products (Leatherhead Food Research, 2011).

According to an estimate, 15% of European consumers avoid dairy products for a variety of reasons, including medical reasons such as lactose intolerance (LI), cow's milk allergy (CMA), cholesterol issues and phenylketonuria, as well as lifestyle choices like a vegetarian/vegan diet or concerns about growth hormone or antibiotic residues in cow's milk (Jago, 2011) (Leatherhead Food Research, 2011). LI is generally an inherited condition (primary hypolactasia) that disables lactose digestion due to lactase deficiency, causing abdominal pain, bloating and flatulence upon the consumption of lactose containing foods (Swagerty et al., 2002). The prevalence of LI varies between ethnic groups, being below 20% only among white Europeans and their descendants. The significantly higher prevalence in other ethnic groups (50-80% among Hispanic and Black and nearly 100% among Asian and Native American populations) has led to a theory that lactase deficiency is a normal condition for adult humans and the frequency of the lactase persistency gene has increased in cultures where milk has offered a selective advantage (Sahi, 1994). LI can also be caused by injuries to the intestinal mucosa (secondary hypolactasia), resulting from diseases such as untreated celiac disease, cystic fibrosis and gastroenteritis (Bode and Gudmand-Høyer, 1988; Swagerty et al., 2002). Sufferers of the inflammatory bowel disease have a higher dairy sensitivity prevalence compared to the average population (10-20%), and are often advised to avoid dairy products (Mishkin, 1997). The main treatment for LI is the avoidance of lactose containing foods and replacing milk and dairy products with lactose free dairy or dairy free alternatives.

CMA is a disorder in which the immune system reacts to one or more milk proteins causing an inflammatory response. Cow's milk is the most common allergen in infants, but 80-90% of sufferers acquire a tolerance by the age of 5 years. The true prevalence of CMA is 2-6% in infants and 0.1-0.5% in adults, but the number of self-diagnosed cases is 10-fold higher possibly due to confusion with LI or misdiagnosis without clinical evaluation (Crittenden and Bennett, 2005). The only treatment for CMA is the complete avoidance of cow's milk antigens. Infants with CMA may be fed with hypoallergenic formulas based on extensively hydrolysed whey or casein (Kneepkens and Meijer, 2009).

This review aims to give an overview on the technological, nutritional and environmental aspects of plant milk substitute production and consumption. Soy milk has been extensively studied and a body of scientific literature on peanut beverage exists and reviewed recently (Diarra et al., 2005; Giri and Mangaraj, 2012), but little has been published on cereal or pseudocereal based beverages.

2. Technology

2.1. Overview of the process

Plant milk substitutes are colloidal suspensions or emulsions consisting of dissolved and disintegrated plant material. They are prepared traditionally by grinding the raw material into a slurry and straining it to remove coarse particles. Although countless variations of the process exist, the general outline of a modern industrial scale process is essentially the same: the plant material is soaked and wet milled to extract the milk constituents, or alternatively the raw material is dry milled and the flour is extracted in water (Figure 1). The grinding waste is separated by filtering or decanting. Depending on the product, standardisation and/or addition of other ingredients such as sugar, oil, flavourings and stabilisers may take place, followed by homogenisation and pasteurisation/UHT treatment to improve suspension and microbial stabilities. These extracts can also be spray dried to produce powders (Diarra et al., 2005).

2.2. Raw material pre-treatments

Plant milk substitutes can be produced by extracting the soluble material directly either ground plant material with water or wet grinding soaked grains or legumes into a slurry (Diarra et al., 2005). Alternatively, the product can be reconstituted using protein isolates or concentrates and other ingredients, e.g. oils, sugars, salts and stabilisers (Debruyne, 2006). This approach also allows the formulation of a range of related products such as pharmaceutical beverages, nutritional supplements, infant formulas, meal replacers, cream alternatives and fruit smoothies (Paulsen et al., 2006).

Possible raw material pre-treatments include dehulling, soaking and blanching (Debruyne, 2006). Blanching is required to inactivate trypsin inhibitors and lipoxygenase that would produce off-flavours in soy milk (Giri and Mangaraj, 2012). Roasting of the raw material enhances the aroma and flavour of the final product, but heating decreases the protein solubility and extraction yield (Hinds et al., 1997a; Rustom et al., 1991).

2.3. Extraction

The extraction step has a profound effect on the composition of the resulting product. To increase the yield of the process, the efficiency of this step may be improved by increasing the pH with bicarbonate or NaOH, elevated temperatures or the use of enzymes. Most cereal and legume proteins have an isoelectric point under 5, translating to the lowest solubility (Wolf, 1970). Alkaline pH during extraction increases the protein extractability, but a neutralisation step may be required in the process (Rustom et al., 1991; Aidoo et al., 2012). A higher extraction temperature increases the extractability of fat, but the denaturation of proteins decreases their solubility and yield (Rustom et al., 1991). Hot water extraction of cowpea milk decreases the yield and protein content compared to cold water extraction, but improves the protein digestibility slightly due to trypsin inhibitor inactivation, and leads to a reduced extraction of phytic acid (Akinyele, 1991).

Partial hydrolysis of proteins and polysaccharides using enzymes is another way to increase the extraction yields (Table 1). Papain and enzymes extracted from *Pestulotiopsis westerdijkii* increased the protein yield of peanut and soy milks (Rustom et al., 1993; Abdo and King 1967). In addition to proteolytic enzymes, a mixture of amyloglucosidase and a cellulase cocktail has been shown to increase the carbohydrate recovery of peanut milk (Rustom et al., 1993). Eriksen (1983) used a variety of enzymes in soy milk extraction, and found out that the highest protein and total solids yield was attained using a neutral or alkaline proteinases at their optimum pH, while pectinase and β-glucanase had little effect. Enzymes with low pH optima may not be the most efficient extraction aids even if the enzyme action *per se* increases the yield, as the pH decrease influences the protein solubility, and thus neutral and alkali proteases may be the best options.

In addition to increasing the extraction yield, proteolytic enzymes improve the suspension stability (Rustom et al., 1991). Also a cellulase treatment after homogenisation has been reported to decrease the particle size and yield a more stable suspension (Rosenthal et al., 2003).

2.4. Separation and starch liquefaction

After the extraction step coarse particles are removed from the slurry by filtration, decanting or centrifugation (Diarra et al., 2005; Lindahl et al., 2001). When using raw materials high in fat, such as peanuts, the excess fat can be removed using a separator as in dairy processing (Diarra et al., 2005).

Soy beans and nuts contain little starch, but when using cereals or pseudocereals the starch forms a thick slurry when heated above the gelatinisation temperature (55-65 °C). To prevent this in the further processing steps, starch can be gelatinised and liquefied with α-amylase or a malt enzyme extract (Mitchell and Mitchell, 1990; Tano-Debrah et al., 2005). The patented process of Lindahl et al. (2001) employs α- and β-amylases to hydrolyse the starch until a desired level of sweetness and viscosity is reached. The liquefaction step may take place before or after the removal of coarse particles. However, according to Mitchell and Mitchell (2010) and Giri and Mangaraj (2012), heating the slurry above 50 °C before filtration compromises the mouthfeel of rice and soy milks.

2.5. Product formulation

Other ingredients can be added to the product base after the removal of coarse plant material. These include vitamins and minerals used for fortification as well as sweeteners, flavourings, salt, oils and stabilisers. As suspension stability is an issue in plant milk substitutes, hydrocolloids are often used to increase the viscosity of the continuous phase, and also emulsifiers have been proven to be beneficial in some beverages. (Rustom et al., 1995) yielded the most stable peanut milk by using a stabiliser mix for dairy products containing mono- and diglycerides, glyceryl monostearate, guar gum and carrageenan, while Hinds et al. (2007b) achieved good results with 0.02-0.04% carrageenan and 0.2-0.4% mono- and diglycerides. Lee and Rhee, (2003) used pine nuts to improve the stability of a rice based beverage, as they contain proteins with good emulsifying properties. Sodium stearoyl-2 lactylate (SSL), a lipid surfactant, has been found to bind specifically to partially hydrolysed oat proteins and thus enhance the stability of oat protein suspensions (Chronakis et al., 2004).

The addition of nutrients in food substitutes may be necessary to ensure the nutritional quality of the product. The nutrients used must be bioavailable and sufficiently stable, and not cause excessive changes in product quality. The stability of vitamins is influenced by several factors during food processing, and may be reduced as a result of e.g. heating oxygen exposure (Richardson, 1990). The challenge in mineral enrichment is the reactivity of metal ions with other food components, and the use of sequestrants such as citric acid may thus be necessary

(Richardson, 1990; Zhang et al., 2007a). Some mineral sources used in plant milk substitutes include ferric ammonium citrate and ferric pyrophosphate as iron sources and tricalcium phosphate and calcium carbonate as calcium sources (Zhang et al., 2007a; Zhao et al., 2005).

2.6. Homogenisation and suspension stability

Plant milk substitutes contain insoluble particles, such as protein, starch, fibre and other cellular material. These particles, being denser than water can sediment, making the product unstable (Durand et al., 2003). The suspension stability can be increased by decreasing the particle size, improving their solubility or by using hydrocolloids and emulsifiers (Durand et al., 2003; Rustom et al., 1995). Many plant milk substitutes coagulate when heating. When proteins unfold as a result of heating, the nonpolar amino acid residues are exposed to water increasing the surface hydrophobicity. This enhances protein-protein interactions that can result in aggregation and sedimentation or gelling (Phillips et al., 1994). The heat stability of proteins depends on the pH, ionic strength and the presence of other compounds such as minerals and carbohydrates (McSweeney et al., 2004).

Homogenisation improves the stability of plant milk substitutes by disrupting aggregates and lipid droplets and thus decreasing the particle size distribution (Malaki Nik et al., 2008). When enough lipids are present, an emulsion is formed resulting in a creamier more homogenous product (Chen, 1989). Homogenisation in the conventional dairy processing pressure range (ca. 20 MPa) increases the suspension stability sufficiently of at least soy, peanut and rice milk substitutes (Hinds et al., 1997b; Lee and Rhee, 2003; Rustom et al., 1995). Ultra high pressure homogenisation (UHPH) of soy milk at 200-300 MPa reduces the particle sizes intensely and improves the stability compared to conventionally processed products. The treatment also reduces microbial counts and can be used for preservation (Cruz et al., 2007). A higher homogenisation temperature has been reported to increase the stability of peanut milk (Hinds et al., 1997a; Rustom et al., 1995).

In soy milk, heat denaturation of proteins is required for suspension stability. Malaki Nik et al. (2008) studied the effect of heat denaturation alone and in combination with homogenisation (69 MPa) by characterising fractions obtained by stepwise centrifugation. The protein and solids content decreased after the first centrifugation (8000 *g*) in the untreated samples, while significant decrease in both treated samples occurred after the third centrifugation (40 000 g), indicating an increase in the resistance to sedimentation upon heating and homogenisation. Also the ratios of β-conglycinin (7S) and glycinin (11S) in the fractions were influenced by the treatments. This indicates that although heating decreased the solubility of β-conglycinin, large glycinin aggregates were disrupted, resulting in suspensions with smaller particles and a narrower size distribution.

2.7. Microbial shelf life extension

Commercial plant milk substitutes are pasteurised or UHT treated to extend the shelf life. However heat may cause changes in protein properties that can influence the stability, as well as changes in flavour, aroma and colour (Kwok and Niranjan, 1995; Rustom et al., 1996). Pasteurisation is carried out at temperatures below 100 °C, and it destroys enough microorganisms to enable a shelf-life of ca. 1 week at refrigerated temperatures. In the UHT treatment the product is heated to 135-150 °C for a few seconds, yielding a commercially sterile product (Kwok and Niranjan, 1995). Rustom et al. (1996) treated a peanut beverage for 4 and 20 s at 137 °C. The longer treatment time decreased the suspension stability slightly, but led to higher taste and acceptability scores. Both treatments were effective in increasing the microbial shelf life: no vegetative bacteria, spores or moulds were detected in the products.

The manufacturing process of Horchata (tiger nut milk) takes another approach: the product is not heated to prevent the starch from gelatinising and the occurrence of other sensory changes resulting from heating. Prepared this way the product has an extremely short shelf-life. In commercial products, pulsed electric fields has been suggested to extend the microbial shelf life (Cortés et al., 2005). Also other non-thermal processes such as ultraviolet sterilisation, high pressure throttling, high pressure processing and ultra-high pressure homogenisation (UHPH) have been explored as methods of soy milk preservation (Bandla et al., 2011; Cruz et al., 2007; Smith et al., 2009; Sharma et al., 2009). Sikhye, a Korean rice beverage, is commonly sold frozen to avoid UHT related changes in flavour. However *Bacillus cereus* spores are a risk, and their number has successfully been reduced by tyndallisation with CO_2 injection, a procedure consisting of heating to 80 °C to activate spore germination, followed by heating to 95 °C (Kim et al., 2012).

3. Fermented products

Fermentation with lactic acid bacteria improves the sensory and nutritional properties, and microbial shelf life of foods (Leroy and De Vuyst, 2004). Plant milk substitutes can be fermented to produce dairy free yoghurt type products while rendering the raw material into a more

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palatable form. For example, the levels of hexanal responsible for the undesired beany flavour in peanut milk are efficiently reduced with fermentation (Lee and Beuchat, 1991). Also, the levels of aflatoxin B1 commonly found in peanuts, is reduced by fermentation with *Flavobacterium aurantiacum* (Hao and Brackett, 1988). Fermentation of soy milk reduced the amount of flatulence inducing oligosaccharides depending on the α-galactosidase activity of the strain, and increased the angiotensin-converting enzyme (ACE) inhibitory activity (Donkor et al., 2007). The storage proteins of various cereals contain known ACE inhibitory peptides that can be released using fermentation and exogenous proteases as has been demonstrated with rye malt (Hu et al., 2011; Loponen, 2004). These cereals may have potential as raw materials for dairy type functional products.

In order to produce fermented products, the starter cultures must be able to grow and dominate the microflora in the plant medium, and produce a desired texture. Lactic acid bacteria have been used for cereal fermentations for centuries and many cereals and pseudocereals are known to support their growth, but low levels of fermentable sugars present in some grains may pose a problem (Zannini et al. 2012). To overcome this, sugars and food grade yeast extract can be added to the media (Diarra et al., 2005). Also, germinating the raw material to increase the amount of fermentable sugars and amino acids before processing improves the growth performance of probiotic strains (Charalampopoulos et al., 2002). Mårtensson et al. (2000) studied the growth and product characteristics of an oat milk medium fermented with a range of starter cultures. They found out, that strains of *Leuconostoc mesenteriodes*, *Leuc. dextranicum*, *Pediococcus damnosus* and *Lactobacillus kefiri* produced the highest levels of lactic acid, resulting in a pleasant flavour. In addition, an EPS producing strain of *L. delbrueckii ssp.*

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bulgaricus yielded a viscosity comparable to dairy yoghurts after 72 h fermentation at 25 °C when glucose was used as a carbon source. Jiménez-Martínez et al. (2003) obtained a product with a viscosity similar to dairy yoghurt but slightly lower hedonic rating by fermenting milk extracted from *Lupinus campestris* seeds with *Streptococcus thermophilus* and *L. delbrueckii ssp. bulgaricus*.

Probiotic dairy products have been available for years, but also non-dairy raw materials can be used as vehicles for probiotic strains for the dairy intolerant or vegetarian/vegan consumers (Prado et al., 2008). Donkor et al. (2007) reached desired therapeutic levels of cells (10^8 cfu/ml) after fermenting soy milk with a range of probiotic strains for 48 h. Mårtensson et al. (2002) reported inhibition of some probiotic strains in an oat product when used in combination with a yoghurt starter culture, as the pH of the medium decreases excessively due to a lower buffering capacity in comparison to cow's milk. However a strain of *L. reuteri* was able to survive at a therapeutic level for at least 30 days (Mårtensson et al., 2002).

Some authors have used additives such as carboxymethyl cellulose, coagulants (calcium citrate), milk powder and gelatin to enhance the texture and reduce syneresis in the final product (Cheng et al., 2006; Yadav et al., 2010). However, the use of animal ingredients in this product category in the Western market excludes the vegetarian/vegan consumer segment. Yazici et al. (1997) aimed to increase the calcium content of peanut yoghurt to the level of fat free dairy yoghurt, but the calcium salts decreased the gel strength and promoted syneresis. In addition to plant milk substitutes, also suspensions of solid grain material can been used as media for fermentation, yielding a gruel like product (Salovaara, 2004). This enables a more economic utilisation of the raw material, as well as better preservation of the nutritional properties such as

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high fibre content.

4. Nutritional properties

Plant milk substitutes are often perceived as healthy, possibly due to negative perceptions about the nutritional properties of cow's milk and the health claims associated with soy (Bus and Worsley, 2003; Patisaul and Jefferson, 2010). In reality the nutritional properties vary greatly, as they depend strongly on the raw material, processing, fortification and the presence of other ingredients such as sweeteners and oil. The nutritional values of plant milk substitutes purchased from a local store in Ireland are presented in Table 2. When comparing the products, it is evident that only soy milk has values comparable to cow's milk with protein contents ranging from 2.9-3.7%. All other products are very low in protein, with only quinoa, hemp and Oatly oat milk containing ≥1% protein. This may pose a risk if plant milk substitutes are used to replace cow's milk without knowledge about the differences, especially when given to young children: several cases of kwashiorkor, a protein-energy malnutrition typical for areas of famine, have been reported in Western countries as a result of using rice milk (0.1-0.2% protein) as a weaning food (Carvalho et al., 2001; Katz et al., 2005). Also milks produced of legumes other than soy, such as peanut and cowpea can have a protein content as high as 4% (Rustom et al., 1991; Tano-Debrah et al., 2005). Although plant milk substitutes are low in saturated fats and most products have caloric counts comparable to skim milk, some products contain as much energy as full milk, originating mostly from sugars and other carbohydrates.

Plant proteins are generally of a lower nutritional quality compared to animal derived proteins due to limiting amino acids (lysine in cereals, methionine in legumes) and poor digestibility (Friedman, 1996). The nutritional value of proteins depends mainly on the amino acid composition and their physiological utilisation, and absorption that is in turn affected by processing. Several methods of evaluating the protein quality have been used, incl. protein efficiency ratio (PER) based on weight gain of an experimental animal and amino acid chemical score based on comparison to a reference protein (Friedman, 1996). The method currently preferred by WHO/FAO is the protein digestibility-corrected amino score (PDCAAS) that compares the concentration of the first limiting amino acid to a reference pattern (child 2-5 years), that is corrected for the digestibility (Schaafsma, 2000). PDCAAS and PER values of some raw materials used in commercially available plant milk substitutes are listed and compared to the values of cow's milk in Table 3. Both values are the highest for cow's milk followed by heat treated soy. PDCAAS values for all other raw materials fall below 67.7%, quinoa and hemp scoring highest, with the exception of amaranth protein concentrate with a value of 83%. PER of cow's milk is 3.1, while the closest plant protein sources are quinoa, amaranth and soy (all heat treated) with values 2.7, 2.6 and 2.28, respectively. The extremely low PER value for raw soy (0.46) reflects the presence of protease inhibitors that are inactivated upon heating (Friedman, 1996).

In addition to containing high value protein, milk and other dairy products provide 30–40% of dietary calcium, iodine, vitamin B_{12} and riboflavin, and population groups with low milk intakes often have a poor status for these nutrients (Millward and Garnett, 2010; Black et al., 2002). To combat these shortcomings, some plant milk substitutes are fortified with calcium and vitamins, mainly B_{12}, B_2, D and E (Table 1). However, consumer awareness is important as many of these products are not fortified.

Calcium absorption depends on the salt used for fortification as well as the food matrix (Rafferty et al., 2007). A comparison between cow's milk and soy milk fortified with tricalcium phosphate revealed a 75% absorption in soy milk compared to cow's milk, while no differences have been observed when calcium carbonate was used (Heaney et al., 2000; Zhao et al., 2005). Ionic calcium precipitates soy proteins especially when subjected to thermal treatments, which may influence the calcium content of the beverage consumed (Pathomrungsiyounggul et al., 2010). Indeed, 82% to 89% of the calcium in soy and rice milks, respectively, are separable by centrifugation at 3740 *g*, whereas the value for cow's milk is 11%, which may indicate a decrease in the calcium content of a beverage not properly shaken before use (Heaney et al., 2005). Despite these shortcomings, fortified plant milk substitutes may be a valuable source of calcium for individuals with medical conditions that prevent the consumption of dairy products, and offering soy milk as an option in elementary schools has been reported to increase the selection of a calcium rich beverage slightly (Reilly et al., 2006).

Some plant derived components have favourable health effects, that may be present in the beverages produced from that raw material. For example, replacing low fat cow's milk with oat or soy milks has been reported to decrease the plasma cholesterol and low density lipoprotein (LDL) concentrations of healthy individuals after a 4 week consumption period (Önning et al., 1998). Soy has been perceived as a health food due to its isoflavone content with reported impacts on the prevention of e.g. cardiovascular diseases, prostate cancer and osteoporosis (Patisaul and Jefferson, 2010). The health benefits of isoflavones have however become increasingly controversial and concerns have been raised especially about maternal soy intake and the use of soy in infant formulas. Isoflavones have a complex interaction in the endocrine network, and the effect of long term effect of a soy based diet in early childhood is not known. The serum isoflavone concentration of infants on soy formula can be as high as 10-fold compared to the concentrations in Japanese adults (Patisaul and Jefferson, 2010; Andres et al., 2011).

Seeds often contain antinutritive compounds, such as inositol phosphates and trypsin inhibitors. Trypsin inhibitors decrease the digestibility of protein, but are inactivated by heat treatments (Friedman, 1996). Inositol penta- and hexaphosphates (phytates) present in all seeds bind divalent cations such as calcium, zinc, iron and magnesium, and reduce their physiological availability (Reddy et al., 1982; Sandberg et al., 2006). Mineral bioavailability of seeds can be improved by germination, fermentation, or by using chelating agents or exogenous phytase (Reddy et al., 1982). Zhang et al. (2007a) obtained the highest increase in iron bioavailability of fortified oat milk using citric acid in combination with exogenous phytase.

Processing influences the nutritional properties of foods. For example, the beneficial effects of oat β-glucan on serum LDL cholesterol and postprandial glucose levels are attributed mainly to the viscosity it forms in aqueous solutions, which is sensitive to processing (Wood, 2010). Both homogenisation and thermal treatments have been reported to alter the molecular properties of oat β-glucan (Kivelä et al., 2011; Kivelä et al., 2010). No significant loss of isoflavones occurs during soy milk processing, but coagulating the soy proteins in tofu processing decreases the total isoflavones by 44% (Wang and Murphy, 1996). Another study reports a recovery of 54% isoflavones during soymilk processing and 36% for tofu production (Jackson et al., 2002).

Water soluble vitamins can be lost if the raw material is soaked and/or blanched before the manufacturing process (Kwok and Niranjan, 1995). Also high amounts of minerals (Ca, Fe, P, Zn) (45-74%) are lost during the decanting step in oat milk production (Zhang et al., 2007b). The destruction of heat sensitive vitamins depends on the time temperature exposure (Kwok and Niranjan, 1995). UHT treatment caused a 60% loss of D_3 after 5 s holding time, while increasing the holding time to 20 s led to a 30% decrease in B_{12} concentration. The loss of thiamine (B_1) can be minimised by favouring high temperature short time heat exposure in the manufacturing process in soy milk production (Kwok and Niranjan, 1995). Significant losses of A, D_3 and B_{12} occurred during the storage of oat milk, while the levels of folic acid and vitamins C, B_6 and B_{12} are reduced in soy milk (Zhang et al., 2007b; Kwok and Niranjan, 1995).

5. Consumer acceptance of plant milk substitutes

Although the demand for plant milk substitutes is increasing, the unwillingness of the mainstream consumer to try unfamiliar foods that are perceived as unappealing may be a limiting factor. Many modern day soy milks and related products may have an improved sensory quality, but the product group carries a stigma because of early less appealing products on the market (Wansink et al., 2005). Legume milks tend to possess "beany" and "painty" off-flavours originating from lipoxygenase activity (Kwok and Niranjan, 1995). The presence and intensity of the "beany" flavour depends on processing and storage conditions of soy milks and varieties with less lipoxygenase have less "beany" character (Chambers et al., 2006; Torres-Penaranda and Reitmeier, 2001). Another problem is a chalky mouthfeel some products have due to large insoluble particles (Durand et al., 2003; Hinds et al., 1997c).

Palacios et al (2009) compared the acceptance of lactose free cow's milks and soymilks on American adults. Lactose free cow's milk was preferred over soy milks, with no interactive effect for ethnicity, gender or dairy intolerances (Palacios et al., 2009). Similar results were obtained in a study with American school children (Palacios et al., 2010). In a Swedish study, oat milk scored higher than medium fat UHT cow's milk in general liking, while soy milk had the lowest score (Önning et al., 1998). The acceptance of peanut milk has been shown to depend on the colour, mouthfeel, the absence of peanut flavour and similarity to cow's milk (Diarra et al., 2005).

Information can increase the willingness to try novel foods. Taste is the most important purchase criteria of foods, and the information about a good and/or familiar taste increase the willingness to try an unfamiliar food most efficiently (Magnusson et al., 2001; Pelchat and Pliner, 1995). Possible health benefits are also an important criteria and health information may increase both the willingness to try and the perceived liking of a food, while the environmental aspect is less relevant (Pelchat and Pliner, 1995; Kihlberg et al., 2005; Magnusson et al., 2001). Repeated exposure has been shown to enhance the liking e.g. in the cases of a bitter beverage and a probiotic beverage (Stein et al., 2003; Luckow et al., 2006). Indeed, a 5 day exposure to rice milk increased both the overall and the pleasantness of the taste (Russell and Delahunty, 2004). Also the liking scores for oat milk increased over 3 weeks of consumption in male subjects who initially gave lower scores, while the scores given by females remained unchanged (Önning et al., 1998).

6. Impact on climate and land use

At the moment climate change is considered one of the most important and serious phenomena caused by human action. Greenhouse gases (GHG) varying in their global warming potential are a very probable cause of global warming (IPCC, 2008). GHG emissions originating from food production are remarkable: In the European Union about 29% of total contributions to global warming are estimated to come from the food chain (Huppes et al., 2008). According to FAO, livestock is responsible of 18% of the global GHG emissions, of which dairy production and processing is estimated to contribute 4% (Steinfeld et al., 2006) (Gerber, 2010). Main contributors to global warming from livestock sector are methane from enteric fermentation, nitrous oxide from manure and fertilizer, carbon dioxide from land use changes and agricultural energy use (Steinfeld et al., 2006). GHGs differ in their radiative properties and lifetimes in the atmosphere. The warming potentials are commonly expressed as CO_2 equivalents (CO_2-eq), the amount of CO_2 emission that would have the same warming effect (IPCC, 2008).

In addition to GHG emissions, another major environmental impact of food production is land use and changes in soil such as eutrophication and acidification. Fertile land is a scarce resource, and foods requiring large production areas are less sustainable even if the direct emissions are low (Sonesson et al., 2010). On a per kg basis, the production of plant foods generally emits less GHG and requires less land than does the production of meat and dairy products (Sonesson et al., 2010; Nijdam et al., 2012).

The global warming potential of cow's milk varies in the range of 0.84–1.3 CO_2-eq/ kg product (De Vries and De Boer, 2010). Studies dealing with the GHG emissions of plant milk substitutes are scarce, but the few reports published suggest lower values compared to cow's milk. According to Smedman et al. (2010) the GHG emissions produced during a life cycle of oat and soy drinks are 0.21 and 0.31 kg CO_2-eq/kg product. The global warming potential for commercial Oatly oat milk is 0.32 g CO_2-eq/l product (Dahllöv and Gustafsson, 2008). Mikkola and Risku-Norja (2008) compared the pre-farm gate GHG emissions from optional milk production systems in Finland. The estimated emissions expressed as kg CO_2-eq per capita per year were 4-8 times higher for cow's milk compared to oat and soy milks.

The nutritional profiles of dairy and plant based products are different, which makes the direct comparison of the GHG emissions challenging. One approach is to relate the environmental impact to the protein content. Nijdam et al. (2012) evaluated the GHG emissions and land use of protein from different sources. The productions of one kg protein from milk emits 28-43 CO_2-eq and requires 26-54 m^2 land, whereas the figures are 4-10 CO_2-eq and 10-43 m^2 for pulse protein and 6-17 CO_2-eq and 4-25 m^2 for vegetable based meat analogue protein. González et al. (2011) estimated so-called protein delivery efficiency GHG values (g protein/kg CO_2-eq) for a range of foodstuffs. The values were 31 g for milk and 505 g, 359 g and 56 g for unprocessed soybean, oat and rice protein, respectively. Smedman et al. (2010) developed a so-called nutrient density to climate impact index (NDCI), aiming to reflect the proportion of daily nutrient requirements and the contribution of each nutrient to the Swedish diet in relation to the GHG emissions. As a result the index for cow's milk was superior to oat and soy drinks. The equation used in this study has however been criticised as biased and the finding questioned by other scientists in the field (Scarborough and Rayner, 2010).

Judging from the very limited literature, plant milk substitutes have a lower impact on the climate and require less land to produce, but the issue is more complex as cow's milk contains several key nutrients that are challenging to replace.

Conclusions and future outlook

Plant based milk substitutes have a reputation of "health foods" but the products on the market vary remarkably in their nutritional profiles, some having very low protein and mineral contents. If these products are to be portrayed as substitutes for cow's milk, protein content and quality as well as fortification has to be considered by manufacturers. Attention should be brought to the possible ways of improving the nutritional properties by processing means e.g. the use of enzymes and the selection of raw materials based on their protein quality. Also a reconstitution approach may allow a more efficient extraction of protein from the material and the formulation of higher protein products. This would however increase the costs and also the environmental impact of the products. More knowledge is required to overcome the mineral fortification related stability issues.

Indication of figures and tables

Figure 1 and **Tables 1, 2** and **3** submitted in separate files are a part of this manuscript.

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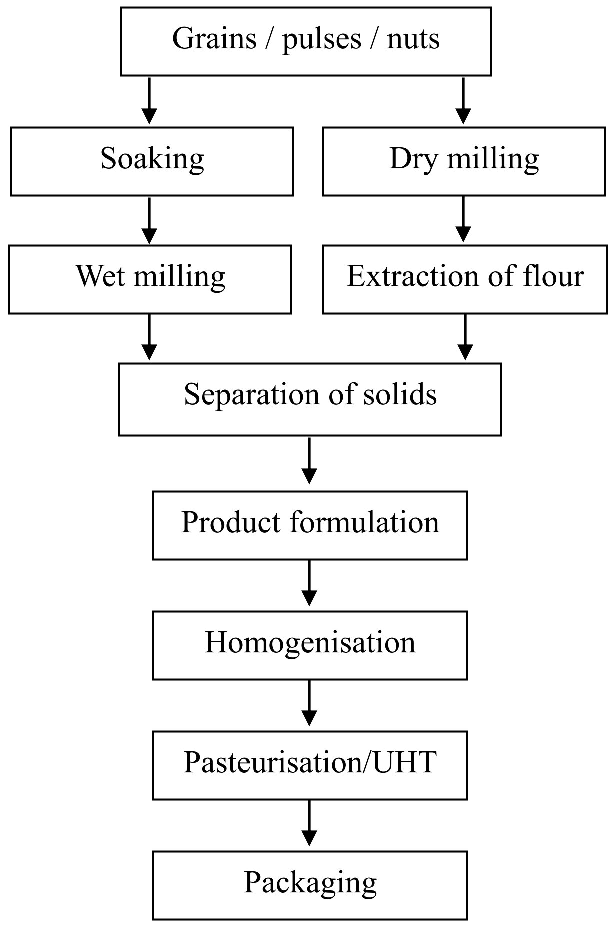
Grains / pulses / nuts
Soaking
Dry milling
Wet milling
Extraction of flour
Separation of solids
Product formulation
Homogenisation
Pasteurisation/UHT
Packaging

Table 1

Raw material	Enzyme	Dosage/pH/T	Increase in yield	Reference
Peanut	Papain	1:50 protein/ 6.95; 8.0/20 °C; 60 °C	"Significant increase in protein extraction"	(Rustom et al., 1991)
Peanut	Cellulase cocktail (Viscozyme) and amyloglucosidase	- / 4.5 / 50°C	13.4% (carbohydrate)	(Rustom et al., 1993)
Soy	Enzyme isolate from *Pestulotiopsis westerdijkii*	- / 4.6 / 37 °C	22% (protein)	(Abdo and King, 1967)
Soy	Neutral protease and β-glucanase cocktail (Neutrase)	0.5%/7.0 / 50 °C	31% (protein) 20% (total solids)	(Eriksen, 1983)
Soy	Pectinase	2% / 5.5 / 50 °C	11% (protein) 7% (total solids)	(Eriksen, 1983)
Soy	Pectinase, cellulase, hemicellulase and protease cocktail (SP-249)	2% / 4.5 / 50 °C	26% (protein) 16% (total solids)	(Eriksen, 1983)

Table 2

Beverage (manufacturer)	Energy (kcal)	Protein	Carbohydrate (sugars)	Fat (saturated)	Fibre	Fortification
Cow's milk (full) [1]	64	3.3	4.6 (4.6)	3.9 (2.5)	-	-
Cow's milk (skim) [1]	33	3.5	4.8 (4.8)	0.3 (0.1)	-	-
Soy (Alpro, UK)	38	2.9	2.8 (2.7)	1.7 (0.3)	0.5	Ca, B_2, B_{12}, D, E
Soy (Tesco, UK)	32	3.4	0.2 (0.1)	1.9 (0.3)	0.6	Ca, E, D, B_{12}
Soy (Triballat Noyal, FR)	45	3.7	3.1 (2.7)	2.0 (0.3)	0.8	Ca *
Oat (Alpro , UK)	66	0.4	12.7 (5.7)	1.5 (0.57)	0.0	-
Oat (Oatly, SE)	35	1	6.5 (4.0)	0.7 (0.1)	0.8	Ca, D_2, B_2, B12
Oat (Hain Europe, BE)	50	0.6	8.6 (4.5)	1.3 (0.2)	1.0	Ca, D_2, B_{12}
Kamut, (La Finestra Sul Cielo,IT)	46	0.7	7.5 (4.6)	1.4 (0.2)	0.5	-
Amaranth (Ecomil, SP)	52	0.6	8 (5.0)	1.9 (0.5)	0.3	-
Sesame (Ecomil, SP)	51	0.6	6.7 (3.4)	2.4 (0.5)	0.2	-
Quino (Ecomil, SP)	46	1.5	3.7 (2.5)	2.8 (0.7)	0.6	-
Hemp (Braham and Murray, UK)	36	1.3	2.2 (2.1)	2.4 (0.3)	0.2	Ca *, D_2
Rice (Hain Europe, BE)	47	0.1	9.4 (4.0)	1.0 (0.1)	0.1	-
Rice (Alpro, UK)	60	0.2	12.2 (5.0)	1.2 (0.2)	0.0	Ca, B_1, B_6, B_{12}
Almond (Alpro, UK)	24	0.5	3.0 (3.0)	1.1 (0.1)	1.6	Ca, B_2, B_{12}, D_2

[1] Food Standards Agency (2002) McCance and Widdowson's The Composition of Foods, Sixth summary edition. Cambridge: Royal Society of Chemistry.

* Seaweed used as a calcium source

Table 3

	PDCAAS (%) [a]	PER	References
Cow's milk	120	3.1	(Schaafsma, 2000)
Soy	91; 93	0.46; *2.28*	(Schaafsma, 2000) (Michaelsen et al., 2009) (Friedman et al., 1991)
Quinoa	*67.7*[b]	2.7	(Ruales, Grijalva, Lopez-Jaramillo, & Nair, 2002) (Ranhotra et al., 1993)
Amaranth	63; 83[c]	1.9 ; *2.6*	(Garcia et al., 1987) (Escudero et al., 2004)
Hemp	63-66	-	(House et al., 2010)
Oat	45-51; 60	2.3	(Michaelsen et al., 2009) (Hischke et al., 1968) (Pedo et al., 1999)
Rice	54	2.0	(Michaelsen et al., 2009) (Juliano et al., 1971)
Wheat	42; 37	1.5	(Schaafsma, 2000) (Michaelsen et al., 2009)
Sesame	-	1.35	(Johnson et al., 1979)
Almond	30	-	(Ahrens et al., 2005)

[a] Nontruncated values

[b] Value from infant food formula

[c] Value from protein concentrate

