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Bioactive compounds and antioxidant activity of cocoa hulls (*Theobroma cacao* L.) from different origins

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Summary

Cocoa hulls are the main by-product of cocoa with a high content of soluble fibres and polyphenols. Cocoa and cocoa-derived products are largely studied because of the antioxidant and antiradical *in vitro* properties of the polyphenolic constituents. These bioactive compounds have beneficial implications to human health (prevention of cardiovascular diseases and cancer) because of their free radical scavenging capacities. Thus, it is an imperative interest to bring this low-priced by-product into a more profitable utilization as possible source of dietary fibre for functional foods. The focus of this work was to characterize the content of bioactive compounds (polyphenols, hydrocolloids) of cocoa hulls from different geographic origins, i.e. Madagascar, Ghana, Trinidad, Venezuela and Ecuador. The antioxidant activity, measured by electron spin resonance (ESR), showed a high correlation between polyphenol content and antioxidant activity. The analyses showed significant differences between the geographic origins. The hulls of Madagascarian cocoa beans had higher contents of polyphenolic compounds and water soluble pectin compared to all other analysed origins. Moreover, they showed the highest antioxidant activity. In contrast the cocoa hulls from Ecuador contained the lowest values of polyphenols as well as of antioxidant activity. The lowest content of water soluble pectin was determined in cocoa hulls from Trinidad. Beside genotype variations, ripeness and fermentation of the cocoa beans, climate and soil conditions as well as stress are significant factors influencing the content of the bioactive compounds. The geographic origin of the cocoa beans (climate, soil quality) substantially influences their quality. Finally, cocoa hulls polyphenol-rich extracts are very interesting raw products with peculiar colorant and functional properties. The interest in functional foods and the focus on potential health benefits of these compounds, invites the speculation that cocoa bean shell could provide a ready source of inexpensive polyphenol and pectin rich additives to human foods, for example chocolate.

Introduction

Cocoa is an important export product of many developing countries for production of cocoa powder and chocolate. The amount of cocoa hulls in the cocoa bean processing industry is very high. Tons of cocoa hulls are disposed as waste every year. They are part of the cocoa bean, separated from the cotyledons after the roasting process. They are commonly used as secondary source of theobromine and caffeine, as fertilizer and as packaging material (ARLORIO et al., 2005). There is a compelling reason to come up with a more useful and profitable outlet for this by-product. Cocoa hulls have a high content in soluble fibre and polyphenolic compounds. The interest in functional foods and the focus on potential health benefits of both compounds, invites the speculation that cocoa bean shell could provide a ready source of inexpensive polyphenol rich dietary fibre (REDGWELL et al., 2003), for example as a supplement to human foods.

Polyphenolic compounds belong to the secondary plant metabolites which have antioxidant activity. They are involved in scavenging free radicals and thus protect the organism from oxidative damages. They have redox properties which allow them to act as reducing agents, hydrogen donators and singlet oxygen quenchers (RICE-EVANS et al., 1997). Polyphenols of cocoa have been reported in many studies as bioactive compounds with antioxidant, anti-radical and anticarcinogenic properties (REN et al., 2003; WOLLGAST and ANKLAM, 2000; SANBONGI et al., 1998). They have been shown to protect against diseases like coronary heart disease, cancer, neurodegenerative disorders, mostly through their antioxidant and free radical scavenging capacities (WAN et al., 2001; BRAVO, 1998). Cocoa polyphenols have been suggested to positively influence cardiovascular health through inhibition of lipid peroxidation, platelet activation or cyclooxygenase and lipoxygenase activities, and enhancing levels of the endothelial-derived relaxing factor, nitric oxide (SCHEWE et al., 2002; KARIM et al., 2000; REIN et al., 2000).

Polyphenolic compounds of cocoa are mainly flavan-3-ols (monomeric epicatechin and catechin, as well as their oligomers from dimers to decamers, the procyanidins), with small amounts of anthocyanins (mainly cyanidin glycosides) and flavonols (quercetin glycosides) (ADAMSON et al., 1999; HAMMERSTONE et al., 1999). (-)Epicatechin is quantitatively the main compound of cocoa polyphenols (approximately 35 % of polyphenol content of unfermented Forastero cocoa beans). Total soluble polyphenol content of good fermented dried cocoa beans ranges from 2 to 6 %, strictly depending on the variety as well as on the geographic origin. Forastero typical content is about 6 %; soluble polyphenols content of Criollo cocoa is about 2/3 of Forastero (ARLORIO et al., 2005).

Despite the changes in polyphenol content during processing a considerable antioxidant activity is preserved in cocoa and chocolate, since epicatechin and catechin are heat-resistant. Especially the low-molecular weight derivatives which consist of only two or three catechin- respectively epicatechin-units have a very good bioavailability in human organism (BITSCH, 2001). They are decomposed as catechin and epicatechin in the upper small intestine which can be absorbed through the intestinal wall for transport via the blood (SPENCER et al., 2001). Evidence exists that catechin is absorbed by the human gut (DAS, 1971), and other investigations involving oral administration of 3-O-methyl-catechin in three volunteers showed that the supplement occurred in plasma (HACKETT et al., 1985). Furthermore, SERAFINI et al. (2003) reported an increase of (-) epicatechin after consumption of dark chocolate in blood plasma of 12 tested volunteers.

Besides flavonoids, the main cocoa hulls component is total dietary fibre, principally composed by insoluble fibre and also containing pectin characterized by high methylation degree. The methoxylation degree was found to be 60.53 % (ARLORIO et al., 2001). Pectin is an important polysaccharide with many applications in the food and pharmaceutical industries.

According to the work of LECUMBERRI et al. (2007) total dietary fibre content of cocoa hulls was extremely high, over 60 % of the dry matter. As for the constituent fractions, soluble dietary fibre accounted for less than 17 % of the total dietary fibre content, made of mainly pectic substances (11.78 % of the total dietary fibre). Quantitatively, insoluble dietary fibre was the main component of the

hulls, accounting for over 80 % of the total dietary fibre and 50 % of the total dry weight.
Dietary fibre is known to be beneficial for the human health and body function, thus a consumption of dietary fibre is associated with a reduced incidence of disorders and diseases common in developed countries such as chronic bowel disorders, obesity, diabetes, cardiovascular disease and cancer (JOHNSON, 2004; KRIS-ETHERTON et al., 2002; BESSESEN, 2001; SUNGSOO CHO and DREHER, 2001).
Considering the health benefits associated to the consumption of dietary fibre and polyphenols in the diet, the presence of both bioactive components in cocoa hulls differing in their origin could highlight the interest of such a product as a potential ingredient for the functional food industry. Therefore, the aim of the study was to determine the contents of polyphenols and water soluble pectins as well as antioxidant activity from cocoa hulls of different proveniences.

Materials and Methods

Samples and chemical analyses

Cocoa hulls were provided by Kakao Verarbeitung Berlin (Berlin, Germany). Different cocoa hull proveniences were obtained from pre-roasted cocoa beans from Madagascar, Ghana, Trinidad, Venezuela and Ecuador. The different cocoa types were Trinitario (Madagascar, Trinidad, Venezuela), Forastero (Ghana) and Nacional „Arriba" (Ecuador). Cocoa hulls were grinded and sieved before the analysis; the powders obtained were directly used for the chemical characterisation (contents of total polyphenols and water soluble pectin, antioxidant activity).

Total polyphenol content and antioxidant activity

For the determination of the antioxidant activity and total phenolic content 0.5 g freeze-dried fruit samples were extracted in 10 mL of the cooled solvent 0.1 % HCl/methanol (15/85, v/v).
The quantitative determination of polyphenols was based on the Folin-Ciocalteu's reagent according to the method by JENNINGS (1981). The samples were spectrophotometrically measured at 765 nm. Polyphenols were expressed as gallic acid equivalents (GAE) using a calibration curve.
The antioxidant activity of the extract was measured by electron spin resonance (ESR) spectroscopy (RÖSCH et al., 2003). The efficiency of an antioxidant compound is expressed as its ability to reduce a synthetic free radical species, Fremy's salt. The antioxidant capacity indicates the quantity of Fremy's salt (mol), which is reduced by 1 mol of antioxidant compound.

Content of water-soluble pectin

Cell wall extraction was conducted as described by BLUMENKRANTZ and ASBOE-HANSEN (1973) and modified by HUYSKENS (1991). Freeze-dried samples were washed with an acetone/ethanol mixture (70/30, v/v) and dried for 24 h at 70 °C. For the extraction of water soluble pectins cell wall material was mixed with destilled water and dissolved by stirring for 1 h. Thereafter samples were brought to a pH value of 4.5 and the enzyme pectinase (20 µg) (Pectinex ultra SP L, Novo Nordisk Ferment, Switzerland) was added. Subsequently samples were stirred for 1 h, centrifuged for 10 min at 4°C and 11000 rpm as well as filtered afterwards. The colorimetric determination of the water soluble pectin fraction was conducted according to the method of MCCOMB and MCCREADY (1952). Thereafter, the amount of galacturonic acid of water soluble pectin was measured spectrophotometrically at 520 nm. D-Galacturonic acid was used as a standard.

Statistics

Each origin was analysed in quadruplicate, except the samples from Ghana (in triplicate). Statistical analysis (ANOVA) was performed by Statgraphics software (2006, Rockville, Maryland, USA). The multiple-range test after Bonferroni was used to assess difference between means respectively the test of Nemenyi and Dunn in the case of variance heterogeneous. For correlation analysis the simple regression analysis was used. A significant difference was considered at the level of $p < 0.05$. All data were expressed as mean ± standard deviation.

Results and Discussion

The average total content of polyphenolic compounds of cocoa hulls ranged from 2.56 to 4.06 mg GAE g^{-1} DM (Fig. 1). The highest polyphenol content revealed the cocoa hulls from Madagascar with 4.06 mg GAE g^{-1} DM, followed by Trinidad, Ghana and Venezuela. There was a significant difference between the hulls from Madagascar and Ecuador which had the lowest polyphenol content with 2.56 mg GAE g^{-1} DM (Fig. 1).

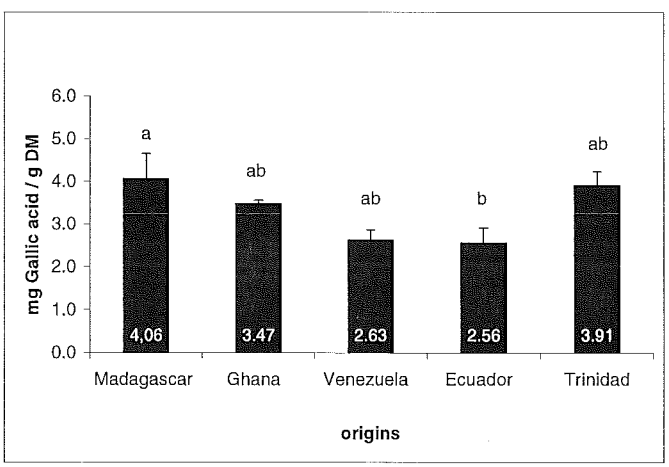

Fig. 1: Total polyphenol content of cocoa hulls extract. Gallic acid was used as standard. Mean values ± standard deviation (n=4). Means with different letters were significantly different at the level of $p < 0.05$ (Bonferroni-test).

Furthermore, the hulls from Venezuela and Madagascar showed the highest pectin content of the water soluble fraction with 25.22 and 24.90 mg GAL g^{-1} DM. They differed significantly from the hulls from Trinidad with a content of only 13.23 mg GAL g^{-1} DM (Fig. 2). The highest antioxidant activity showed the cocoa hulls from Madagascar and Trinidad with 0.035 mmol Fremy's salt g^{-1} DM. They differed significantly from the hulls from Venezuela and Ecuador. The hulls from Ecuador had the lowest value with 0.023 mmol Fremy's salt g^{-1} DM (Fig. 3). There was a strong correlation between antioxidant activity of the methanolic extract and total polyphenol content (r=0.87) suggesting that polyphenolic compounds are responsible for the antioxidant activity.

There are several internal and external factors affecting the quality and quantity of polyphenolic compounds in plants. These include the genetic (varietal and regional) diversity as well as many environmental factors, i.e. growing conditions such as light intensity, humidity, temperature, the use of fertilizers, wounding, infections or other stress factors (STINTZING and CARLE, 2004; VALLEJO et al.,

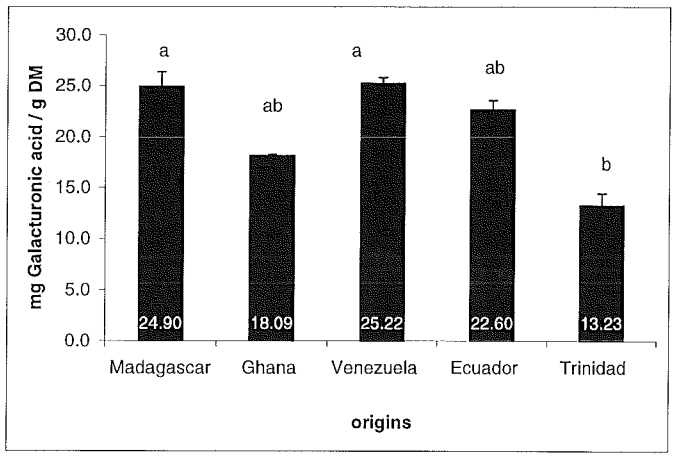

Fig. 2: Content of water-soluble pectin in cocoa hulls. Galacturonic acid was used as standard. Mean values ± standard deviation (n=4). Means with different letters were significantly different at the level of $p < 0.05$ (Bonferroni-test).

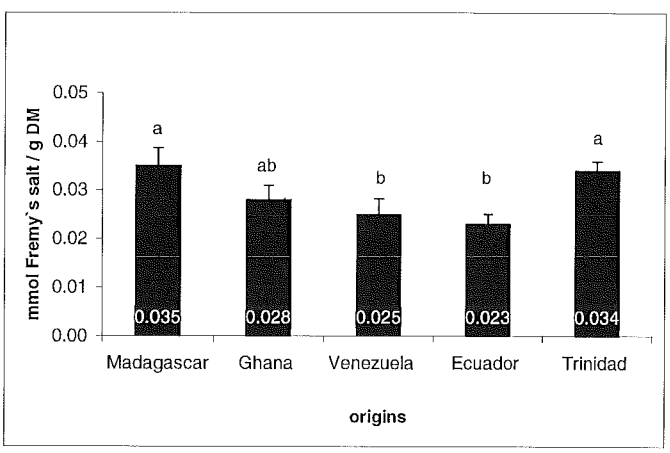

Fig. 3: Antioxidant activity of cocoa hulls extract measured by using ESR spectroscopy. Mean values ± standard deviation (n=4). Means with different letters were significantly different at the level of $p < 0.05$ (Bonferroni-test).

2003; CABRITA et al., 2000; CHALKER-SCOTT, 1999; MACHEIX et al., 1990). Flavonoids are beneficial for the plant itself as physiological active compounds, as stress protecting agents, as attractants or as feeding deterrents, and, in general by their significant role in plant resistance. The defence-related flavonoids are synthesised by plants in response to physical injury, infection, or stress. They may also be constitutively synthesised but, additionally, their biosynthesis is often enhanced under the influence of several types of stress. These preformed compounds are often stored at strategically important sites where they may play a signalling and/or a direct role in defence (TREUTTER, 2006).

The climate on the east and north-west coast of Madagascar is characterized by strong precipitation (2000-4000 mm). Cocoa trees react to water stress more sensitive than other tropical plants, like coffee, bananas or citrus (WOOD and LASS, 1985, p.71). High precipitation causes a strong washing out of the soil and an accumulation of aluminium and ferric oxides as well as an increased danger of fungal diseases like phytophtora fruit rot. Flavonoids may help plants to live on soils which are rich in toxic metals such as aluminium (BARCELÓ and POSCHENRIEDER, 2002).

Although polyphenolic compounds usually accumulate in the outer parts of plants such as shells and skins (BRAVO, 1998) there is scarce information on the polyphenol content of cocoa hulls. In previous studies (ARLORIO et al., 2001) a mean of 1.8 % of total polyphenol compounds was detected by means of Folin Ciocalteu's method. In a recent study by ARLORIO et al. (2005) the polyphenol composition of the hulls was determined. The major components in hulls were epicatechin and p-hydroxybenzoic acid (2753 and 2252 ppm). The HPLC method used in this study showed the presence of some minor "unknown" compounds, supposed to be anthocyanins and proanthocyanidins. Also these compounds are reported as strong *in vitro* antioxidants (ADAMSON et al., 1999).

The high polyphenol content of the cocoa hulls from Madagascar (4.06 mg GAE g^{-1} DM) could be attributed to the stress factors the cocoa tree was exposed to. Also permanently high day temperatures over 30 °C are a stress factor for the cocoa tree. This is reported by MURRAY and SPURLING (1964) conducting experiments on Trinidad which showed that a constant temperature of 31 °C leads to a loss of apical dominance. This does not occur when the temperatures vary during the day, i.e. in Ghana (WOOD and LASS, 1985, p. 54). Thus the high day temperature of about 30 °C could have caused the high polyphenol content (3.91 mg GAE g^{-1} DM) in the cocoa hulls from Trinidad.

Fermentation and roasting conditions also have a great influence on the content of polyphenols. A high content of polyphenolic compounds in the cocoa hull is probably attributed to an optimal fermentation time. During fermentation polyphenols diffuse with cell liquids from their storage sites and are subjected to oxidation, polymerization, and binding to proteins; so the content of catechins and soluble polyphenols is lowered during fermentation (CALIGIANI et al., 2007). Therefore, major content of soluble polyphenols of dried cocoa beans are often an index of bad fermentation (ARLORIO et al., 2005). In the present study the cocoa hulls from Ecuador had the lowest content of polyphenols. This could be due to the short fermentation applied (WOOD and LASS, 1985). So the origin of the cocoa beans is an important influencing factor. A study done by NATSUME et al. (2000) reported that polyphenol content in cocoa liquor varied with the country of origin. Furthermore, the polyphenol content of cocoa depends on cocoa bean variety (genotype). Fine varieties with short fermentation processes (Madagascar, Trinidad, Venezuela) proved to contain more procyanidins, while bulk cocoa liquors extracts from Africa (Ghana) which are highly fermented proved very poor in such compounds (COUNET et al., 2004).

Water soluble pectins are affected by the interaction of genetic, physiological, agronomic (position of pods on the tree) and environmental factors. The analyses showed that the content of water soluble pectin was not dependent on the cocoa type because the hulls from Venezuela (25.22 mg GAL g^{-1} DM) and the hulls from Trinidad (13.23 mg GAL g^{-1} DM), which differed significantly in their pectin content, belong to Trinitario cocoa types. Comparable literature values for water soluble pectin in cocoa hulls are scare. ARLORIO et al. (2001) detected a content of water soluble pectin in raw hulls of 46.67 g kg^{-1}. The content of water soluble pectin is mainly influenced by external factors (climate and soil). Intensive radiation, high day temperature and low night temperature and sufficient water supply promote an accumulation of pectic substances (CAESAR, 1986). Such climate conditions exist in Venezuela and Madagascar. The hulls from Venezuela showed with 25.22 mg GAL g^{-1} DM the highest content of water soluble pectin. The cocoa hulls from Trinidad had the lowest content of water soluble pectin (13.23 mg GAL g^{-1} DM). In contrast to the other isles of this region Trinidad is not of volcanic origin and fertile soils are limited (WOOD and LASS 1985). This might be one reason for the low values.

Another reason for different contents of pectic and also polyphenolic substances is the high heterogeneity between individuals derived from one cross, and heterogeneous transmission of genetic traits to the progeny, especially in Ghana. Uniform seeds from firms are often too expensive. In most cases seeds are heterogeneous because of the generative propagation with seeds of cocoa fruits from the plantation and from other growers of the neighbourhood. These regional adaptations result in differences for trade and production. There are differences in cocoa types and varieties as well as in isolated populations. However, the raw cocoa products are typical for the growing country itself (EICHHOLZ, 2000). Therefore, the cocoa provenience is an important quality parameter.

In the present study antioxidant activity was influenced by the polyphenol content. The analysis showed a strong correlation between antioxidant activity and polyphenol content (r=0.87). Also other studies showed a correlation between antioxidant activity and polyphenol content (NAGAI et al., 2003; YANG et al., 2002; VELIOGLU et al., 1998). Thus, it is suggested that polyphenolic compounds present in cocoa extracts have strong scavenging ability. The polyphenol composition of hulls extract was determined in previous studies. All polyphenolic compounds identified in hulls extract were described as antioxidant or antiradical molecules (COISSON et al., 2003; AZIZAH et al., 1999). This could be due to the antioxidant mechanisms of polyphenolic compounds towards free radicals. The redox potential of polyphenolic compounds plays an important role in determining the antioxidant capacity (RICE-EVANS et al., 1997). In cocoa beans, antioxidant compounds soluble in water could be (-)-epicatechin, (+)-catechin, and quercetin (SANBOGI et al., 1997). RÖSCH et al. (2003) reported that the flavan-3-ols, catechin and epicatechin, were excellent radical scavengers because of their chemical structure. ARLORIO et al. (2005) supposed that some oligomeric polyphenols as procyanidins (as well as some not-polymerized anthocyanins) could be the bioactive molecules involved in damage protection.

Conclusion

Cocoa hulls exhibited a high content of total polyphenols and water-soluble pectins. They appear to be a good and inexpensive source of these bioactive compounds that might be isolated and added to chocolate with higher antioxidant activity. Moreover, they have a low content of fat compared to cocoa beans with a fat content of about 50 %. Together with a low content of soluble sugars cocoa hulls are an optimal ingredient for functional food low in calories and rich in dietary fibre (LECUMBERRI et al., 2007). For this reason, we suggest to perform further analysis (HPLC) aiming to identify all polyphenolic compounds in the cocoa hulls extract for their safe use in food technology.

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References

ADAMSON, G.E., LAZARUS, S.A., MITCHELL, A.E., PRIOR, R.L., CAO, G., JACOBS, P.H., 1999: HPLC method for the quantification of procyanidins in cocoa and chocolate samples and correlation to total antioxidant capacity. J. Agric. Food Chem. 47, 4184-4188.

ARLORIO, M., COISSON, J.D., RESTANI, P., MARTELLI, A., 2001: Characterization of pectin and some secondary compounds from *Theobroma cacao* hulls. J. Food Sci. 66, 653-656.

ARLORIO, M., COISSON, J.D., TRAVAGLIA, F., VARSALDI, F., MIGLIO, G., LOMBARDI, G., MARTELLI, A., 2005: Antioxidant and biological activity of phenolic pigments from *Theobroma cacao* hulls extracted with supercritical CO_2. Food Res. Int. 38, 1009-1014.

AZIZAH, A.H., NIK RUSLAWATI, N.M., SWEE TEE, T., 1999: Extraction and characterization of antioxidant from cocoa by-products. Food Chem. 64, 199-202.

BARCELÓ, J., POSCHENRIEDER, C., 2002: Fast root growth responses, root exudates, and internal detoxification as clues to the mechanism of aluminium toxicity and resistance: a review. Environ. Exp. Bot. 48, 75-92.

BESSESEN, D.H., 2001: The role of carbohydrates in insulin resistance. J. Nutr. 131, 2782-2786.

BITSCH, I., 2001: Kakao und Schokolade: gut für die Gesundheit. Moderne Ernährung heute 3, 1-4.

BLUMENKRANTZ, N., ASBOE-HANSEN, G., 1973: New method for quantitative determination of uronic acids. Anal. Biochem. 34, 484-489.

BRAVO, L., 1998: Polyphenols: chemistry, dietary sources, metabolism and nutritional significance. Nutr. Rev. 56, 317-333.

CABRITA, L., FOSSEN,T., ANDERSEN, O.M., 2000: Colour and stability of the six common anthocyanidin 3-glucosides in aqueous solutions. Food Chem. 68, 101-107.

CAESAR, K., 1986: Einführung in den tropischen und subtropischen Pflanzenbau. DLG-Verlag, Frankfurt (Main).

CALIGIANI, A., CIRLINI, M., PALLA, G., RAVAGLIA, R., ARLORIO, M., 2007: GC-MS detection of chiral markers in cocoa beans of different quality and geographic origin. Chirality 19, 329-334.

CHALKER-SCOTT, L., 1999: Environmental significance of anthocyanins in plant stress responses. Photochem. Photobiol. 70, 1-9.

COISSON, J.D., PAPASSO, M., TRAVAGLIA, F., PIANA, G., ARLORIO, M., MARTELLI, A., 2003: Proprietà antiossidanti di estratti fenolici da sottoprodotti di lavorazione di cacao e nocciola. Proceedings of 5th National Congress of Food Chemistry, Parma, Morgan Edizioni tecniche, Milano, 154-158.

COUNET, C., OUWERX, C., DELPHINE, R., COLLIN, S., 2004: Relationship between procyanidin and flavor contents of cocoa liquors from different origins. J. Agric. Food Chem. 52, 6243-6249.

DAS, N.P., 1971: Studies on flavonoid metabolism: absorption and metabolism of (+)-catechin in man. Biochem. Pharmacol. 20, 3435-3445.

EICHHOLZ, I., 2000: Die wirtschaftliche Bedeutung des Kakaoanbaus und ausgewählter Nutzhölzer in Ghana unter phytosanitären Gesichtspunkten. Unpublished Master's thesis, Humboldt Universität zu Berlin, 2000.

HACKETT, A.M., GRIFFITHS, L.A., WERMEILE, M., 1985: The quantitative disposition of 3-O-methyl-(+)-(U-^{14}C) catechin in man following oral administration, Xenobiotica 15, 907-914.

HAMMERSTONE, J.F., LAZARUS, S.A., MITCHELL, A.E., RUCKER, R., SCHMITZ, H.H., 1999: Identification of procyanidins in cocoa (*Theobroma cacao*) and chocolate using high-performance liquid chromatography/mass spectrometry. J. Agric. Food Chem. 47, 490-496.

JENNINGS, A.C., 1981: The determination of dihydroxy phenolic compounds in extracts of plant tissues. Anal. Biochem. 118, 396-398.

JOHNSON, I., 2004: New approaches to the role of diet in the prevention of cancers of the alimentary tract. Mutat. Res. 551, 9-28.

KARIM, M., MC CORMICK, K., KAPPAGODA, T.C., 2000: Effects of cocoa extracts on endothelium dependent relaxation. J. Nutr. 130, 2105-2108.

KRIS-ETHERTON, P.M., HECKER, K.D., BONANOME, A., COVAL, S.M., BINKOSKI, A.E., HILPERT, K.F., 2002: Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. Am. J. Med. 113, 71-88.

LECUMBERRI, E., MATEOS, R., IZQUIERDO-PULIDO, M., RUPÉREZ, P., GOYA, L., BRAVO, L., 2007: Dietary fibre composition, antioxidant capacity and physico-chemical properties of a fibre-rich product from cocoa (*Theobroma cacao* L.). Food Chem. 104, 948-954.

MACHEIX, J.J., FLEURIET, A., BILLOT, J., 1990: Fruit phenolics. CRC Press, Boca Raton, FL, 192-217.

MC COMB, E.A., MC CREADY, R.M., 1952: Colorimetric determination of pectic substances. Anal. Chem. 24, 1630-1632.

NATSUME, M., OSAKABE, N., YAMAGISHI, M., TAKIZAWA, T., NAKAMURA, T.,

MIYATAKE, H. et al., 2000: Analyses of polyphenols in cacao liquor, cocoa and chocolate by normal-phase and reversed-phase HPLC. Bioscience Biotech. Biochem. 64, 2581-2587.

REDGWELL, R., TROVATO, V., MERINAT, S., CURTI, D., HEDIGER, S., MANEZ, A., 2003: Dietary fibre in cocoa shell: characterization of component polysaccharides. Food Chem. 81, 103-112.

REIN, R.D., PAGLIERONI, T.G., WUN, T., PEARSON, D.A., SCHMITZ, H.H., GOSSELIN, R., KEEN, C.L., 2000: Cocoa inhibits platelet activation and function. Am. J. Clin. Nutr. 72, 30-35.

REN, W., QIAO, Z., WANG, H., ZHU, L., ZHANG, L., 2003: Flavonoids: promising anticancer agents. Med. Res. Rev. 23, 519-534.

RICE-EVANS, C.A., MILLER, N.J., PAGANGA, G., 1997: Antioxidant properties of phenolic compounds. Trends in Plant Sci. 4, 152-159.

RÖSCH, D., BERGMANN, M., KNORR, D., KROH, L.W., 2003: Structure-antioxidant efficiency relationships of phenolic compounds and their contribution to the antioxidant activity of Sea Buckthorn juice. J. Agric. Food Chem. 51, 4233-4239.

SANBONGI, C., OSAKABE, N., NATSUME, M., TAKIZAWA, T., GOMI, S., OSAWA, T., 1998: Antioxidative polyphenols isolated from *Theobroma cacao*. J. Agric. Food Chem. 46, 454-457.

SANBOGI, C., SUZUKI, N., SAKANE, T., 1997: Polyphenols in chocolate, which have antioxidant activity, modulate immune function in human in vitro. Cell. Immun. 177, 129-136.

SCHEWE, T., KUHN, H., SIES, H., 2002: Flavonoids of cocoa inhibit recombinant human 5-lipoxygenase. J. Nutr. 132, 1825-1829.

SERAFINI, M., BUGIANESI, R., MAIANI, G., VALTUENA, S., DE SANTIS, S., CROZIER, A., 2003: Plasma antioxidants from chocolate. Nature 424, 1013.

SPENCER, J.P., SCHROETER, H., SHENOY, B., SRAI, S.K., DEBNAM, E.S., RICE-EVANS, C., 2001: Epicatechin is the primary bioavailable form of the procyanidin dimers B2 and B5 after transfer across the small intestine. Biochem. Biophys. Res. Commun. 285, 588-593.

STINTZING, F.C., CARLE, R., 2004: Functional properties of anthocyanins and betalains in plants, food, and in human nutrition. Trends Food Sci. Techn. 15, 19-38.

SUNGSOO CHO, S., DREHER, M.L., 2001: Handbook of dietary fibre. New York, Marcel Dekker Inc.

TREUTTER, D., 2006: Control and optimisation of secondary plant metabolites; accompanying reading, Bologna, 2006: significance of flavonoids in plant resistance.

VALLEJO, F., TOMÁS-BAEBERÁN, F.A., GARCIA-VIGUERA, C., 2003: Effect of climatic and sulphur fertilisation conditions on phenolic compounds and vitamin C in the inflorescences of eight broccoli cultivars. Eur. Food Res. Techn. 216, 395-401.

VELIOGLU, Y.S., MAZZA, G., GAO, L., OOMAH, B.D., 1998: Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. J. Agric. Food Chem. 46, 4113-4117.

WAN, Y., VINSON, J.A., ETHERTON, T.D., PROCH, J., LAZARUS, S.A., KRIS-ETHERTON, P.M., 2001: Effects of cocoa powder and dark chocolate on LDL oxidative susceptibility and prostaglandin concentrations in humans. Am. J. Clin. Nutr. 74, 596-602.

WOLLGAST, J., ANKLAM, E., 2000: Polyphenols in chocolate: is there a contribution to human health? Food Res. Int. 33, 449-459.

WOOD, G.A.R., LASS, R.A., 1985: Cocoa. Fourth Edition, Longman Group Limited, London.

YANG, J.H., LIN, H.C., MAU, J.L., 2002: Antioxidant properties of several commercial mushrooms. Food Chem. 77, 229-235.

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