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ANALYSIS OF THE EFFECT OF TECHNOLOGICAL PROCESSING ON CHANGES IN ANTIOXIDANT PROPERTIES OF COCOA PROCESSED PRODUCTS

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Key words: antioxidants, cocoa, chocolate, chocolate production

Antioxidant properties were studied by determining the ability to deactivate stable two chromogen radicals – 1.1-dipheny-2-picrylhydrazyl (DPPH·), [2.2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] ($ABTS^{\bullet+}$) as well as inhibition of reactions that yield peroxides in emulsions of linoleic acid and chelation of iron.

The study demonstrated the influence of technological processing on changes in antioxidant properties of cocoa processed products. Cocoa beans were characterised by the highest whereas cocoa butter by the lowest content of polyphenols. This study demonstrated that the products under investigation exhibited antioxidant properties in relation to $ABTS^{\bullet+}$ and $DPPH^{\bullet}$. Extracts were more effective in scavenging $ABTS^{\bullet+}$ than $DPPH^{\bullet}$. Extracts from cocoa beans, cocoa mass as well as dark chocolate were characterised by very high capacity (at 100% level) to inhibit the formation of peroxides in emulsion systems. Dilution of extracts had an impact on the antioxidant properties of the products under investigation. Correlations were found between the total content of polyphenols in cocoa products and the ability of extracts to scavenge $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ as well as to chelate iron and inhibit peroxide production in emulsion systems.

INTRODUCTION

Natural antioxidants are compounds found in edible products, mainly of plant origin. Tocopherols, tocotrienols, carotenoids, ascorbic acid, polyphenols, some amino acids and dipeptides are some of the antioxidants present in foods. The presence of antioxidants in foods favours the maintenance of sensory properties and limits losses of nutrients in products.

Their use is particularly important in fat-containing products. The final step of fat peroxidation, *i.e*. autooxidation, which is accompanied by the production of rancid flavours can be delayed by the addition of antioxidants to oils and fats.

A large interest in cocoa processed products, mainly chocolate, is related to its content of natural antioxidants [Zumbe, 1998]. These compounds are responsible not only for biochemical processes and the development of sensory properties of chocolate during production, but also play significant physiological functions. The most abundant flavonoids in cocoa and chocolate are flavanoles present in the form of monomers (catechin and epicatechin) and oligomers, known as proanthocyanidins. The content of polyphenols in cocoa products depends on the degree of cocoa ripeness, variety, technological processes as well as storage; they account for 6-8% of cocoa bean dried weight. A large reduction in the amount of polyphenols is seen during the process of chocolate production from cocoa beans. The change in polyphenol content is related mainly to cocoa bean fermentation and the process of its drying. Fermentation and drying of cocoa beans leads to the hydrolysis of anthocyanins to anthocyanidins that later condensed with catechins, yielding tannins, which give cocoa beans their characteristic brown colour. Appropriate process of fermentation and drying plays an important role in the development of desirable precursors of taste and flavour [Hansen *et al*., 1998; Knight, 2001].

Changes in the content and composition of polyphenols during the production of chocolate, mainly during roasting, bruising, milling and conging process, are a result of high temperature and the presence of oxygen which accelerate oxidative processes. High temperatures and/or long exposure to heat as well as alkalization process decrease the content of polyphenols in cocoa processed products.

Polyphenols are responsible for the characteristic pungent and bitter tastes developed during roasting [Luna *et al*., 2000]. Depending on the method of production applied during production, cocoa powder may contain about 10% flavonoids per dry matter. Dark chocolate contains more cocoa mass than white chocolate and it is the reason why it has more flavonoids.

Likewise in the case of many vitamins, time and temperature in combination with other production processes (for example, alkalization) can decrease the amount of flavonoids in chocolate. However, the application of appropriate technological processes can decrease the loss of these compounds in semi-finished and final products [Becket, 1994].

The aim of the study was to analyse the influence of technological processing on changes in antioxidant properties of

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TABLE 1. The amount of polyphenols calculated as gallate (standard), (mg/100 g product).

Total amount of polyphenols (mg) in 100 g product					
Study series	Cocoa beans	Cocoa mash	Cocoa butter	Dark chocolate	Milk chocolate
1	2153.3 ± 0.073	2023.5 ± 0.118	8.8 ± 0.004	1222.2 ± 0.004	561.2 ± 0.0029

cocoa processed products. The scope of the study involved the determination of the total amount of polyphenols, the ability of preparations to deactivate stabile free radicals ($DPPH^{\bullet}$), cationic radicals ($ABTS^{\bullet+}$) as well as inhibition of reactions that yield peroxides in emulsions of linoleic acid and chelation of iron.

MATERIALS AND METHODS

The studied material included cocoa processed products: cocoa beans, cocoa butter, cocoa mash as well as white and dark chocolate obtained from the same batch. Cocoa beans were obtained from Ivory Coast. Products were stored in original sealed containers (white and dark chocolate) or in polyethylene bags (cocoa beans, cocoa mash and cocoa butter) at a temperature of about 15°C and relative humidity of 71% without access to light. Characteristics of the studied material were carried out in extracts obtained from the abovementioned cocoa processed products. Chemical analysis was performed at least in triplicate.

Before the appropriate estimation, a preliminary test was conducted to determine the optimal amount of solvent needed for extraction. To this end water, acetone as well as methanol were used, and the antioxidant properties of the extracts were analysed against $DPPH^{\bullet}$. On the basis of the results of preliminary tests, acetone was chosen as the solvent.

The determination of the amount of total polyphenols was carried out according to Folin-Ciocalteu assay was used as well. Extraction was done with the aid of water and acetone. Absorbance was measured with a Shimadzu spectrophotometer UV-1200 V at the wavelength of 750 nm.

Free radical scavenging of the extracts against $DPPH^{\bullet}$ was performed according to Saint Criq de Gaulejac *et al.* [1999]. Absorbance was measured with a Shimadzu spectrophotometer UV-1200 V at the wavelength of 750 nm.

Antioxidant activity of extracts against $ABTS^{\bullet+}$ was determined according to Re *et al.* [1999]. Absorbance was measured with a Shimadzu spectrophotometer UV-1200 V at the wavelength of 734 nm. The spectrophotometer was calibrated with acetone. The activity of extracts was expressed as mmol/L Trolox with the capacity to deactivate $ABTS^{\bullet+}$ by the extracts.

The determination of the preparations capacity to inhibit the formation of peroxides in emulsion of linoleic acid was conducted according to Kuo *et al*. [1999]. Absorbance was measured at the wavelength of 480 nm against the blank test.

The capacity of the extracts to chelate iron was assessed according to Lai *et al*. [2001]. Absorbance was measured with a Shimadzu spectrophotometer UV-1200 V at the wavelength of 562 nm. Results were expressed in μg of chelated Fe^{2+} by 100 mL of the baseline infusion.

Mean values and standard deviation were calculated with the use of Microsoft Excel 2003. Statgraphics plus ver. 4.0 for Windows was used for the estimation of the influence of a technological process on changes in the examined products.

RESULTS AND DISCUSSION

Changes in the amount and composition of polyphenols during the production of chocolate, mainly during roasting, bruising, milling and conging process are a result of high temperature and the presence of oxygen which accelerate oxidative processes.

The polyphenol content of extracts were calculated based on the final values of absorbance as well as standard curves prepared from gallate solution and later converted per 100 g of product (Table 1). The extract obtained from cocoa beans was characterised by the highest amount of polyphenols, ranging from 2153.3 to 2234.7 mg/100 g product.

Wollgast & Anklam [2000] showed a decrease in the polyphenol content of cocoa beans and chocolate as a result of technological process of production. Generally, the amount of polyphenols in cocoa beans after fermentation and drying is about 6-8% [Zumbe, 1998]. A rise in temperature during roasting, from 127°C to 181°C brought about a decline, from 24.618 to 12.786 mg/g in the polyphenol content of cocoa mash in studies by Kealey *et al*. [1998]. Statistical analysis did not reveal any significant changes in the content of polyphenols in the examined products as a result of storage.

Radical scavenging activity of extracts was assessed in relation to $DPPH^{\bullet}$. The capacity to deactivate $DPPH^{\bullet}$ was determined for products which were prepared as a baseline extract (characterised by a high content of compounds responsible for radical scavenging) as well as diluted extract 1:10 and 1:100 (to demonstrate a significant effect of extract dilution on radical scavenging). Extracts prepared in such a way were used in subsequent analyses of their effects in scavenging $ABTS^{\bullet+}$.

Radical scavenging activity of extracts was calculated on the basis of the final values of absorbance. Cocoa butter was characterised by the lowest capacity to scavenge $DPPH^{\bullet}$. Figure 1 shows changes in the $DPPH^{\bullet}$ content measured with a spectrophotometer at the wavelength of 517 nm in the presence of baseline extracts as well as dilutions in the ratio 1:10 and 1:100.

Higher radical scavenging activity was found for baseline extracts compared to diluted extracts.

After dilution at the ratio of 1:10, the extract obtained from cocoa beans was the most effective (70%) in scavenging chromogen radicals. Dilution brought about a decrease by 50% in radical scavenging activity of the extract from dark chocolate and by 60% in the case of milk chocolate. The capacity of cocoa butter extract to deactivate $DPPH^{\bullet}$ decreased by 55% after dilution of the baseline extract.

Further dilution of the baseline extracts, that is, 1:100 induced a greater fall in their radical scavenging activity, and

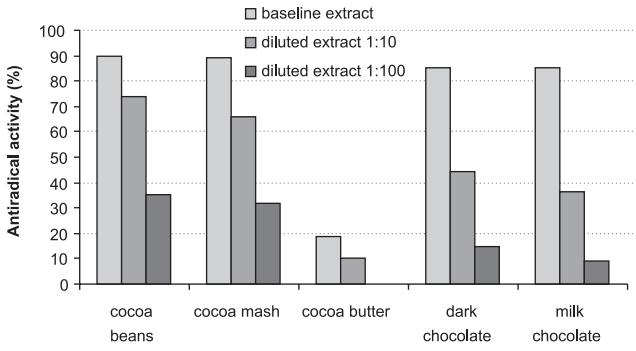

FIGURE 1. Changes in scavenging activities of extracts against DPPH$^{\bullet}$ depending on the degree of dilution.

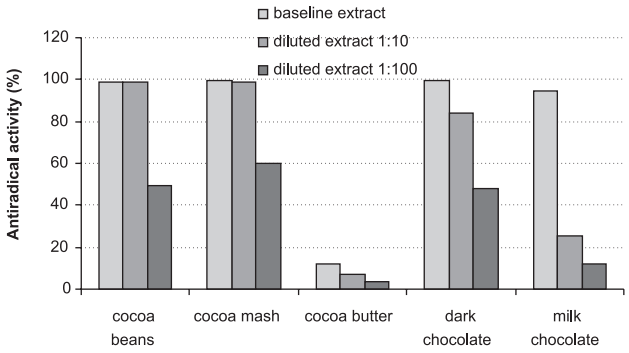

FIGURE 2. Changes in scavenging activities of extracts against ABTS$^{\bullet+}$ depending on the degree of dilution.

cocoa butter at this degree of dilution did not demonstrate any antioxidant properties. Cocoa bean extracts were characterised by the highest capacity to scavenge chromogen radicals (36%) followed by cocoa mash (31.9%). Extracts obtained from dark chocolate and milk chocolate scavenged DPPH$^{\bullet}$ at the level of 15 and 9%, respectively.

The radical scavenging activity of cocoa and cocoa processed products, except for cocoa butter, is high and similar to that of infusions of semi-fermented Oolong and Pu-erh tea, which was found to be at the level of 70 and 67.1-67.5%, respectively [Fik & Zawiślak, 2004]. Duration of the storage of cocoa beans, cocoa butter as well as milk chocolate did not have a significant on their ability to scavenge DPPH$^{\bullet}$ However, it affected the ability of cocoa mash and dark chocolate to scavenge this chromagen radical.

The capacity of cocoa processed products to scavenge ABTS$^{\bullet+}$ was also determined in the present study. Figure 2 depicts changes in the content of ABTS$^{\bullet+}$ measured with a spectrophotometer at the wavelength of 734 nm in the presence of baseline extracts as well as their corresponding dilutions in the ratio of 1:10 and 1:100.

Baseline extracts of cocoa beans, cocoa mash as well as dark and milk chocolate had a higher radical scavenging capacities, almost at the level of 100%. Extract from cocoa butter (independent of its source, baseline extract or dilution) was characterised by the lowest antioxidant properties with radical scavenging activity of about 11%. It was noticed that the higher the dilution of cocoa butter, the lower was its antiradical activity.

Dilution of extracts of cocoa beans, cocoa mash as well as dark chocolate did not have any significant impact on their capacity to scavenge free radicals. Antiradical activity of cocoa beans and cocoa mash decreased by 1% after dilution of baseline extract at the level of 1:10; antioxidant activities of dark chocolate dropped by about 15% after dilution (1:10). Extracts of milk chocolate were characterised by the lowest radical scavenging capacities after dilution.

Dilution of extracts at the ratio of 1:100 decreased their capacity to scavenge radicals. At this level of dilution three of the examined extracts, namely, cocoa beans, cocoa mash, and dark chocolate, demonstrated a 45-60% capacity to scavenge radicals. A further decrease in antiradical activity was found for the extract of milk chocolate and cocoa butter at the level of 12 and 3.6%, respectively.

In the present study, the ability of extracts to inhibit the formation of peroxides in emulsions of linoleic acid was determined as well.

The ability to inhibit the formation of peroxides was measured on the basis of the final values of absorbance of baseline extracts and their dilution (1:10) at the wavelength of 480 nm (Figure 3).

Out of the examined extracts two were chosen: baseline extract as well as 1:10 dilution. Because the results obtained for the dilution 1:10 enabled obtaining antioxidant activity at the level of 20-80% for almost all examined products (only cocoa mash had a value above 80%) further dilution was not performed.

Baseline extracts of cocoa beans, cocoa mash as well as dark chocolate were characterised by the highest capacity to inhibit (100%) the formation of peroxides in emulsions. The lowest antiradical activity was found for Cocoa butter.

Cocoa mash extract at 1:10 dilution showed the highest antioxidant activity (about 83%). Extracts of cocoa beans and dark chocolate inhibited the formation of peroxides at the level of 75%. Dilution of 1:10 brought about the greatest changes in milk chocolate extract, where the ability to inhibit peroxide formation decreased by about 60%.

Analysis of the capacity of extracts to chelate iron was carried out by interaction of the different products with $FeCl_2$ as well as by spectrophotometric measurement of non-chelated iron after the formation of coloured complex with ferrozine. On the basis of absorbance values and standard curve, the amount of chelated iron (μmol) was calculated and extrapolated to 100 g of each the products examined.

Results for cocoa beans, cocoa mash and dark chocolate were similar and ranged from 188.97 to 192.4 μmol Fe^{2+}/100 g product. Cocoa butter had lower values, about 157 μmol Fe^{2+}/100 g product (Figure 4).

Higher iron chelating activity was found for cocoa beans extract, cocoa mash as well as dark and milk chocolate compared to cocoa butter, which may be related to their higher polyphenol content. Flavonoids in cocoa beans and cocoa processed products can chelate iron because of the hydroxyl group located in the B ring.

Autooxidation of foods, besides their microbial spoilage, has an impact on their shelf life [Alaiz *et al*., 1999]. Although other macronutrients, such as protein and carbohydrates can be oxidized, the peroxidation of fats and lipids evokes

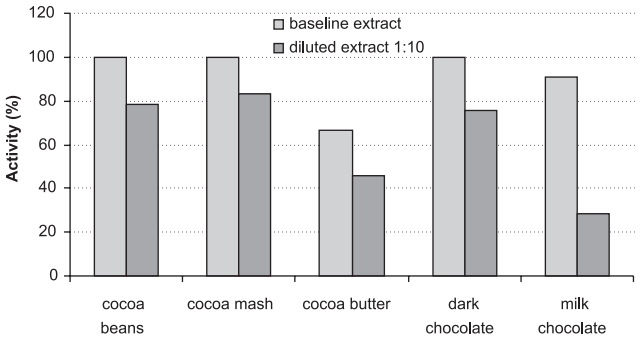

FIGURE 3. Activity of extracts to inhibit peroxide formation in emulsion system depending on the degree of dilution.

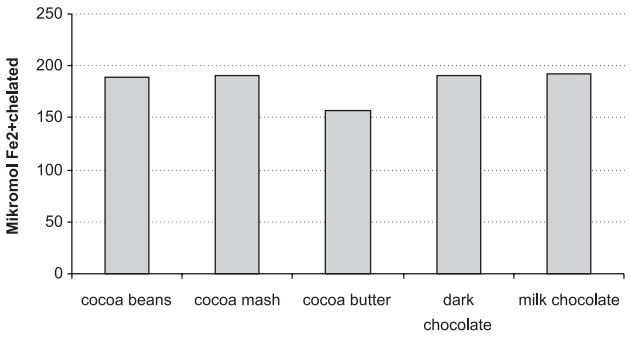

FIGURE 4. The capacity of products to chelate Fe^{2+}/100 g.

more unfavourable changes [Zieliński & Kozłowska, 1999]. Fat oxidation induces changes in sensory attributes, such as aroma, taste, colour and texture, as well as a decrease in the content of nutrients due to degradation of vitamins and essential fatty acids [Alaiz *et al*., 1999]. Consumption of polyphenol-containing products, such as grape juice, chocolate as well as soy products, has been shown to decrease oxidative damage to LDL-cholesterol [Gaspar *et al*., 1994]. Duration of the storage of the examined products did not have any significant influence on their ability to inhibit peroxide formation.

A relationship between the polyphenol content of cocoa beans, cocoa mash as well as dark and milk chocolate and their capacity to scavenge strong chromagen radicals was demonstrated in this study. These products were more effective in scavenging $DPPH^{\bullet}$ than $ABTS^{\bullet+}$. No such a relation was found for cocoa butter, which may be related to its lower flavoinoid content.

CONCLUSIONS

1. Technological processes induced changes in polyphenol content of the examined products. The highest total content of polyphenols was found in cocoa beans and the least in cocoa butter.

2. The examined products exhibited, at different degree, the ability to scavenge chromogen radicals $ABTS^{\bullet+}$ and $DPPH^{\bullet}$. Extracts of the products were more effective in scavenging $ABTS^{\bullet+}$ than $DPPH^{\bullet}$.

3. The ability of cocoa bean extracts to chelate iron was demonstrated at the level of 157.83-192.4 μmol Fe^{2+}/100 g product.

4. Extracts of cocoa beans, cocoa mash and dark chocolate were characterised by a high capacity (100%) to inhibit the formation of peroxides. The lowest capacity was found for cocoa butter (64%).

5. Dilution of extracts brought about changes in their antiradical activity. Extracts with higher concentrations were characterised by higher antioxidant properties.

6. This study showed a correlation between the content of total polyphenols in cocoa beans, cocoa mash as well as dark and milk chocolate and the capcity of extracts to scavenge chromogen radicals $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ as well as to chelate iron and inhibit the formation of peroxides.

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ANALIZA WPŁYWU PROCESU TECHNOLOGICZNEGO NA ZMIANĘ WŁAŚCIWOŚCI PRZECIWUTLENIAJĄCYCH PRODUKTÓW PRZEROBU ZIARNA KAKAOWEGO

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Właściwości przeciwutleniające badano poprzez oznaczenie zdolności do dezaktywacji stabilnych rodników $DPPH^{\bullet}$, kationorodników $ABTS^{\bullet+}$ oraz hamowania reakcji tworzenia nadtlenków w emulsjach kwasu linolenowego i chelatowania jonów żelaza.

Stwierdzono wpływ procesu technologicznego na zmianę zawartość polifenoli w ziarnie kakaowym i produktach jego przerobu. Najwięcej polifenoli zawierało ziarno kakaowe, a najmniej tłuszcz kakaowy. Badane produkty wykazywały wyraźne, choć niejednakowe właściwości przeciwrodnikowe wobec kationorodników $ABTS^{\bullet+}$ i stabilnych rodników $DPPH^{\bullet}$. Ekstrakty wykazywały większą efektywność działania wobec kationorodników $ABTS^{\bullet+}$ w porównaniu do stabilnych rodników $DPPH^{\bullet}$. Ekstrakty ziarna kakaowego, miazgi kakaowej oraz czekolady gorzkiej posiadały bardzo wysoką aktywność (na poziomie 100%) do hamowania tworzenia nadtlenków w układzie emulsyjnym. Rozcieńczenie ekstraktów wpłynęło na aktywność przeciwutleniającą badanych produktów. Wykazano, że istnieje zależność pomiędzy zawartością polifenoli ogółem w badanych produktach, a zdolnością ekstraktów do zmiatania rodników $ABTS^{\bullet+}$ i $DPPH^{\bullet}$ oraz chelatowania jonów Fe^{2+} i hamowania tworzenia nadtlenków w układzie emulsyjnym.