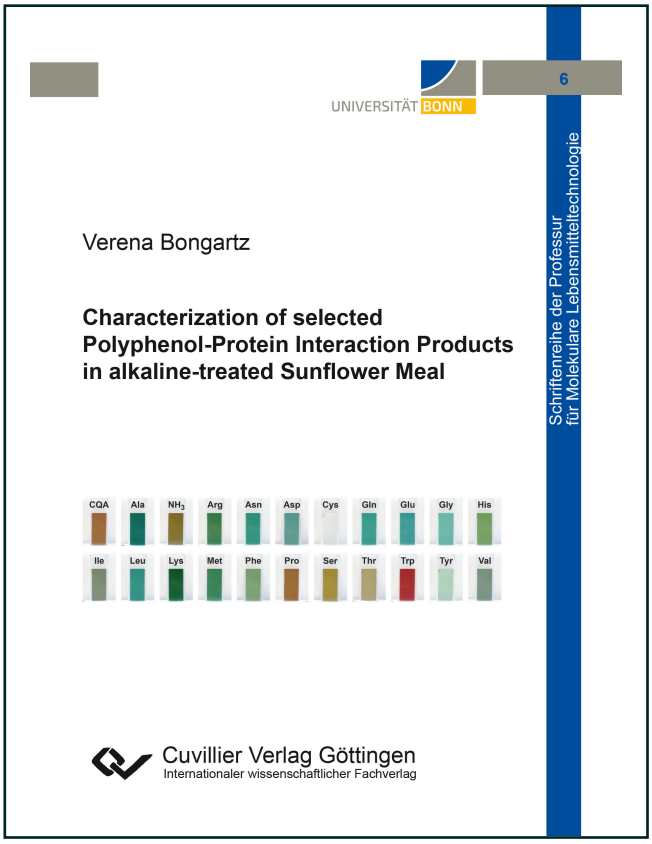
Verena Bongartz (Autor)

Characterization of selected Polyphenol-Protein Interaction Products in alkaline-treated Sunflower Meal



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Chapter 1: **General Introduction**

1 The sunflower (*Helianthus annuus* L)

1.1 Origin and proliferation

Helianthus (formerly Annui) is a genus of plants that contains approximately 70 species of sunflower in the Asteraceae. Although most species are perennial, section *Helianthus* includes more than 10 species most of which are annuals. The domesticated sunflower is derived from the wild form of *Helianthus annuus* L, or common sunflower [Schilling et al. 1998; Roth & Kormann, 2000].

Helianthus annuus L is native to North America. The wild form occurs throughout the continental United States, southern Canada, and northern Mexico. However, its prehistoric distribution is poorly understood. It is speculated that, prior to the arrival of Homo sapiens, the species was restricted to the south western United States. Native Americans used wild forms of *Helianthus annuus* L as a food, suggesting it became a camp-following weed and was thereby introduced to the central and eastern United States, where it was then domesticated [Heiser, 1951; Heiser et al., 1969].

Another theory states that buffaloes were the primary dispersal agents and that wild *Helianthus annuus* L had been widely distributed before the colonization of North America by humans. All modern domesticated sunflowers can be traced to a single center of domestication in eastern North America predating 3000 B.C. [Asch, 1993; Smith, 2011].

In the late 16th century, *Helianthus annuus* L – alongside beans and corn – was introduced to Europe by Spanish explorers, where it was cultivated as an ornamental plant. The commercial cultivation as an oil crop first started in Russia, around 1830. It resulted in high-oil lines with increasing oil contents from 28% to almost 50%. Today, Helianthus annuus L is cultivated throughout North and South Amercia, Europe and Asia [Roth & Kormann, 2000; Lieberei & Reisdorff, 2007; Department of Agriculture, Forestry and Fisheries, 2010; FAOSTAT, 2016].

1.2 Botanic characteristics

Helianthus annuus L is an annual plant. Fully grown, the erect plant is characterized by broad leaves, a strong taproot and a prolific lateral spread of surface roots. In cultivation, sunflowers are usually unbranched and grow to a height of 300 cm or more. They bear one wide flower head, up to a size of 40 cm in diameter, with yellow ray florets at the outside and yellow or maroon disc florets inside. The outer florets are sexually sterile, whereas the inner florets ("disc flowers") mature into fruits. Typically, crop sunflower varieties are self-incompatible. Therefore, pollen movement among plants by insects is important, and bee colonies are often used to increase yields. Each disc flower can produce up to 2000 achenes, single-seeded indehiscent fruit, which are commonly referred to as "sunflower seeds" [Dorrel & Vick, 1997; Department of Agriculture, Forestry and Fisheries, 2010].

1.3 Oil production

The sunflower has one of the shortest growing seasons of the major crops of the world. Early maturing varieties are ready for harvest 90 to 120 days after planting: they require approximately 11 days from planting to emergence, 33 days from emergence to head visibility, and 65 days from head visibility to maturity. Depending on climatic and cultivation conditions, yields can vary from as little as 600 to as much as 3.000 kg/ha [Department of Agriculture, Forestry and Fisheries, 2010; FAOSTAT, 2016].

Ripe fruiting heads are harvested either manually or mechanically. The oil containing seeds are four-sided and flat and are generally 0.8 cm to 1.7 cm long and 0.4 cm to 0.9 cm wide. Additionally, they are wrapped by a hull consisting of a single layer of skin, which can easily be separated [Roth & Kormann, 2000; Lieberei & Reisdorff, 2007].

With an average harvest of 33 million t, sunflower seeds represent 8% of the total global production of oilseeds, following soybean (55%), rapeseed (14%) and cottonseeds (10%). The Ukraine, Russia and Argentina are the main producers of sunflower seeds and sunflower by-products, as well as suppliers

to the global market. They account for 52% of the global production of sunflower and 40% of the world exports of sunflower seeds. Therefore, these countries are often referred to as the “Sunflower Triangle” [faostat, 2016].

Sunflower oil is extracted mainly from oil-type sunflower seed varieties and hybrids. Solvent-extracted sunflower meal (SEM), a by-product of the oil extraction process, is used primarily as an ingredient in livestock feed rations. Oil-type sunflower seeds contain 38% to 50% oil and about 20% protein. More than 90% of the sunflower seeds produced are processed into edible oil. Per 100 kg of seeds, about 40 kg of oil, 35 kg of high-protein meal and 20 kg to 25 kg of by-products are produced. Sunflower seeds are processed in industrial oil mills with processing capacities of up to 15.000 t oilseed per day. In order to ensure good storage properties, the water content of the seeds is generally reduced to a maximum of 10%. Sunflower oil manufacture involves different stages: cleaning and grinding the seeds, pressing and extracting the crude oil, and further refining of the oil. For oil extraction, volatile hydrocarbons are used as a solvent [Remmele, 2009; faostat, 2016].

Incoming oil seeds show a high degree of contamination with stones, dirt and metals. To remove any extraneous material, the seeds are passed both over magnets and through differently sized sieves. The dehulled seeds are ground into coarse meal to provide more surface area. Mechanic grooved rollers or hammer mills crush the seeds before the resulting meal is heated to facilitate the extraction of the oil. While this procedure permits the recovery of a higher amount of oil, more impurities are released with the oil, which in turn need to be removed. The heated meal is continuously fed into a screw press, which increases the pressure progressively as the meal passes through a slotted barrel. The remaining oil cake still contains about one third of its original amount of oil. Hexane or a comparable volatile hydrocarbon dissolves the oil out of the oil cake before it is separated by distillation. About 90% of the hydrocarbon in the extracted oil simply evaporates and is collected for reuse. The remaining hydrocarbon is retrieved and recollected by a stripping column [Bockisch, 1993; Remmele, 2009; faostat, 2016].

In order to remove color, odor, and bitterness, the oil is refined at temperatures between 40°C and 85°C in the presence of alkaline substances such as sodium hydroxide or sodium carbonate. Additionally, the oil is treated by water steam or a water/acid mixture and subsequently centrifuged, to eliminate the arising gums (phosphatides, precipitate, and dregs). Oil which is intended to undergo high temperatures ("cooking oil") is usually bleached by filtering it through Fuller's earth, activated carbon, or activated clays that absorb pigmented material from the oil. In contrast, oil that will undergo low temperatures (in the refrigerator, for example) is winterized – rapidly chilled and filtered to remove any waxes. This procedure ensures that the oil will not partially solidify during storage at low temperatures [Bockisch, 1993; Remmele, 2009; faostat, 2016].

During deodorization, steam is passed through the hot oil in a vacuum at between 225°C and 250°C. This process allows any volatile taste and odor components to distil from the oil. After deodorization, citric acid at 1% is added to chelate any trace metals that might promote oxidation within the oil (and hence shorten its shelf life) [Bockisch, 1993; Remmele, 2009; faostat, 2016].

In addition to its final oil content of 3%, the remaining SEM contains hydrocarbon residues. To minimize the risk of explosion during transport and storage, the final content of hydrocarbon must be kept to a maximum 300 mg/kg, which is usually achieved by desolvation at temperatures of up to 108°C [Bockisch, 1993; Münch, 2009].

2 Solvent-extracted sunflower meal

Solvent-extracted sunflower meal (SEM) is the co-product that results from solvent extraction of sunflower oil from the seeds. It contains 400 to 500 g/ kg protein (dry matter basis), making SEM an economically interesting source of protein. For further usage as animal feed, the press cake that results from oil production is milled and commonly pressed into pellets. However, excessive microbial protein degradation in the rumen decreases the efficiency of protein utilization in the small intestine [Bockisch, 1993; González-Pérez et al., 2002; Münch, 2009; Calsamiglia et al., 2010; Weisz et al., 2010; Lomascolo et al., 2012].

Thus, ruminants such as goats or cows benefit from ruminally undegraded protein sources that escape degradation but can still be hydrolyzed (leaving the released amino acids to post-ruminal absorption). Previous attempts aimed at the preparation of high-protein sources include, but are not limited to, the use of polymeric coatings, heat, addition of ionophores, treatment of proteins with chemicals such as formaldehyde or different acids, and the addition of secondary plant metabolites [Antoniewicz et al., 1992; Wu and Papas, 1997; Yu et al., 2002; Patra and Saxena, 2009].

However, some phytogenic additives may induce adverse effects such as decreased feed intake due to sensory effects. The use of formaldehyde and other chemicals has raised safety concerns. Heat treatment facilitates Maillard reactions, which can be poorly controlled and may lead to decreased intestinal digestibility and, consequently, availability of some amino acids. Therefore, alternative approaches for the preparation of feedstuffs with elevated crude protein concentrations are needed. A promising approach may be using naturally occurring compounds in feedstuffs such as chlorogenic acid (CQA).

2.1 Composition

The exact composition of SEM depends on the characteristics of the cultivar and on the method of processing, specifically the degree of dehulling (Table 1.1).

Table 1.1: Composition of SEM (modified from DLG, 1997)

SEM	Dry matter (DM)	Crude ash	Crude protein	Crude fat	Crude fibre	N-free extractive matter
[g · kg^{-1}]	[g · kg DM^{-1}]	[g · kg DM^{-1}]	[g · kg DM^{-1}]	[g · kg DM^{-1}]	[g · kg DM^{-1}]	[g · kg DM^{-1}]
Dehulled	910	79	439	20	135	327
Partly dehulled	900	70	379	24	223	304
Unhulled (low hull cultivar)	880	64	324	25	287	300
Unhulled (High hull cultivar)	890	50	237	13	404	296

The residual water content of SEM ranges from 9% to 12%. The crude fiber mainly consists of cellulose, lignin and pentosans. The precise amount depends on the degree of dehulling and ranges from 15% to 40%. Similarily, the protein content varies from 25% to 45%. The content of crude fat figures between 1% and 3%. The dry matter ranges between 5% and 8%. Nitrogen-free extractive matter includes all organic components that are neither crude protein, crude fat, nor crude fiber, such as the soluble components of cellulose, lignin or pentosans or hemicellulose, pectins and carbohydrates. The content of carbohydrates in SEM ranges from 5% to 10% [Gassmann, 1983; DLG, 1997; González-Pérez & Vereijken, 2007].

2.2 Protein

Unprocessed sunflower seeds exhibit a crude protein content of 17% to 22%, depending on the cultivar. Dehulled sunflower seeds contain between 25% and 45% crude protein. The total amount of crude protein includes peptides, free amino acids and further nitrogen-containing compounds that account for up to 13%. Mainly depending on the extraction method, the protein content in SEM varies from 30% to 50% [Gassmann, 1983; González-Pérez & Vereijken, 2007; Weisz et al., 2009].

Furthermore, except for their low lysine content, sunflower proteins match the FAO (Food and Agriculture Organization) reference protein patterns for humans in terms of amino acid composition and are low in antinutritive compounds. In addition to their relatively high nutritive value, sunflower proteins display various interesting technological characteristics comparable to those of soybean and other legume proteins, such as emulsifying or foaming properties [Weisz et al., 2010].

The classification of sunflower proteins is based on either the Osborne fractionation or the Svedberg sedimentation coefficient. The Osborne fractionation classifies the single proteins according to their solubility behavior. In accordance to that, the main part of sunflower protein (50% to 70%) consists of salt-soluble globulines, whereas water-soluble albumins account for only 18% to 35% [Raymond et al., 1995].

Sunflower protein is naturally low in both alkaline-soluble glutelins and alcohol-soluble prolamines. The Svedberg sedimentation coefficient classifies SEM protein into two major fractions of globular proteins, with helianthinin being the predominant one. Helianthinin is an 11S-globuline with a molecular weight of 300 to 350 kDa. Depending on pH, ionic strength, temperature and protein concentration, its hexamer 11S form readily dissociates into its 7S form, which, under extreme conditions, dissociates into its 2─3S form. Additionally, in high concentrations and under moderate alkaline conditions (pH 8.5 to 9), helianthinin aggregates to 15─18S forms. The second major Svedberg fraction includes 2S albumins, which account for 10% to 30% of the total SEM protein content. They are characterized by a molecular weight of 10 to 18 kDa and a high degree of stability towards pH changes as well as under high temperatures [Sabir et al., 1974; Schwenke et al., 1975; Gassmann, 1983; González-Pérez et al., 2004; González-Pérez & Vereijken, 2007].

2.3 Phenolic content

In addition to its high protein content, SEM is also rich in phenolic compounds, with chlorogenic acid (CQA) being the predominant component. Depending on

environmental and genetic factors, total phenolics in SEM may range from 1% to 4%, with 5-*O*-caffeoylquinic acid (chlorogenic acid, CQA) being the predominant compound. Along with its main isomers 3-*O*-caffeoylquinic acid and 4-*O*-caffeoylquinic acid, CQA accounts for 62% to 92% of the total polyphenol content in SEM [Sabir et al., 1974; Weisz et al., 2009].

3 Polyphenols

3.1 General overview

Plant phenolic compounds, or polyphenols, are characterized as compounds possessing one or more aromatic rings bearing hydroxyl substituent(s). The name derives from the Ancient Greek word πολύς (polus, meaning “many, much”), and the word phenol, which refers to the chemical structure formed by attaching to an aromatic benzenoid (phenyl) ring, a hydroxyl (-OH) group identical to that found in alcohols (hence the -ol suffix). The term polyphenol appears to have been in use since 1894 [Prigent et al., 2007; Merriam-Webster Thesaurus, 2017].

Polyphenols form a diverse structural class of natural compounds that are synthesized in plant secondary metabolism. They play a role in numerous processes, such as plant growth and reactions to stress and pathogen attack. The main effects of phenolic compounds in plant tissues can be divided into the following categories [Engelhardt & Galensa, 1997; Parr and Bolwell, 2000; Weisz et al., 2010; Le Bourvellec & Renard, 2012]:

- Release and suppression of growth hormones
- UV screens to protect against ionizing radiation and to provide coloration
- Deterrence of herbivores
- Prevention of microbial infections
- Signaling molecules in ripening and other growth processes

The content and profile of phenolic compounds depend on the cultivar and cultivation conditions of the plant. Also, the degree of maturation significantly affects the total phenolic content. The highest concentration of phenolic compounds can be observed in the exterior layer of the single plant parts. Phenolic compounds contribute to the organoleptic properties of plant foods, especially by their astringency, bitter taste and color. They occur naturally in many foods and drinks from plant origin, e.g. fruits, vegetables, coffee, tea,

beer, wine and chocolate. The total content of phenolic compounds in foods may change during storage, due to effects induced by light and temperature. Their presence can be easily observed due to the chromophoric groups that some phenolic compounds bear, e.g., the red-purple anthocyanins, or by the brown reaction products of polyphenols when fruits are damaged [Robards et al. 1999; Friedman & Jürgens, 2000; Yabuta et al., 2001; Prigent, 2005].

Owing to their antioxidative properties, phenolic compounds are of great interest for the food industry both nutritionally and technologically. They have been associated with various health-promoting effects such as antioxidative, antibiotic or anti-inflammatory properties, which possibly prevent diseases associated with oxidative stress. Because of their presumed positive effects on human health, polyphenols are increasingly used in functional foods [Prigent, 2005; Kammerer et al., 2007; Weisz et al., 2010].

Phenolic compounds represent a wide range of molecules with a molecular mass from about 100 to 4.000 Da. They can be divided into the following groups [O'Connell & Fox, 2001; Prigent, 2005]:

- the C_6 group, comprising simple phenols and benzoquinones,
- the C_6C_n group, which consists of phenolic acid derivatives and hydroxycinnamic acid derivatives,
- the C_6-C_n-C_6 group, including flavonoids (C_6-C_3-C_6),
- the $(C_6$-$C_3)_n$ group consisting of lignans and lignins, and
- the tannin group, which itself in turn is divided into hydrolyzable tannins, condensed tannins and phlorotannins. The hydrolyzable tannins are formed by gallic acid, or hexahydroxydiphenic acid, esterified to a polyol such as glucose or quinic acid. The condensed tannins mainly consist of the proanthocyanidins. Proanthocyanidins are oligomers and polymers of flavanols, which are members of the flavonoid sub-class. Therefore, some authors classify proanthocyanidins into the same class as their monomeric units, i.e. the C_6-C_n-C_6 class. Among these monomeric units,