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„Sensory evaluation of commercial dark origin and non-origin chocolates with different cocoa contents using Temporal Dominance of Sensations (TDS)"

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Abbreviations

AOC	Antioxidative Capacity
BMI	Body Mass Index
FMD	Flow-Mediated Dilatation
ICCO	International Cocoa Organization
LDL	Low-density lipoprotein
NFCS	Non-Fat Cocoa Solids
N-OR	Non-Origin
OR	Origin
RDI	Reference Daily Intake
TI	Time-Intensity
TDS	Temporal Dominance of Sensations

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1 Introduction and objectives

Cocoa beans (processed seeds of *Theobroma cacao L.*) and derived products such as chocolate are concentrated sources of flavonoids, a subclass of polyphenols with flavan-3-ols and procyanidins representing the major fractions (Belitz et al, 2008; Steinberg et al, 2003). These compounds are widely acknowledged for eliciting multiple beneficial health effects shown in epidemiological and clinical studies mainly due to their high antioxidative capacity (Dreosti, 2000; Gu et al, 2006; Lee et al, 2003; Miller et al., 2006). The effects include free radical scavenging (Heim et al, 2002), the suppression of plasma lipid oxidation (Vinson et al., 2006), blood pressure lowering (Allen et al., 2008; Buijsse et al., 2010; Grassi et al., 2005; Hooper et al., 2012; Persson et al., 2011), reduction of pulse wave velocity (Crichton et al., 2016; Grassi et al., 2015), improvement in endothelial function/flow-mediated dilation (Grassi et al., 2015; Heiss et al, 2010; Hooper et al., 2012; Schroeter et al., 2006), inhibition of platelet activation and function (Khawaja et al, 2011; Rein et al., 2000) and increased insulin sensitivity (Alkerwi et al, 2016; Grassi, et al., 2005; Hooper et al., 2012). Furthermore, immune-modulating effects were observed (Steinberg et al., 2003). These mechanisms potentially contribute to the prevention or delay of development of several chronic diseases such as cardiovascular disease (Dong et al, 2017; Larsson et al, 2016; Mostofsky et al, 2010), diabetes (Crichton et al, 2017), cancer (Katz et al, 2011) and other age-related diseases afflicting industrialized societies such as cognitive decline (Crichton et al., 2016; Moreira et al., 2016).

Since polyphenols are stored in the non-fat cocoa solids (NFCS), chocolates with higher cocoa contents are considered as richer sources of these phytochemicals, revealing a higher antioxidative capacity (Cooper et al., 2008; Miller et al., 2006). In the EU, milk chocolate formulations contain a min. proportion of 25 % total cocoa solids whereas in dark chocolates a minimum proportion of 43 % is required. White chocolate does not contain any non-fat cocoa solids at all (European Parliament and Council of the European Union “Directive 2000/36/EC”). The beneficial health effects are thus attributed to moderate habitual consumption of dark chocolates with high cocoa (%cc) and polyphenol

contents (Grassi et al., 2015; Mostofsky et al., 2010; Persson et al., 2011; Steinberg et al., 2003).

Polyphenols represent the main source of antioxidants in human nutrition (Graf et al., 2005). In the United States, chocolate is considered the third highest contributor of antioxidants (100-107 mg/day) after fruits (255 mg/day) and vegetables (233 mg/day) (Vinson et al., 2006). Although, the consumption of fruits and vegetables is of more relevance to ensure polyphenol supply, taking into account the overall nutritional profile including energy density, the recommendation of moderate amounts of chocolate with high cocoa and preferably standardized polyphenol contents seems reasonable within a well-balanced diet addressing the general population (Rusconi & Conti, 2010).

Although dark chocolate accounts now for 31% of the global market share, still being significantly less popular than milk chocolate (51%), increasing attention in the general public has been recorded in response to these positive findings (Afoakwa, 2016d). Due to compositional requirements (such as the absence of dairy ingredients, lower sugar contents), the sensory profile of dark chocolates differs considerably from that of milk chocolate (Guinard & Mazzucchelli, 1999; Liu et al., 2015; Sørensen & Astrup, 2011). Higher cocoa content in dark chocolates is associated with bitter taste and astringency (Kennedy & Heymann, 2009) elicited by a series of flavan-3-ols (procyanidins, (-)-epicatechin, (+)-catechin), caffeine and theobromine (Stark et al, 2006) naturally occurring in the non-fat cocoa solids (Belitz et al., 2008) potentially limiting consumers' acceptance and preference (Komes et al., 2012; Lesschaeve & Noble, 2005; Sun-Waterhouse & Wadhwa, 2013; Torres-Moreno et al., 2012).

However, there are strong variations in amounts and profiles of polyphenols in cocoa beans and chocolate ascribed to several factors. The chemical composition of raw cocoa beans is influenced by the cocoa bean genotype (*Criollo*, *Forastero*, *Trinitario, Nacional*), environmental conditions such as climate, sun exposure, rainfall and soil conditions in the region of origin, ripening, time of harvesting as well as time between harvesting and bean fermentation. Furthermore, post-harvest treatments such as pulp-preconditioning, fermentation and drying, usually carried out by farmers in the region of origin, as well as industrial processing such as roasting lead to drastic polyphenol losses and

chemical modifications attributed to diffusion, browning and oxidative polymerization (Albertini et al., 2015; Aprotosoaie et al, 2016; Bertazzo et al, 2013; Counet et al, 2004; Kongor et al., 2016). In addition, chocolate manufacturing stages such as grinding, refining and conching seem to affect polyphenol content and composition in the final chocolate product ascribed primarily to heat exposure and the presence of oxygen (Wollgast & Anklam, 2000). Therefore, cocoa contents (%cc) of chocolate products appear to be no reliable and standardized indicator for polyphenol contents and thus health benefits and sensory properties (Albertini et al., 2015; Rusconi & Conti, 2010).

Sensory evaluation methods commonly used to describe the sensory characteristics of chocolates for various research purposes are conventional profiling techniques (Guinard & Mazzucchelli, 1999; Sim et al, 2016; Sune et al, 2002). However, these approaches produce time-averaged data (Lee & Pangborn, 1986) not taking into consideration the temporality of sensory perception as a function of time. Sensory perception is not happening at a constant rate but as a series of events, occurring at all stages of oral processing from the first bite until swallowing of the bolus (Dijksterhuis & Piggott, 2001; Foster et al., 2011; Piggott, 1994) involving changes in physical properties, release of volatiles and non-volatiles followed by the time-dependent mechanisms of perception (Overbosch et al, 1991). Contributing factors include mastication, interaction with saliva, heat exchanges and pH changes (Boland et al, 2006). Furthermore, some stimuli induce very short sensory signals while other compounds may induce persisting sensations, including bitter taste (Delarue & Blumenthal, 2015) and astringency (Courregelongue et al, 1999). As sensory perception is thus not simply the result of the present stimuli, but a dynamic phenomenon, sensory profiling techniques acknowledging the dynamics and nature of human perception have been established (Pineau et al., 2009). Studies on chocolate using such methods as Time-Intensity (Lenfant et al., 2013; Palazzo & Bolini, 2014; Ziegler et al, 2001) and Temporal Dominance of Sensations (TDS) (Rodrigues et al, 2016), which are nowadays well-established sensory tools, are limited.

Thus, the objective of this study was to explore the kinetics of dominant sensations of polyphenol-associated sensory attributes bitter taste and

astringency as well as sweet taste as covering agent in commercial dark origin and non-origin chocolates with different cocoa contents (%cc) over the course of evaluation. For this purpose, Temporal Dominance of Sensations (TDS) as a dynamic sensory approach was applied (Pineau et al., 2009). This method proved as a useful tool in terms of product positioning and discrimination providing complementary information on temporal aspects (Albert et al, 2012; Meillon et al, 2009) likely to be more accurate in terms of interpretation of consumer responses (Dinnella et al, 2012).

In addition, hedonic data on consumers' preference and acceptance of commercial dark origin and non-origin chocolates was collected. Linking the temporal dominant sensations in the samples containing different amounts of cocoa solids with liking data could provide useful information for chocolate manufacturers and stakeholders aiming at the development of international standards for cocoa quality and flavour assessment.

2 Literature review

2.1 Definition of Chocolate

Generally, the term chocolate refers to a uniform, semisolid luxury product consisting of fine solid cocoa and sugar particles suspended in a continuous fat phase (Awua, 2002) with high popularity in many cultures and countries (Rusconi & Conti, 2010). Generally, chocolate is composed of cocoa ingredients (cocoa nibs or non-alkalized cocoa liquor with or without additional cocoa butter), milk ingredients (in the case of milk and white chocolate) and sugar (all kinds). Furthermore, various other ingredients such as natural spices, vanillin or ethylvanillin, coffee, fruits, nuts (Belitz et al., 2008), and emulsifiers (e.g. soy lecithin) find application in the chocolate manufacture. The percentages of cocoa solids, cocoa butter, milks solids, milk fat and vegetable fats (as cocoa butter equivalents) vary among different chocolate types according to legislation, which is not consistent throughout the world (Yates, 2009)

According to “Directive 2000/36/EC” on cocoa and chocolate products intended for human consumption (European Parliament and Counsel of the European Union, 2000) as well as the Codex Alimentarius Austriacus (BMG, 2010), the term “chocolate” refers to a product manufactured from cocoa-based ingredients and sugar (various types), containing 35% total dry cocoa solids (min) including 18% cocoa butter (min) and 14% non-fat dry cocoa solids (min). In addition, vegetable fats (cocoa butter equivalents) have permission for usage up to 5% (max) in the final product, if declared appropriately and not reducing the required minimum proportion of cocoa butter.

For variations of chocolate formulations and thus, different chocolate types apply specific requirements. The composition of the major general types - dark chocolate, milk chocolate and white chocolate - are described in the following.

2.1.1 Dark chocolate

Compositional requirements for chocolates declared as “dark” are a minimum proportion of 43% total dry cocoa solids (dry) including 26% cocoa butter (BMG, 2010).

2.1.2 Milk chocolate

Besides cocoa-based ingredients and sugar (various types), milk chocolate contains milk, milk powder (semi-skimmed or skimmed), cream (pure or dried), butter or milk fat. It consists of 25% total cocoa solids (dry) (min), 2.5% non-fat cocoa solids (dry) (min), 14% total milk solids (dry) (min), 3.5% milk fat (dry) (min) and a minimum proportion of 25% total fat (cocoa butter and milk fat) (BMG, 2010).

2.1.3 White chocolate

White chocolate formulations (also referred to as “cocoa butter confectionery”) are manufactured from cocoa butter (the only cocoa-based ingredient), sugar (various types), milk or milk powder (semi-skimmed or skimmed), cream (pure or dried), butter or milk fat. It contains a minimum proportion of 20% of cocoa butter, 14% total milk solids (dry) (%) and 3.5% milk fat (BMG, 2010).

2.1.4 Specialty products

Besides standard products, consumer’s demand for special flavours, sustainability, better trading conditions and health-promoting formulations is increasing. The market for specialty products such as origin, organic and fairtrade chocolates as well as sugar-reduced formulations is therefore expanding (Yates, 2009)

2.1.4.1 Origin chocolates

Origin chocolates (usually dark types) are manufactured from cocoa beans from one specific geographical region, either of one cocoa variety (“single-origin”) or blends of different cocoa varieties. As cocoa flavour is not only depending on the cocoa variety, but also on climate and soil as well as post-harvest treatment (fermentation, drying, roasting) conditions, these chocolates exhibit unique sensory properties and are used for manufacturing premium chocolate products. Produced in smaller growing areas, these *“fine or flavour”* cocoa beans (often *Criollo*) (Beckett, 2008) are of limited availability and thus even more exclusive (Yates, 2009).

2.1.4.2 Organic chocolate products

Organic chocolate formulations do not differ from standard products in terms of compositional requirements. However, the ingredients used for chocolate

manufacturing must be produced organically, representing a minimum of 95% in the final chocolate product. Non-organic ingredients can be used to an extend of 5% wherever organic alternatives are not available (Yates, 2009).

2.1.4.3 Fairtrade

Another area of growth is the fairtrade sector focusing on export products from developing to developed countries such as chocolate, cocoa, coffee, tea and bananas (Yates, 2009). As an alternative approach to conventional international trade, fairtrade is defined as a trading partnership, based on transparency, dialogue and respect that seeks greater equity in international trade. It offers better trading conditions to and ensures the rights of disadvantaged producers and workers, and thus contributes to sustainable development (Krier, 2001). However, fairtrade certified cocoa still captures a very low share of 0.5% on the cocoa market (ICCO, 2016b).

2.1.4.4 Chocolate with sweeteners

Chocolates consists of appr. 30-55% sugar (principally sucrose obtained from sugar cane or sugar beet) depending on the chocolate type. It is considered to be an integral part of chocolate both as a key flavouring as well as bulking agent (Yates, 2009). In order to meet consumers demand for calorie- and sucrose-reduced/-free products, several sugar alcohols (e.g. xylitol, sorbitol, mannitol, erythritol, maltitol, maltitol syrup, isomalt and lactitol) are used in chocolate manufacturing. With respect to the rheological and flavour properties and thus chocolate quality, maltitol is most suitable as sucrose replacement (Afoakwa, 2010).

2.1.5 Applications of chocolate

Chocolate is applied in a variety of different types of products such as pastries (e.g. coated biscuits and cakes), a wide range of confectionary from moulded bars to hand-crafted assortments, ice cream or as inclusions (chips or chunks) in muffins or cookies (Yates, 2009).

2.2 Historical background

Chocolate, a product widely appreciated nowadays by people all over the world, was firstly described in history as *Xocolatl*, which was a rather unpleasant tasting, astringent and fatty drink made of roasted and ground cocoa nibs mixed with

water and often flavoured with spices, honey and vanilla (Beckett, 2008). Firstly invented by the Mayas and Aztecs (Talbot, 2009) in 1000 B.C. (Henderson et al, 2007) in Mesoamerica, these ancient cultures appreciated this drink primarily for its nourishing, fortifying and aphrodisiac qualities (Murray et al, 2005). After discovering Central America, Columbus was the first returning to Europe with some cocoa beans, however the *Xocolatl* recipe was introduced to the Spanish courts by Hernandos Cortés in 1528 (ICCO, 2013). The drink gained popularity among the aristocracy families throughout Europe not only before sugar was added, suppressing some of the bitter taste and astringent mouthfeel (Beckett, 2008). In order to reduce the high fat content and thus to improve palatability, the Dutch developed the cocoa press in 1828 that enables the separation of cocoa solids from cocoa butter, a process bringing two advantages at a time. The defatted cocoa solids milled into cocoa powder were easier to dissolve in liquids and more preferred by consumers. Unexpectedly, the remaining fat mixed with ground cocoa beans and sugar turned out as suitable to produce uniform chocolate bars (Beckett, 1999), which were solid at room temperature (20-25°C/70-75°F), but melt smoothly during consumption at body temperature (37°C/98.5°F) exhibiting desirable flavour and texture properties. Throughout the decades, chocolate gained worldwide popularity thanks to continuous improvement of knowledge, technologies and innovations and thus sensory and nutritional properties (Afoakwa, 2010).

2.3 Cocoa beans

2.3.1 Botanical characteristics

Cocoa beans, basic raw material of chocolate and various other cocoa products, are the fermented and dried seeds of the fruits of Theobroma cacao L. (Theobroma = gr. “food of the gods”), the cocoa or cacao tree (family: Sterculiaceae) (Bertazzo et al., 2013). Originally grown in northern South America, it is now cultivated commercially in tropical areas all over the world with suitable conditions such as high average temperatures (24-28°C) and constant high humidity. Since it is susceptible to direct sun exposure and wind, it thrives at max. 600 m above sea level. Thus growing naturally in the lower storeys of the evergreen rain forest, it reaches a maximum height of 10-15 m (Belitz et al., 2008)

whereas commercially cultivated cocoa plants reach heights of only 4-8 m (Afoakwa, 2016b) even though often shaded by intercropping trees such as banana and coconut. Each cocoa tree provides 20-50 mature fruits (pods) 25-50 cm in length consisting of a relatively thick husk, each containing 25-50 seeds embedded in a mucilaginous white fruit pulp. Fig. 1 shows the cocoa seed anatomy: two cotyledons, the radicle (embryo), the testa and the surrounding pulp (Belitz et al., 2008). The cotyledons (also referred to as “cocoa nibs”) provide nutrients for the developing plant and consists mainly of fat (30-32%; cocoa butter). These storage cells also contain the phytochemicals theobromine, caffeine and polyphenols as well as organic acids (Afoakwa, 2015).

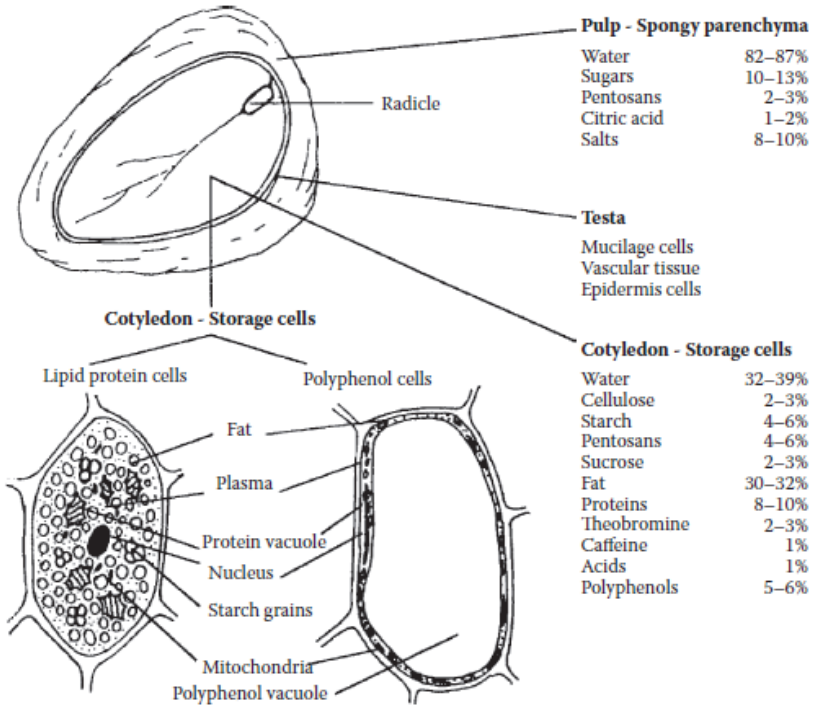


Fig. 1: Cocoa seed anatomy (Afoakwa, 2015)

2.3.2 Classification of cocoa beans

Due to genetic variations of *Theobroma cacao L.*, cocoa beans used for chocolate confectionary are classified into four principal varieties *Criollo*, *Forastero*, *Trinitario* and *Nacional*. These types are further classified into *“fine or flavour”* and *“bulk”/”basic”/”ordinary”* cocoa beans on the world cocoa market due to the individual flavour potential and specific quality criteria (Afoakwa, 2016b; Elwers et al., 2009; ICCO, 2017a).

2.3.2.1 Cocoa varieties

The four principal varieties *Criollo*, *Forastero*, *Trinitario* and *Nacional* differ either in chemical and thus sensory properties as well as vulnerability and yields. In general, the 'fine' varieties *Criollo*, *Trinitario* and *Nacional* show aromatic, mild and distinct flavour characteristics, whereas 'bulk' cocoa beans are made from *Forastero* types exhibiting strong basic cocoa notes (Afoakwa, 2010). Typical characteristics of each varieties are summarized in Tab. 1.

Tab. 1: Basic characteristics of principal cocoa varieties and corresponding categories (Afoakwa, 2016b; Beckett, 2008).

Variety	Characteristics	Category
Criollo	white cotyledons, mild cocoa and nutty flavour, susceptible to diseases, low in yields, rarely grown	"fine and flavour"
Forastero	From the Amazonas region, mainly grown in West Africa, violet cotyledons, slightly bitter, strongest cocoa flavour, vigorous and less susceptible to diseases, widespread application in the chocolate industry	"bulk"/"basic"/"ordinary"
Trinitario	disease-resistent hybrid of Criollo and Forastero, strong basic chocolate characters and some wine-like flavour	"fine and flavour" or "bulk"/"basic"/"ordinary"
Nacional	grown in Ecuador, full cocoa flavour with additional floral and spicy flavours, less cultivated	"fine and flavour"

2.3.2.1.1 Criollo

Cocoa beans derived from *Criollo* (span. "native") varieties are highly appreciated aromatic *"fine or flavour"* cocoa beans. Due to their high susceptibility to diseases, insects and stress this type is rarely grown compared to *Forastero* varieties, resulting in lower yields and poor productivity (Hebbar et al, 2011). They can be found e.g. in old and small plantations in Venezuela, Central America, Madagascar, Sri Lanka and Samoa (Afoakwa, 2016b). Characterized by the strongest aromatic flavour profile on the international market with mild and nutty cocoa notes these cocoa beans are highly prized and mainly used for manufacturing premium quality chocolates (Fowler, 1999).

2.3.2.1.2 Forastero

Forastero (span. “foreigner”) refers to any cocoa varieties that are not *Criollo* or a hybrid (Hebbar et al., 2011). Originally from the Amazonas region, *Forastero* varieties (mainly the ‘Amelonado’ type) is now cultivated largely in West Africa particularly Côte d'Ivoire, Ghana, Nigeria and Cameroon (Fowler, 1999; Kongor et al., 2016). It is more vigorous and less susceptible to diseases than *Criollo*, resulting in higher yields and thus widespread application in the chocolate industry. Its violet cotyledons are slightly bitter and exhibit the strongest cocoa flavour whilst being low in complex and fruity notes (Hebbar et al., 2011). Due to its high productivity and less distinct flavour profile, it represents the *“bulk”* cocoa sector, which accounts for 95% of the world cocoa production (Fowler, 1999).

2.3.2.1.3 Trinitario

Trinitario is a disease-resistent hybrid of *Criollo* and *Forastero* and therefore displays characteristics of both varieties (Fowler, 1999). Developed in Trinidad, it is now mainly grown in Venezuela, Ecuador, Cameroon, Sri Lanka and Papa New Guinea. Some varieties produce *“fine or flavour”* cocoa beans with special flavours (e.g. strong basic chocolate characters and some wine-like flavour whereas some *Trinitarios* (e.g. from Cameron) do not have desirable flavour characteristics and are traded as *“bulk”* (Afoakwa, 2016b; Beckett, 1999; Giacometti et al., 2015; ICCO, 2017a).

2.3.2.1.4 Nacional

Cultivated in Ecuador, the *Nacional* cocoa beans (especially ‘Arriba’, a hybrid of *Nacional* with Venezuelan *Trinitario*) are acknowledged for their full cocoa flavour with additional floral, spicy and green notes. However, the availability of these cocoa beans is limited and chocolate manufacturers often pay premiums for good quality Arriba beans for manufacturing speciality dark chocolates (Afoakwa, 2010).

2.3.2.2 “Fine or flavour” chocolates

Accounting for 40-50% of the total world cocoa production at the beginning of the 20th century, the share of *“fine or flavour”* cocoa is currently about 5% (ICCO, 2017a) with rising tendency as recently the demand for premium, origin and dark chocolate products, characterized by distinct flavour profiles, started to grow very rapidly. Whereas *“bulk”* varieties are grown on a large scale, susceptible *“fine or*

flavour" types are cultivated in small plantations with Ecuador, Trinidad and Tobago being the major producers. Quality criteria for *"fine or flavour"* cocoa beans involve the genetic origin, morphological and chemical characteristics, degree of fermentation and drying, percentage of internal mould, insect infestation and other impurities however mainly the sensory properties such as colour and especially flavour characteristics of processed cocoa beans including acidity and off-flavours (ICCO, 2017a). Thus, the main difference to *"bulk"* cocoa beans is the distinct flavour profile, characterized by floral, fruity, herbal, nutty, woody and caramelic notes and balanced cocoa bases (Afoakwa, 2016b). However, no harmonized international assessment standards of these criteria are established at present, which is the focus of discussion within the global cocoa and chocolate sector (Sukha, 2017).

2.3.3 World cocoa production

Due to ideal climatic conditions, West Africa dominates the world cocoa production since the 1930s (Afoakwa, 2010), still accounting for estimated 72.5 % in 2014/15, followed by America (18,0 %) and Asia and Ozeania (9.5 %) as shown in Tab. 2 (ICCO, 2016a). Although Malaysia and Indonesia emerged in the 1980s, giving a more balanced geographical distribution of cocoa production (Afoakwa, 2010), the largest cocoa suppliers remained Côte d'Ivoire and Ghana with an estimated share of 42,5% and 17,5%, respectively (in total, 60% of net cocoa exports) followed by Indonesia with 7.7 % in the cocoa year 2014/15. Whereas the world cocoa production declined by estimated 3.7% to 3.931 mio. tonnes in 2012/2013, compared to 2011/12 (ICCO, 2014), in 2013/14 the world cocoa supply reached a surplus of 11 % with an all-time record output of 4.372 mio. tonnes arising from excellent weather conditions with another slight decrease of estimated 3.25 % (to 4.230 mio. tonnes) in 2014/15. In 2015/16, world cocoa production is forecast to further decline to 4.154 mio. tonnes with a shift towards the West African region (up to 73.7 %) and a decrease in net exports in the Americas and Asia and Ozeania to 17.2% and 9.1%, respectively (Afoakwa, 2016d; ICCO, 2016a).

Tab. 2: World cocoa production in thousand tonnes (2013/14, estimates: 2014/25, forecast: 2015/16) (ICCO, 2016a).

	2013/14		**2014/15 (estimates)**		**2015/16 (forecast)**	
Africa	3.199	73.2 %	3.068	72.5 %	3.063	73.7 %
Côte d'Ivoire	1.746	39.9 %	1.796	42,5 %	1.690	40,7 %
Ghana	897	20.5 %	740	17,5 %	840	20,2 %
Nigeria	248	5.7 %	195	4,6 %	200	4,8 %
Cameroon	211	4.8 %	232	5,5 %	230	5,5 %
Others	97	2.2 %	105	2,5 %	103	2,5 %
America	726	16.6 %	760	18.0 %	714	17.2 %
Ecuador	234	5.4 %	250	5.9	230	5.5 %
Brazil	228	5.2 %	230	5.4	210	5.1 %
Others	264	6.0 %	280	6.6	274	6.6 %
Asia & Oceania	447	10.2 %	401	9.5 %	377	9.1 %
Indonesia	375	8.6 %	325	7.7	300	7.2 %
Papa New Guinea	36	0.8 %	36	0.9	36	0.9 %
Others	36	0.8 %	40	0.9	41	1.0 %
World total	4.372	100.0%	4.230	100.0%	4.154	100.0%

"Fine or flavour" cocoa beans account for approximately 5% of the global cocoa production (tendency rising). The major producing regions are Bolivia, Costa Rica, Dominica, Grenada, Madagascar, Mexico, Nicaragua, Saint Lucia, Venezuela, Trinidad and Tobago (100% of total exports). Furthermore, Colombia and Jamaica (95%), Papa New Guinea (90%), Peru and Ecuador (75%), Belize, Guatemala, Honduras and Panama (50%), the Dominian Republic and Vietnam (40%), São Tomé and Principe (35%) as well as Indonesia (1%) are recognized by the ICCO as *"fine or flavour"* producing countries (ICCO, 2017a).

2.3.4 Chocolate manufacturer worldwide

At present, the world's leading manufacturer of high-quality chocolate and other cocoa products is the Barry Callebaut group (head office Switzerland), followed by Cargill (USA), Blommer (USA), Fuji Oil (Belgium), Puratos (Germany), Cémoi (France), Irca (Italy), Clasen (USA), Kerry Group (Ireland) and Guittard (USA) (Barry Callebaut Group, 2017). These companies supply and cooperate with the major confectionary companies worldwide with Mars Inc (USA), Mondelēz International (USA), Ferrero Group (Luxembourg/Italy), Meiji Co Ltd (Japan), Nestlé SA (Switzerland), Hershey Co (USA), Pladis (UK), Schokoladenfabriken Lindt & Sprüngli AG (Switzerland), Ezaki Glico Co Ltd (Japan) and Arcor (Argentina) representing the top 10 in 2016 based on net confectionery sales values (ICCO, 2017b).

With rising demand for premium cocoa and chocolate products, new, small processors are emerging within the market specifically for the processing and manufacturing of *"fine or flavour"* cocoa and derived products. Among these companies are Crown of Holland (Netherlands), Original Beans (Netherlands), Marou (Vietnam), Idilio Origins (Switzerland) and Amma (Brazil), combined with single-origin and bean-to-bar concepts. In addition, larger companies such as Valrhona (France), Daarnhouwer (Netherlands) and Ecom Dutch Cocoa (Netherlands) as well as mainstream chocolate manufacturers such as the Barry Callebaut group trade and process *"fine or flavour"* cocoa beans. In order to produce specialty products with distinct colour and flavour profiles, these chocolate manufacturers use *"fine or flavour"* cocoa beans from specific origins such as Grenada, São Tomé, Madagascar and Java or Ecuadorian Arriba *Nacional* (CBI Ministry of Foreign Affairs, 2016). In Austria, chocolate manufacturer Zotter specializes on organic and fairtrade products with a range of dark, single-origin chocolates produced from *"fine or flavour"* cocoa beans from bean-to-bar.

2.3.5 Chocolate consumption

Cocoa beans are mainly consumed as chocolate confectionary, chocolate-coated products (e.g. biskuits or ice cream) or products containing cocoa powder such as beverages, cakes and snacks (ICCO, 2012). The worldwide consumption of

chocolate confectionary increased from 6,946[3] in 2012/13 to 7,314[1] in 2015/16 and this trend is forcast to continue with estimated 7,696[1] in 2018/19 (Statista, 2016c). According to the *Association of Chocolate, Bisukuits and Confectionery Industries of Europe (CAOBISCO)*, between 2002 and 2015 the consumption of chocolate confectionary in the 20 leading chocolate-consuming countries worldwide increased by 10 % on average (ICCO, 2015a).

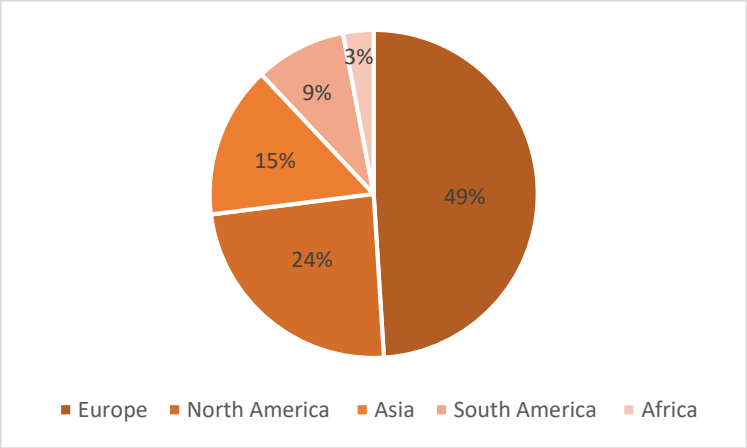


Fig. 2: World consumption of chocolate products by region (Afoakwa, 2016d).

The chocolate market is dominated by Western Europe and North America (Afoakwa, 2016d; ICCO, 2012) (Fig. 2) with the major chocolate consuming countries Switzerland, Germany, Russia, the United Kingdom, the United States, Austria, Poland, France and China in 2015 as shown in Fig. 3 (Statista, 2016b). However, young markets such as Brazil, India, China and Indonesia are a major driving force in the growth of chocolate consumption whereby the Asian region is expected to share 20% of the global market by 2016 (ICCO, 2012).

[3] in 1,000 metric tonnes

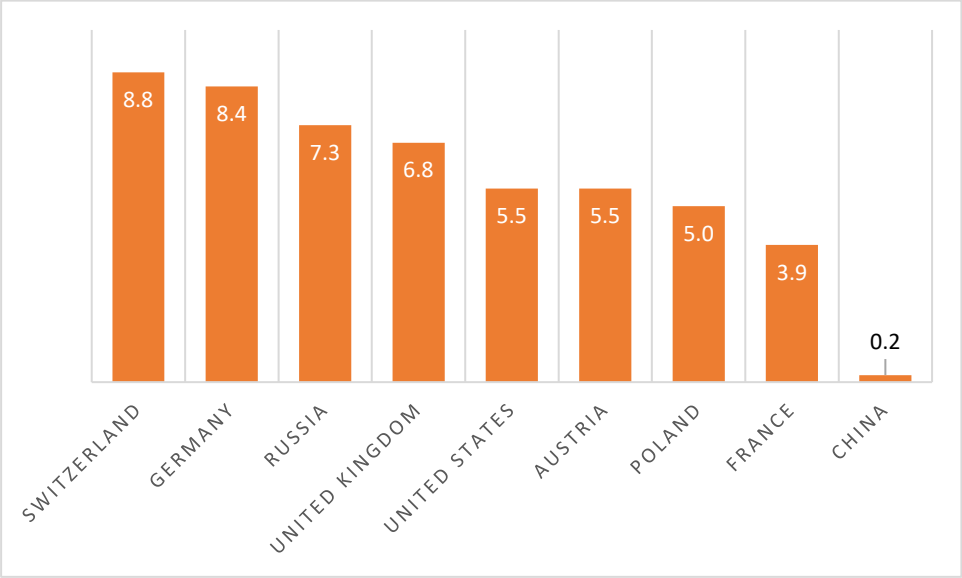


Fig. 3: Chocolate confectionary consumption worldwide by key markets in 2015 (kg/capita) (Statista, 2016b)

Besides increasing consumption in emerging and newly industrialized countries, dynamic changes in chocolate consumption patterns were observed in mature markets. Those changes are characterized by a broad shift towards premium chocolate products including single-origin high cocoa content chocolates with distinctive flavours (ICCO, 2012) as well as organic, fair trade and sugar-reduced chocolate products. This trend reflects increasing consumers demand for high quality, special tastes, good health and nutrition associated with those products as well as sustainability, traceability, ethical and environmental concerns (Afoakwa, 2016d). In response, many companies have increased levels of new product activity with launches of new lines including dark, origin, organic and fairtrade chocolate (Afoakwa, 2010), which is supported by data of *Mintel* revealing that in 2015, more premium chocolate products were on offer than ever before, with a global 72% increase of launched products as of 2011 (Mintel, 2016). As an example, Fig. 4 shows chocolate consumption patterns with respect to major chocolate types among U.S. consumers in 2012 revealing that milk chocolate is still predominant (51%), followed by dark (35%) and white chocolate (8%) (Statista, 2016a).

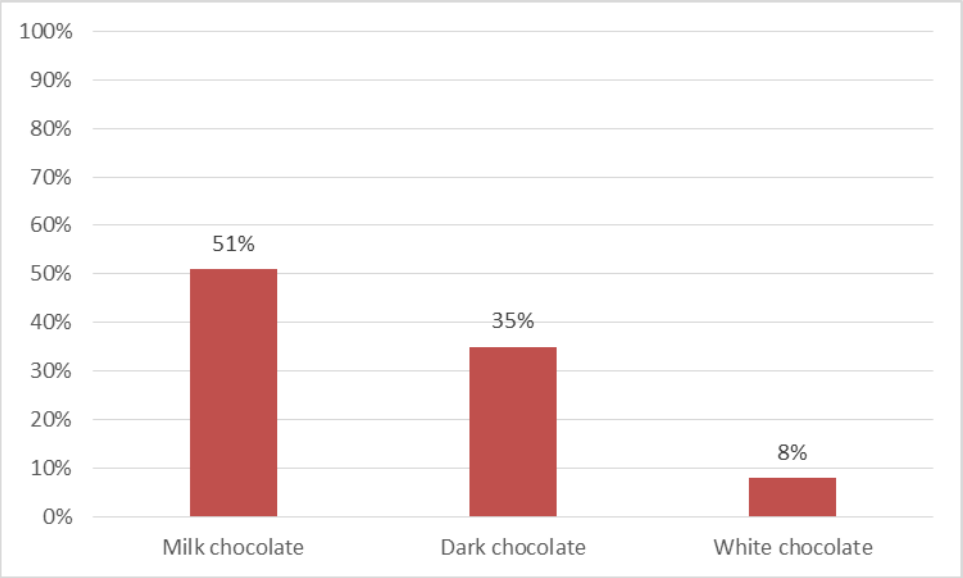


Fig. 4: Chocolate consumption share by type in the USA (2012) (Statista, 2016a).

2.4 Chocolate manufacturing process

2.4.1 Treatment of cocoa beans

Chocolate manufacturing starts with preparation of the cocoa nibs used for producing cocoa liquor either in the cocoa growing country or in the country of chocolate manufacturing (Beckett, 2008). The first stages of cocoa bean processing, which are essential for the desired cocoa flavour development, are correct fermentation and subsequent drying (in order to produce stable, non-perishable commodities), alkalization and roasting (Afoakwa, 2016a).

2.4.1.1 Fermentation

After harvesting, the mature cocoa pods are opened and the cocoa seeds with the adhering pulp are transferred into heaps, boxes or baskets for fermentation, which lasts 1-3 days (*Criollo* varieties) or 5-6 days (*Forastero* types). Fermentation is essential for several reasons: major flavour precursors are formed through a variety of chemical processes giving the final chocolate product its unique flavour and brown colour (Afoakwa, 2016a). Furthermore, non-perishable commodities are produced since the radicle dies under the conditions of fermentation. Thus, fermentation represents a critical step in cocoa bean processing and failures at this stage cannot be rectified at subsequent steps (drying, roasting) (Beckett, 2008).

At the beginning of the fermentation process, the mucilaginous pulp, consisting of water (82-87%), fermentable sugars (glucose, fructose, sucrose) (10-13%), pentosans (2-3%), citric acid (1-2%) and salts (8-10%) (Fig. 5) (Afoakwa, 2015) is degraded by ethanoic, acetic and lactic fermentation through microbiological activity under anaerobic conditions ("external fermentation"). The resulting liquid consists of ethanol and acetic as well as lactic acid. The reduced pH value (6.5 to 4.5 on average), accompanied by the steadily rising temperatures up to 45-50°C, inhibits germination and contributes to subsequent structural changes of cocoa beans by breaking down cell walls and membranes. Thus, compartmentalization of enzymes and substrate is removed, enabling movements through the cotyledons and flavour precursor formation ("internal fermentation") (Afoakwa, 2016a; Albertini et al., 2015; Zach, 2017). Fig. 5 explains systematically these reactions and movements taking place during fermentation. Compounds formed or modified responsible for sensory properties of chocolate are (Belitz et al., 2008):

- Polyphenols (bitter taste, astringency)
- Methylxanthines: caffeine and theobromine (bitter taste)
- Maillard reaction precursors (typical chocolate aroma and flavour)
- Organic acids: citric, acetic, lactic acid (acidic taste)

During fermentation, the level of caffeine and theobromine declines by around 30% possibly as a result of diffusion from the cotyledons into fermentation sweating (Fowler, 1999). Polyphenol content and thus, bitter taste and astringency significantly drops during fermentation and subsequent drying, generally ascribed to multiple factors: diffusion into the shell and fermentation liquid from the storage cells, enzymatic and non-enzymatic oxidation (Albertini et al., 2015; Kim & Keeney, 1984).

In addition, Maillard reaction precursors are formed from storage proteins and sucrose. Sucrose (a non-reducing sugar) is converted into reducing sugars (glucose and fructose) by invertase and proteins are hydrolysed into short-chain oligopeptides and amino acids by peptidase enzymes. The resulting compounds

serve as precursors for the Maillard reaction during roasting of the cocoa beans to form typical cocoa flavour compounds (Fowler, 1999).

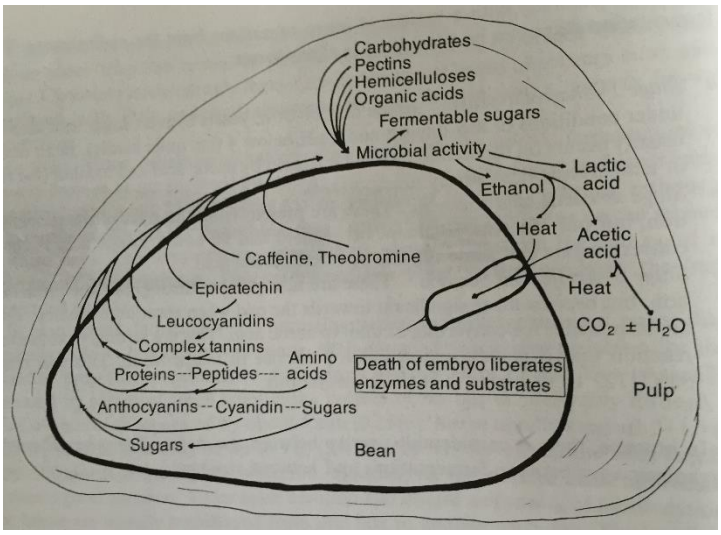


Fig. 5: Chemical changes within a cocoa bean during fermentation (Fowler, 1999).

2.4.1.2 Drying

Following fermentation, cocoa beans are washed (e.g. in Java, Sri Lanka) and dried to a moisture content of 6 - 8% (Belitz et al., 2008) before transported to the chocolate making factories. Correct drying is crucial since an appropriate moisture content (< 8%) prevents mould growth during storage and transportation. In contrary, lower moisture contents (< 6%) result in a brittle texture, negatively affecting subsequent handling and processing (Beckett, 2008). In regions with comparatively dry weather conditions at harvest time, cocoa beans are usually sun dried. For this purpose, the beans are spread out in layers during the day on mats, trays or a terrace on the ground. The layers are heaped up and raked over in the course of the day and protected by night or when it rains (Fowler, 1999). Drying results in reduced levels of acidity, bitter taste and astringency due to releasing volatile acids and non-enzymatic oxidation of polyphenols through sun drying (Afoakwa, 2010; Albertini et al., 2015). Due to high rainfall and humidity, in some areas artificial drying is necessary (Wollgast & Anklam, 2000) such as open fire potentially leading to off-flavour formation described as ‘burnt’ (Zach, 2017).

2.4.1.3 Storage

The fermented and dried beans must be stored and transported to chocolate manufacturers under controlled conditions in order to meet quality assurance standards (Afoakwa, 2010). Traditionally, cocoa beans are stored and shipped in 60-65 kg jute sacks due to a number of positive properties. It is a strong, stackable and breathable fabric made from natural biodegradable fibre. The warehouses storing bagged cocoa must be dry, ventilated and free from vermin and infestation. Other commodities releasing odorous compounds (e.g. spices) must be stored separately since cocoa beans are susceptible to uptake extraneous odours resulting in off-flavours in the chocolate. Furthermore, cocoa bags should be stored off the floor and away from walls preferably on wooden pallets (Beckett, 2008).

2.4.1.4 Cleaning, Breaking, Winnowing

To obtain nibs of consistent quality for chocolate manufacturing, the cocoa beans have to undergo the processes of cleaning, breaking and winnowing, resulting in clean, well broken and deshelled nibs of uniform size. After drying on the ground, impurities such as sand, stones, strings, nails, plant material etc. as well as infestations have to be removed from the beans using sieves, suction and magnets. This procedure is carried out not only for product safety, but for two other major reasons. Firstly, hard particles can potentially damage the machinery used for grinding the beans subsequently and secondly, organic contaminants would burn during the roasting procedure, releasing gases likely to spoil the final cocoa flavour (Afoakwa, 2016a; Beckett, 2008).

2.4.1.5 Sterilization

The procedure of sterilization is either carried out before or after roasting, depending on the factory or equipment used. Microorganisms that might have contaminated the nibs in previous process stages (fermentation, drying, storage, transport), are destroyed by wetting or heating with steam. After sterilization, the beans can either be roasted directly (natural approach) or undergo alkalization by the Dutch process first (Afoakwa, 2016a).

2.4.1.6 Alkalization (Dutching)

Cocoa beans, nibs or liquor can be treated with an alkali solution (usually potassium or sodium carbonate) in order to produce cocoa powder with modified

flavour/colour properties (darker ingredients, reduced acidity and bitterness) and improved dispersibility and suspension in liquids for various applications, mostly beverages (Gu et al., 2006; Miller et al., 2008). The use of alkali causes the pH to raise from 5.2 – 5.6 towards a neutral pH range (6.8 – 7.5 on average) (Awua, 2002). This procedure is called “alkalization” or “dutching” since it was first invented by the Dutch manufacturer known as Van Houten (Afoakwa, 2010). Alkalization leads to a gradual loss of flavan-3-ol. However, the degree of polyphenol reduction depends on the intensity of alkali treatment: ~40% of the natural flavan-3-ol content retains in lightly dutched cocoa powders (pH 6.5 – 7.2) and ~22% in moderately dutched products (pH 7.21 – 7.6) (Miller et al., 2008).

2.4.1.7 Roasting

In order to develop the typical cocoa flavour and colour from existing precursors generated in previous process stages (fermentation, drying), cocoa beans, nibs or liquor are roasted. Several physical and chemical changes appear within this step, depending on time and temperature of roasting (90-170°C;120–150°C, 20-40 min.) and the rate of moisture loss (preferable ~ 2% final content). Nib roasting involves two stages: first, breaking of dry cocoa beans and removal of shells and radicles and second the actual roasting of the remaining cocoa nibs (Afoakwa, 2010; Ebermann & Elmadfa, 2011). Besides shell loss, further darkening of cocoa nibs and texture alteration towards a more friable status, these changes involve evaporation of volatile acids (acetic acid) and losses of polyphenols contributing to reduced acidity, bitter taste and astringency. In addition, the key flavour compounds are formed within the non-enzymatic browing reaction (Maillard) by degradation of peptides, free amino acids and reducing sugars (fructose, glucose) (Afoakwa, 2010). The roasting procedure yields in 78 – 80% cocoa nibs, 10 – 12% shells and radicles and 4 % cocoa waste on average (Belitz et al., 2008).

2.4.1.8 Grinding

The roasted cocoa beans are then ground into a homogenous suspension called cocoa or chocolate liquor consisting of cocoa butter and fine, brown non-fat cocoa particles. Using stone mills, disc mills, pin or hammer mills and bead or ball mills, cocoa nib cells are crushed, releasing the melting cocoa butter (~ 55% of the nib),

which gets mixed with fine cocoa particles resulting a smooth paste suitable for chocolate manufacturing (Afoakwa, 2010).

2.4.2 Chocolate manufacturing processes

Chocolate manufacturing stages are depicted in Fig. 6.

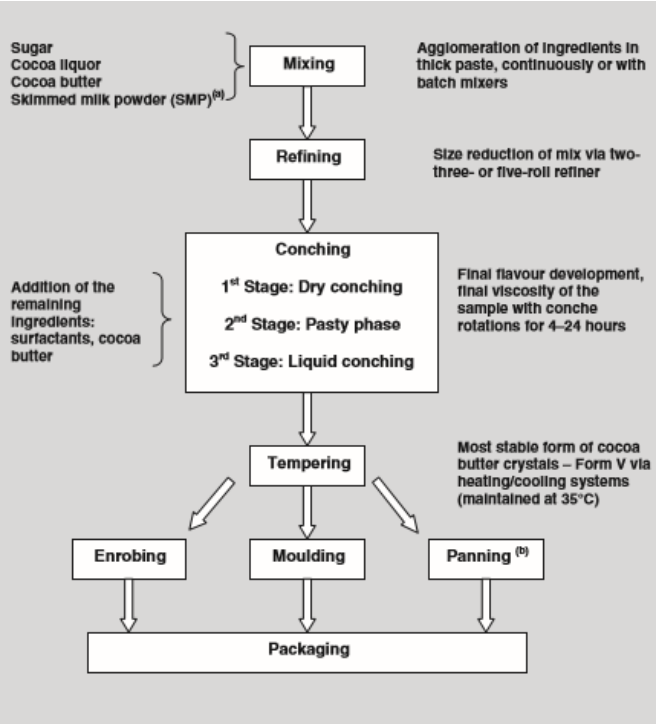


Fig. 6: Stages of chocolate manufacturing (Afoakwa, 2010)

2.4.2.1 Mixing

The first step of chocolate manufacturing consists of mixing the ingredients (cocoa liquor, cocoa butter, sugar, milk fat, milk powder – depending on the type of chocolate produced) for 12-15 Minutes at 40-50°C in order to produce a homogenous mass. Time-temperature procedures are applied using continuous or batch mixer in order to obtain consistent formulations (Afoakwa, 2016a).

2.4.2.2 Refining

Refining the obtained mixture to a particle size of < 30 µg is a crucial step in chocolate production since it affects both rheological and flavour properties. Using a combination of two- and five-roll refiners, the solid particles are further fragmented. The new surfaces are then coated with cocoa butter, which actively absorbs volatile flavour compounds from cocoa particles. Furthermore, the solid

particles are distributed homogeneously throughout the continuous fat phase (Afoakwa, 2010).

2.4.2.3 Conching

Conching represents the final process in flavour formation. The aim is to remove undesirable flavour-active compounds (e.g. acetic acid and methanol), while retaining or developing pleasant ones, depending on the requirements of the final product (Beckett, 1999). Homogenization of the mass through heat and movement also contributes to the development of a smooth texture (Giacometti et al., 2015). The refined chocolate mass, which is dry and powdery when it enters the conche (= shell), is stored there for 24 h at 45 – 50°C before the actual conching process starts resulting in a pasty consistency. In order to achieve a fine and smooth paste for producing high quality chocolates, the paste is then agitated and kneaded in conches (Belitz et al., 2008), which are large tanks with intermeshing mixer blades providing shearing and mixing (Afoakwa, 2010). It is usually carried out as a three-step-approach, although not all stages occur in each recipe. The three phases are (Afoakwa, 2010; Beckett, 1999; Belitz et al., 2008):

- dry-phase (6 - 12 h): moisture and volatile compounds are removed, lipids are finely distributed
- pasty phase (6 - 40 h): central stage; addition of cocoa butter and high work input to obtain a smooth, homogeneous mass
- liquid phase (2 - 3 h): high speed stirring to mix in emulsifiers (e.g. lecithine) and fat. Viscosity sinks to a threshold of 0.5 % providing suitable flow properties.

Temperatures used are varying, depending on the type of chocolate: milk chocolates max. 65°C, chocolates without milk ingredients max. 75°C (Belitz et al., 2008).

2.4.2.4 Tempering

In order to achieve suitable appearance and texture properties, chocolate undergoes the process of fat pre-crystallisation (= tempering) inducing the most stable form of cocoa butter. It consists of shearing the liquid chocolate mass at

controlled temperatures (50°C at the beginning, cooling down to 18°C within 10 min., kept at 18°C for further 10 min., re-heated to 29 – 31°C within 5 min.) This procedure results in preferably fine fat crystals with high melting points ensuring a homogenous, micro crystalline and heat resistant fat matrix after the final cooling procedure (Belitz et al., 2008) and thus good setting characteristics, foam stability, demoulding properties, product snap, contraction, gloss and shelf-life characteristics (Afoakwa et al, 2008).

2.4.2.5 Moulding, Enrobing, Panning

Tempering is followed by the process of forming the final chocolate product. Different approaches exist for this purpose such as moulding (e.g. solid tablets), enrobing (of a central material, e.g. nougat, biscuit or caramel) or panning (chocolate-coated or sugar-coated chocolate items) depending on the final chocolate product. For moulding, chocolate mass is fed into pre-warmed moulds at 30-32°C using a depositing head. In order to avoid airbubbles in the final product, the moulds are vigorously vibrating. Cooling down on appropriate paths, the chocolate bars demould at 10°C, followed by packaging (Belitz et al., 2008).

2.5 Nutritional values

Despite the high energy density and content of saturated fatty acids and sucrose, chocolate consumption is associated with several positive health effects in humans due to the provision of nutrients with primarily antioxidative properties such as polyphenols. Located within the cocoa nibs, dark chocolates with higher cocoa contents are consequently richer sources than those with lower if any cocoa nib ingredients (milk or white chocolate) (Steinberg et al., 2003).

The chemical composition of the cocoa beans and thus, nutritional values and sensory properties of cocoa beans change throughout the beans' life cycle. It is strongly influenced by the genetic origin, which determines its chemical composition, specifically the contents of storage proteins, polysaccharides, and polyphenols (Kongor et al., 2016), as well as geographical origin, post-harvest and chocolate manufacturing processes.

2.5.1 Composition of cocoa beans

2.5.1.1 Macronutrients

2.5.1.1.1 Lipids

The lipid fraction (cocoa butter), representing the main component in cocoa beans, accounts for 37% to 58% of the dry weight (Ebermann & Elmadfa, 2011), mainly constituted by neutral lipids with the predominant fraction of triglyceride molecules. It is composed of the saturated stearic (18:0; 37%) and palmitic (16:0; 25%) as well as the monounsaturated oleic (18:1; 34%) fatty acid, the remaining fat being primarily the polyunsaturated linoleic acid (18:2; 3%) (Belitz et al., 2008; Bracco, 1994; Ebermann & Elmadfa, 2011) (Tab. 3).

2.5.1.1.2 Proteins

The second most abundant constituents in fermented cocoa seeds are proteins with a dry weight of 12.0-18.0% (Ebermann & Elmadfa, 2011) (Tab. 3) representing 60% of the total nitrogen content. In addition, free amino acids, partly in amide form as well as ammonia (being formed during fermentation) and methylxanthines such as caffeine and theobromine are present, constituting the non-protein nitrogen fraction (Bertazzo et al., 2013). Proteins in cocoa beans consists mainly of hydrophobic amino acids such as leucine, isoleucine, valine (0.5% each). Furthermore, serine, alanine and glutamic acid (1%, protein bound) are present (Ebermann & Elmadfa, 2011). During fermentation, extensive degradation of cocoa proteins caused by the microbiological and enzymatic reactions take place with an enhancement of free amino acids, which are essential compounds within the Maillard reaction and thus cocoa flavour precursors (Adeyeye, 2010).

2.5.1.1.3 Carbohydrates

Carbohydrates in cocoa beans, 35.0-44.0% of the dry weight (Ebermann & Elmadfa, 2011) (Tab. 3), consists of starch (6.0 %), cellulose (9.0%) (Belitz et al., 2008) and soluble carbohydrates such as stachyose, raffinose, verbascose and sucrose ranging from 0.4% to 3.5% as well as glucose and fructose (Redgwell et al., 2003). Glucose and fructose, partly as a result from sucrose hydrolysis occurring during fermentation, are reducing sugars and, together with the free amino acids, involved in the Maillard reaction taking place in the subsequent roasting procedure (Bertazzo et al., 2013).

2.5.1.2 Micronutrients

The mineral content in cocoa beans is approx. 3.0% (Tab. 3) of which magnesium, iron, copper, phosphorus, potassium and zinc are the most abundant (Tab. 6). In addition, niacin, nicotinamide and traces of riboflavin (vitamine B_2) und pyridoxine (vitamine B_6) are also present (Ebermann & Elmadfa, 2011).

Tab. 3: Composition (%) of fermented and dried cocoa beans (Belitz et al., 2008; Ebermann & Elmadfa, 2011; Redgwell et al., 2003)

Components	%
Lipids	37.0 - 58.0
- Triglycerids	7.5 - 9.0
- C 16:0	25.0
- C 18:0	37.0
- C 18:1 (9)	34.0
- C 18:2 (9, 12)	3.0
Carbohydrates	35.0 - 44.0
- Dietary fibre	6.0 - 9.0
- Starch	6.0
- Cellulose	9.0
- Stachyose, raffinose, verbascose, sucrose	0.4 - 3.5
Protein	12.0 - 18.0
Water	5.0
Phenolic compounds	6.0 - 8.0
Minerals	3.0
Purine alkaloids	
- Theobromine	1.0 - 3.5
- Caffeine	0.05 - 1.3
- Theophylline	0.3 - 0.5
Organic acids	1.2 - 1.6

2.5.1.3 Phytochemicals

2.5.1.3.1 Polyphenols

Polyphenols constitute one of the most numerous groups of phytochemicals with more than 8000 chemical structures currently known (Bravo, 1998) and represent the major source of antioxidants in the human diet (Graf et al., 2005). Flavonoids, the most important subclass and major fraction present in cocoa beans, can be further divided into 13 classes with more than 5000 compounds (Wollgast & Anklam, 2000).

In cocoa beans, polyphenols are located in the cotyledons or “nibs” which are composed of two types of parenchyma storage cells. More than 90% consist of small cells containing protoplasm, protein and lipid vacuoles and other components such as starch granules. Located between these types of cells are the larger “pigment cells” containing polyphenols as well as purine alkaloids as shown in Fig. 7. Depending on the amount of anthocyanins present in those cells, the colour ranges from white to deep purple (Belitz et al., 2008). The polyphenol content in fresh, dried cocoa seeds is approx. 15.0 -20.0% polyphenols (Afoakwa, 2010), whereas fermented and dried cocoa beans contain approx. 6.0% (Belitz et al., 2008). The peculiarity of cocoa is the high content of polyphenols per dry weight that is superior to other beverages and foods including tea and wine (Arts et al, 1999; Arts et al, 2000).

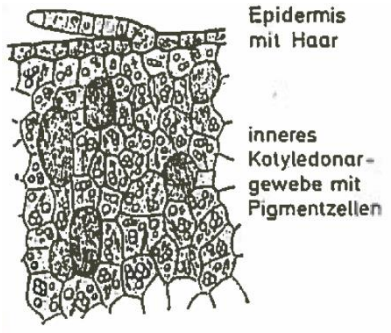


Fig. 7: Cocoa bean cotyledon showing pigment cells containing polyphenols and purine alkaloids (Belitz et al., 2008)

The flavonoids present in cocoa beans are (Belitz et al., 2008):

- Catechins (37%) (Tab. 4): flavan-3-ols with the predominant monomer (-)-epicatechin, less abundant (+)-catechin and in traces, (+)-gallocatechin and (-)-epigallocatechin (Fig. 8).

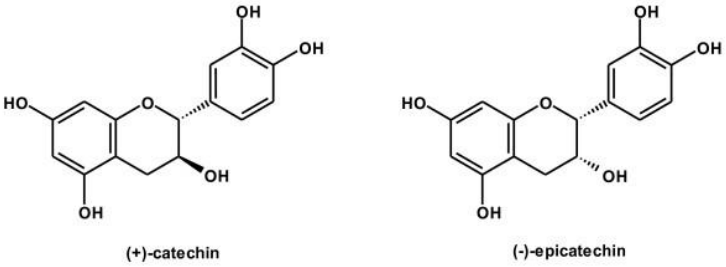


Fig. 8: Structures of (+)-catechin and (-)-epicatechin (Hurst et al., 2011)

Condensation of (-)-epicatechin and (+)-catechin (C4 → C8; C4 → C6) leads to the formation of oligomeric and polymeric, high-molecular weight, insoluable procyanidines also refered to as 'condensed tannins' (Belitz et al., 2008; Ma et al., 2014; Wollgast & Anklam, 2000).

Tab. 4: Polyphenols in cocoa beans (Afoakwa, 2010; Belitz et al., 2008).

Components	%
Catechins (flavan-3-ols)	37.0
- (-)-epicatechin	35.0
Procyanidins	58.0
Anthocyanins	4.0

- Anthocyanins (4%) (Tab. 4): predominantly the pigments cyanidin-3-α-L-arabinosid and cyanidin-3-β-D-galactosid (Fig. 9).

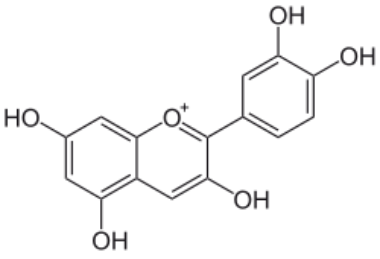


Fig. 9: Cyanidin [https://en.wikipedia.org/wiki/Catechin (accessed: Oct 24 2016)]

- Procyanidines (58%) (Tab. 4): flavan-3,4-diol

The most abundant polyphenolic compounds in cocoa beans are oligo- or polymeric procyanidines formed from flavan-3-ol monomers (Gu et al., 2006) (Fig. 10). These complex tannins are highly hydroxylated molecules and can form insoluable complexes with carbohydrates and proteins. This functionality is responsible for eliciting astringency in tannin-rich foods as they complex with and thus prepicitate saliva proteins and cell wall polysaccharides (Wollgast & Anklam, 2000).

In type B procyanidines such as B_1, B_2, B_3, B_4 and B_5 dimers, condensation of (-)-epicatechin and (+)-catechin occurs between C4 → C6 or C4 → C8.

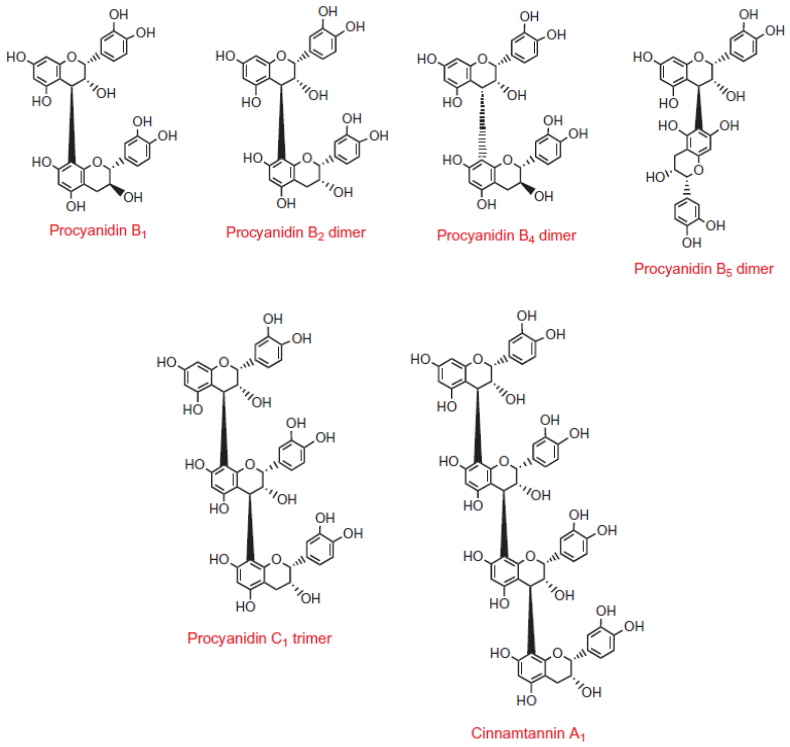


Fig. 10: Procyanidines in cocoa beans (Tomás-Barbéran et al., 2011).

Further procyanidines identified in cocoa include the C_1 trimer and tetramers such as cinnamtannin A_1 (Fig. 10) (Porter et al, 1991; Tomás-Barbéran et al, 2011; Wollgast & Anklam, 2000).

To a significantly lesser extend, flavonols (quercetin-*O*-glycosides) including quercetin-3-*O*-galactoside, quercetin-3-*O*-arabinoside and quercetin-3-*O*-glucoside also occur in cocoa and cocoa powder (Tomás-Barbéran et al., 2011).

Other minor polyphenol constituents include the flavone isovitexin, the polyphenolic amide clovamide and deoxyclovamide (Dreosti, 2000).

Furthermore, the stilbene *trans*-resveratrol and its 3-*O*-glucoside piceid have also been detected in cocoa powders, dark chocolate and cocoa liquor (Counet et al, 2006; Hurst et al., 2008).

Additional phenolic substances found in cocoa beans are coumarins including coumarin and esculetin (6,7-dihydroxycoumarin) as well as phenolic acids such as caffeic acid, ferulic acid, syringic acid, vanillic acid, *o*-hydroxyphenyl-acetic acid, protocatechuic acid, *p*-hydroxybenzoic acid (Ebermann & Elmadfa, 2011), chlorogenic acid, *p*-coumaric acid and phloretic acid (Borchers et al, 2000).

Furthermore, phenolic acid derivates including N-caffeoyl-3-O-hydroxytyrosine (clovamide) and N-p-coumaroyl-tyrosine (deoxyclovamide) occur in cocoa beans (Tomás-Barbéran et al., 2011).

2.5.1.3.2 Purine alkaloids/methylxanthines:

Cocoa beans are sources of purine alkaloids including theobromine (3,7-dimethylxanthine), caffeine (1,3,7-trimethylxanthine) and theophylline (1,3-dimethylxanthine) of which theobromine occurs in physiologically significant amounts (1.0-3.5%) (Tab. 3). It stimulates the nervous system, however this effect is known to be weaker compared to caffeine consumed in coffee beverages (Belitz et al., 2008). Furthermore, the isochinolinalkaloids salsolinol (1-methyl-6,7-dihydroxy-tetrahydroisochinoline) and salsolin (1-methyl-6-methoxy-7-hydroxy-tetrahydroisochinoline) are worth mentioning, since these substances are acknowledged not only for their stimulating properties but also as addiction promoters (Ebermann & Elmadfa, 2011).

2.5.1.4 Organic acids

The most common organic acids in cocoa beans (1.2-1.6% in total) are citric, acetic, oxalic, malic and formic (Fig. 11). Acetic acid, which is formed during fermentation of lactic acid and ethanol, is the most important one due to its contribution to the typical cocoa taste. It diffuses into the cocoa nibs after destroying the cell matrix (Holm et al, 1993). The amount of acetic acid in the cocoa nibs depend therefore on fermentation duration as well as the subsequent drying procedure (Belitz et al., 2008). In general, organic acid composition in cocoa beans is highly depend on the geographical origin (Bertazzo et al., 2013).

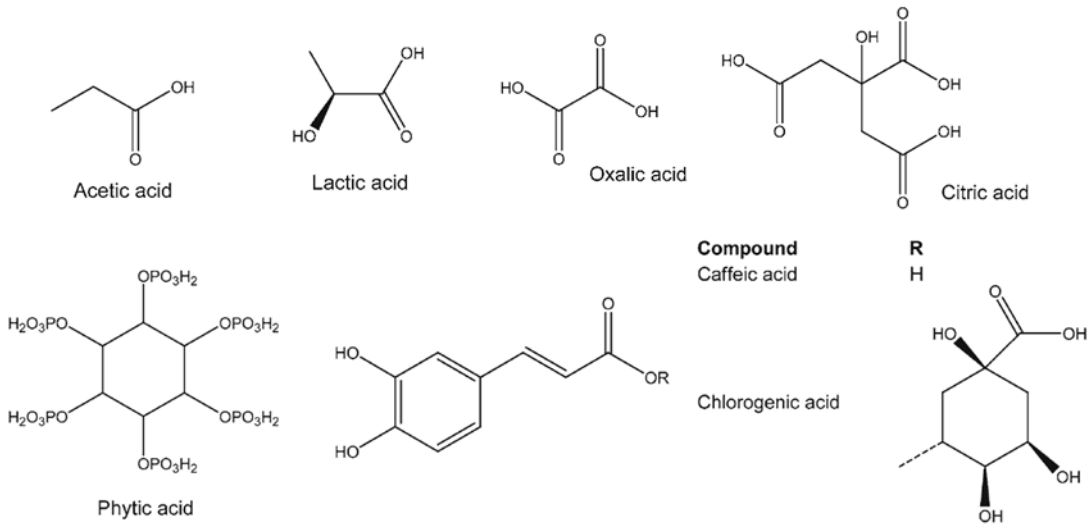


Fig. 11: Chemical structures of common organic acids in cocoa (Bertazzo et al., 2013).

2.5.2 Composition of dark chocolate

Dark (also referred to as “semisweet”, “bittersweet”) chocolates basically consists of cocoa-based products (cocoa liquor, cocoa powder, cocoa butter) and sugar (various types) with a minimum proportion of 43% total dry cocoa solids (dry) including 26% cocoa butter (BMG, 2010).

2.5.2.1 Macronutrients

Dark chocolates (70-85% cocoa content) provide 598 kcal/100 g or 149.5 kcal/serving [25 g] (USDA, 2016) (Tab. 5). It covers 7.5% of the daily energy requirement calculated for an average adult (25 - < 50 years old, physical activity level/PAL 1,4, gender averaged) (D-A-CH, 2016) and are thus classified as high energy-dense foods (Schusdziarra & Erdmann, 2010). The high energy density results from the high fat (10.7g/serving [25 g] or 16.1%) and sugar (6.0g/ serving

[25 g] or 12.0%) contents. However, the predominant lipid fraction in dark chocolates is stearic acid (C 18:0) known to exert a neutral cholesterolemic response in humans (Steinberg et al., 2003) as well as MUFAs (11.1%/serving [25 g]) and PUFAs (1.9%/serving [25 g]) and only traces of cholesterol (Tab. 5). In contrary, milk chocolates contain higher levels of medium-chain saturated fatty acids, cholesterol as well as 3-5% *trans* fatty acids from milk fat (Beckett, 2008). Total carbohydrate and specifically sugar contents decline with increasing proportions of cocoa solids (12.0% sugar/serving [25 g] in dark chocolate and 27.0% sugar/serving [25 g] in milk chocolate). Whereas milk chocolates consist mainly of sucrose as well as lactose, dark chocolates are characterized by lower sucrose however higher dietary fibre contents (milk chocolate: 0.3%/serving [25 g]; dark chocolate (70-85%): 9.0%/serving [25 g]). Milk chocolates are richer total protein sources with greater proportions of essential amino acids compared to dark chocolates (Beckett, 2008).

Tab. 5: Nutritive information on dark chocolate (70-85% cocoa) (MRI, 2016; D-A-CH, 2016; USDA, 2016).

	Dark chocolate (70 – 85%)			**Milk chocolate**		
	100 g	**1 serving [25 g]**	**% D-A-CH*/ serving [25 g]**	**100 g**	**1 serving [25 g]**	**% D-A-CH*/ serving [25 g]**
Energy (kcal)	598.0	149.5	7.5	539.0	134.8	6.7
Total Fat (g)	42.6	10.7	16.1	31.5	7.9	11.9
SFA (g)	24.5	6.1	27.5	18.9	4.7	21.2
Palmitic acid (16:0)	-	-	-	8.1	2.0	-
Stearic acid (18:0)	-	-	-	9.9	2.5	-
MUFA (g)	12.7	3.2	11.1	10.2	2.6	9.0
Oleic acid (18:1, n-9)	-	-	-	10.2	2.6	-
PUFA (g)	1.3	0.3	1.9	1.0	0.25	1.6
Linoleic acid (18:2, n-6)	-	-	-	1.0	0.25	-
Linolenic acid (18:3, n-3)	-	-	-	0.0	0.0	-
Cholesterol (mg)	3.0	0.8	0.3	9.0	2.3	0.8
Total Carbohydrate (g)	45.9	11.5	4.6	54.1	13.5	5.4
Sugar (g)	24.0	6.0	12.0	54.1	13.5	27.0

Fiber (g)	10.9	2.7	9.0	1.3	0.3	1.0
Protein (g)	7.8	1.9	3.8	9.2	2.3	2.3
Water (g)	1.4	0.4	-	1.4	0.4	-

*D-A-CH reference value: 2,000 kcal/day (gender averaged); - no value

2.5.2.2 Micronutrients

Cocoa and chocolate can contribute a significant amount of minerals in the human diet, that are necessary for optimum functioning of biologic systems and vascular tone, such as calcium, copper, iron, magnesium, phosphorus, potassium, sodium and zinc (Afoakwa, 2010; Borchers et al., 2000; Cinquanta et al., 2016; Steinberg et al., 2003). As displayed in Tab. 6, higher cocoa content chocolates are typically richer sources of these elements than milk chocolate, since the mineral content depends on the proportion of cocoa solids in the product except for calcium and sodium, which naturally occur in milk derived products. A serving size [25 g] of dark chocolate (90 % cocoa content) provides approx. 20% magnesium, 18.0 (f) - 27.0% (m) iron, 33.3 - 50.0% copper, 14.0% phosphorus, 4.5% potassium, 9.0 (m) – 12.9% (f) zinc, 0.1% sodium and 2.3% calcium (D-A-CH, 2016). Adequate amounts of magnesium, copper, potassium and calcium are essential for preventing high blood pressure and reducing the risk of cardiovascular diseases (Steinberg et al., 2003).

Tab. 6: Mineral content of different cocoa products in mg/serving size (25 g) (Cinquanta et al., 2016)

	Milk choco-late	**Dark choco-late (60%)**	**Dark choco-late (70%)**	**Dark choco-late (90%)**	**Cocoa beans**	**D-A-CH***
Calcium	45.1	16.0	19.8	22.7	17.5	1000
Copper	0.1	0.4	0.4	0.5	0.8	1.0–1.5
Iron	0.3	2.4	2.5	2.7	0.8	10.0 (m), 15.0 (f)
Magnesium	13.0	39.7	48.1	63.1	72.7	350 (m), 300 (f)
Phosphorus	49.7	55.5	70.0	99.1	129.0	700
Potassium	94.8	116.4	135.1	180.0	180.0	4000
Sodium	18.2	1.3	1.1	0.9	0.9	1500
Zinc	0.1	0.6	0.8	0.9	1.2	10.0 (m), 7.0 (f)

* D-A-CH reference values for adults ≥ 25 y.

Even though the phytate content of cocoa seeds is relatively high, several processing steps such as fermentation and heat treatments cause hydrolysis of phytates resulting in good availability of minerals from chocolate products (Aremu & Abara, 1992).

2.5.2.3 Phytochemicals

2.5.2.3.1 Polyphenols

As shown in Tab. 7, dark chocolate represents a concentrated source of polyphenols (flavan-3-ol monomers and procyanidins) with higher concentrations than is found in milk chocolate and other plant-based foods and beverages such as apples, black tea, green tea and red wine (Arts et al., 1999; Steinberg et al., 2003). Furthermore, cocoa exerts the highest antioxidative capacity, which is highly correlated with phenolic and flavonoid content (Lee et al., 2003).

Tab. 7: Polyphenol content and antioxidative capacity of various foods and beverages (Steinberg et al., 2003)

Source	Flavan-3-ol monomers + procyanidins (mg)	ORAC, mmol Trolox equivalents*
Cocoa liquor 100g	 1,400.0	 40.0
Dark (semisweet) chocolate 100g 100 kcal	 170.0 85.0	 13.1 2.7
Milk chocolate 100g 100kcal	 70.0 14.0	 6.7 1.3
Apples 100g 100kcal	 106.0 130.0	 0.2 0.3
Cranberry juice cocktail 100g 100kcal	 12.6 20.0	 0.2 0.3
Red wine 100g 100kcal	 22.0 25.0	 0.7 0.9
Brewed black tea (2g/200 ml water, 5 min. infusion)	40.0	1.6

*Antioxidant activity expressed as Oxygen Radical Absorbance Capacity (ORAC)

A portion size of 5 g of dark chocolate provides as much polyphenols as 50 ml red wine and 200 ml green tea (Ebermann & Elmadfa, 2011). Major flavonoids present in chocolate are the flavan-3-ol monomers catechin and epicatechin as well as the oligomeric procyanidins (Steinberg et al., 2003). However, the polyphenol content and/or profile (monomeric flavan-3-ol epimers and oligomeric

procyanidins) in chocolate and other cocoa-containing products differ substantially from that in raw cocoa seeds and can be highly variable between chocolate samples due to various factors such as bean variety, origin, postharvest treatments and manufacturing steps (see 2.5.3) (Alañón et al., 2016; Cooper et al., 2007). At present, no standardized certifying system is available aiming at controlling these factors in order to provide products of consistent quality and polyphenol content (Albertini et al., 2015). In addition, no established analytical methods to quantify individual phenolic compounds on a standardized basis exists and further research is required in order to classify products based on their polyphenol content and thus, their health-promoting properties (Rusconi & Conti, 2010) and impact on the sensory profile.

2.5.2.3.2 Purine alkaloids/xanthines

In dark chocolates, the predominant methylxanthine is theobromine (3,7-dimethylxanthine) with contents ranging from 5000 - 7500 mg/kg. Caffeine is present in significantly lower concentrations ranging from 625 – 875 mg/kg, while milk chocolate contains about 1000 mg/kg theobromine and 56 mg/kg caffeine (Franco et al, 2013).

2.5.3 Factors influencing polyphenol content in cocoa beans and chocolates

Amounts and profiles of polyphenols in cocoa beans are strongly influenced by bean genotype, but also environmental conditions such as climate, sun exposure, rainfall, soil conditions, ripening, time of harvesting as well as time between harvesting and bean fermentation. Furthermore, post-harvest treatments such as pulp pre-conditioning, fermentation, drying as well as industrial processing such as roasting - crucial processing steps in terms of colour, taste and flavour development - lead to polyphenol losses and chemical modifications (Afoakwa, 2010; Albertini et al., 2015; Aprotosoaie et al., 2016; Bertazzo et al., 2013; Kongor et al., 2016). In addition, chocolate manufacturing processes such as grinding, refining and conching also seem to affect polyphenol content and composition in the final chocolate product (Wollgast & Anklam, 2000).

2.5.3.1 Effect of cocoa bean genotype

Cocoa bean genotype strongly influences the the polyphenol content and profile in processed cocoa beans and chocolates since the initial chemical composition (polysaccharides, proteins, polyphenols) as well as activity of enzymes determines the quantities and type of compounds generated during post-harvest processing (pulp-preconditioning, fermentation, drying). Thus, each cultivar (*Criollo*, *Forastero*, *Trinitario*, *Nacional*) exhibits specific flavours (Afoakwa, 2010; Clapperton et al., 1994; Kongor et al., 2016). Tab. 8 shows (-)-epicatechin concentrations in cocoa beans from different varieties unaffected by post-harvest treatments. Concentrations ranged from 34.65-43.27 mg/g in the freshly harvested, defatted cocoa bean samples. Although significant, these differences are not considered as of practical importance (Kim & Keeney, 1984). Furthermore, Elwers et al., (2009) and Griffiths, (1960) found no significant differences in total polyphenol and (-)-epicatechin contents between *Criollo*, *Forastero*, *Trinitario* and *Nacional* varieties.

However, the highly appreciated *"fine or flavour"* cocoa variety *Criollo* was shown to contain few or no anthocyanins due to an anthocyanin inhibitor gene compared to *Forastero* and *Trinitario* varieties (Fowler, 1999; Kongor et al., 2016). Amounts of procyanidins were shown to exceed those of *Forastero* and *Trinitario* varieties (Giacometti et al., 2015). However, Lange & Fincke, (1970) found the total polyphenol content in *Criollo* seeds to be appr. 2/3 of the amount in *Forastero* varieties, which is in contrary to the findings of Elwers et al., (2009) and Griffiths, (1960). Although often less fermented (Counet et al., 2004), the decrement of catechins during post-harvest treatments (fermentation, drying) is reported to be much stronger in *Criollo* seeds than in other genotypes (Elwers et al., 2009). Thus, bitter taste and astringency were found to be less intense compared to other varieties resulting in highly prized cocoa beans with the strongest aromatic flavour profile on the international market characterized by mild and nutty cocoa notes (Ascrizzi et al, 2017; Fowler, 1999; Wolters, 1999).

Clapperton et al., (1994) however found *Criollo* beans grown in Malaysia to be more bitter and astringent compared to "*bulk*" Forastero varieties (West African *Amelonado* and Upper Amazonas clones), which is suggested to be the result of higher pH values after fermentation and drying of *Forastero* varieties. Chocolates

produced from the *Forastero* beans were thus reported to be less bitter, less astringent and less acidic than chocolate produced from either *Criollo* beans or *Trinitario* beans (Clapperton et al., 1994; Kongor et al., 2016). The "*bulk*" *Forastero* cocoa beans are considered as *slightly* bitter with the strongest cocoa flavour whilst being low in complex and fruity flavour notes (Hebbar et al., 2011).

Tab. 8: Concentration of (-)-epicatechin in cocoa beans unaffected by post-harvest treatment (Kim & Keeney, 1984).

Varietal type	**Clone**	**(-)-epicatechin concentration in defatted sample (mg/g)**
Nacional	NA-22-23	37.63 ± 0.22
Nacional	N-22-A3	34.65 ± 0.20
Trinitario	UP-667	41.33 ± 0.54
Trinitario	UF-11	39.33 ± 0.71
Trinitario	UF-296	36.33 ± 1.30
Amazon Forastero	EEG-29	35.33 ± 1.18
Amazon Forastero	SIC-250	43.27 ± 0.44

Even though a comprehensive analysis of the genetic influence on the chemical reactions during cocoa seed processing is not available yet (Elwers et al., 2009; Luna et al, 2002), it is widely acknowledged that the chemical composition of the bean, specifically the contents of storage proteins, polysaccharides, and polyphenols are genotype-dependent, determining the quantities and types of compounds formed cocoa bean processing (Kongor et al., 2016). However, these differences are also attributed to different environmental conditions as well as post-harvest treatment practices (ICCO, 2015b).

2.5.3.2 Effect of postharvest treatment

Post-harvest treatments include pulp-preconditioning, fermentation and drying. The process of fermentation has been shown to be highly affected by pre-conditioning of the mucilaginous pulp, which serves as substrate for microbiological activity (Ostovar & Keeney, 1973). Changes in its composition, such as moisture and sugar content, volume of pulp per seed as well as pH and acidity, may thus affect the production of alcohols by yeasts and subsequent production of acids by lactic and acetic acid bacteria with significant effects on acidity and polyphenol content and thus, cocoa bean flavour. It can be achieved through pod storage, depulping (mechanical or enzymatic depulping) and bean

spreading (Kongor et al., 2016). Pod storage significantly reduces the content of polyphenols (especially (-)-epicatechin). Furthermore, pod storage affects the degradation of (-)-epicatechin and (+)-catechin during subsequent fermentation, resulting in reduced astringency and bitter taste in cocoa beans and cocoa products. (Nazaruddin et al., 2006).

The process of fermentation is crucial in terms of colour and flavour generation as it promotes drastic biochemical changes and thus in the type and concentration of aroma precursors and polyphenols (Kongor et al., 2016). During fermentation, the total content of soluable polyphenols decreases ~ 50% in total, from 13-20% in freshly harvested cocoa beans to 5-8% in fermented cocoa beans (Ebermann & Elmadfa, 2011). 10% of polyphenols in cocoa beans or more are considered as a sign of pour fermentation (Wollgast & Anklam, 2000). The maximum loss takes place within the first two days of fermentation and is attributed to multiple factors. This includes diffusion of soluble polyphenols from their storage cells into the shell and fermentation sweating as well as enzymatic and non-enzymatic oxidation to high-molecular weight, mostly insoluable tannins. Furthermore, condensation with other compounds (such as proteins) occurs resulting in the formation of complexes (such as brown, water insoluable pigments). Oxidation is catalyzed by polyphenol oxidase, which is mostly inactivated during the first days of fermentation. By polymerizing into tannins, (-)-epicatechin content decreases drastically. Furthermore, procyanidin levels have been shown to decrease 3- to 5-fold during fermentation (Di Mattia et al., 2013; Niemenak et al., 2006; Wollgast & Anklam, 2000). Degree and duration of fermentation are thus critical factors in terms of polyphenol content (Albertini et al., 2015; Hansen et al., 1998; Kim & Keeney, 1984; Wollgast & Anklam, 2000).

Further losses of polyphenols take place during subsequent drying, which is particularly ascribed to non-enzymatic oxidation events. It has been shown that two days of sun-drying of fresh unfermented cocoa beans alone causes a 50% decrease in epicatechin content. Thus, in order to obtain high polyphenol content chocolates, much attention should be paid to these two manufacturing steps, which are far from being standardized throughout the world resulting in 6-fold variations in flavan-3-ol content as shown in Tab. 9 (Albertini et al., 2015; Hansen et al., 1998; Kim & Keeney, 1984; Wollgast & Anklam, 2000).

The decrement of polyphenols during these stages results in reduced bitter taste and astringency in the final product at acceptable levels, enhancing the overall flavour of cocoa beans (Kongor et al., 2016; Misnawi, 2008). Further steps in the processing chain such as alkalization used in cocoa powder manufacturing (Miller et al., 2008) and roasting cause losses in flavan-3-ol contents of cocoa beans (Miller et al., 2006). Roasting results in additional losses of 10-40%, especially if water or alkali are added due to oxidation of the arising phenolates when exposed to oxygen (Ebermann & Elmadfa, 2011). The impact of roasting on the polyphenol content depends on roasting duration and temperature. With increasing roasting duration, the heat labile polyphenols alter into *o*-quinones through thermal oxidation, which then polymerize with proteins, amino acids and sugar to form the typical brown colour and reduce astringency and bitter taste (Afoakwa et al., 2015). In addition, roasting affects the stereochemical properties of polyphenols especially the epimerization of (-)-epicatechin to (-)-catechin (Alañón et al., 2016; Hurst et al., 2011; Kothe et al., 2013).

Chocolate manufacturing stages such as grinding, refining and conching also seem to affect polyphenol content and composition in the final chocolate product, which is mainly attributed to high temperatures and the presence of oxygen. However, in-depth knowledge of these changes is limited, even though it has been shown that generally, higher processing temperatures and/or longer processing times reduce the amount of polyphenols available in cocoa components (Wollgast & Anklam, 2000).

2.5.3.3 Effect of cocoa bean origin

In addition, the region of origin contributes to the polyphenol profile and content in cocoa beans due to environmental factors including light intensity, humidity, temperature and the use of fertilizers (Niemenak et al., 2006). Soil fertilization may lead to significantly smaller amounts of total polyphenols, flavan-3-ols and anthocyanins (Elwers et al., 2009). Furthermore, different post-harvest treatment practices (pulp-preconditioning, fermentation, drying) usually carried out by farmers in the country of origin explain differences in polyphenol content from different origins. Contents of (-)-epicatechin were shown to be highest in cocoa beans from regions where fermentation is less extensive (e.g. 2.66 mg/g in Jamaican beans to 16.52 mg/g in Costa Rican beans, Tab. 9) (Kim & Keeney,

1984; Krähmer et al., 2015). Regarding fermentation durations, Rohan & Steward, (1967), reported the following order on a scale from shorter to longer fermentation times: Machala (Ecuador) < Sanchez (Dominican Republic) < Para (Brazil) < Costa Rica < Carupano (Venezuela) < Indonesia < Grenada < Jamaica < Fernando Po (Republic of Ecuadorial Guinea) < Puerto Cabello (Venezuela) < Trinidad < Arriba (Ecuador) < Ivory Coast < Accra (Ghana) < New Guinea < Cameroon < Lagos (Nigeria) < San Thome < Bahia (Brazil). Thus, some bean origins such as Madagascar and Java were shown to have double the amount of flavan-3-ols and procyanidins. Less fermented cocoa beans are considered as of lower quality due to their high bitterness as well as low cocoa flavour in comparison to well-fermented, flavourful cocoa beans (e.g. from Ghana) with appropriate bitterness and astringency (Miller et al., 2006).

Tab. 9: (-)-epicatechin concentrations in fermented and dry cocoa beans from different origin (Kim & Keeney, 1984)

Cocoa bean origin	(-)-epicatechin content in fermented defatted samples (mg/g)
Ivory Coast	6.22
Maracaibo (Venezuela)	3.62
Samoa	10.64
Trinidad	4.68
Bahia (Brazil)	8.23
Ghana	4.52
Lagos (Nigeria)	4.68
Costa Rica	16.52
Arriba (Ecuador)	8.08
Jamaica	2.66

2.6 Nutritional and health benefits of chocolate consumption

Chocolate is appreciated for its flavour and texture properties by many cultures and countries, but is typically considered as a non-nutritive luxury food containing mostly energy, fat and sugar. However, certain types of chocolate can provide significant amounts of essential nutrients associated with multiple beneficial health effects, when consumed in moderation within a well-balanced diet.

These health effects are attributed particularly to the polyphenols present in the non-fat cocoa solids (NFCS) of chocolates (Cooper et al., 2008). The bioactivity of these flavanols and thus, their nutritional relevance, was shown to be

significantly influenced by their stereochemical configuration. The monomeric flavan-3-ols (-)-epicatechin (Hooper et al., 2012; Schroeter et al., 2006) and (+)-catechin as well as oligomeric procyanidins (Rusconi & Conti, 2010; Tomás-Barbéran et al., 2007) possess a high antioxidative capacity (AOC) (Dreosti, 2000; Lee et al., 2003; Steinberg et al., 2003), which was found to be stronger in cocoa-containing products than those in green tea, black tea and red wine per serving (Lee et al., 2003). Thus, cocoa polyphenols are associated with protective effects against arteriovascular diseases, cancer and inflammatory events in the human body (Elwers et al., 2009).

Dark chocolate formulations with higher cocoa contents are richer sources of flavan-3-ol compounds compared to milk chocolates (Tab. 7) eliciting a stronger AOC (Miller et al., 2006). Even though, milk proteins have been suggested to interfere with polyphenol absorption in tea (Serafini et al., 1996), this has not been found the case in cocoa-containing products (Richelle et al., 1999; Roura et al., 2008). Thus, the high AOC in dark chocolate types is affected by the NFCS content (Miller et al., 2006) rather than the absence of milk ingredients. Whereas the AOC and thus, the potential human nutritional benefits is known to significantly correlate with the total polyphenol content (Miller et al., 2006), the functional significance of the various polyphenolic compounds present in chocolates, influenced by factors such as genetic origin, growing region and variations in cocoa processing and chocolate manufacturing, has not been entirely clarified. Furthermore, no official analytical methodology for the quantification of phenolic compounds in cocoa and chocolate exists at present (Rusconi & Conti, 2010).

Besides the high polyphenol content and AOC, the overall nutrient profile of dark chocolates is suggested to be more beneficial than that of milk chocolate considering the lower sugar as well as higher mineral content. More so, the lipid profile of cocoa, consists mainly of stearic acid (C18:0), which exerts a neutral cholesteremic response in humans, despite the relatively high fat content. Taking into consideration the high energy density, moderate amounts of dark chocolate can be part of a healthy diet (Crichton et al., 2016; Steinberg et al., 2003).

Among the preventive and therapeutic effects observed in association with high-polyphenol cocoa and chocolate consumption are the following:

- suppression of LDL-oxidation (Vinson et al., 2006)
- blood pressure lowering (Allen et al., 2008; Grassi et al., 2005; Hooper et al., 2012; Persson et al., 2011)
- reduction of pulse wave velocity (Crichton et al., 2016; Grassi et al., 2015)
- improvement in endothelial function/flow-mediated dilation (Grassi et al., 2015; Heiss et al., 2010; Hooper et al., 2012; Schroeter et al., 2006)
- inhibition of platelet activation and function (Khawaja et al., 2011; Rein et al., 2000)
- increased insulin sensitivity (Alkerwi et al., 2016; Grassi, Lippi, et al., 2005; Hooper et al., 2012)
- Immune modulation (Steinberg et al., 2003)

Through these mechanisms, chocolate can contribute to prevent or delay the development of several chronic diseases such as cardiovascular disease (CVD) (Dong et al., 2017; Larsson et al., 2016; Mostofsky et al., 2010), diabetes (Crichton et al., 2017) and other age-related diseases afflicting industrialized societies (Afoakwa, 2010; Steinberg et al., 2003).

Due to the small amount of studies, more clear evidence is needed on the bioavailability of the cocoa polyphenols in humans, likely affected by product processing, interactions with other components of the diet as well as host factors (Albertini et al., 2015).

Furthermore, a higher frequency of chocolate consumption was found to be associated with a lower BMI, lower levels of total and central fatness as well as waist circumference – factors potentially promoting above-mentioned diseases (Cuenca-García, Ruiz et al., 2014). This finding cannot be explained by sugar, fat and calorie intake - as frequent chocolate consumption was associated with more overall calories, physical activity or other potential confounders. It is suggested, that polyphenols (cocoa-derived epicatechin) could exert this effect by increasing mitochondrial biogenesis and capillarity, lean muscle mass, muscular performance and reducing weight without changing calorie intake or exercise practices as shown in rodent studies (Golomb et al., 2012).

In addition, it has been shown that regular intake of cocoa-related products and thus, cocoa flavanols may have beneficial effects on neuropsychologial health and potentially protect against age-related cognitive decline (Crichton et al., 2016; Moreira et al., 2016) in a dose-dependent manner (Nurk et al., 2009).

Polyphenols represent the main source of antioxidants in human nutrition (Graf et al., 2005). In America, chocolate is considered the third highest contributor of antioxidants (100–107 mg/day) after fruits (255 mg/day) and vegetables (233 mg/day) (Vinson et al., 2006) and the Dutch population is supplied with catechins up to 20% from chocolate (Arts et al., 1999). Although, the consumption of fruits and vegetables is of more relevance to ensure polyphenol supply, taking into account the overall nutritional profile including energy density, the recommendation of moderate amounts of chocolate with high cocoa and preferably standardized polyphenol contents seems reasonable within a well-balanced diet addressing the general population (Rusconi & Conti, 2010). Furthermore, various chocolate products with increased amounts of polyphenols were launched recently due to these health-protecting properties (Elwers et al., 2009).

2.7 Sensory evaluation of chocolate

Chocolate is a semi-solid, luxury food and highly appreciated for its complex sensory properties by individuals all over the world (Liu et al., 2015). Its distinct set of sensory characteristics (*appearance*, *flavour* (=olfaction, gustation and trigeminal responses (ASTM E253 - 17, 2017) and *texture*) is the result of cocoa beans' inherent factors, processing and manufacturing as well as the chocolate formulation (Guinard & Mazzucchelli, 1999). Flavour precursors are derived from chemical compounds present in the raw cocoa beans, which are affected by bean genotype, age of cocoa tree, environmental conditions such as climate, Sun exposure, rainfall, soil conditions, ripening, time of harvesting as well as time between harvesting and bean fermentation. Furthermore, complex biochemical and chemical reactions taking place during post-harvest treatments such as pulp pre-conditioning, fermentation, drying and roasting (Afoakwa, 2010; Kongor et al., 2016) as well as the transformation of the beans into semi-solid chocolate

matrices during chocolate manufacturing highly affect the chemical composition and thus, nutritional and sensory properties (Afoakwa, 2010).

Due to compositional requirements (such as the absence of dairy ingredients, lower sugar contents), the sensory profile of dark chocolates differs considerably from that of milk chocolate (Guinard & Mazzucchelli, 1999; Kennedy & Heymann, 2009; Liu et al., 2015; Sørensen & Astrup, 2011). Higher cocoa contents are associated with bitter taste and astringent mouthfeel sensations (Kennedy & Heymann, 2009) elicited by a series of flavan-3-ols ((-)-epicatechin, (+)-catechin), procyanidins, caffeine and theobromine (Stark et al., 2006) naturally occurring in the non-fat cocoa solids (Belitz et al., 2008) potentially limiting consumers' acceptance and preference (Lesschaeve & Noble, 2005). In order to satisfy consumers expectations, a balanced sensory profile in terms of bitterness and astringency is necessary (Ma et al., 2014; Stark et al., 2006).

As sensory perception is not simply the result of the present stimuli, but a multi-modal phenomenon involving oral processing and subsequent flavour release, sensory profiing techniques acknowledging these dynamics are recommended (Afoakwa, 2016c).

2.7.1 Sensory profile of dark chocolate

In general, the sensory characteristics *appearance*, *flavour* and *texture* are the major determinants of chocolate quality (Afoakwa, 2016c). Tab. 10 displays common sensory descriptors for the evaluation of chocolates.

Tab. 10: Set of common sensory descriptors for the evaluation of chocolate (Afoakwa, 2010).

Sensory attribute	Description
Appearance	
Colour	
intensity	the intensity (tint) of typical chocolate colour ranging from light brown to dark brown.
brightness	luminescence of colour ranging from dull to shiny.
Texture	
on surface	amount of regular cavities or holes on surface to bottom of chocolate ranging from even to gritty.
on snap	

melting in hand	level of chocolate melting after 30 seconds of contact in the hand ranging from melted little to melted very much
Flavour/aroma	
Chocolate/cocoa	This descriptor is reminiscent of the aroma and flavour of cocoa powder and chocolate (including dark chocolate and milk chocolate).
Fruity/citrus/berry	flavour/aroma associated with the odour and flavour of fruits. The natural aroma of berries is highly associated with this attribute. The perception of high acidity in some chocolates is correlated with citrus characteristic.
Floral/fragrant	flavour/aroma associated with the slight scent of different types of flowers including honeysuckle, jasmine, dandelion and nettles
Taste	
Bitter taste	A basic taste factor characterised by the solution of caffeine, quinine and certain alkaloids.
Sweet taste	A basic taste factor solutions of sucrose.
Acidic/acidity	A basic taste factor characterised by the solution of an organic acid. A desirable sharp and pleasing taste particularly strong with certain origins as opposed to an overfermented sour taste.
Texture/Mouthfeel	
Astringency	The feeling of a puckering or a tingling sensation on the surface and/or edges of the tongue and mouth.
Gritty	This is the perception of roughness of chocolate matrix due to detection of solid particles of sizes >35 µm by the tongue during mastication. Grittiness is normally detected by rolling the sample between the tongue and the palate. Descriptions used vary from very gritty to very smooth.

2.7.1.1 Appearance

Appearance, which is often the first impression of a product, affects other sensory modalities (odour, flavour, taste) and thus product acceptance, choice and enjoyment. Good quality chocolate is characterized by a continuous light to dark brown colour (depending on the chocolate formulation) generated during fermentation, drying and roasting, and a glossy appearance. Undesireable characteristics include white specks, patches or blotches attributed to the migration of fat or sugar to the product surface as a result of unproper tempering. ‘Fat bloom’ or ‘sugar bloom’ affect the quality and acceptability of chocolates dramatically (Afoakwa, 2010; Afoakwa et al., 2008).

2.7.1.2 Flavour

2.7.1.2.1 Aroma-active volatile compounds

Chocolate flavour is ascribed to an extremely rich and complex mixture of 550 (Camu et al., 2008) to 600 or even more aroma-active, volatile compounds including alcohols, carboxylic acids, aldehydes, ketones, esters and pyrazines, likely to play a role in chocolate flavour (Aprotosoaie et al., 2016). These compounds are derived from flavour precursors, which are formed during fermentation and drying, and finally generated during the roasting stage through Maillard reactions and Strecker degradation of these precursors and their intermediates (Afoakwa et al. 2008). Most of these compounds possess particular flavour characteristics. Whereas most esters confer fruity/flowery notes, pyrazines usually evoke rather earthy/roasted flavour impressions. In addition to cocoa bean processing, the initial chemical composition of the cocoa seeds affects the final cocoa flavour, which is highly dependent on the cocoa genotype as well as the region of origin. However, the relationship between these sensory characteristics, the odour-active volatile compounds and the sources and mechanisms of flavour formation are not yet fully understood (Aprotosoaie et al., 2016; Kongor et al., 2016).

In addition to volatile aroma-compounds affecting the sensory profile of chocolates, various non-volatile components present in the raw cocoa beans play major roles in the formation of the unique cocoa flavour primarily eliciting taste and mouthfeel sensations.

2.7.1.2.2 Non-volatile compounds

The non-volatile compounds present in cocoa beans are carbohydrates, proteins, polyphenols and purine alkaloids (methylxanthines) (Aprotosoaie et al., 2016). Carbohydrates and proteins act mainly as precursors of aroma-active volatiles within the Maillard reaction during roasting yielding in characteristic cocoa flavour components as described above. Some residual proteins participate also in phenol–protein interactions contributing to the typical brown colour as well as reduced bitterness and astringency. On the other hand, diketopiperazines formed during roasting through thermal decompositions of proteins as well as free amino acids present in the non-fat cocoa solids contributing to bitter taste sensations in

cocoa-derived products (Afoakwa, 2015; Belitz et al., 2008; Kongor et al., 2016; Stark et al., 2006).

It is widely acknowledged that polyphenols are key inducers of bitter taste and astringency in cocoa and chocolate. Although important components of cocoa flavour, these compounds can affect the chocolate flavour detrimentally if present in excess amount (Elwers et al., 2009; Jinap et al., 1995). A series of flavan-3-ols ((-)-epicatechin, (+)-catechin) and procyanidins present in the non-fat cocoa solids confer these sensations (Bravo, 1998; Kim & Keeney, 1984; Lesschaeve & Noble, 2005; Noor-Soffalina et al., 2009; Stark et al., 2005; Stark et al., 2006), which are thus stronger in cocoa-derived products with higher cocoa contents such as dark chocolates (Kennedy & Heymann, 2009). However, the polyphenol content and profile present in the final chocolate product is influential by several factors as described previously. Fig. 12 displays the mechanisms of flavour precursor formation (volatiles and non-volatiles) and major impact factors during post-harvest treatment.

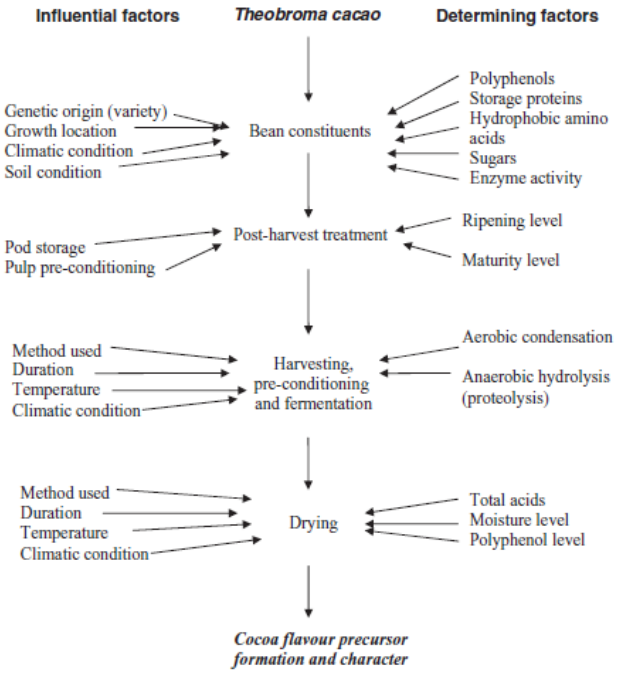


Fig. 12: Mechanism of chocolate flavour precursor formation (volatile and non-volatile compounds) (Afoakwa, 2010)

In addition, metylxanthines, with theobromine (3,7- dimethylxanthine) being the most abundant alkaloid of cocoa followed by small amount of caffeine (1,3,7-trimethylxanthine) and traces of theophylline (1,3-dimethylxanthine) (Tab. 3), contribute to bitter taste (Franco et al., 2013).

Tab. 11 contains non-volatile compounds contributing to bitter taste and/or astringency in roasted cocoa nibs.

Tab. 11: Compounds eliciting bitter taste and astringency in roasted cocoa nibs (Belitz et al., 2008; Stark et al., 2006)

Compound	concentration (µmol/kg)
Group I: Bitter tasting compounds	
Theobromine	63 564.4
Caffeine	5218.3
cis-cyclo (L-Pro-L-Val)	8877.8
cis-cyclo (L-Val-L-Leu)	817.1
cis-cyclo (L-Ala-L-Ile)	734.0
cis-cyclo (L-Ile-L-Pro)	537.0
Group II: Bitter and astringent compounds	
(-) - epicatechin	8613.1
procyanidin B2	2082.8
procyanidin C1	1628.0
[epicatechin-(4β → 8)]$_3$-epicatechin	1158.9
[epicatechin-(4β → 8)]$_4$-epicatechin	802.1
(+) - catechin	2363.9
procyanidin B5	791.5
[epicatechin-(4β → 8)]$_{5\text{-}9}$-epicatechin	623.3
Group III: Purely astringent compounds	
γ-aminobutyric acid	5011.4
Quercetin-3-0-β-D-glucopyranosid	101.4
Quercetin-3-0-β-D-galactopyranosid	33.8
N-[3',4'-Dihydroxy-(E)-cinnamoyl]-3-hydroxy-L-tyrosine	851.5

2.7.1.2.3 Bitter taste

Bitter sensations are elicited by structurally heterogenic compounds including amino acids and peptides, sulfimides (saccharin), ureas and thioureas (6-n-

propylthiouracil (PROP) and phenylthiocarbamide (PTC), esters and lactones, terpenoids, phenols and polyphenols (Drewnowski & Gomez-Carneros, 2000). Receptor cells responsible for evoking bitter taste are TAS2Rs (Chandrashekar et al., 2000) located in the papillae on the tongue. Several transduction mechanisms for bitterness perception have been identified and appear to be compound specific. However, a clear definition of molecular characteristics conferring bitterness has not yet been proposed and is in the focus of intense research (Lesschaeve & Noble, 2005).

In general, humans are extraordinary sensitive to bitter sensations and instinctively reject foods eliciting this taste sensation as it is primarily attributed to poisonous and toxic substances (Drewnowski & Gomez-Carneros, 2000). However, large individual differences exist (Tepper, 2008).

2.7.1.2.4 Astringency

Astringency is a chemical induced complex of tactile sensations perceived via mechanoreceptors. It is defined as “complex of sensations due to shrinking, drawing or puckering of the epithelium as a result of exposure to substances such as alums or tannins” (ASTM, 1989). These compounds evoke a rough and dry mouthfeel and cause a drawing, puckery, or tightening sensation in the cheeks and muscles of the face (Lawless & Heymann, 2010c).

Due to increasing intensity of the sensation through repeated stimulation rather than adaption, which is characteristic for taste sensations, as well as the fact that astringency can also be sensed from areas in the mouth lacking in taste receptors, astringency has been classified as a tactile sensation (Breslin et al., 1993; Lee & Lawless, 1991).

Polyphenolic compounds, present in cocoa derived products such as chocolate and other foods and beverages such as wine, tea, nuts and fruits (Lesschaeve & Noble, 2005), are chemical stimuli of astringent sensations due to their capacity to bind salivary proteins and mucins, causing them to precipitate or aggregate. Thus, saliva loses its ability to coat and lubricate oral tissues. Individuals with higher salivary flow rates are thus less reactive and might give lower astringency ratings due to greater protection of oral surfaces against astringent compounds and a faster “clean up” after of the astringent stimulant (Lawless & Heymann,

2010c). Product preferences and acceptance thus may vary tremendously among individuals and may not increase with decreasing astringency perception (Lesschaeve & Noble, 2005).

2.7.1.2.5 Sweet taste

It has been reported that the compounds present in roasted cocoa nibs eliciting sweet taste (monosaccharides, disaccharides, oligosaccharides and some L-amino acids) are in concentrations lower than the recognition threshold of panelists. Thus, these compounds might not contribute significantly to the typical taste of roasted cocoa beans and related products (Stark et al., 2006). Sweetness in chocolates is therefore attributed to the content of sucrose or other sweeteners.

2.7.1.2.6 Impact of cocoa bean genotype and region of origin on the sensory properties of dark chocolate

As already mentioned, the sensory profile of dark chocolates differs substantially depending on the cocoa bean variety, region of origin, environmental factors, farming and post-harvest treatment practices. Tab. 12 shows flavour characteristics of different cocoa bean genotypes (and blends thereof) from different origins (Reed, 2010).

Tab. 12: Flavour profile of different cocoa beans from different origins (Reed, 2010) .

Origin	Cocoa type	Flavour profile
Côte d'Ivoire	Forastero	Good cocoa impact, low bitterness, low acid, fruity, nutty
Ghana	Forastero hybrids	Strong chocolate flavour
Nigeria	Forastero hybrids	Medium cacao, occasional off-notes
São Tomé & Principe	Forastero	Good cocoa flavour, bitter, spicy, fruity, earthy
Madagascar	Criollo	Winey, putrid, citrus
Venezuela	Criollo "Porcelana"	Mild chocolate, slightly bitter, distinct fruity notes (plum and cherry)
Brazil	Forastero	Cocoa impact, bitter, acid, astringent (sometimes rubber, hammy, smoky), some fruitiness, no nutty notes

Colombia	Trinitario and Criollo	Fruity, bitter, cacao
Peru	Forastero	Slightly bitter and fruity
Ecuador (home of *Arriba*)	Forastero (Nacional)	Balanced profile, low chocolate, floral, fruity, grass, earthy notes
Mexico (Tabasco)	Criollo/Forastero hybrids	Low chocolate, strong acid, low fruitiness
Panama	Forastero	Moderate chocolate, acidic, fruit and nut notes
Jamaica	Forastero	Fruity
Dominican Republic (Sanchez)	Criollo/Forastero hybrids	Low cacao, flavourless, bitter
Dominican Republic (Hispaniola)	Criollo/Forastero hybrids	Winey, earthy, can have tobacco notes
Costa Rica	Forastero	Fruity, balanced cocoa flavour
Trinidad & Tobago	Trinitario (birthplace)	High cacao, nutty and winey notes, aromatic
Grenada	Trinitario	Chocolate, fruity, floral, grassy, woody
Indonesia	Criollo/Forastero hybrids	Low chocolate, acidic, fruity
Sulawesi	Criollo/Forastero hybrids	High bitter, low sour, low cacao, astringent
Java	Criollo/Forastero hybrids	Mild, bland profile, acid, low cacao, light colour
Papua New Guinea	Hybrids/pure Criollo and Forastero	Variable strong acid, floral, mild, nutty
Malaysian	Forastero hybrids	Low to medium cacao, medium to high acidity, astringent (due to fermentation level) phenolic

In addition to the volatiles and non-volatiles present in the cocoa liquor, the chocolate flavour profile is characterized by the recipe and chocolate formulation. Due to the presence of dairy ingredients as well as the higher sugar content, milk chocolate is mainly perceived as sweet, milky, and honey-like with coconut notes (Liu et al., 2015). This impression is also attributed to the presence of milk fat, which was shown to induce a significant decrease in the release of volatiles (Keršiene et al., 2008) from cocoa solids. Dark chocolate is thus characterized by an intense chocolate flavour with malty, nutty, and praline notes (Liu et al., 2015). Furthermore, low sugar contents in chocolates are associated with higher bitter

and roasted notes (Guinard & Mazzucchelli, 1999) contributing to distinct flavour profile of dark chocolates.

2.7.1.3 Texture

The texture of chocolates is the most complex of its physical characteristics. It is dependent not only on the chocolate formulation (milk or dark chocolate; w/o lecithins), but also on the particle size of cocoa solids as well as distribution of solid particles within the continuous fat phase consisting of cocoa butter (Afoakwa, 2010). Proper grinding, refining and conching are thus crucial steps in chocolate manufacturing as inappropriate particle sizes affect both the rheological characteristics as well as flavour/taste and thus the overall sensory quality of chocolates. It has been shown that particle sizes <15 µg tend to produce undesirable peanut-butter-like flavours, whereas great proportions of particles sized 30-35 µg elicit a rather undesired sandy, gritty and rough sensation (Afoakwa et al. 2007; Zach, 2017). The three main quality criteria are defined as smoothness, meltiness and hardness, which are affected not only by above-mentioned manufacturing procedures but also by the cocoa butter in the formulation. The crystalline structure of cocoa butter influences i.a. gloss and stability of the product, but also its unique melt-in-mouth characteristics. Dark chocolates show greater bite firmness, melt more slowly (Afoakwa, 2010) and elicit rather dry, sticky and mealy sensations in comparison to milk chocolates which are perceived as more smooth, creamy and melting (Thamke et al., 2009).

Overall, chocolate is most desired as a firm solid product with a good snap, a glossy appearance that melts easily in the mouth producing smooth mouthfeel sensations. Defects in texture are therefore a poor snap, a sticky surface, a soft or hard texture, not melting readily in the mouth and a gritty and sandy mouthfeel (Afoakwa, 2010).

2.8 Sensory evaluation techniques

In order to determine chocolates quality in terms of sensory characteristics (*appearance*, *taste*, *flavour* (=olfaction, gustation and trigeminal responses), *texture*, *aftertaste*), a set of sensory evaluation techniques exist for accurate measurement of human responses. Sensory evaluation has been defined as a

scientific approach used to evoke, measure, analyze and interpret those responses to products as perceived through the senses of sight, smell, touch, taste and hearing (Stone & Sidel, 2004). Two major testing categories exist in sensory science: analytical (objective) and hedonic (subjective) (Kemp et al. 2009). Analytical approaches aim at comparing products based their specific sensory properties using descriptive or discriminating techniques (in order to evaluate subtle differences between products). Such objective methods require trained (or even highly trained) panelists (Lawless & Heymann, 2010b).

On the other hand, hedonic (affective) approaches measure subjective responses of preferably untrained consumers to the sensory properties of a product introspectively or observationally (Duerrschmid, 2010).

2.8.1 Dynamics of sensory perception

Sensory evaluation methods commonly used to describe the sensory characteristics of chocolates for various research purposes are conventional profiling techniques (Guinard & Mazzucchelli, 1999; Sim et al., 2016; Sune et al., 2002). These approaches aim at quantifying sensory responses in terms of attribute intensities using a static, single-point measurement, where judges must time-average or integrate their responses to provide single intensity values (Lee & Pangborn, 1986). However, sensory perception, which depends not only on product-inherent characteristics but also on consumer-related aspects (such as chewing pattern, efficiency, saliva flow rate), is not happening at a constant rate but as a series of events, occurring at all stages of oral processing from the first bite/sip until swallowing of the bolus (Dijksterhuis & Piggott, 2001; Foster et al., 2011; Piggott, 1994). This complex dynamic and time-dependent process, affecting sensory perception and thus presumably consumers' hedonic response (Delarue & Blumenthal, 2015; Wang & Chen, 2017), is determined by several factors (Overbosch et al., 1991):

- The nature and amounts (ratios) of volatile and non-volatile compounds present in the food product
- The availability of these compounds to the sensory system as a function of time, which is affected by two types of influences:

    - The breakdown of the food matrix through oral processing such as mastication, enabling flavour release, and
    - the subsequent transfer of the released compounds to the corresponding receptors
- Mechanisms of perception
- Strategies of scaling (consumer inherent)

Thus, the food matrix plays an important role in controlling flavour release at each step of product consumption (Druaux & Voilley, 1997). Factors contributing to the alteration of the physical properties of the product include mastication, interaction with saliva, heat exchanges and pH changes (Boland et al., 2006).

In terms of chocolate, these factors of oral processing lead to the transformation of the structure from a composite semi-solid state at room temperature (20-25°C) to an oil/water emulsion, consisting of cocoa solids in dissolved sugar crystals and cocoa butter, at oral temperature (37°C) prior to swallowing. This dynamic process is defined by the physical characteristics of the lipid phase as well as the particle size of cocoa and sugar solids, which facilitate the perception of its typical taste, flavour and textural attributes (Rodrigues et al., 2017; Ziegler et al., 2001). Volatile compounds, detected retronasally from the olfactory system during tasting, as well as other compounds such as polyphenols, eliciting bitter and astringent sensations on the tongue and the oral cavity, are inherent in the insoluable non-fat cocoa solids. Released and suspended in the fat phase through melting, mixed with saliva and fragmented through mastication (extending the surface area), the cocoa particles are then transferred to the corresponding receptors followed by the mechanisms of perception. In addition, saliva acts as a solvent for sugar crystals as well as carrier to the receptors, where bitter taste and sweet taste are detected time-dependently (Afoakwa, 2016c; Rodrigues et al., 2017). Furthermore, some stimuli induce very short sensory signals while other compounds may induce persisting sensations, including bitter taste (Delarue & Blumenthal, 2015) and astringency (Courregelongue et al., 1999).

These highly complex processes are not captured by conventional descriptive methods of sensory analysis, which produce time-averaged data unconsidering the multidimensionality of sensory perception. In order to overcome this limitation multiple sensory evaluation techniques have been established in recent decades acknowledging the temporality of sensory perception as a function of time (Castura et al., 2016; Clark & Lawless, 1994; Duizer et al., 1996; Kuesten et al., 2013; Lee & Pangborn, 1986; Pineau et al., 2009).

Only a few studies are performed on chocolate applying these techniques, such as Time-Intensity (TI) (Lenfant et al., 2013; Palazzo & Bolini, 2014; Ziegler et al., 2001) and Temporal Dominance of Sensations (TDS) (Rodrigues et al., 2016).

Whereas Time-Intensity (TI) enables the evaluation of changes in intensity of a given attribute continuously through the course of tasting quantitatively (Cliff & Heymann, 1993), is the aim of Temporal Dominance of Sensations (TDS), which is a relatively young, however very popular and well-established dynamic method, to record the evolution of dominant descriptors along the tasting period (Pineau et al., 2009).

2.8.2 Temporal Dominance of Sensations (TDS)

The TDS method consists of selecting among a list of attributes the one perceived as dominant at every single point of time during the tasting of the product (from intake to swallowing and/or aftertaste until perception ends). The “dominant” attribute is thereby defined as the sensation “popping up” (Pineau et al., 2009) or the one “which triggers the most your attention at a point of time” (Lenfant et al., 2009), likely to be the one with raising intensity, allowing you to suddenly perceive it, but the dominant attribute does not have to be very or the most intense in the product” (Pineau & Schlich, 2015).

The assessors are instructed to put the sample (a defined portion size) in the mouth, click the “START”-button on the screen presented for TDS evaluation simultaneously and immediately start the evaluation by selecting the first dominant attribute. The moment a new sensation catches a panellist’s attention, he/she is supposed to click the respective button on the screen and thereby inactivate the previous one since a selected attribute is considered as dominant until the next one is chosen. Along the tasting period, attributes can be chosen

several times or not at all. Depending on the study objective and thus the testing protocol, the evaluation ends e.g. with swallowing of the sample or when perception ends by clicking the “STOP”-Button. The list of attributes is suggested to not exceed 8-10 attributes per evaluation to ensure good quality data since panelists tend to use only a subset of attributes if provided more, which differs across subjects. To avoid any attribute order effects it is further recommended to counterbalance the attribute order among subjects, but the same order should be kept in each replication for a given assessor (Pineau & Schlich, 2015; Pineau et al., 2012). Depending on the research target, TDS can be conducted as single-intake or full portion size experiment (Galmarini et al., 2017; Pineau & Schlich, 2015; Thomas et al., 2017; Zorn et al., 2014).

The procedure results in individual sequences of dominant sensations (one dominant sensation follows the other) considering different durations for each dominant attribute (active attributes = ‘1’; inactive attributes = `0`) as shown in Fig. 13.

	0					5					10					15					20
sweet	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
bitter	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	0	0
astringent	0	0	0	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	1	1	1
	sweet			astringent							bitter								astringent		

Fig. 13: Sequence of dominant attributes for one panelist and one product with corresponding data matrix (own illustration).

Typically, the individual sequences are summarized and displayed as TDS curves to get a descriptive picture of the evolution of dominant perceptions at panel level. This procedure considers each attribute separately as shown in Fig. 14. At each point of time, the proportion of evaluations (panellist x replication) for which the given attribute was selected as dominant is computed. The dominance rates (DR %) representing the y-axis of the graph are calculated as follows:

$$DR\ (\%) = NE/NE_{max}$$

NE…number of citations of a given attribute at a given time

NE_{max}… total number of evaluations (panellists x replications)

Thus, high DR (%) indicate a high consensus among panellists to quote a specific attribute as dominant at a given point of time. As illustrated in Fig. 14, the DR (%) are plotted against time (x-axis) (3) and smoothed (4). The resulting curves are then depicted on one graph displaying the evolving dominant sensations in the course of time at panel level (5). The product evaluated in Fig. 14 is perceived as bitter at the beginning of the tasting period with the highest panel consensus, followed by sweet taste, astringency and again sweet taste towards the end of assessment (Labbe et al., 2009; Pineau et al., 2009; Pineau & Schlich, 2015).

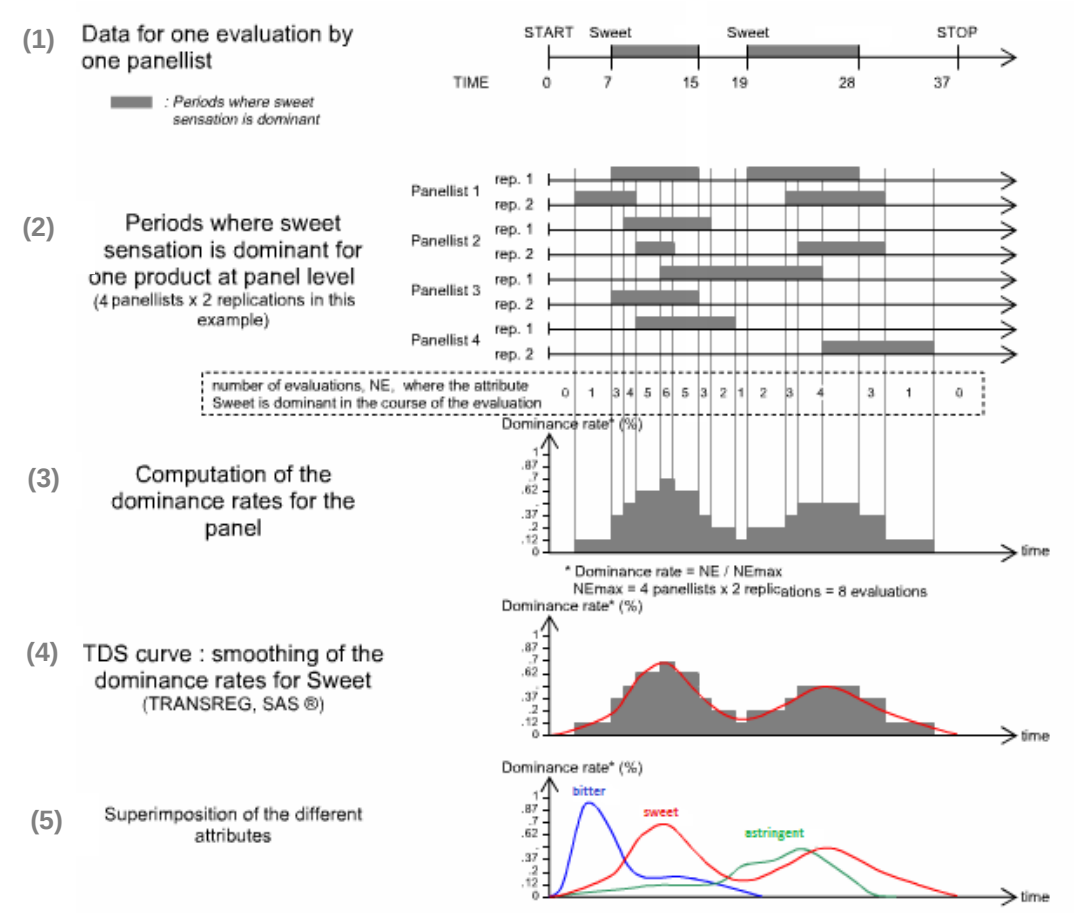


Fig. 14: Data processing to build TDS curves (Pineau et al., 2009).

A testing protocol consisting of individual rather than a predefined evaluation durations (which are more elaborated for beverages) requires data standardization procedures for the computation of the TDS curves in order to align the data among assessors and thus obtain higher levels of consensus (DR%) as proposed by Lenfant et al., (2009). Standardization turns the

dominance duration (s) of each attribute into a proportion of the total duration (%) as shown in Fig. 15, easily showing the contribution of each attribute to the complete TDS evaluation. Thus, the x-axis does not represent real time (s) anymore, but the testing period (%) from first scoring (x = 0) until perception ends (x = 100).

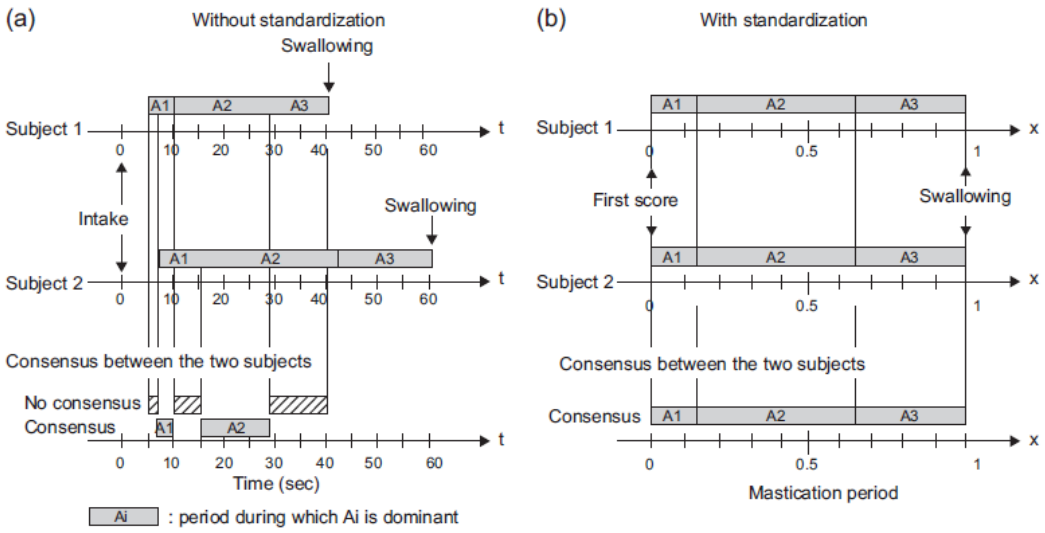


Fig. 15: Explanation of data standardization) by comparing TDS sequences between two subjects. (a) shows the unstandardized data (real time), (b) with standardization (%) (Lenfant et al., 2009).

Fig. 16 illustrates the differences in TDS-graphs obtained from non-standardized (a) and standardized (b) data (evaluation of a breakfast cereal sample), clearly showing increased DR(%) in (b) (Lenfant et al., 2009; Pineau & Schlich, 2015).

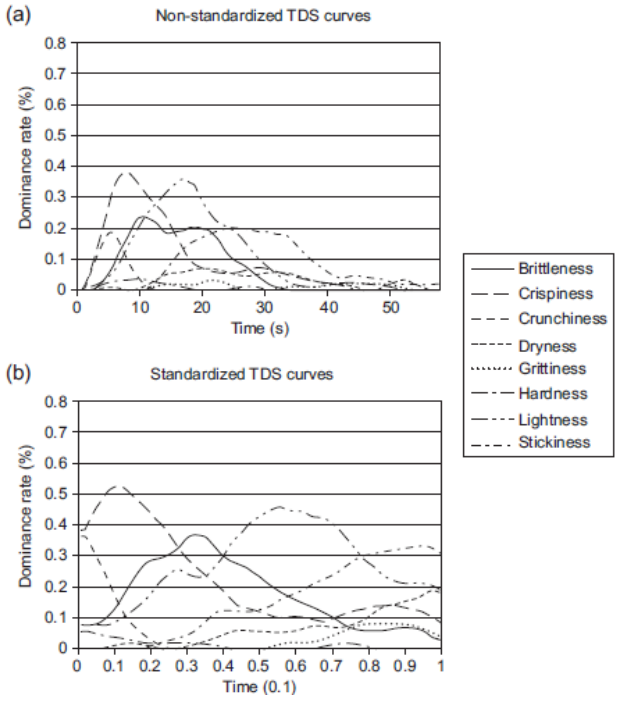


Fig. 16: Example for non-standardized (a) and standardized (b) TDS curves (texture evaluation of a breakfast cereal sample) (Pineau & Schlich, 2015).

In addition to the visual inspection of the TDS curves, some authors made the attempt to analyse curve-extracted parameters (such as maximum value for dominance rate (V_{ma}), time to reach V_{max} from the beginning of the testing period (T_{max}), duration period when dominance rate is higher than 0,9 x V_{max} (D_{max}), first time at which an attribute reaches significant dominance (T_{first}), last time at which an attribute reaches significant dominance (T_{last}), area under the curve AUC or area above significance (AAS) by Principal Component Analysis (PCA) (Pineau & Schlich, 2015) or the TDS-curves by Parallel Factor Analysis (PARAFRAC) (Rodrigues et al., 2016) in order to get an overview of the product space. However, this idea is not supported by Schlich, (2017) as these curves are not produced by individuals, but are an approximate visual summary of the panel.

Instead of analyzing curve-extracted parameters, Galmarini et al., (2017) propose the duration of attribute dominances as additional key information on TDS data in order to characterize and distinguish products.

2.8.3 Consumer surveys

The investigation of consumers' subjective responses to products enables researchers to gain insight in consumer's preferences, behaviours, attitudes, opinions and perceptions. Hedonic surveys are key elements in product development or improvement and marketing (Kemp et al., 2009). Two commonly used quantitative techniques are preference and acceptance measurements. In preference testing, the consumer is offered a choice among alternative products in order to see if there is a clear preference from the majority of respondents. This procedure however gives no information on the degree of liking of the product individually while acceptance tests are designed to exactly measure this aspect by rating the liking of a product on a scale (Lawless & Heymann, 2010b).

2.8.3.1 Preference test 1: Paired preference test

Product discrimination techniques suitable for preference testing are the paired preference test and the ranking test for preference (Kemp et al., 2009). In paired preference tests, the panelist is provided with two coded samples simultaneously. The task is to indicate the sample that is preferred over the other. In order to simplify data analysis and interpretation, the tests are usually conducted as "forced-choice" procedures, although a "no-preference" option theoretically also exists (Lawless & Heymann, 2010d).

2.8.3.2 Preference test 2: Ranking test for preference

The ranking method consists of presenting panelists with 3 or more products to be rank-ordered for preference with one sample being preferred over the next (Hein et al., 2008). This forced-choice approach is intuitively simple for the consumer, however, the multiple comparisons involved in ranking can be very fatiguing minimizing the number of products included (Lawless & Heymann, 2010d).

2.8.3.3 Acceptance test

Preference tests are popular due to their simplicity, but also because they are suggested to effectively reflect consumers purchasing habits (choosing among alternatives). They are regarded to be more sensitive than scaled product acceptance, since two products are likely to receive the same score on a scale, but one could yet be slightly preferred over the other. However, a product could also win the forced-choice test, but is still perceived unappealing on its own.

Acceptance tests are designed to record hedonic responses elicited without direct comparison to other samples using rating scales (Lawless & Heymann, 2010a). Traditionally, a 9-point hedonic scale or “degree of liking scale” (horizontally or vertically) is used to obtain acceptability data (Tab. 18) This type of scale has gained wide popularity in the food research area since its invention in the 1940s (Peryam & Girardot, 1952) and is still regarded as “key tool” due to practicality, reliability and high stability of responses. However, various other line scales and magnitude estimation have been used in order to gauge consumer’s appealing to products to deal with some concerns related to the 9-point hedonic scale such as unequal interval spacing, central tendency and lack of freedom to elicit responses due to predefined categories (Lawless & Heymann, 2010a).

Acceptability data are advantageous for various other purposes. It can be used in preference mapping techniques. These multivariate approaches of data analysis enable the illustration of the relationship between the sensory characteristics of a product evaluated by trained subjects (descriptive data) and consumers’ hedonic responses providing deeper knowledge on potential drivers of liking (Kemp et al., 2009).

3 Materials and methods

3.1 Samples

The aim of the present study was to explore the temporality of polyphenol-associated characteristics of dark chocolates. Five origin (OR) and six non-origin (N-OR) chocolate samples with different cocoa contents (66.8-80.1%cc and 54.5-80.0%, respectively) were investigated within the shelf-life period. All 11 samples were sponsored by Barry Callebaut N.V. (Wieze, Belgium). As shown in Tab. 13, OR chocolates are manufactured primarily from “fine or flavour” cocoa varieties grown in specified geographical regions, whereas cocoa beans used for manufacturing N-OR chocolates are blends of “bulk” cocoa beans from different and non-specified origins. The samples were compared within each group considering different cocoa content and between the groups, i.e. OR with N-OR.

The study procedure adhered to the guidelines of the Declaration of Helsinki in its revised form 2008.

Tab. 13: Characterization of the investigated chocolate samples.

Cocoa content (cc%)	Region of origin	Genotype
Origin chocolates (OR)		
OR 66.8	Brazil (single origin)	Trinitario, Forastero
OR 67.4	Madagascar (single origin)	mainly Forastero, Criollo, Trinitario
OR 70.0	São Tomé (single origin)	Amelonado (Forastero)
OR 70.4	Ecuador (single origin)	Arriba Nacional
OR 80.1	Tanzania, Ghana, São Tomé (blend of origins)	Trinitario, Forastero
Non-origin chocolates (N-OR)		
N-OR 54.5	mainly West Africa	mostly Forastero
N-OR 60.0	mainly West Africa	mostly Forastero
N-OR 65.0*	n.s.	n.s.
N-OR 70.3	mainly West Africa	mostly Forastero
N-OR 70.5	mainly West Africa	mostly Forastero
N-OR 80.0	mainly West Africa	mostly Forastero

n.s. = not specified
*high-polyphenol chocolate (2,200 mg flavanols/100 g).

3.2 Methods

3.2.1 Temporal Dominance of Sensations (TDS)

The TDS evaluation was performed according to Pineau et al., (2009).

3.2.1.1 Selection of attributes

The attributes selected for the TDS investigation are the two main descriptors associated with polyphenols: *bitter taste* and *astringency*. In addition, *sweet taste* was chosen as potential covering agent, since sweetness was shown to potentially suppress both bitterness (Green et al., 2010) and astringency perception (Courregelongue et al., 1999).

3.2.1.2 Evaluation conditions

The TDS investigation was conducted at the sensory laboratory of Barry Callebaut N.V. (Wieze, Belgium) in individual taste booths in July 2015 (Fig. 17). The 11 samples were divided into two sets consisting of OR and N-OR chocolates (Tab. 14). The products were administrated in coded form (3-digit random numbers), randomized across all 13 panelists following a William Latin square design and evaluated in three replicates as recommended by Pineau & Schlich, (2015) on three different days. A portion size of 5 g, representing one bite, as determined in pre-tests by five individuals, was served in small plastic cups at room temperature (20°C). A 1-minute break was provided between each evaluation in order to neutralize the mouth with unsalted crackers and tap water. Data acquisition was carried out using EyeQuestion® sensory software.

Tab. 14: Investigated samples in the TDS evaluation.

	Cocoa Content (%)	Type
Set 1	66.8 %	OR
	70.4 %	OR
	70.5 %	N-OR
	54.5 %	N-OR
	65.0 %	N-OR
Set 2	67.4 %	OR
	70.0 %	OR
	80.1 %	OR
	60.0 %	N-OR
	70.5 %	N-OR
	80.0 %	N-OR

Fig. 17: Sensory booths during investigation at Barry Callebaut, Wieze, Belgium (own illustration).

3.2.1.3 Testing protocol

In order to ensure homogeneity among subject responses and good reliability of the data a tasting protocol has been defined, as recommended by Pineau & Schlich, (2015), based on pretest experiences. According to the protocol, panelists were instructed to put the sample in their mouth, masticate 2x before bringing the sample to the top of the tongue and immediately initiate the evaluation of the dominant sensation by clicking the START button (Fig. 18). The assessors were asked to indicate the dominant attribute by clicking the according attribute-button (intensity was not recorded) while manipulating the sample between the tongue and palate. As mastication behavior and mastication duration in particular tend to differ from one subject to another (Lenfant et al., 2009), assessors were free to swallow the sample whenever a proper bolus was formed in order to reflect the real consumption situation. After swallowing, panelists were instructed to continue evaluating the dominant aftertaste sensations until perception ends and click the STOP button (Fig. 19).

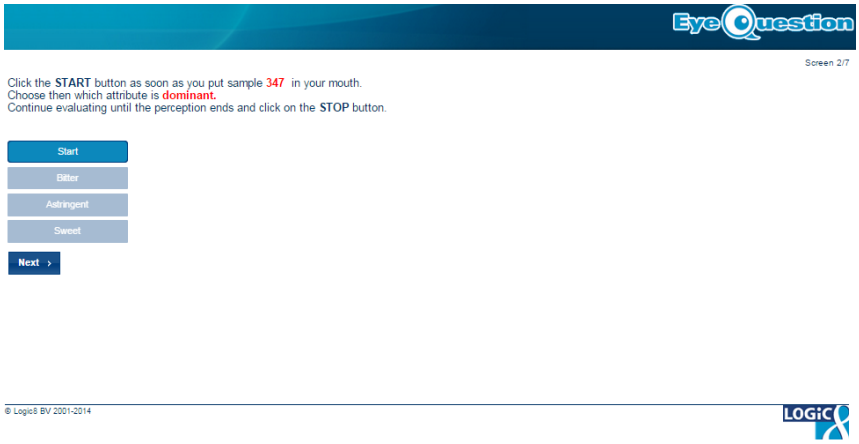


Fig. 18: Screen presented for TDS evaluation (with START- and attribute-buttons) (own illustration).

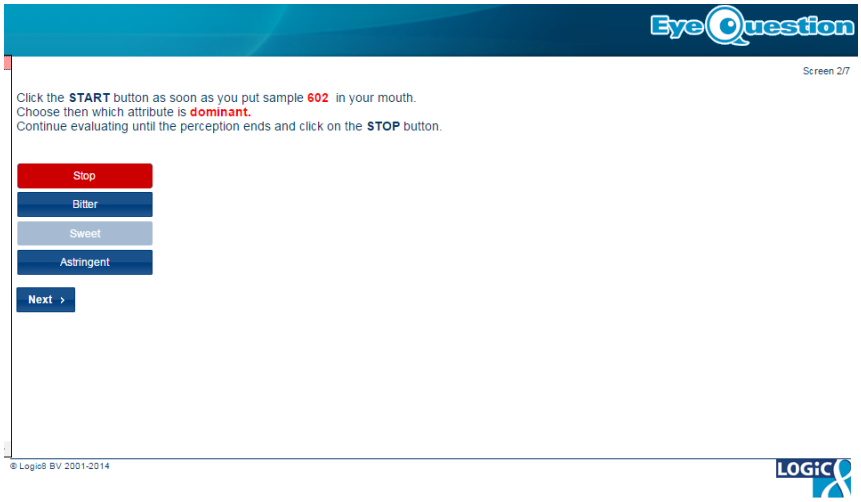


Fig. 19: Screen presented for TDS evaluation (with STOP- and attribute-buttons) (own illustration).

3.2.1.4 Selection of panelists

The TDS evaluation was carried out by 13 trained panelists, selected according to the guidelines of the ISO 8586:2012 standard as well as 6-n-propylthiouracile taster status, ages ranging from 30 to 50 years old (female: n = 11; male: n = 2). All participants were consumers of chocolate and highly familiar with the sensory evaluation of different kinds of chocolate products using different approaches, however unfamiliar to the TDS task. Thus, prior to testing, a three-step training was mandatory.

3.2.1.5 Training of panelists

The three-step-training of panellists was conducted as five 1h-sessions including the following:

1) A theoretical introduction on dynamic methods of sensory analysis (TI and TDS), starting with the role of oral processing on sensory perception, and a detailed description of TDS (key elements, concepts of “dominance” and “sequence”, data analysis and interpretation).

2) Selection and characterization of attributes using references shown in Tab. 15. Since the entire methodology relies on the ability of panelists to choose the dominant attribute among the list of attributes (Pineau & Schlich, 2015), the notion of “dominance” was major part of this session.

3) The next three sessions were devoted to familiarize the panellists with the utilization of the TDS tool in EyeQuestion® sensory software, the list of attributes and the testing protocol. The procedure was explained and demonstrated by the researcher. At first, plain and slightly sweetened, strong black tea (200 ml water, 1 teabag, 20 min. brew time, 5 g sugar) was used for this aim. In addition, reference chocolate samples for *bitterness*, *sweetness* and *astringency* were provided for familiarization with the TDS task and the list of attributes (Tab. 15). At last, 8 dark chocolate samples with different cocoa contents and regions of origin were evaluated by the panelists for training purposes.

Tab. 15: Sensory glossary for panel-training with references.

Attributes	Definition	References
BASIC TASTE		
Bitter taste	A basic taste factor of which caffeine or quinine is typical	Caffeine solution (c = 0,5 g/l)/ Sample N-OR 65.0%cc
Sweet taste	A basic taste factor of which sucrose is typical	Sucrose solution (c = 10 g/l)/ Sample N-OR 54.4%cc
MOUTHFEEL		
Astringency	The feeling of a puckering, shrinking or a tingling sensation on the surface and/or edges of the tongue and mouth	Strong black tea (infusion time 20 min.)/ pure cocoa liquor

3.2.1.5.1 Exclusion criteria

In addition to the requirements the ISO 8586:2012 standard (including a good health status), 6-n-propylthiouracile taster status was defined as exclusion criterion in the present investigation.

Bitter taste, sweet taste (Gent & Bartoshuk, 1983) and astringency (Pickering et al., 2004) sensitivity have been linked with the genetically-determined ability to perceive bitter thiourea compounds such as phenylthiocarbamide (PTC) and the less toxic 6-n-propylthiouracil (PROP) (Fig. 20).

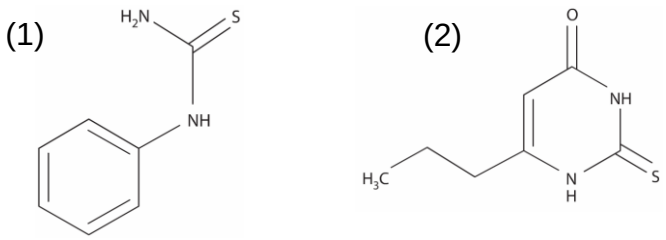


Fig. 20: Phenylthiouracile/PTC (1) and 6-n-propylthiouracile/PROP (2) (Keller & Adise, 2016).

PTC and PROP elicit moderate-to-intensive bitterness considering the majority of individuals across the globe. Based on their responsiveness to either of these compounds, individuals are classified as “tasters” and “non-tasters”, representing

~70% (50% medium-tasters, 20% super-tasters) and ~30% of the population, respectively (percentages vary due to age, gender and ethnicity) (Bartoshuk et al., 1994; Guo & Reed, 2001; McAnally et al., 2007; Tepper et al., 2009). Polymorphisms in the *TAS2R38* gene, encoding for a bitter taste receptor, seem to explain the majority of phenotypic variation in the PROP phenotype. In addition, a relationship between PROP taster status and density of fungiform papillae has been found, with PROP taster showing a greater number of fungiform papillae, even though not all studies support this finding (Keller & Adise, 2016).

Since these perceptual variabilities in PROP responsiveness were found to affect food acceptance, choice and thus eating patterns (Kaminski et al., 2000; Keller & Adise, 2016), PROP non-taster were excluded in the present study. It was identified using a combined threshold determination and intensity rating procedure. Participants received a PROP solution concentration series (0 mM, 0,00293 mM, 0,01170 mM, 0,04690 mM, 0,188 mM, 0,7375 mM) coded with three-digit random numbers and were asked to taste the samples from left to right and to indicate the perceived taste quality and intensity of each sample. Participants, who were able to identify PROP as a bitter tasting substance at 0.188 mM, were classified as “tasters”, those who did not as “non-tasters”. Except one employee, all other individuals were able to detect PROP and thus classified as “tasters”. They were invited to participate in the TDS investigation.

3.2.1.6 Statistical analysis of TDS data

3.2.1.6.1 Dominance rates (%)

The dominance rates (DR%) were calculated as proportions of evaluations (panellist x replication) for which the given attribute was choosen as dominant at each point of time. In order to create the TDS curves, the DR%, representing the y-axis, were plotted against time (x-axis) according to Pineau et al., (2009). As the testing protocol considered individual evaluation durations, the time was standardized for the computation of the TDS curves as recommended by Lenfant et al., (2009). Thus, the x-axis does not represent real time (in seconds), but percentages (%) of time.

Each graph includes two lines: the “chance level” and “significance level”. The “chance level” represents the DR% an attribute can obtain by chance:

Chance level: $P_0 = 1/n$

n…number of attributes

In the present study, 3 attributes were investigated, the chance level was thus set to 33.3% ($p = 1/3$).

Furthermore, the “significance level” was calculated as 45.9% using the following equation:

Significance level:

$$P_S = P_0 + 1.645\sqrt{\frac{P_0(1 - P_0)}{n}}$$

P_s……	lowest significant proportion value (α = 5%) at any time point
P_0……..	probability of success
1.645…	one-tailed normal law *z*-value for α = 5%
n……..	number of trials (subject x replication)

The significance level represents the minimum dominance rate (DR%) being considered as significantly ($p < 0.05$) higher than the “chance level” (P_0).

DR (%) above the “significance level” were thus considered as dominant at that specific time% (Pineau et al., 2009) whereas DR(%) between “chance” and “significance” level are considered as having a tendency towards significant ($p < 0.05$) dominance (Galmarini et al., 2017).

The computation of the TDS graphs was done using plot2 (a scientific 2D plotting program for OS X).

3.2.1.6.2 Attribute dominance duration

The *“attribute dominance durations”* were obtained at individual level by adding all the time periods (sequences) during which each attribute (bitter, sweet, astringent) was cited as dominant, regardless of the moment of perception. In this way, sequentiality gets lost, however statistical power is gained while keeping a dominat variable.The data was standardized by turning dominance duration of each attribute (s) into a proportion of the total duration (%). The variable of interest

is thus, the relative duration (%) of a given dominant attribute along the testing period (mastication and aftertaste) (Galmarini et al., 2017).

In order to explain differences among chocolate products quantitatively, a linear mixed model was used with “attribute dominance duration% (DD%)” as dependent variable, product as fixed and subject as random effect. For multiple comparisons, a Sidak post hoc test was applied. In addition, the impact of cocoa contents% on the sensory perception of investigated attributes (sweet taste, bitter taste, astringency) was investigated using a Pearson correlation coefficient (r). Furthermore, a Principal Component Analysis (PCA) was performed to study the product configuration in relation to the “attribute dominance duration” proportions (DD%) of each attribute.

3.2.1.6.3 Total duration of evaluation duration

The downside of data standardization (TDS curves) is that access to real time (s) gets lost. To overcome this gap, analyzing the duration of each product evaluations in order to assess whether some products generally need a significantly ($p < 0.05$) longer evaluation than others provides useful complementary information (Lenfant et al., 2009). For this purpose, mean values of the total TDS evaluation duration (s) of each product were calculated and compared in order to determine significant ($p < 0.05$) differences between products. For this purpose, a linear mixed model (product as fixed effect, subject as random effect) was applied for “total TDS evaluation duration” as dependent response variable.

Data analysis of “attribute dominance duration” and “total duration of evaluation duration” was performed using IBM SPSS statistics (version 24) and Senstools.

3.2.2 Consumer survey

The consumer survey (paired preference test, ranking test for preference and acceptance test) was conducted at the Department of Nutritional Sciences (University of Vienna, Austria). All chocolate samples ($n = 11$) analyzed at Barry Callebaut chocolate factory (Wieze, Belgium) were evaluated by naïve consumers recruited at the University. Recruitment was performed via several student platforms, e-mail invitations and personal communication. Initially, 114

subjects were invited to participate, which is in agreement with the required minimum for consumer surveys (*n* = 112) (Hough et al., 2006). Since a general liking of dark chocolate was required (inclusion criterion), some participants had to be excluded. Finally *n* = 96 female individuals aged 19-33 years old participated in the survey. They were asked to fill in a simple questionnaire in order to collect data on gender, age, consumption behavior (frequency of chocolate consumption, preferred type of chocolate: milk or dark chocolate).

The tests were carried out at the sensory laboratory (designed in accordance with DIN EN ISO 8589:2014-10) at the Department of Nutritional Sciences, Vienna, in individual sensory booths under red light conditions in order to avoid colour identification. The samples (three chocolate callets/~5 g of weight) were coded with three-digit-numbers and served in randomized (following a Williams Latin Square experimental design) and counterbalanced order on small, labelled silver trays at room temperature (20°C). For neutralization, the participants were asked to rinse their mouth with water between each sample or set (in accordance to the respective tasting protocol). Samples were evaluated at individual speed. Data were captured and analyzed by FIZZ Biosystèmes v2.51 a 86. Further data analysis was performed using IBM SPSS statistics (version 24).

3.2.2.1 Preference test 1: Paired preference test

As preference test 1 in the consumer survey, a forced-choice paired preference test was conducted with 4 pairs/sets, each pair consisting of an origin and a non-origin sample with similar cocoa contents (Tab. 16).

Tab. 16: Paired samples (origin vs. non-origin) investigated in paired preference test.

	Type	Cocoa content (%)
Set 1	OR	66.8
	N-OR	65.0
Set 2	OR	70.4
	N-OR	70.5
Set 3	OR	70.0
	N-OR	70.3
Set 4	OR	80.1
	N-OR	80.0

The task was to taste each sample in a pair and to select the one that is preferred over the other. The assessors were asked to use tap water as palate cleanser between each set of pairs, however neutralizing was not allowed between the samples within a pair. Fig. 21 shows the evaluation screen on FIZZ including clear instructions.

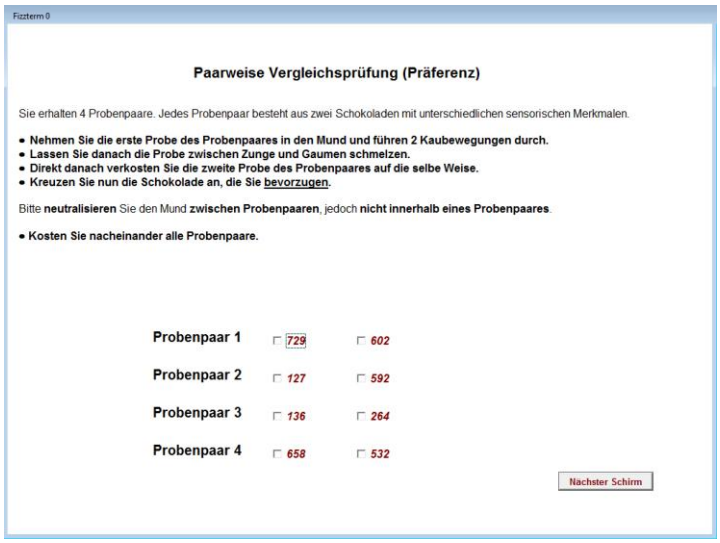


Fig. 21: Paired Preference Test (FIZZ v 2.51 a 86) (own illustration).

3.2.2.1.1 Statistical analysis: Preference test 1

The probability of selecting a specific product in the paired preference test is one chance in two. The null hypothesis (referring to the underlying population) states when consumers do not prefer one product, they will pick each product an equal number of times. Thus, $H_0 = p(A) = p(B) = ½$ with a probability of $P_{prop} = 0.5$. The test is two-tailed, no right answer exists as it is a matter of opinion.

The alternative hypothesis is $H_A = p(A) \neq p(B)$. For data analysis, three approaches based on the binominal, chi-square or normal distributions can be used (Lawless & Heymann, 2010d).

In order to determine whether the number of judges preferring one product over the another was obtained by chance or whether it is of statistical significance ($p < 0.05$), data of this two-tailed test were analyzed in FIZZ v2.51a86 based on the binomial law. The significance threshold (α-risk) was set to 5%.

3.2.2.2 Preference test 2: Ranking test for preference

The second approach to obtain data on consumer's preferences was ranking of products according to their preference. The participants received two sets of chocolate samples with different cocoa contents (%), the first set consisting of origin, the second of non-origin chocolates (Tab. 17). As shown in Fig. 22, the assessors were instructed to rank the samples within each set from most preferred (1) to least preferred (set 1: 5; set 2: 6) via "drag-and-drop" on the screen. Neutralizing the mouth with water after each sample within one set was not allowed; however, between the two sets a 5-minutes break was set up for recovering and neutralizing. Re-tasting of samples was permitted, as recommended by Hein et al., (2008), to enable comparisons between the samples and confirm the ranking order.

Tab. 17: Sets of samples (origin, non-origin) investigated in ranking test for preference.

	Type	Cocoa contents (%)
Set 1	OR	66.8
	OR	67.4
	OR	70.0
	OR	70.4
	OR	80.1
Set 2	N-OR	54.5
	N-OR	60.0
	N-OR	65.0
	N-OR	70.3
	N-OR	70.5
	N-OR	80.0

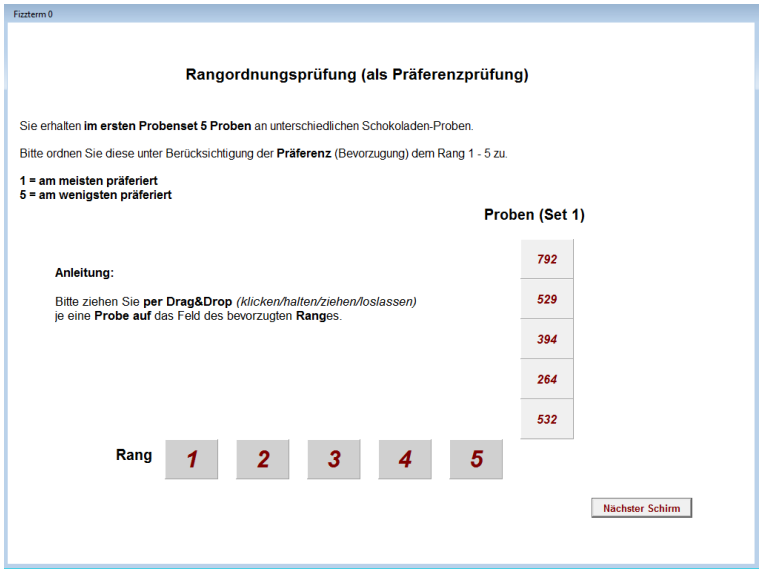


Fig. 22: Ranking test for preference (Set 1) (FIZZ v 2.51 a 86) (own illustration).

3.2.2.2.1 Statistical analysis: Preference test 2

Ranking data were again analyzed in FIZZ v2.51a86. First, based on consumers' responses, rank sums were calculated for each product separately. In order to identify wheater there is a significant ($p < 0.05$) difference between at least two products, a Friedman's chi-square non-parametric equivalent of the two-way analysis of variance (ANOVA) without interactions was performed. The level of significance (α-risk) was set to 5%.

For multiple comparisons, least significant ranked difference (LSRD) values were computed using Bonferroni correction (test at global risk) in order to determine which rank sums differed significantly ($p < 0.05$) from each other. Products sharing the same letter next to their rank sums are not regarded as significantly ($p < 0.05$) different.

3.2.2.3 Acceptance test

The degree of product acceptability in an absolut sense was assessed by presenting the consumers with 2 sets of coded samples (all samples - origin, non-origin with different cocoa contents - randomized according Latine Square as shown in Tab. 19) and instructing them to indicate their overall liking for each chocolate on the 9-point-hedonic scale anchored with ‘‘dislike extremely’’ (1 point) and ‘‘like extremely’’ (9 points) (Fig. 23) using phrases as shown in Tab. 18.

Tab. 18: Terminology for a 9-point hedonic scale (Lawless & Heymann, 2010b).

1	dislike extremely
2	dislike very much
3	dislike moderately
4	dislike slightly
5	neither like nor dislike
6	like slightly
7	like moderately
8	like very much
9	like extremely

A 30 seconds break was introduced between each sample and 5 minutes between set 1 and set 2 (Tab. 19) in order to provide space for neutralizing the mouth and taste buds with tap water.

Tab. 19.: Sets of samples (origin, non-origin) investigated in acceptance test

	Type	**Cocoa contents (%)**
Set 1	OR	80.1
	N-OR	60.0
	OR	70.0
	N-OR	65.0
	OR	67.4
Set 2	N-OR	80.0
	OR	70.4
	N-OR	70.5
	OR	66.8
	N-OR	54.5
	N-OR	70.3

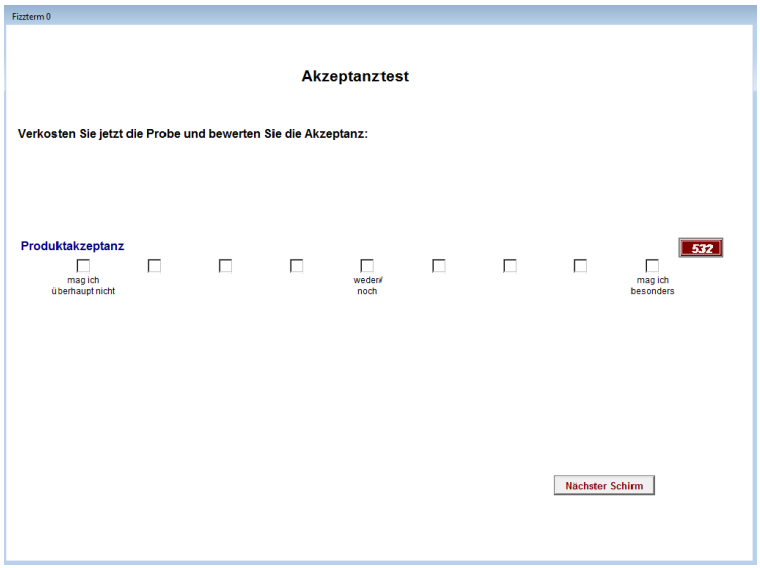


Fig. 23: Acceptance test (FIZZ v 2.51 a 86) (own illustration).

3.2.2.3.1 Statistical analysis: Acceptance test

Significant ($p < 0.05$) differences in product acceptance between the chocolate samples were determined applying a linear mixed model with products as fixed and subject as random effect for “product acceptance” as dependent response variable. In addition, consumption frequency (daily [several times/day], frequently [several times/week], regularly [1x/week], occasionally [several times/month], sporadicly [1x/month], rarely [<1x/month]), preferred type of chocolate (dark chocolate, milk chocolate) and age were included into the model as covariates. A Sidak post-hoc test was used for multiple comparisons of the products. In order to gain better insight into consumer’s behaviour, the influence of both attribute dominance duration (DD%) and cocoa content% on product acceptance was assessed using Pearson correlation coefficient (r).

In order to determine the association between product acceptance and the consumers buying intention, a Spearman's non-parametric correlation coefficient was calculated.

4 Results

4.1 TDS profiles

The following TDS graphs are created based on the DR (%) (y-axis) and standardized time points (T0-T100%) (x-axis). Due to curve smoothing (FFT smooth in plot2), the DR (%) displayed in the corresponding graphs are not consistently coherent with the calculated DR (%) as listed in the appendix, however will be discussed as such.

Each curve shows the evolution of the DR (%) of each attribute (bitter taste, sweet taste, astringency) through the course of tasting (evaluation of mastication and aftertaste). DR (%) above the significance level ($p < 0.05$) are considered as relevant as they reflect a high panel consensus.

4.1.1 TDS curves - origin chocolates (OR)

4.1.1.1 OR 66.8%cc

As shown in Fig. 24, sample OR 66.8%cc (Trinitario, Forastero; Brazil) was perceived as significantly ($p < 0.05$) bitter from T18% (DR = 46.2%) to T77% (DR = 46.2%) with peaks at T20% (DR = 59.0%), T39% to T41% (DR = 69.2%), T55% (DR = 69.2%) and T70% to T73% (DR = 51.3%). At T78%, this impression declined beneath the level of significant dominance ($p < 0.05$; DR 43.6%), taken over from the rising dominant astringency perception with significance ($p < 0.05$) starting from T74% (DR = 46.2%) and a maximum DR = 53.8% from T87% until the end of the evaluation period. This sample was hardly perceived as sweet with a maximum DR of 20.5% at the beginning of the testing period (T12%), however, the sweetness dominance declined alongside with bitterness at the end of the evaluation period (Fig. 24).

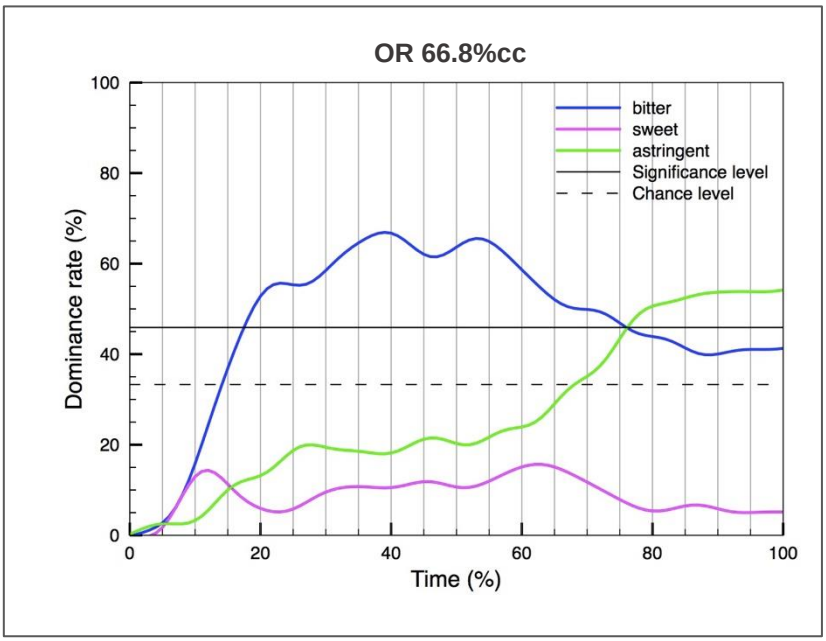


Fig. 24: TDS curves for sample "OR 66.8%cc".

4.1.1.2 OR 67.4%cc

The dominant attribute of sample OR 67.4%cc (Forastero, Criollo, Trinitario; Madagascar) was sweetness along the entire testing period. Significant ($p < 0.05$) dominance started at T15% for 48.7% of the panelists and lasted until the end of the evaluation with the highest panel consensus (DR = 64.1%) at T50-51%, T64% and T66-68%. Bitter taste never reached the level of significance ($p < 0.05$), however briefly exceeded the chance level at T63-64% (maximum value DR 35.9%). Astringency neither reached significance ($p < 0.05$) nor chance level with a maximum value of DR 30.8% at T97-100%. However, at the end of the testing period (after approx. T75%), it was observed that sweet and bitter taste dominances declined and astringency DR (%) rose, being even superior to bitter taste DR (%) (Fig. 25).

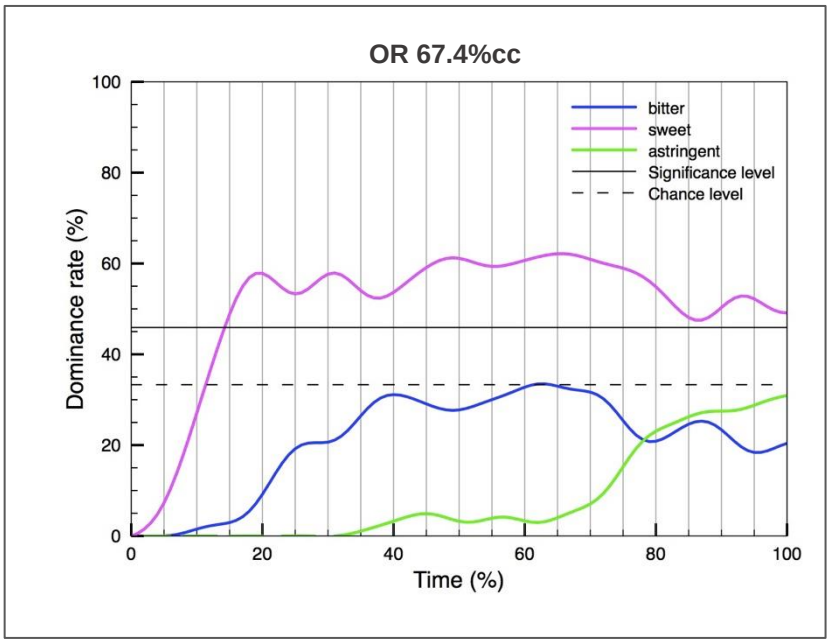


Fig. 25: TDS curves for sample "OR 67.4%cc".

4.1.1.3 OR 70.0%cc

The significantly ($p < 0.05$) dominant sensation in sample OR 70.0%cc (Amelonado Forastero; São Tomé) was bitter taste along the entire evaluation period. It exceeded the level of significance ($p < 0.05$) the first time at T22% (DR = 46.2%) and reached the maximum DR (= 61.5%) at T41%. After falling below the level of significance ($p < 0.05$) at T71%, bitter taste was again perceived as significantly ($p < 0.05$) dominant from T76-88%. Along with the significant ($p < 0.05$) decline of bitterness dominance at T71%, sweet taste, which never reached even the chance level (maximum DR 30.8%), also declined at approx. T75%. The decrease of both sensations (bitter and sweet taste) was accompanied by rising astringency perception. This mouthfeel sensation never reached the level of significance ($p < 0.05$), however showed max. DR = 41.0% at the end of the evaluation period (T80%, T89-100%) with a clear tendency towards significant ($p < 0.05$) dominance (Fig. 26).

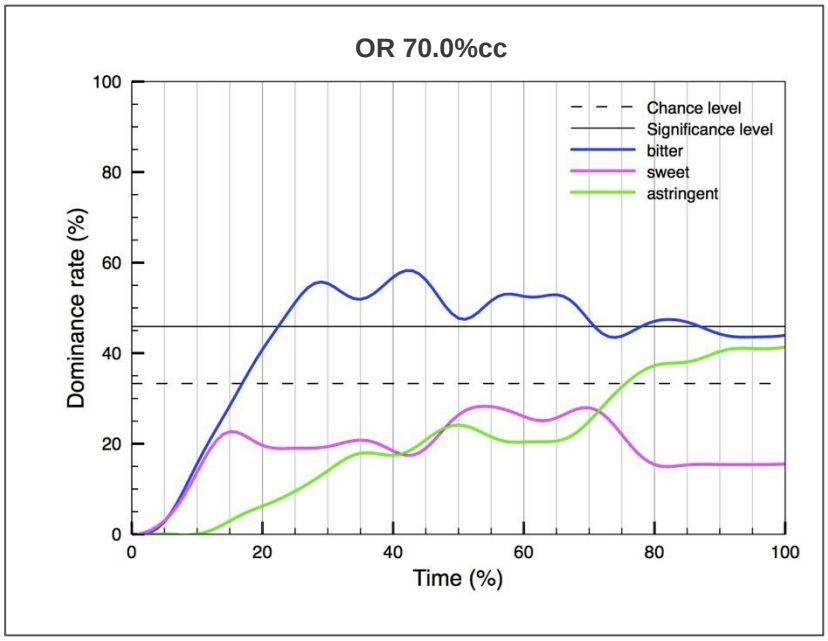


Fig. 26: TDS curves for sample "OR 70.0%cc".

4.1.1.4 OR 70.4%cc

Sample OR 70.4%cc (Arriba Nacional; Ecuador) was significantly ($p < 0.05$) perceived as bitter from T32% (DR = 47.4%) to T72% (DR = 52.6%), reaching the strongest panel consensus of 63.2% at T41%. From T60-63% as well as T73-79%, bitterness sensation dropped under the significance level ($p < 0.05$) (DR from 42.1% to 44.7%), however still represented the most dominant perception until the end of the evaluation. At T73-78% sweet taste was dominant to the same extent (DR 42.1% to 44.7% = max. sweetness DR%). This sample hardly showed astringency at all. This sensation started to rise at the end of the evaluation period (along with a decline of bitter and sweet taste), however did not reach a significant ($p < 0.05$) DR% (max. DR = 28.9% at T95-100%) indicating a weak panel consensus and thus low relevance in the present sample (Fig. 27).

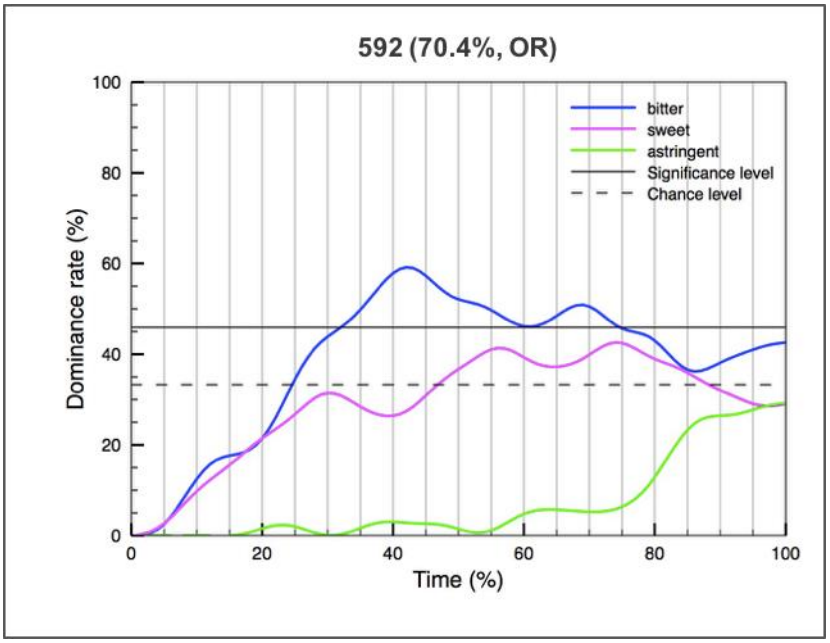


Fig. 27: TDS curves for sample "OR 70.4%cc".

4.1.1.5 OR 80.1%cc

The significantly ($p < 0.05$) dominant sensation in sample OR 80.1%cc (Trinitario, Forastero; blend of origins: Tanzania, Ghana, São Tomé) was also bitterness, as depicted in Fig. 28. It started at T20% (DR = 46.2%) with the first peak and highest DR (= 79.5%) at T47-50% and the second DR(%) peak at T66-68% (DR = 74.4%). It dropped beneath the significance level ($p < 0.05$) at T85% (DR = 41.0%) and thereof showed a similar temporal profile as astringency (bitterness-DR = 38.5%; astringency-DR = 43.6%) until perception ended. However, the astringent sensation did not reach the level of significant ($p < 0.05$) dominance at all. Sweet taste neither reached chance nor significance level ($p < 0.05$) with a maximum DR% of 20.5% at T55-56% and T88-100% (Fig. 28).

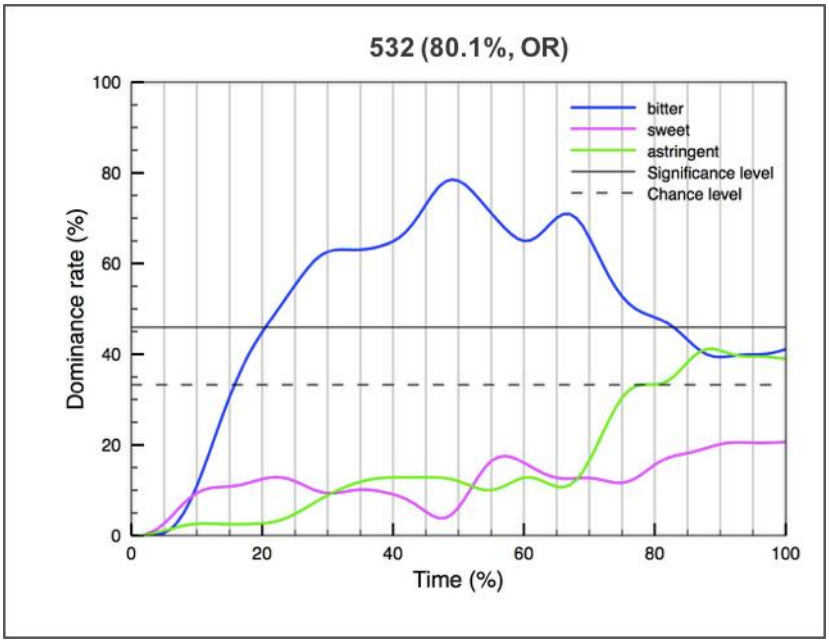


Fig. 28: TDS curves for sample "OR 80.1%cc".

4.1.2 TDS curves - non-origin chocolates (N-OR)

4.1.2.1 N-OR 54.5%cc

As shown in Fig. 29, sample N-OR 54.5%cc, representing the sample with the lowest cocoa content (%) among both origin and non-origin chocolates, was significantly ($p < 0.05$) perceived as sweet along the entire testing period starting from T16% (DR = 46.2%) until the end of evaluation (DR = 76.9%), with a maximum DR of 89.7% (T46-68%), exceeding the significance level ($p < 0.05$) by far indicating a very high panel consensus. Astringency perception, which was not indicated as dominant at all until T72% (DR = 2.6%), started to rise to some extent, however reached a maximum DR of not more than 17.9% (T87-93%) and thus remained far beyond both chance and significance level ($p < 0.05$). The same was observed with bitter taste, which occurred with highest DR of 12.8% at T33-34% and T70-71% short before astringency perception started to rise (Fig. 29).

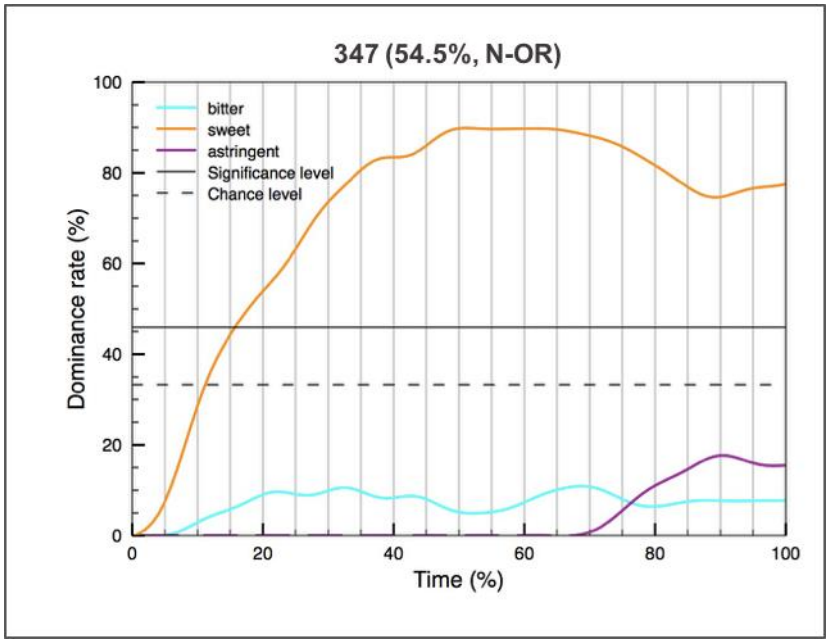


Fig. 29: TDS curves for sample "N-OR 54.5%cc".

4.1.2.2 N-OR 60.0%cc

The significantly ($p < 0.05$) dominant sensation in sample N-OR 60.0%cc, with a slightly higher cocoa content (%) than sample N-OR 54.5%cc, was sweetness along the entire evaluation period starting from T12% (DR = 47.4%) and reaching maximum DR of 94.7% (T59-66%, T80-87%), which reflects an extremely high consensus among panelists. In contrary, bitterness and astringency were hardly perceived as dominant, reaching the highest DR = 5.3% and 7,9%, respectively, towards the end of the evaluation period (Fig. 30).

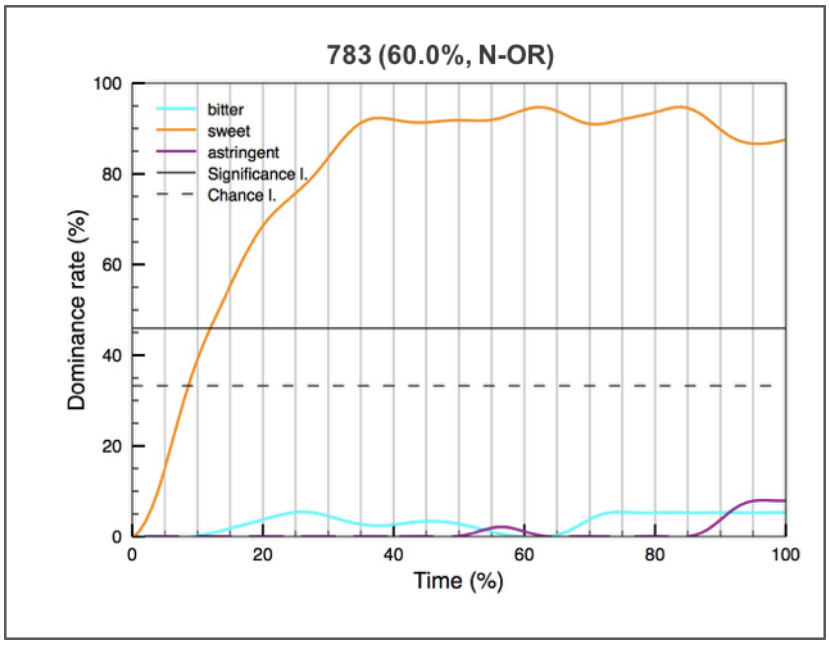


Fig. 30: TDS curves for sample "N-OR 60.0%cc".

4.1.2.3 N-OR 65.0%cc

In contrary to samples N-OR 54.5%cc and N-OR 60.0%cc, sample N-OR 65.0%cc was significantly ($p < 0.05$) dominated by bitter taste and astringency. Bitterness reached the significance level ($p < 0.05$) at T17% (DR = 46,2%) showing peaks at T22% (DR = 51.3%) and T32% (DR = 53.8%). At T39% (DR = 41.0%), bitterness fell under the level of significant dominance ($p < 0.05$) remaining until the end of the evaluation period, differentiating this sample from the previous two as the decline took place at an earlier stage of consumption. At the same time, astringency perception started to be dominant, reaching significance ($p < 0.05$) at T41% with DR = 46.2% and after that increasing rapidly through the course of testing to obtain the DR of 74.4% at T79% and T97-100%, the highest astringency-DR% of all samples. Sweet taste was not identified at a significant ($p < 0.05$) rate at all, reaching the highest value of DR = 15.4% at T13-14% and T25% (Fig. 31).

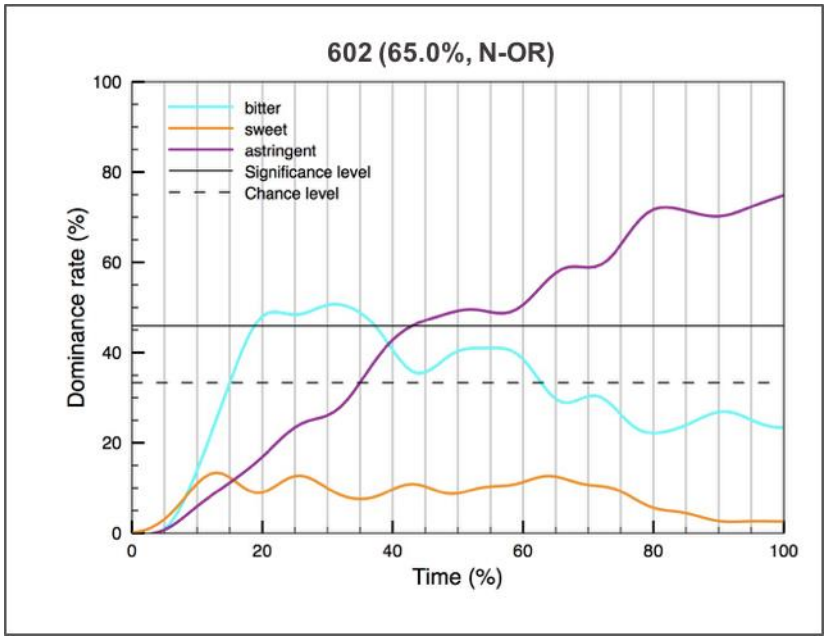


Fig. 31: TDS curves for sample "N-OR 65.0%cc".

4.1.2.4 N-OR 70.3%cc

The significantly ($p < 0.05$) dominant sensation in sample N-OR 70.3%cc was sweet taste along the entire testing period starting at T34% (DR = 51.3%) until T87% (DR = 46.2%). From T41% to T46%, it shortly dropped beneath the level of significance ($p < 0.05$), and at T47% being equal with the DR(%) of bitter taste sensation (DR 46.2%). The DR(%) of sweet taste increased again at T47% (DR 51.3%) above significance level ($p < 0.05$) reaching the maximum DR of 56.4% at T61% and T74%. Bitterness hardly reached significance level ($p < 0.05$) in sample N-OR 70.3%cc, but showed DR% above chance level, starting from T28% (DR 33.3%) almost until the end of the evaluation period (T100%, DR 38.5%), reflecting a considerable tendency towards significance ($p < 0.05$). Sample N-OR 70.3%cc was hardly perceived as astringent with maximum DR of 20.5% at the end of the evaluation period (T92-100%) as bitterness declined (Fig. 32).

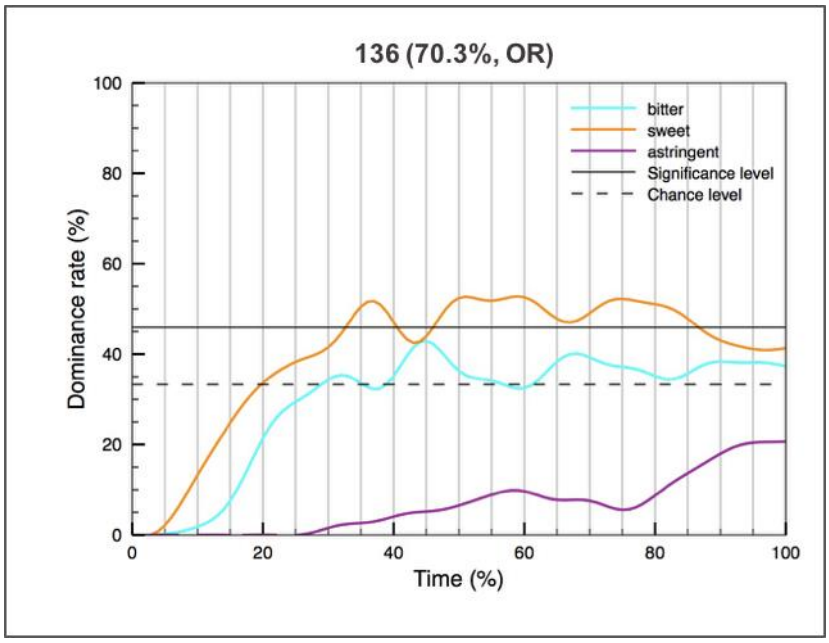


Fig. 32: TDS curves for sample "N-OR 70.3%cc".

4.1.2.5 N-OR 70.5%cc

As depicted in Fig. 33, sample N-OR 70.5%cc was significantly ($p < 0.05$) perceived as dominantly bitter along the entire evaluation period, starting from T27% (DR = 46.2%) until T100% (DR = 56.4%) with the highest DR of 69.2% at T49-50%. Sweetness was hardly perceived as dominant in this sample reaching the chance level only for a short period of time at T72% (DR 33.3%) as well as T72-78% with max. DR 38.5% and significance level not at all. Astringency perception started to be dominant at the end of the evaluation period, alongside with decreasing sweetness perception, however did not reach the level of significance ($p < 0.05$; highest DR = 23.1% at T84-100%). The simultaneous drop of bitter taste (T74-78%) as well as astringency (T71-78%) and increase of sweet taste (T72-78%) gives an interesting picture of the taste and mouthfeel evolution in this sample, suggesting the potential point of swallowing (Fig. 33).

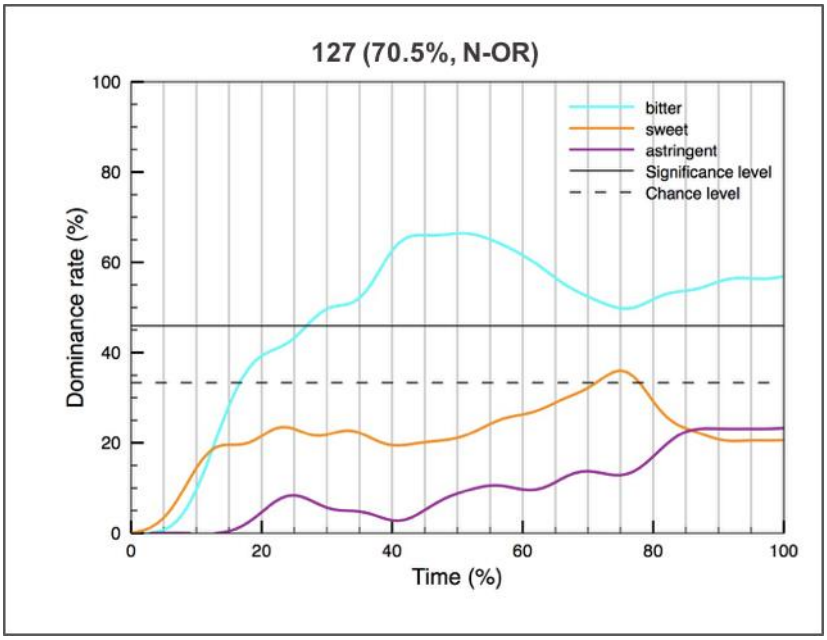


Fig. 33: TDS curves for sample "N-OR 70.5%cc".

4.1.2.6 N-OR 80.0%cc

Sample N-OR 80.0%cc was significantly ($p < 0.05$) dominated by bitter taste along the entire evaluation period starting from T15% (DR = 51.3%) until T77% (DR = 46.2%) with the highest panel consensus of 74.4% at T62-63% and T65%. Towards the end of the evaluation period starting from T76%, astringency perception was significantly ($p < 0.05$) dominant (DR = 46.2%) as indicated by the panel. From T92-100% of the evaluation period, astringency reached its maximum value of DR = 53.8%. Sweet taste was hardly identified as dominant along the entire evaluation period at all, with the highest DR of 7.7% at the end of consumption (T86-100%) (Fig. 34).

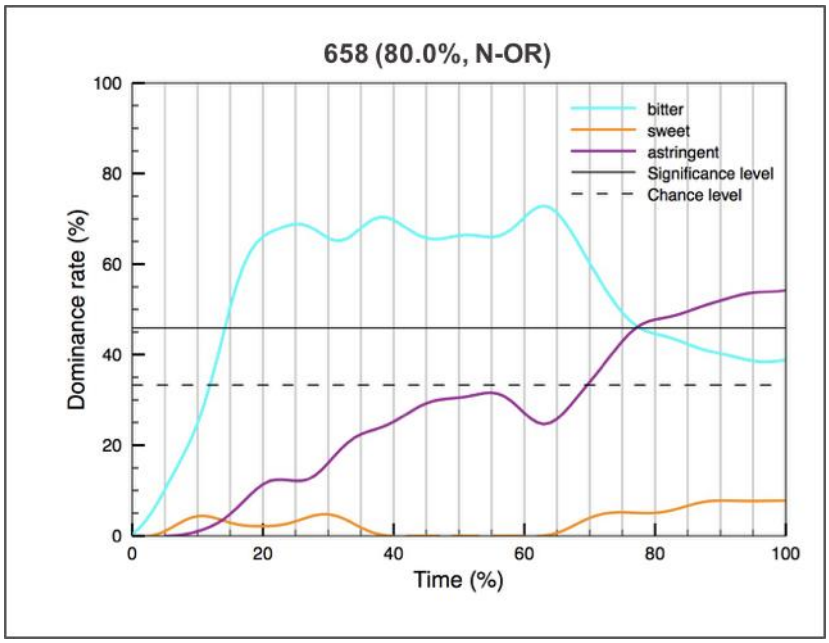


Fig. 34: TDS curves for sample "N-OR 80.0%cc".

4.1.3 TDS curves by attribute

4.1.3.1 TDS curves – „sweet taste"

4.1.3.1.1 N-OR chocolates

Visual inspection of the TDS curves showed that in N-OR 80.0%cc, sweet taste DR(%) was lowest (max. DR% 7.7), followed by N-OR 65.0%cc (max. DR% 15.4), both never reaching the level of significance ($p > 0.05$). Samples N-OR 70.3%cc and N-OR 70.5%cc with relatively similar cocoa content showed differences in sweetness DR%. While in N-OR 70.3%cc, the DR(%) exceeded the significance level ($p < 0.05$) (max. DR 53.8% starting from T34% and ending at T87%), DR(%) of sample N-OR 70.5%cc was only between chance and significance level (max. DR 38.5% between T67%-78%). As expected, the samples N-OR 54.5%cc and N-OR 60.0%cc containing the lowest cc% and thus highest sugar contents within the investigated non-origin chocolates showed the highest sweet taste DR%, far exceeding the level of significance ($p < 0.05$) along the entire testing period. Despite of the higher cc%, sample N-OR 60.0%cc revealed higher max. sweetness DR% compared to N-OR 54.5%cc (89.7% and 94.7% for N-OR 54.5%cc and N-OR 60.0%cc, respectively) (Fig. 35).

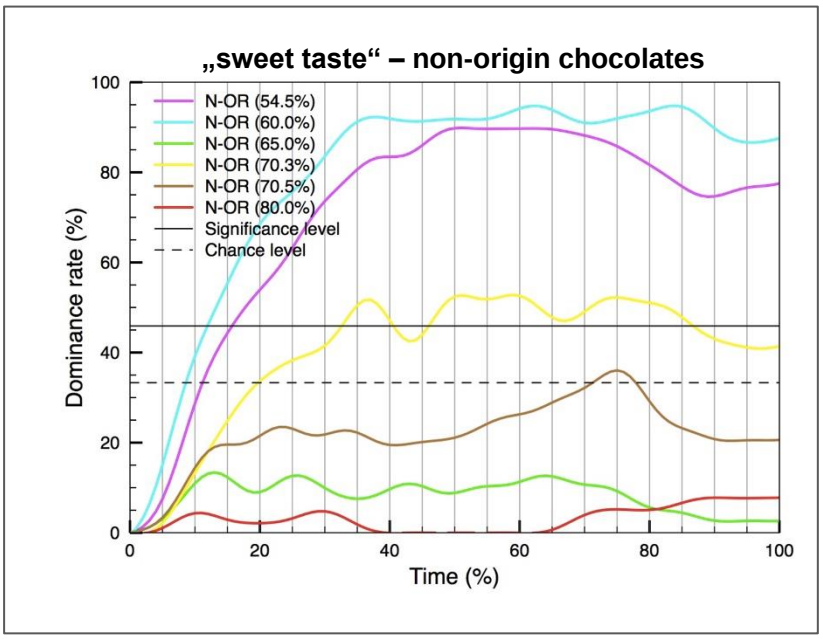


Fig. 35: DR (%) for „sweet taste“ in non-origin chocolates.

4.1.3.1.2 OR chocolates

DR(%) of sweet taste exceeded the significance level ($p < 0.05$) in OR 67.4%cc, a single-origin chocolate from Madagascar (max. DR% 64.1) starting from T15% until the end of perception. This sample was followed by OR 70.4%cc from Ecuador, revealing sweet taste DR% between chance and significance level at the time% T47%-91% (max. DR% 44.7). The samples OR 70.0%cc (single-origin from São Tomé), OR 80.1%cc (blend of origins from Tansania, Ghana, São Tomé) and OR 66.8%cc (single-origin from Brazil) amounted in sweet taste DR(%) of 30.8%, 20.5% and 17.9%, respectively, and never reached the level of significance (Fig. 36).

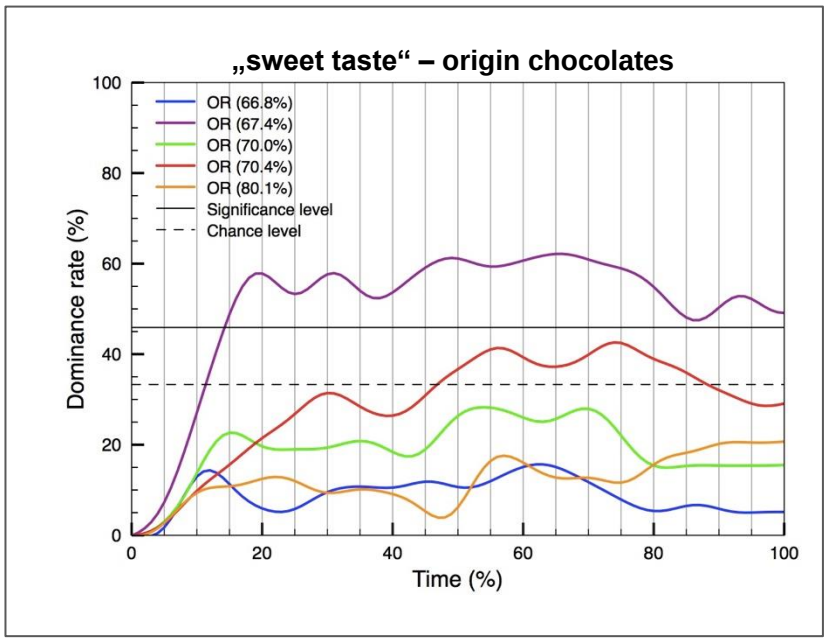


Fig. 36: DR (%) for „sweet taste“ in origin chocolates.

4.1.3.2 TDS curves – „bitter taste“

4.1.3.2.1 N-OR chocolates

In N-OR samples, bitter taste DR% was highest in N-OR 80.0%cc (max. DR% 74.7 between T15%-77%) and N-OR 70.5%cc (max DR% 69.2 from T27%-100%), exceeding the level of significance ($p < 0.05$). At T50%, the DR(%) of N-OR 70.5%cc and N-OR 80.0%cc reached similar values (DR% 69.2 and 66.7, respectively). At the beginning of the evaluation period (T17%-38%), the bitter taste of N-OR 65.0%cc was also perceived as significantly ($p < 0.05$) dominant with max. DR of 53.8%. N-OR 70.3%cc showed a tendency towards significance ($p < 0.05$) from T28-100% (max. DR% 46.2), but never reached the level of significance ($p < 0.05$). In N-OR 60.0%cc and N-OR 54.5%cc, bitter taste was less pronounced along the entire evaluation period (max. DR% of 5.3 and 12.8, respectively) (Fig. 37).

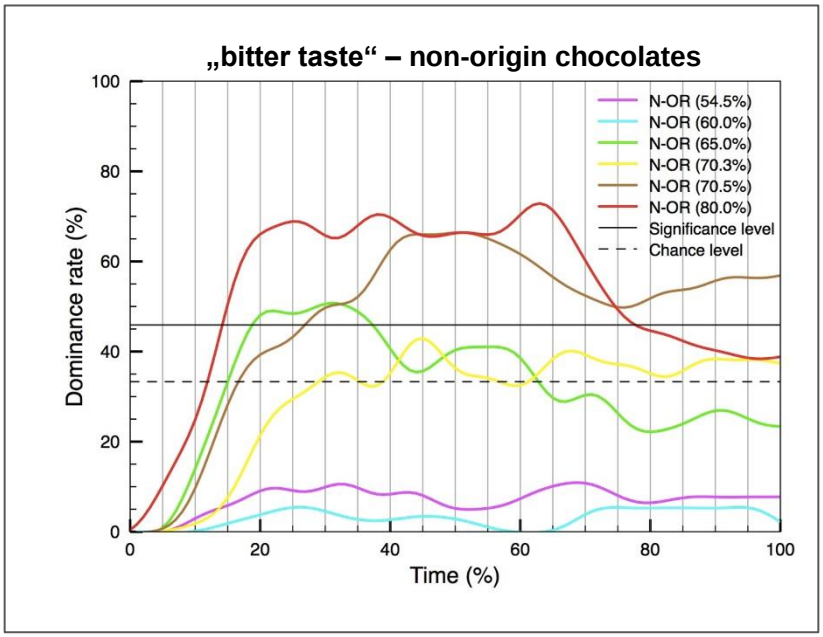


Fig. 37: DR (%) for „bitter taste“ in non-origin chocolates.

4.1.3.2.2 OR chocolates

Visual inspection of bitter taste TDS curves of OR chocolates showed different pattern as in all samples independent of cocoa contents, except for one (OR 67.4%cc, max DR% 35.9), DR(%) exceeded the significance level ($p < 0.05$). Sample OR 80.1%cc received the highest bitter taste DR% (79.9%) starting from T20% until T84%, followed by OR 66.8%cc with max. DR% = 69.2 at T18%-77%. Samples OR 70.0%cc and OR 70.4%cc showed relatively similar temporal profiles of bitter taste. OR 70.0%cc was perceived as significantly ($p < 0.05$) bitter from T22% until T70% (max. DR% 61.5) and from T76% until T88% (max. DR% 48.7). Bitter taste dominance in OR 70.4%cc started to rise slower and reached the significance level ($p < 0.05$) at T32% (max. DR% 63.2). It dropped below the significance level ($p < 0.05$) at approx. T60% and was significantly ($p < 0.05$) dominant again from T64% until T72% (max. DR% 52.6). Sample OR 67.4%cc exhibited a different temporal profile of bitterness compared to the other OR chocolates. DR% only reached the chance level for a short period of time (T63%-64%; DR% 35.9) (Fig. 38).

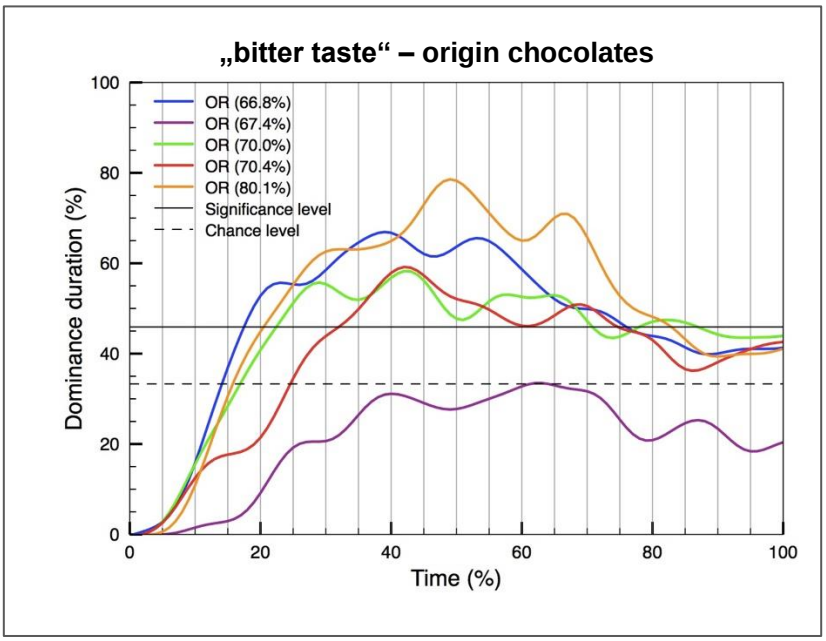


Fig. 38: DR (%) for „bitter taste“ in origin chocolates.

4.1.3.3 TDS curves – astringency

4.1.3.3.1 N-OR chocolates

Among N-OR chocolates, N-OR 65.0%cc was characterized by the highest dominance of astringency, significant for the longest period of evaluation (T41-100%), reaching maximum DR% of 74.4%. In N-OR 80.0%cc, the dominance of astringency also exceeded the level of significance ($p < 0.05$), but only at the end of evaluation (T76%-100%; max. DR% 53.8). The samples N-OR 70.5%cc, N-OR 70.3%cc, N-OR 54.5%cc and N-OR 60.0%cc never reached chance or significance level in terms of astringency (max. DR% of 23.1%, 20.5%, 17.9% and 7.9%, respectively) (Fig. 39).

4.1.3.3.2 OR chocolates

Among OR chocolates, the only sample perceived as significantly ($p < 0.05$) astringent was OR 66.8%cc, reaching max. DR% 53.8 at the end of the evaluation period. This chocolate was followed by OR 70.0%cc and OR 80.1%cc, which showed similar temporal profiles of astringency. These two samples never reached the level of significance ($p > 0.05$), however a tendency towards significance was observed (T74%-100%, max. DR% 41.0 and T76%-100%, max. DR% 43.6, respectively). Samples OR 67.4%cc and 70.4%cc reached neither

chance nor significance level with max. DR% of 30.8% and 28.9%, respectively at the end of the evaluation period (Fig. 40).

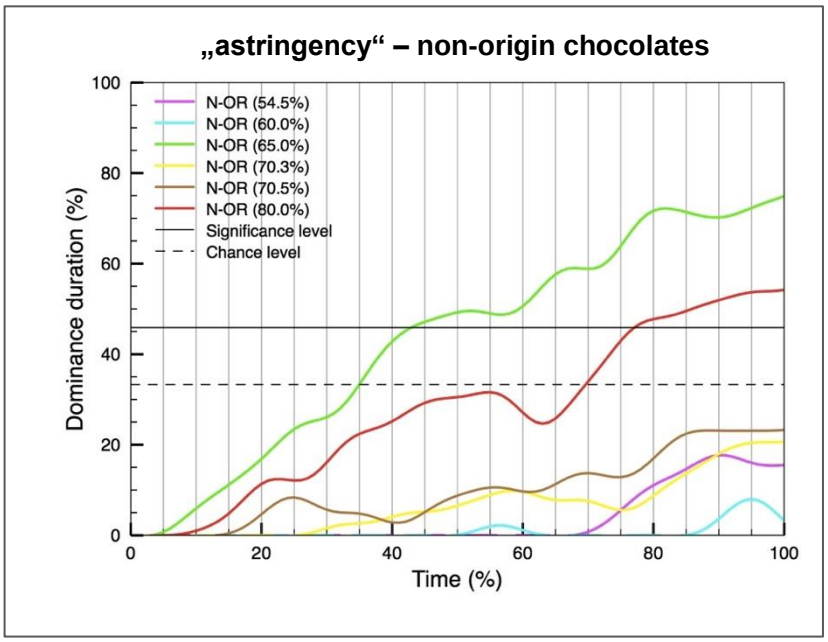


Fig. 39: DR (%) for „astringency“ in non-origin chocolates.

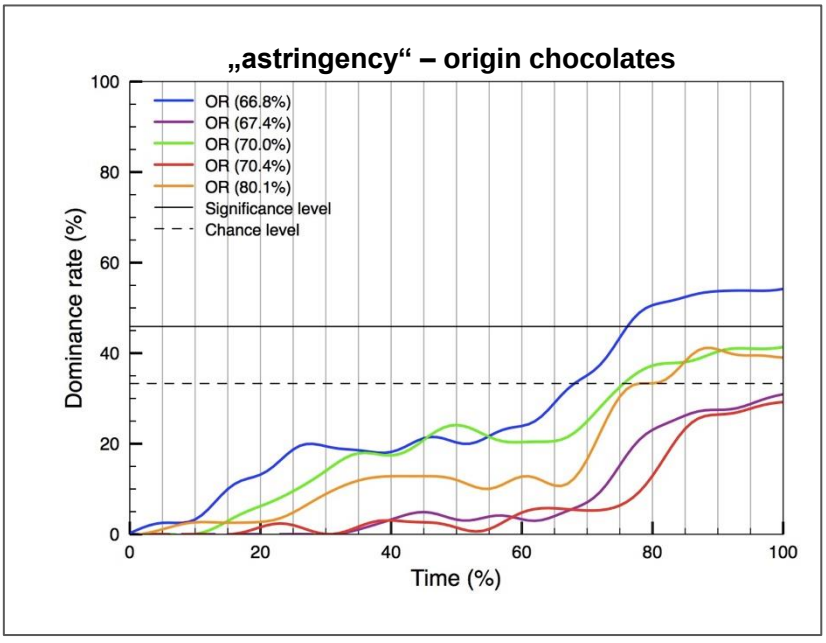


Fig. 40: DR (%) for „astringency“ in origin chocolates.

4.1.4 Attribute dominance duration

In addition to the visual inspection of the TDS curves, quantitative comparison of the products based on the TDS parameter "*attribute dominance duration*" (DD%) (as a proportion% of total TDS sequences) was performed using a linear mixed model with DD% as dependent variable, product as fixed and subject as random effect. For multiple comparisons, a Sidak post hoc test was applied.

Results (displayed in Tab. 20; mean, standard error, 95%-CI) revealed that products differed significantly ($p < 0.001$) in terms of "*attribute dominance duration*" considering all investigated attributes (*sweet taste, bitter taste, astringency*). Product groups were identified for each attribute according to Sidak post hoc test for multiple comparisons. Products within one group sharing the same letter do not differ significantly ($p > 0.05$). However, different letters indicate significant ($p < 0.05$) differences in "*attribute dominance duration*" (DD%).

4.1.4.1 Sweet taste

Considering all investigated products (OR, N-OR), the longest *sweet taste* DD%, not differing significantly ($p < 0.05$), was observed in the samples N-OR 60.0%cc (95.3%) and N-OR 54.5%cc (85.1%) both with the lowest content of cocoa liquor (%) among all investigated chocolates (Tab. 20). These samples were followed by the single-origin chocolate OR 67.4%cc (Forastero, Criollo, Trinitario; Madagascar) with the second lowest cocoa content within the origin chocolates (DD% of 61.7). This chocolate differed significantly ($p < 0.05$) from N-OR 60.0%cc, however not from N-OR 54.5%cc, N-OR 70.3%cc and OR 70.4%cc ($p > 0.05$). The samples N-OR 70.3%cc and OR 70.4%cc (Arriba Nacional; Ecuador) with similar cocoa contents revealed DD% of 53.1% and 41.3%, respectively, without significant ($p > 0.05$) difference. The chocolates N-OR 70.5%cc and the single-origin OR 70.0%cc (Amelonado Forastero; São Tomé und Príncipe) with DD% of 26.6% and 22.7%, respectively, without significant difference ($p > 0.05$), showed also similar cocoa contents, however differed in origins and quality of cocoa beans used. These last two samples differed significantly ($p < 0.05$) from N-OR 70.3%cc, OR 67.4%cc, N-OR 54.5%cc and N-OR 60.0%cc, but not from OR 70.4%cc as well as from the products with the highest cocoa contents OR 80.1%cc (Trinitario, Forastero; blend of origins: Tanzania, Ghana, São Tomé) and N-OR 80.0%cc and from the sample N-OR

65.0%cc with the highest polyphenol content (*sweet taste* DD% of 14.5%, 3.4% and 9.8%, respectively). The single origin chocolate OR 66.8%cc (Trinitario, Forastero; Brazil) with the lowest %cc within the origin chocolates (66.8%cc) was also part of this group with a *sweet taste* DD% of 10.4%, differing significantly ($p < 0.05$) from N-OR 60.0%cc, N-OR 54.5%cc, OR 67.4%cc, N-OR 70.3%cc and OR 70.4%cc.

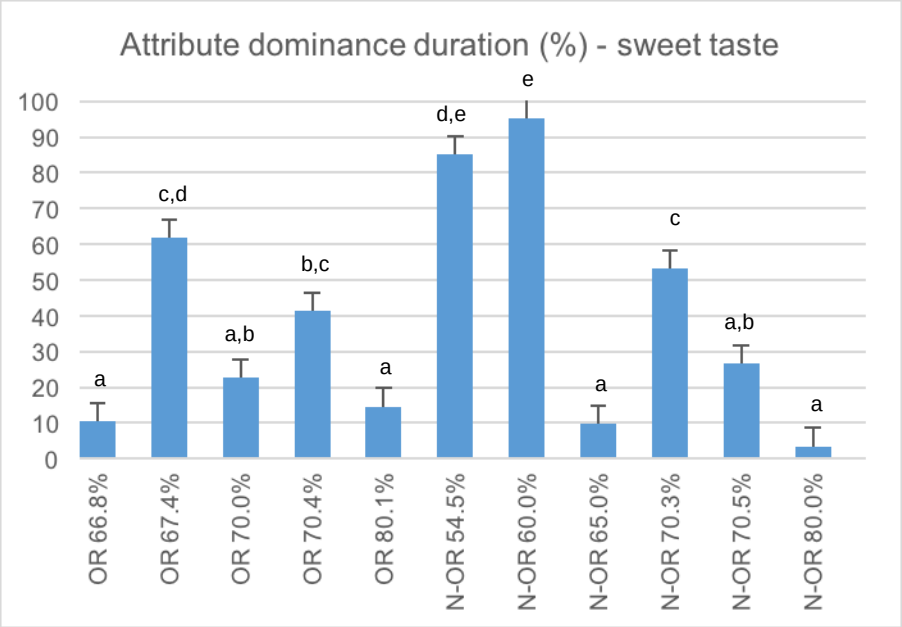


Fig. 41: Sweet taste DD% (mean values and standard errors) including results of Sidak post hoc test (all investigated products).

In OR chocolates, the sample with the longest sweet taste DD% was OR 67.4%cc (61.7%). It was followed by OR 70.4%cc, OR 70.0%cc, OR 80.1%cc and OR 66.8%cc with DD% of 41.3%, 22.7%, 14.5% and 10.4%, respectively. No significant ($p > 0.05$) differences were observed between OR 67.4%cc and 70.4%cc as well as between OR 70.0%cc, OR 80.1%cc and 66.8%cc.

As expected, in N-OR chocolates, the samples with the lowest cocoa and thus highest sugar contents, N-OR 54.5%cc and N-OR 60.0%cc, elicited the longest sweet taste DD% (85.1% and 95.3%, respectively). No statistically significant ($p > 0.05$) difference between these two samples was observed. However, these samples were found to differ significantly ($p < .05$) as compared to the other N-OR chocolates. Although the TDS graph (Fig. 35) indidcates significant differences in DR(%) between N-OR 70.3%cc and N-OR 70.5%cc, these two samples did not differ significantly ($p > 0.05$) in terms of sweet taste DD% (53.1%

and 26.6%, respectively), however from N-OR 54.5%cc and N-OR 60.0%cc as well as N-OR 65.0%cc and 80.0%cc (DD% 9.8 and 3.4, respectively; no significant difference at $p > 0.05$) (Fig. 41).

4.1.4.2 Bitter taste

Considering all investigated products (OR and N-OR), the longest *bitter taste dominance* duration (DD%) exhibit the samples with the highest cocoa contents OR 80.1%cc, (Trinitario, Forastero; blend of origins: Tanzania, Ghana, São Tomé) and N-OR 80.0%cc with 63.1% and 62.5%, respectively. These products were followed by N-OR 70.5%cc and OR 66.8%cc (Trinitario, Forastero; Brazil), OR 70.0%cc (Amelonado Forastero; São Tomé und Príncipe) and OR 70.4%cc, (Arriba Nacional; Ecuador) with *bitter taste* DD% of 61.0%, 57.1%, 50.7% and 47.1%, respectively. Also within this subset was the high-polyphenol chocolate sample N-OR 65.0%cc as well as sample N-OR 70.3%cc with *bitter taste* DD% of 38.6% each. This subset of products differed significantly ($p < 0.05$) from the samples OR 67.4%cc (Forastero, Criollo, Trinitario; Madagascar), N-OR 54.5%cc and N-OR 60.0%cc in terms of of *bitter taste* DD% (26.8%, 10.9% and 3.8%, respectively) (Fig. 42).

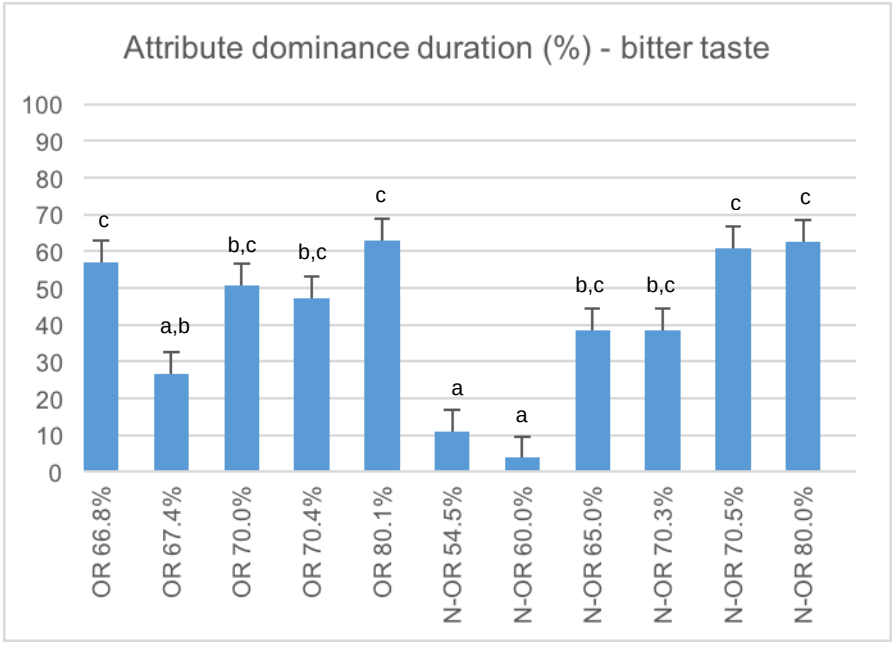


Fig. 42: Bitter taste DD% (mean values and standard errors) including results of Sidak post hoc test (all investigated products).

In N-OR samples, bitter taste was perceived as dominant for the longest period of time (DD%) in the sample with the highest cocoa content N-OR 80.0%cc

(62.5%), followed by the sample with the second highest cocoa content N-OR 70.5%cc (61.0%). Although showing differences in bitter taste DR% (Fig. 37), bitter taste DD% was found to be similar in N-OR 70.3%cc and N-OR 65.0%cc (38.6% both). The differences between these samples in terms of bitter taste DD% was found to be not significant ($p < 0.05$) as shown in Tab. 20. The shortest bitter taste DD% were observed in N-OR 60.0%cc and N-OR 54.5%cc, the samples with the lowest cocoa contents (3.8% and 10.9%, respectively). N-OR 54.5%cc showed either higher bitter taste DR% and longer DD% compared to N-OR 60.0%cc despite of the slightly higher cocoa content, however this result was not statistically significant ($p < 0.05$). In terms of bitter taste DD%, these two samples differed significantly ($p < 0.05$) from all other N-OR chocolate samples.

In accordance to the “bitter taste” DR% in the TDS curves (Fig. 38), OR chocolates showed a different pattern in terms of DD% as the samples are perceived as dominantly ($p < 0.05$) bitter for longer periods of time (%) regardless of cocoa contents (Tab. 20). The highest DD% value received the sample with the highest cocoa content OR 80.1% (blend of origins from Tanzania, Ghana, São Tomé, *Trinitario* and *Forastero* varieties) (63.1%). It was followed by OR 66.8%cc, OR 70.0%cc and OR 70.4%cc with bitter taste DD% of 57.1%, 50.7% and 47.1%, respectively. The sample with the shortest period of bitter taste dominance was OR 67.4%cc (26.8%) differing significantly ($p < 0.05$) from OR 80.1%cc and OR 66.8%cc, however not from OR 70.0%cc and OR 70.4%cc (Fig. 42).

4.1.4.3 Astringency

Astringency dominance duration was highest in the high-polyphenol sample N-OR 65.0%cc (DD% of 51.6) when considering all investigated products (OR, N-OR) (Tab. 20). This sample was followed by N-OR 80.0%cc, the sample with the highest cocoa content among the N-OR chocolates as well as OR 66.8%cc with *astringency* DD% of 34.1% and 32.4%, respectively. Sample N-OR 65.0%cc differed significantly ($p < 0.05$) from all other evaluated products (except from N-OR 80.0%cc and OR 66.8%cc) in terms of *astringency* DD%. The chocolates with the lowest duration of *astringency* dominance% were N-OR 60.0%cc and N-OR 54.5%cc (0.9% and 4.0%, respectively), however not being significantly different ($p > 0.05$) from N-OR 70.3%cc, OR 67.4%cc, OR 70.4%cc, N-OR 70.5%cc and

OR, 80.1%cc (DD 8.3%, 11.4%, 11.6%, 12.5% and 22.4%, respectively). A significant ($p < 0.05$) difference in DD% was observed between samples N-OR 60.0%cc and OR 70.0%cc (*astringency* DD% of 0.9 and 26.5%, respectively).

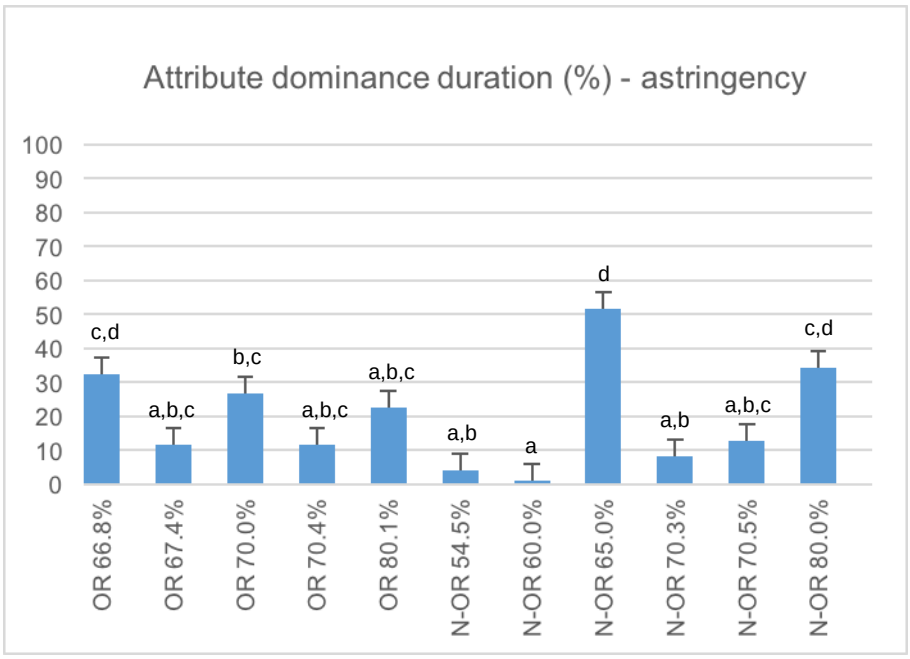


Fig. 43: Astringency DD% (mean values and standard errors) including results of Sidak post hoc test (all investigated products).

In OR chocolates, astringency DD% was longest in OR 66.8%cc (32.4%), the only OR sample exceeding the level of significance in terms of astringency DR%. However, it did not differ significantly ($p < 0.05$) from OR 70.0%cc, OR 80.1%cc, OR 70.4%cc and even OR 67.4%cc with DD% of 26.5%, 22.4%, 11.6% and 11.4%, respectively.

In N-OR chocolates, the sample with the highest astringency DD% (51.6%) was N-OR 65.0%cc, which did not differ significantly ($p > 0.05$) from N-OR 80.0%cc (34.1%), although both samples reached significant astringency DR%. These samples were followed by N-OR 70.5%cc, N-OR 70.3%cc, 54.5%cc and N-OR% 60.0%cc with astringency DD% of 12.5%, 8.3%, 4.0% and 0.9%, respectively, without significant differences ($p > 0.05$). Significant different ($p < 0.05$) was the astringency DD% within N-OR samples between N-OR 70.3%cc, N-OR 54.5%cc and N-OR 60.0%cc and N-OR 65.0%cc and N-OR 80.0%cc. Sample N-OR 70.5%cc did not differ significantly ($p > 0.05$) from all other products (Fig. 43).

Upon the Pearson correlation coefficient, sweet taste DD% was highly significant ($p < 0.001$) negative correlated with bitter taste DD% (r = - 0.812) and astringency

DD% (r = -0.693), whereas between bitter taste and astringency DD% no significant correlation was observed (r = 0.142; *p* = 0.213).

4.1.4.4 Impact of cocoa content (%) on attribute dominance duration

A negative correlation was found between cocoa content% and sweet taste DD% in both N-OR (r = - 0.709; *p* < 0.001) and OR chocolates (r = - 0.263; *p* = 0.035) upon the Pearson correlation coefficient. The correlation was even more pronounced in N-OR chocolates without taking into account N-OR 65.0%cc (r = - 0.831; *p* < 0.001). When considering all evaluated products (OR and N-OR), Pearson correlation revealed a significant (*p* < 0.001) strong negative (r = - 0.617) association between cocoa contents% and sweet taste DD%.

Bitter taste DD% was strongly positive correlated with cocoa contents% (r = 0.697; *p* < 0.001) in N-OR chocolates. Without N-OR 65.0%, this correlation was slightly higher (r = 0.727; *p* < 0.001). A weak (r = 0.272), however significant (*p* = 0.028) positive correlation between cocoa contents% and bitter taste DD% was also found in the investigated OR chocolate samples. Considering all investigated samples (OR, N-OR), the correlation of cocoa content% and bitter taste DD% was significantly (p < 0.001) positive (r = 0.580).

The correlation between cocoa contents and astringency DD% was found to be significantly (*p* = 0.002) positive (r = 0.342) in N-OR chocolates. Without N-OR 65.0%cc, the correlation was strong positive (r = 0.628) and significant (*p* < 0.001). In contrary, OR chocolates showed no significant correlation of cocoa contents% with astringency DD% (r = - 0.001; *p* = 0.993). Considering all chocolate products (OR, N-OR), a weak positive (r = 0.233), however significant (*p* = 0.005) correlation was observed.

Tab. 20: Results of the linear mixed model for dominant attribute duration (DD%).

Products	Sweet taste				Bitter taste				Astringency			
	Mean*	s.e.**	95%-CI***	*p* –value	Mean*	s.e.**	95%-CI***	*p* –value	Mean*	s.e.**	95%-CI***	*p* –value
OR 66.8%cc	10.4^{a}	5.2	(1.0 - 20.8)	< 0.001	57.1^{c}	5.9	(45.4 - 68.9)	< 0.001	$32.4^{c,d}$	4.9	(22.7 - 42.1)	< 0.001
OR 67.4%cc	$61.7^{c,d}$	5.2	(51.3 - 72.1)		$26.8^{a,b}$	5.9	(15.1 - 38.6)		$11.4^{a,b,c}$	4.9	(1.8 - 21.1)	
OR 70.0%cc	$22.7^{a,b}$	5.2	(12.4 - 33.1)		$50.7^{b,c}$	5.9	(39 - 62.5)		$26.5^{b,c}$	4.9	(16.8 - 36.2)	
OR 70.4%cc	$41.3^{b,c}$	5.2	(30.9 - 51.7)		$47.1^{b,c}$	5.9	(35.4 - 58.9)		$11.6^{a,b,c}$	4.9	(1.9 - 21.3)	
OR 80.1%cc	14.5^{a}	5.2	(4.1 - 24.9)		63.1^{c}	5.9	(51.4 - 74.8)		$22.4^{a,b,c}$	4.9	(12.7 - 32.1)	
N-OR 54.5%cc	$85.1^{d,e}$	5.2	(74.7 - 95.5)		10.9^{a}	5.9	(0.0 - 22.7)		$4.0^{a,b}$	4.9	(0.0 - 13.7)	
N-OR 60.0%cc	95.3^{e}	5.2	(84.9 – 100)		3.8^{a}	5.9	(0.0 - 15.5)		0.9^{a}	4.9	(0.0 - 10.6)	
N-OR 65.0%cc	9.8^{a}	5.2	(0.0 - 20.1)		$38.6^{b,c}$	5.9	(26.9 - 50.4)		51.6^{d}	4.9	(41.9 - 61.3)	
N-OR 70.3%cc	53.1^{c}	5.2	(42.7 - 63.5)		$38.6^{b,c}$	5.9	(26.9 - 50.3)		$8.3^{a,b}$	4.9	(0.0 - 18)	
N-OR 70.5%cc	$26.6^{a,b}$	5.2	(16.2 - 37)		61.0^{c}	5.9	(49.2 - 72.7)		$12.5^{a,b,c}$	4.9	(2.8 - 22.2)	
N-OR 80.0%cc	3.4^{a}	5.2	(0.0 - 13.8)		62.5^{c}	5.9	(50.8 - 74.2)		$34.1^{c,d}$	4.9	(24.4 - 43.8)	

Different letters identify product groups according to Sidak post hoc test.
*Mean value: proportion (%) of attribute dominance duration
**Standard error
***Confidence interval: 95%

4.1.5 Principal component analysis for “attribute dominance durations”

The factor analysis PCA plot showed that 33% and 21% of the total variance were explained by the first two principal components (PC1, PC2). N-OR 60.0%cc and N-OR 54.5%cc were located closely together being highly positive associated with sweet taste DD%, followed by OR 67.4%cc. The products N-OR 70.3%cc, OR 70.4%cc and N-OR 70.5%cc were located between sweet taste and bitter taste DD%, with increasing cocoa contents(%) showing a tendency towards bitterness. The samples N-OR 70.3%cc and OR 70.4%cc were thus positively associated by sweet taste whereas N-OR 70.5%cc together with OR 80.1%cc showed the strongest positive association with bitter taste, however N-OR 70.5%cc still being more sweet in terms of DD% than OR 80.1%cc. Samples positively associated with bitter taste and astringency DD% were OR 70.0%cc, OR 66.8%cc and N-OR 80.0%cc. The strongest association with astringency showed N-OR 65.0%cc upon the factor analysis PCA plot (Fig. 44).

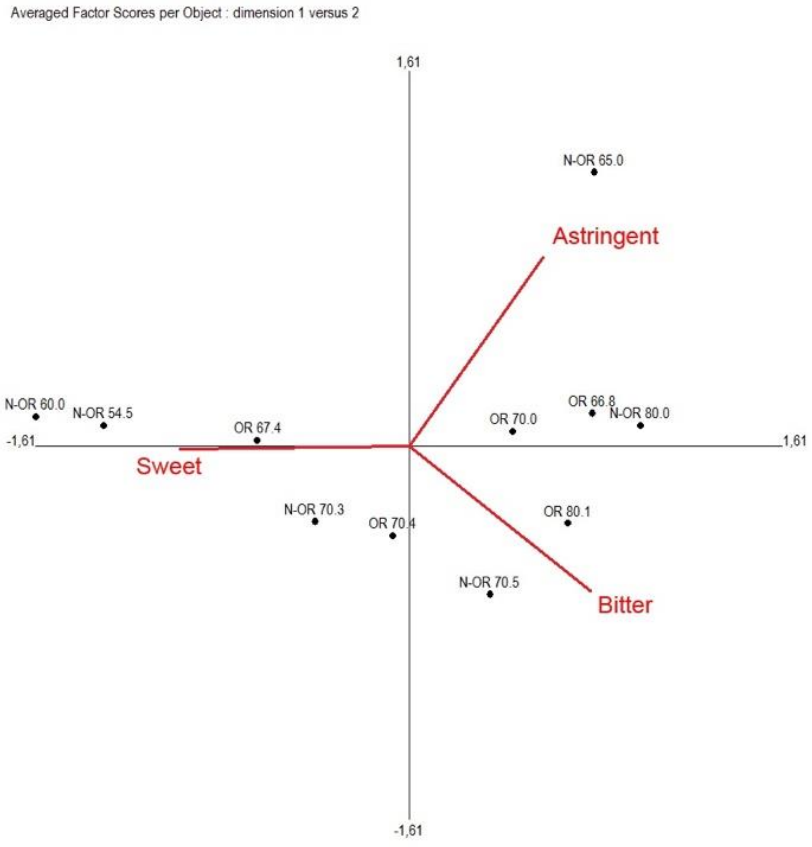


Fig. 44: PCA plot for the TDS-parameter “attribute dominance duration" (DD%) of sweet taste, bitter taste and astringency in commercial dark origin and non-origin chocolates with different cocoa contents (%cc).

4.1.6 Total duration of TDS evaluation

As panelists were free to decide when to swallow the samples (see 3.2.1.3), this procedure resulted in different evaluation durations. However, the total duration of TDS evaluation did not differ significantly (p = 0.430) between all 11 investigated products (OR as well as N-OR) according to the linear mixed model.

4.2 Consumer survey

4.2.1 Questionnaire

96 female individuals aged 19-33 years old were invited to participate in the consumer survey. According to the questionnaire, the frequency of chocolate consumption varied greatly among participants with the group of occasional (several times/month) consumers representing the greatest proportion (28.1 %) followed by regular (1x/week; 22.9%) and rare (<1x/month; 20.8%) consumption (Fig. 45). 57.3% of the participants preferred dark chocolate, while 42.7% indicated milk chocolate as the preferred product.

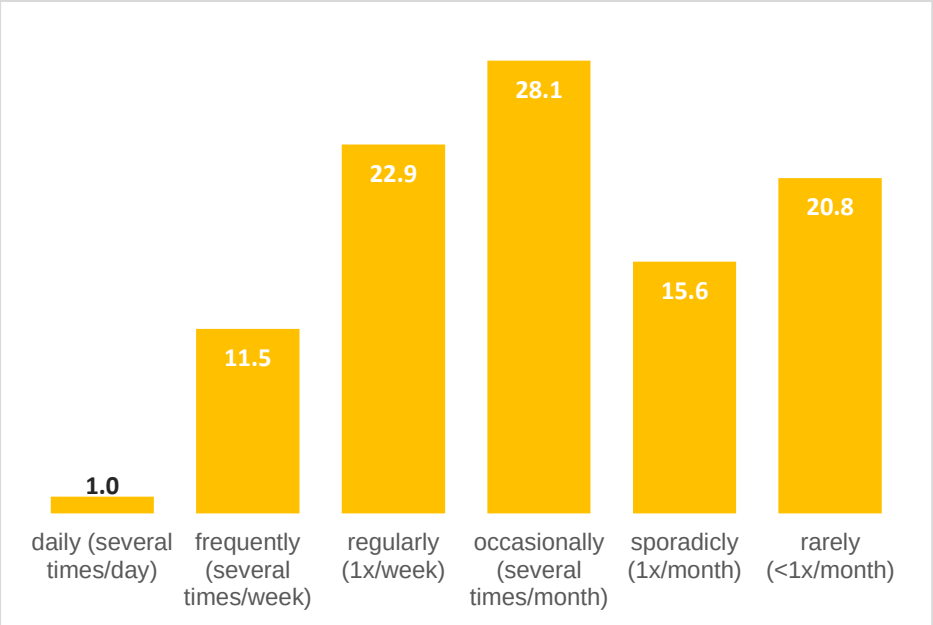


Fig. 45: Demographic data: consumption frequency (%).

4.2.2 Preference test 1: Paired preference test

As shown in Tab. 21, in set 2, OR 70.4%cc (Arriba Nacional; Ecuador) was significantly (p < 0.001) preferred over N-OR 70.5%cc. However, in set 3, N-OR 70.3%cc was significantly (p < 0.001) chosen over OR 70.0%cc (Amelonado Forastero; São Tomé). Within the other sets the same trend was observed, i.e.

N-OR chocolates were selected over OR chocolates (in set 1 N-OR 65.0%cc vs. OR 66.8%cc (Trinitario, Forastero; Brazil) and set 4 N-OR 80.0%cc vs. OR 80.1%cc (Trinitario, Forastero; blend of origins: Tanzania, Ghana, São Tomé), however without being statistically significant ($p > 0.05$).

Tab. 21: Data set of paired preference test (binomial distribution) (FIZZ v2.51 a 86).

Set #	Answers taken	Product	Answers	Significance (Risk)
1	96	OR 66.8%cc	39	0.082
		N-OR 65.0%cc	57	
2	96	OR 70.4%cc	70	<0.001***
		N-OR 70.5%cc	26	
3	96	OR 70.0%cc	29	<0.001***
		N-OR 70.3%cc	67	
4	96	OR 80.1%cc	38	0.052
		N-OR 80.0%cc	58	

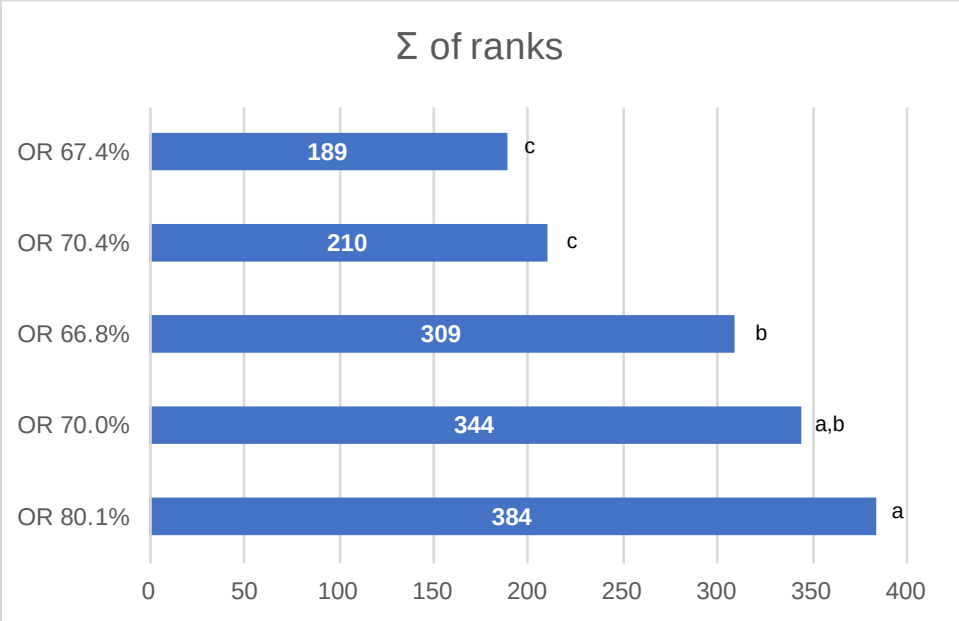
* Signif. (5%): $p < 0.05$
**Signif. (1%): $p < 0.01$
***Signif. (0.1%): $p < 0.001$

4.2.3 Preference test 2: Ranking test for preference

4.2.3.1 Origin chocolates (different cocoa contents %)

As shown in Fig. 46, the ranking for preference of origin samples (1 = most preferred; 5 = least preferred) resulted in the following order based on rank sums: sample OR 67.4%cc was most preferred (Σ 189.0) followed by the samples OR 70.4%cc **(**Σ 210.0), OR 66.8%cc (Σ 309.0), OR 70.0%cc (Σ 344.0) and OR 80.1%cc (Σ 384.0). According to the Friedman test, a significant ($p < 0.001$) difference in product preference was observed between the evaluated OR products.

Multiple comparisons of rank sums (Bonferroni correction) revealed that the most preferred samples OR 67.4%cc and OR 70.4%cc differed significantly ($p < 0.05$) from the other OR chocolates. No significant ($p > 0.05$) differences were observed between OR 66.8%cc and OR 70.0%cc as well as OR 70.0%cc and the least preferred OR 80.1%cc. Cocoa contents (%) of the evaluated chocolates with approx. 70.0% did not seem to affect preference for a certain chocolate sample.



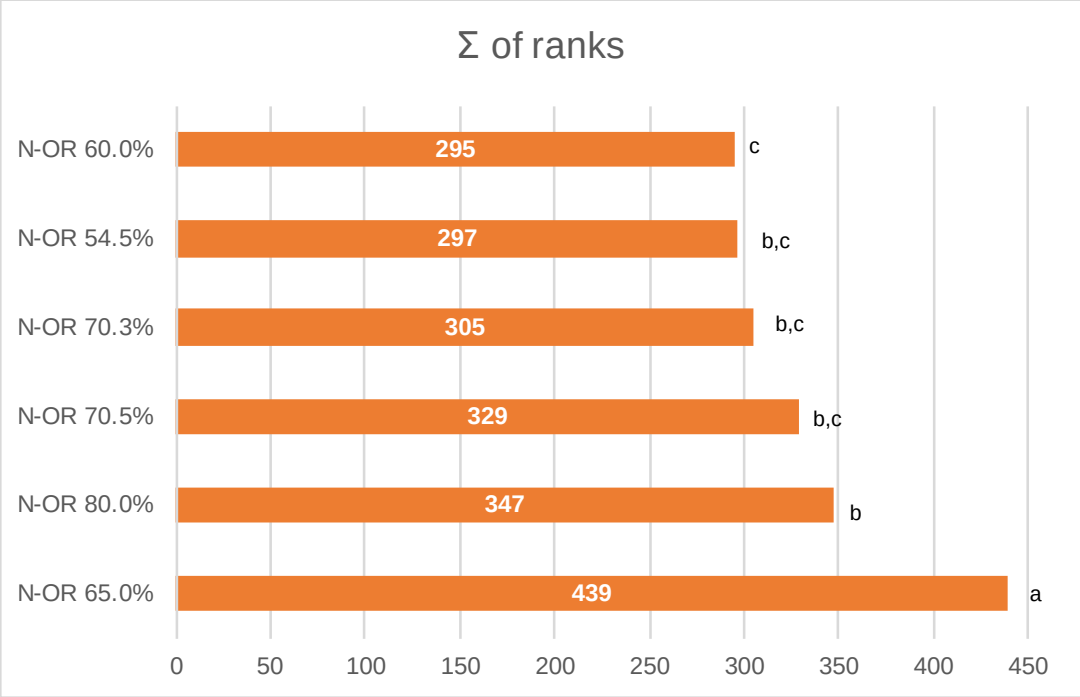
Different letters identify product groups according to Bonferroni correction.

Fig. 46: Sum of ranks (OR chocolates).

4.2.3.2 Non-origin chocolates (different cocoa contents %)

As shown in Fig. 47, N-OR chocolate samples were ranked as follows (1 = most preferred; 6 = least preferred): most preferred was the sample N-OR 60.0%cc (Σ 295.0), closely followed by the sample N-OR 54.5%cc (Σ 297.0) and N-OR 70.3% (Σ 305.0). On the 4th and 5th rank position were samples N-OR 70.5%cc (Σ 329.0) and N-OR 80.0%cc (Σ 347.0). The highest rank sum obtained the sample N-OR 65.0%cc (Σ 439.0) and is thus, considered as least preferred. The non-parametric Friedman test revealed significant ($p < 0.001$) differences in product preference among the evaluated N-OR samples.

According to the Bonferroni correction for multiple comparisons of rank sums, the least preferred sample N-OR 65.0%cc differed significantly ($p < 0.05$) from all other N-OR products. The sample N-OR 80.0%cc, with the highest cocoa content% (5th rank position), showed no significant ($p > 0.05$) differences compared to N-OR 70.5%cc, N-OR 70.3%cc and N-OR 54.5%cc, however differed significantly ($p < 0.05$) from N-OR 60.0%cc. Furthermore, no significant ($p > 0.05$) differences were observed between N-OR 70.5%cc, N-OR 70.3%cc, N-OR 54.5%cc and N-OR 60.0%cc.

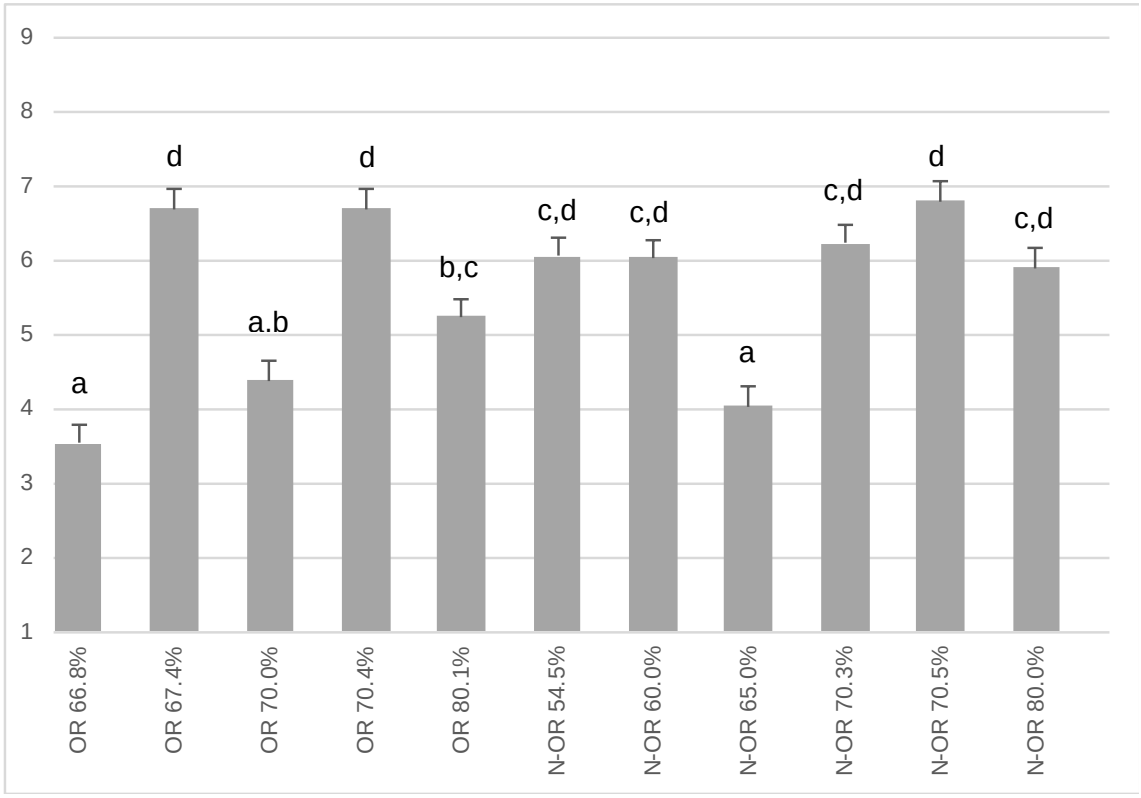


Different letters identify product groups according to Bonferroni correction.

Fig. 47: Sum of ranks (N-OR chocolates).

4.2.4 Acceptance test

The linear mixed model revealed that product acceptance differed significantly ($p < 0.001$) between all chocolate samples. It was shown, that frequency of chocolate consumption (daily [several times/day], frequently [several times/week], regularly [1x/week], occasionally [several times/month], sporadicly [1x/month], rarely [<1x/month]), the preference for a certain chocolate type (dark or milk chocolate) and age (data obtained by a questionnaire) did not affect product acceptance significantly ($p = 0.183$, $p = 0.585$ and $p = 0.082$, respectively).



Different letters identify product groups according to Sidak post hoc test.

Fig. 48: Product acceptance data (mean values and standard error) of all investigated products including results of Sidak post hoc test.

Tab. 22: Results for product acceptance (9-point-hedonic scale) upon the linear mixed model.

Product	Mean*	s.e.**	95%-CI***	*p* – value****
OR 66.8%cc	3.5[a]	0.25	3.04 - 4.03	< 0.001
OR 67.4%cc	6.7[d]	0.25	6.20 - 7.18	
OR 70.0%cc	4.4[a,b]	0.25	3.89 - 4.87	
OR 70.4%cc	6.7[d]	0.25	6.20 - 7.18	
OR 80.1%cc	5.2[b,c]	0.25	4.74 - 5.72	
N-OR 54.5%cc	6.0[c,d]	0.25	5.55 - 6.54	
N-OR 60.0%cc	6.0[c,d]	0.25	5.53 - 6.51	
N-OR 65.0%cc	4.0[a]	0.25	3.54 - 4.53	
N-OR 70.3%cc	6.2[c,d]	0.25	5.73 - 6.71	
N-OR 70.5%cc	6.8[d]	0.25	6.30 - 7.29	
N-OR 80.0%cc	5.9[c,d]	0.25	5.40 - 6.38	

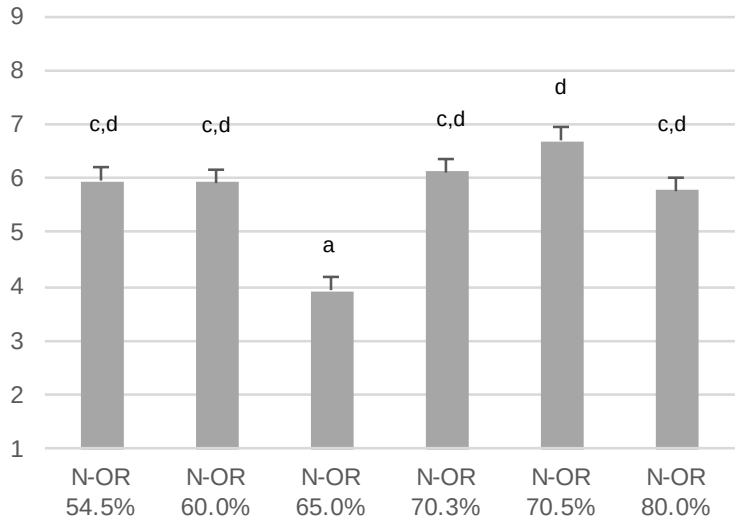
Different letters identify product groups according to Sidak post hoc test.
*Mean value: proportion (%) of attribute dominance duration
**Standard error
***Confidence interval (95%)
****Significance level: 5%

As shown in Fig. 48 and Tab. 22, the most liked product was N-OR 70.5%cc (6.8 points in the 9-point hedonic scale), followed by OR 67.4%cc and OR 70.4%cc (6.7 points both) as well as N-OR 70.3%cc (6.2 points). The acceptance of the N-OR samples with the lowest cocoa contents N-OR 54.5%cc and N-OR 60.0%cc was slightly lower with a similar mean acceptance value of 6.0 points. The lowest acceptance was observed in the products with the highest cocoa contents (%) from both groups: N-OR 80.0%cc and OR 80.1%cc, receiving mean acceptance values of 5.9 and 5.2 points, respectively.

The comparison of the samples N-OR 70.5%cc, OR 70.4%cc, OR 67.4%cc, N-OR 70.3%cc, N-OR 54.5%cc, N-OR 60.0%cc and N-OR 80.0%cc, showed no significant ($p > 0.05$) differences in product acceptance upon the Sidak post hoc test for multiple comparisons.

However, the acceptance of the samples N-OR 70.5%cc, OR 70.4%cc and OR 67.4%cc differed significantly ($p < 0.05$) from OR 80.1%cc (5.2) as well as from OR 70.0%cc (4.4), N-OR 65.0%cc (4.0) and OR 66.8%cc (3.5). The last three samples did not differ significantly ($p > 0.05$) in terms of consumers acceptance.

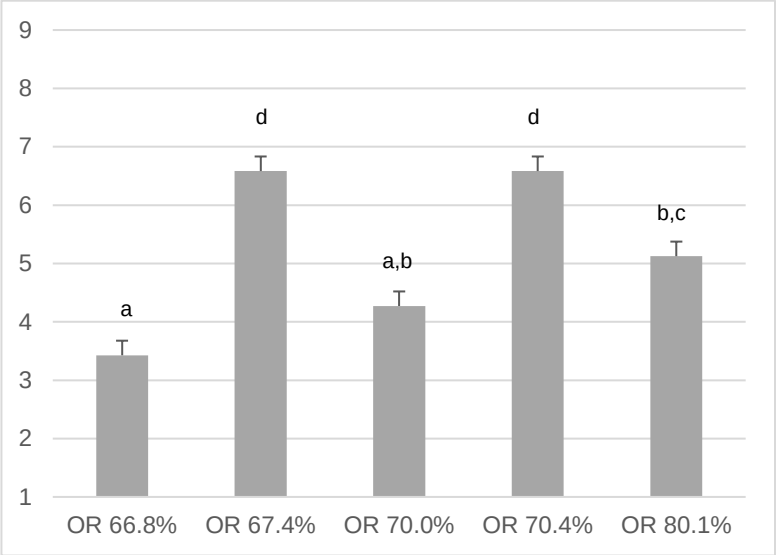
Within the group of N-OR chocolates (Fig. 49), the product acceptance was rated as follows: the most accepted product N-OR 70.5%cc (6.8) was followed by N-OR 70.3%cc (6.2), N-OR 54.5%cc (6.0), N-OR 60.0%cc (6.0) and N-OR 80.0%cc (5.9) without being stastically significant ($p > 0.05$) according to Sidak post hoc test. However, all five products mentioned above differed significantly ($p < 0.05$) from N-OR 65.0%cc (4.0), which received the lowest mean acceptance value.



Different letters identify product groups according to Sidak post hoc test.

Fig. 49: Product acceptance data (mean values and standard error) of N-OR products including results of Sidak post hoc test.

The most accepted OR samples were OR 67.4%cc and OR 70.4%cc, both liked equally (6.7 points in the 9-point hedonic scale). Thus, these samples did not differ significantly ($p > 0.05$). Significant ($p < 0.05$) differences (Sidak post hoc test) were found comparing the two above mentioned samples with OR 80.1%cc (5.2), OR 70.0%cc (4.4) and OR 66.8%cc (3.5) (in decreasing order of acceptance rating). However no significant ($p > 0.05$) differences were observed between OR 80.1%cc and OR 70.0%cc as well as OR 70.0%cc and OR 66.8%cc (Fig. 50).



Different letters identify product groups according to Sidak post hoc test.

Fig. 50: Product acceptance data (mean values and standard error) of OR products including results of Sidak post hoc test.

4.2.4.1 Impact of cocoa contents (%) on product acceptance

According to the Pearson correlation coefficient, cocoa contents% were shown to not affect consumers overall liking of all tested products (r = 0.009; *p* = 0.979), i. e. N-OR (r = 0.046; *p* = 0.272) as well as OR chocolates (r = 0.039; *p* = 0.401) significantly.

4.2.4.2 Impact of attribute dominance duration (DD%) on product acceptance

Generally, product acceptance was positively correlated with sweet taste DD%, however not significantly (r = 0.525; *p* = 0.097). The impact of bitter taste DD% on acceptance rating was also not significant (r = - 0.251; *p* = 0.457). However, a strong significant negative correlation was observed between astringency DD% and product acceptance with r = - 0.743; *p* = 0.009.

In N-OR chocolates, sweet and bitter taste DD% did not affect product acceptance significantly (r = 0.355; *p* = 0.489 and r = 0.090, *p* = 0.866 for sweet taste and bitter taste, respectively) upon the Pearson correlation coefficient. However, product acceptance was negatively correlated with astringency DD%, although not being significant (r = 0.799; *p* = 0.057).

Product acceptance in the group of OR chocolates was strongly positive correlated with sweet taste DD% (r = 0.857) and negatively correlated with bitter taste DD% (r = -0.651), however not significantly (p = 0.063 and 0.234, respectively). Astringency DD% strongly affected consumers acceptance negatively (r = - 0.998; $p < 0.001$).

Based upon the Spearman's correlation coefficient, buying intention was highly correlated with product acceptance (r_S =0,906; $p < 0.001$).

5 Discussion

5.1 TDS profiles

The results of the present study indicate a strong influence of cocoa bean genotype and region of origin on the evolution of dominance of polyphenol-associated descriptors bitter taste and astringency as well as sweet taste as covering agent during consumption (mastication and aftertaste period) in addition to cocoa contents.

“Bulk” Forastero cocoa beans produced in West Africa were characterized previously by a strong chocolate flavour, low bitterness and astringency, which is typical for well-fermented cocoa beans in this geographical region (Clapperton et al., 1994; Kongor et al., 2016; Miller et al., 2006). The present evaluation confirms this observation considering bitter taste and astringency in non-origin chocolates produced mostly from Forastero cocoa beans in West Africa, with exception of N-OR 65.0%cc, containing cocoa beans of unidentified genotype and region of origin, however high polyphenol content due to preservative processing procedures as claimed by the manufacturer. Whereas the temporal profile in N-OR 80.0%cc and 70.5%cc was characterized by the dominance of bitterness for the longest time% of evaluation, in N-OR 54.5%cc and 60.0%cc sweet taste was most dominant. Thus, in non-origin chocolates both attributes were correlated with cocoa contents (%). In addition, a weak positive correlation of cocoa contents and astringency was observed.

In origin chocolates manufactured from “fine grades” (Forastero, Criollo, Trinitario, Nacional) cultivated in specified growing regions, bitter taste dominated significantly in the sample with the highest cc% (OR 80.1%cc) for the longest period of evaluation, however a similar pattern was found in the sample with the lowest cc% (OR 66.8%cc). While OR 80.1%cc was manufactured from a blend of origins (Tanzania, Ghana, São Tomé), the single-origin chocolate OR 66.8%cc was produced from the same blend of cocoa bean genotypes (Trinitario, Forastero), however in Brazil. As reported by Reed (2010), Forastero beans cultivated in Brazil are characterized by bitterness and astringency, although other authors found significant differences depending on the Brazilian region (e.g. Bahia and Para) (Jinap et al., 1995).

Sample OR 67.4%cc (Madagascar; Forastero, Criollo, Trinitario) with similar cc% as OR 66.8%cc (Brazil; Trinitario, Forastero), however different country of origin and blends of different varieties, was not perceived as dominantly bitter at any time during the entire evaluation period, on the contrary was characterized by a strong significant dominance of sweetness. Mild Criollo types in the blend might have caused this impression. These “fine” grades have been described as low in bitterness and high aromatic with mild flavours (Ascrizzi et al., 2017; Wolters, 1999). The initial polyphenol content in Criollo seeds was found to be 2/3 of the amount of Forastero varieties (Lange & Fincke, 1970). Although often less fermented (Counet et al., 2004), the decrement of catechins during post-harvest treatments (fermentation, drying) was reported to be much stronger in Criollo seeds than in other genotypes (Elwers et al., 2009). However, significant differences in bitter taste in Criollo varieties have been shown even if cultivated in the same country, but in different regions (Vázquez-Ovando, 2015). They have also been found to be even more bitter and astringent than Forastero varieties (Clapperton et al., 1994) indicating a strong impact of other factors in addition to the genetic constitution.

The sample OR 70.0%cc from São Tomé (Amelonado Forastero cocoa beans) was dominated by bitterness (during mastication), which is also supported by Reed (2010) who used bitter taste as a descriptor in order to characterize this type of cocoa beans produced in São Tomé. The chocolate OR 70.4%cc with similar cc%, however produced from Arriba Nacional in Ecuador showed a balanced temporary profile considering the polyphenol-related attributes with periods of dominant bitter sensations as well as a tendency towards sweetness and low astringency after swallowing.

Thus, the impact of cc% on bitter and sweet taste as well as astringency on the temporal profile was found to be rather minor in OR chocolates. Other factors such as climate, sun exposure, rainfall and humidity, soil conditions, age of the cocoa tree, time of harvesting and time between harvesting and fermentation might have caused these differences. Especially the use of fertilizers was shown to affect amounts and profiles of polyphenols and thus bitter taste and astringency (Elwers et al., 2009; Kongor et al., 2016; Niemenak et al., 2006). Further major contributing factors on the polyphenol contents are degree and duration of pod

storage, fermentation, drying and roasting of the cocoa beans. Differenes in these postharvest treatment practices, which are usually carried out by the farmers in the region of origin, might have led to these variations in the sensory profile of origin chocolate samples (Afoakwa, 2015; Albertini et al., 2015; Nazaruddin et al., 2006).

Astringency differed considerably from the evaluated taste descriptors bitterness and sweetness. In both N-OR and OR chocolates, astringency started to rise congruently as the dominant attribute at the end of the evaluation period resulting in significant changes in the temporary profile as bitter and sweet taste declined simultaneously. Therefore, it is hypothesized that the samples were swallowed at approximately 75% of the evaluation period at panel level. The tactile sensation of astringency arises through precipitation and aggregation of salivary proteins and mucins (coating of epithelium tissues) with polyphenols. Swallowing causes a loss of saliva (and dissolved taste stimulants) in the oral cavity and thus oral lubricating. This leads to the exposure of the mucosal epithelium to the astringent stimuli, enabling it to interact directly with the oral tissue (Ma et al., 2014) explaining the emergence of astringency dominance in the after-taste period. Among N-OR chocolates, this impact was strongest in the sample N-OR 65.0%cc, where astringency started to rise at a very early stage of evaluation, suggesting a strong polyphenol-protein-binding effect. This sample generally differed from the other N-OR chocolates in terms of the evaluated sensory attributes reaching significant dominance of bitter taste at the beginning of evaluation despite of its medium cc%, whereas sweet taste was almost absent.

5.2 Consumer survey

In the present study, product acceptability was found to be not affected by cocoa contents of the evaluated chocolates. In addition, no impact of dominance durations of sweet and bitter taste on liking was observed. However, the longer astringency dominated the temporal sensory profile during product evaluation the lower the acceptability scores given by the consumers. Thus, among the least accepted products were OR 66.8%cc, N-OR 65.0%cc, OR 70.0%cc, OR 80.1%cc and N-OR 80.0%cc (in increasing order of product acceptance), which elicited astringency dominances for the longest evaluation periods among all tested chocolate products. Interestingly, OR 66.8%cc from Brazil received the lowest

acceptability score, although astringency was perceived as dominant longer time during evaluation in N-OR 65.0%cc (the difference is not significant). This finding is likely the result of other sensory attributes typical for the region of origin besides the influence of polyphenol-associated descriptors. Due to high rainfall and humidity, in some areas artificial drying (e.g. open fire) is necessary potentially leading to off-flavour formation described as 'burnt' (Wollgast & Anklam, 2000; Zach, 2017). High concentrations of phenol, guaiacol, 2-phenylbutenal and γ-butyrolactone were reported to characterize Brazilian cocoa beans known for smoky and hammy notes (Dimick & Hoskin, 1999; Reed, 2010) potentially limiting consumers' acceptance.

The most accepted products N-OR 70.5%cc, OR 67.4%cc, OR 70.4%cc and N-OR 70.3%cc (in decreasing order of product acceptance) were located between sweet and bitter taste dominance durations on the PCA plot with higher acceptance scores in products with a tendency towards longer bitter rather than sweet taste dominance durations. In addition, the temporal sensory profiles of chocolates with explicitly high sweet taste dominances as in low cocoa content chocolates N-OR 54.5%cc and N-OR 60.0%cc were not shown to increase consumers acceptability. This finding indicates that liking of cocoa products is strongly driven by a balanced sensory profile in terms of bitterness, as previously reported by Stark et al (2006) rather than sweetness dominance.

Pairwise comparison of chocolates with similar cc%, however different country of origin and genetic constitution indicates that product preference was driven by sensory characteristics other than sweetness, bitterness and even astringency. The high-polyphenol sample N-OR 65.0%cc was chosen over OR 66.8%cc, although astringency was most pronounced. This observation, still not statistically significant, might again be attributed to the presents of hammy, smokey, rubber notes in addition to the temporal profile of bitter taste and astringency in the Brazilian sample (Dimick & Hoskin, 1999; Reed, 2010). The same trend was observed when comparing the samples with the highest cocoa contents (N-OR 80.0%cc vs. OR 80.1%cc). Although N-OR 80.0%cc elicited stronger astringency sensations and lower temporal sweetness, it was preferred over OR 80.1%cc with a tendency toward statistical significance. Comparing OR 70.0%cc from São Tomé and N-OR 70.3%cc, the non-origin sample was significantly preferred over

the origin chocolate. This finding confirms the results of the individual acceptance test and can be attributed to higher astringency and lower sweetness ratings in the origin sample as well as the presence of e.g. earthy notes as described previously (Reed, 2010).

When compared directly, the Ecuadorian specialty Arriba Nacional chocolate OR 70.4%cc was significantly preferred over the most liked sample N-OR 70.5%cc as revealed in the acceptance test. Taking into consideration the evaluated polyphenol-associated descriptors as well as sweet taste, these products show similar temporal profiles. However, the “fine and flavour” Arriba Nacional cocoa beans are acknowledged for their full cocoa flavour with additional floral, fruity, spicy and green notes (Afoakwa, 2010; Reed, 2010) assumed to play a role in product preference. As these descriptors were not evaluated in the present study, further research is necessary in order to verify this hypothesis.

Ranking test for preference of origin chocolates resulted in a higher preference for products showing the strongest sweetness dominances in their temporal profile: OR 67.4%cc (Forastero, Criollo, Trinitario from Madagascar) and OR 70.4%cc (Arriba Nacional from Ecuador). These chocolates were also among the most accepted. They were followed by the samples OR 66.8%cc from Brazil, OR 70.0%cc from São Tomé and OR 80.1%cc (from Tanzania, Ghana and São Tomé) characterized by bitterness and astringency. Interestingly, the least accepted sample OR 66.8%cc was not the least preferred when compared directly with the other origin samples. Significantly least preferred was OR 80.1%cc.

The same trend was observed in ranking of non-origin chocolates. The low cocoa content samples (N-OR 54.5%cc and N-OR 60.0%cc), eliciting the strongest sweet taste dominances, were most preferred when compared directly to the other non-origin samples, although product acceptance was found to be moderate. The high-polyphenol chocolate N-OR 65.0%cc was significantly least preferred, followed by the sample with the highest cocoa content N-OR 80.0%cc, both associated with strong bitterness and astringency dominances. The medium cocoa content samples N-OR 70.5%cc and N-OR 70.3%cc, which were among the most accepted products when evaluated individually, were not preferred over the other non-origin chocolates. These results confirm that acceptability of

products evaluated in a single-product test does not necessarily reflect preferences when products are compared directly in multiple-product tests (Lawless & Heymann, 2010a). As the consumers' purchasing intent strongly correlates with product acceptance, these results do not strengthen the idea that choosing among alternatives mimic consumers purchasing habits more appropriately (Lawless & Heymann, 2010a), which could be of relevance for chocolates manufacturers as well as consumers.

6 Conclusion

In the present study, the temporal sensory profile of commercial dark origin and non-origin chocolates was investigated. It was shown to differ substantially depending not only on cocoa contents (%) but also on cocoa bean variety and region of cocoa bean origin. Whereas in non-origin chocolates, cocoa content (%) significantly affects the polyphenol-associated descriptors bitter taste and astringency as well as sweet taste, this was not observed in premium quality origin chocolates. Single or blends of origins exhibit generally higher bitter taste dominances for a longer evaluation period regardless of cocoa contents. Astringency was most pronounced towards the end of evaluation showing good congruency among all evaluated samples (origin and non-origin).

Although, most major chocolate manufacturers produce a wide range of premium quality, single-origin dark chocolates with different cocoa contents, only few studies exist at present examining the polyphenol content and related sensory characteristics in cocoa-derived products from different origins and genotype (Counet et al., 2004; Luna et al., 2002). Jinap et al., (1995) evaluated the flavour of chocolates manufactured from beans from different countries including bitterness indicating a strong influence of post-harvest treatments (fermentation, drying). However, no research exists at present exploring and comparing polyphenol-associated attributes bitter taste and astringency in commercial dark chocolates with different cocoa contents (%cc) taking into account different origins and genotypes of cocoa beans using dynamic, descriptive methods of sensory analysis. The present study allowed the identification of differences in the temporality of these sensory characteristics showing dominant sensations during consumption.

In order to identify potential drivers of liking, a consumer survey was conducted including the evaluation of individual product acceptance as well as preference in direct comparison. It revealed that acceptance of chocolate products with different cocoa contents, cocoa bean variety and region of origin was driven by a balanced temporal profile with respect to bitterness and sweetness rather than cocoa contents, which was found to not affect consumers liking at all. In contrary, the emergence of astringeny dominance towards the end of evaluation clearly

elicited negative consumer responses. However, the results suggest that not only polyphenol-associated descriptors and sweetness, but also flavour attributes typical for certain cocoa bean varieties and growing regions might have affected product acceptance and preference of origin chocolates. As these descriptors were not subject of focus in the present evaluation, further research is needed in order to verify this hypothesis.

The present study provides useful information for both consumers and chocolate manufacturers. The results of the TDS evaluation suggest that in non-origin chocolates, the general recommendation to consume moderate amounts of especially high cocoa content chocolates in order to contribute to polyphenol-intake might be reasonable. In N-OR chocolates, the polyphenol-associated attributes bitterness and astringency were strongly correlated with cocoa contens. As medium cocoa contents (70.0%cc) showed higher acceptance scores, nutrition experts should advise consumers to focus on these types of non-origin chocolates as a public health approach. In origin chocolates, cocoa contents were shown to be no indicator of temporal bitterness and astringency sensations. Polyphenol content might vary due to the genetic constitution of ‘fine and flavour’ cocoa beans and conditions in the region of origin. However, polyphenol contents had not been measured in the present study. In order to draw conclusions, with respect to public health concerns, further investigations are necessary linking chemical analysis of polyphenols with the temporal sensory profile of these descriptors.

Knowing the target market preferences enables manufacturers to carefully select cocoa bean varieties, region of origin (with respective farming and post-harvest treatment practices) as well as roasting and conching conditions for producing dark origin chocolates (Torres-Moreno et al. (2012).

7 Abstract

Dark chocolates are rich sources of polyphenols, widely acknowledged for eliciting several beneficial health effects related to cardiovascular diseases, metabolic disorders, inflammatory events, cancer and cognitive dysfunctions. However, these compounds are key inducers of bitter taste and astringency, potentially limiting consumers' acceptance of chocolates with higher cocoa contents. In order to gain better insight in consumers' choices, the present study investigated the temporal profile of bitterness and astringency as well as sweet taste as covering agent, during the testing period (mastication, aftertaste) in 5 dark origin (OR) (66.8-80.1% cocoa) and 6 non-origin (N-OR) (54.5-80.0% cocoa) chocolates with different cocoa contents, applying Temporal Dominance of Sensations (TDS). The temporal profile of the evaluated OR chocolates was characterized by the dominance of bitterness independent of cocoa contents (%cc), reaching maximum dominance rates (DR%) between 60.0-80.0% over approximately 75.0% of the testing period, i.e. prior to swallowing. After swallowing, astringency dominated, however not significantly, except for the sample OR 66.8%cc. Sweetness also dominated the temporal profile of OR chocolates, but reached the level of significance only in one sample (OR 67.4%cc).

N-OR chocolates with 54.5-60.0%cc were characterized by significant dominances of sweet taste along the entire evaluation period, reaching maximum DR% between 90-95%. The increase of cocoa contents (70.3, 65.0, 70.5 and 80.0%cc) was combined with higher DR% of bitter taste (46.0, 54.0, 70.0 and 74.0%, respectively). Astringency dominated in N-OR samples at the end of the evaluation period presumably after swallowing. Finally, in N-OR chocolates, cc% highly affected the dominance of the evaluated attributes. This impact was found to be rather minor or absent in OR chocolates. Thus, the TDS-parameters showed variations in attribute`s dominance in OR and N-OR chocolates indicating a strong influence of cocoa bean variety and region of origin in addition to cocoa contents.

The consumer survey indicates that acceptance of chocolate products with different cocoa contents, cocoa bean variety and region of origin was driven by a balanced temporal profile with respect to bitterness and sweetness. In contrary, the emergence of astringeny dominance towards the end of evaluation elicited negative consumer responses. Cocoa contents (%) were found to not affect consumers liking and preference at all. Furthermore, the results suggest that in addition to polyphenol-associated descriptors and sweetness, flavour attributes typical for certain cocoa bean varieties and growing regions might have affected product acceptance and preference of origin chocolates. Further research is needed in order to verify this hypothesis.

8 Zusammenfassung

Dunkle Schokoladen sind konzentrierte Aufnahmequellen von Polyphenolen, deren positive Wirkung auf das Herzkreislauf-System, metabolische und inflammatorische Vorgänge, Krebserkrankungen sowie kognitive Prozesse in einer Vielzahl an klinischen Studien bestätigt wurde. Der gesundheitliche Benefit dieser Verbindungen geht allerdings mit typischen sensorischen Eigenschaften wie Bitterkeit und Adstringenz einher, die die Konsumenten-Akzeptanz potentiell limitieren.

Zum vertiefenden Verständnis von Verbraucherverhalten wurde in der vorliegenden Studie die zeitliche Entwicklung der polyphenol-assoziierten, sensorischen Deskriptoren Bitterkeit und Astringenz sowie der Süße, als maskierender Geschmacksqualität, während des Verkostens (Manipulation im Mund, Nachgeschmack) von dunklen Schokoladen mit unterschiedlichem Kakaoanteil und unterschiedlicher Herkunft und Qualität der Kakaobohne untersucht. Evaluiert wurden fünf dunkle Schokoladen, hergestellt aus hochwertigen Kakaobohnen definierten Anbaugebiets (OR = „origin“ Schokoladen; 66.8-80.1% Kakaoanteil) und sechs dunkle Schokoladen, hergestellt aus Mischungen von gewöhnlichen Kakaobohnen aus unterschiedlichen Anbaugebieten (N-OR = „non-oigin“ Schokoladen; 54.5-80.0% Kakaoanteil) mittels Temporal Dominance of Sensations (TDS), einer dynamischen Analysemethode. Die Ergebnisse der TDS-Analyse zeigten Unterschiede in den Dominanzraten (DR%) der untersuchten Attribute in Abhängigkeit des Kakaoanteils, der Qualität und geographischen Herkunft der verarbeiteten Kakaobohne.

Während die Dominanz des bitteren Geschmacks in den N-OR-Schokoladen erwartungsgemäß mit steigendem Kakaoanteil (%) sig. ($p < 0.05$) anstieg, dominierte die Bitterkeit in den OR-Schokoladen unabhängig des Kakaoanteils mit den höchsten DR zwischen Zeitpunkt T0% – 75%. Etwa zum Zeitpunkt T75% sank der dominante bittere Geschmack unter das Signifikanz-Niveau ($p < 0.05$), gefolgt von Adstringenz. Dieser Trend ließ sich in beiden Kategorien (OR und N-OR) erkennen. Die Süße dominierte sig. ($p < 0.05$) in N-OR-Schokoladen mit geringstem Kakaoanteil über den gesamten Analyse-Zeitraum. Auch dieser

Eindruck nahm erwartungsgemäß mit steigendem Kakaoanteil (65.0, 70.5 bzw. 80.0%) ab und wurde von der ansteigenden Bitterkeit (DR% 53.8, 69.2 bzw. 74.4) abgelöst. Unter den OR-Schokoladen wurde die Süße zu keinem Zeitpunkt der Analyse als sig. ($p < 0.05$) dominant empfunden. Aufgrund der Ergebnisse lässt sich feststellen, dass das temporäre sensorische Produktprofil auf Basis polyphenol-assoziierter Eigenschaften von dunklen Schokoladen nicht primär nur vom Kakaoanteil (%) sondern auch stark von der Qualität sowie der Herkunft der Kakaobohne beeinflusst wird. Während OR-Schokoladen unabhängig des Kakaoanteils (%) als dominant bitter (T0 - 75%) und adstringierend (ab etwa T75%) empfunden wurden, dominierte in den N-OR-Schokoladen in Abhängigkeit des Kakaoanteils (%) der süße (54.5, 60.0% Kakao) bzw. bittere (65.0, 70.5, 80.0% Kakao) Geschmack. Des Weiteren lässt sich die Tendenz erkennen, dass nach dem Schlucken der Proben die Adstringenz dominiert unabhängig davon, ob es sich um origin oder non-origin Schokoladen handelte.

Der KonsumentInnen-Test zeigte eine hohe Akzeptanz für dunkle Schokoladen, mit einem ausgeglichenen, dynamischen sensorischen Profil bezogen auf die Attribute Bitterkeit und Süße. Im Gegensatz dazu wurden Schokoladen mit stark dominanter Adstringenz von den KonsumentInnen weniger akzeptiert. Der Kakaoanteil (%) der evaluierten Schokoladen beeinflusste die KonsumentInnen-Akzeptanz und -Präferenz nicht. Die Ergebnisse weisen darauf hin, dass die hedonische Bewertung zusätzlich zu den polyphenol-assoziierten Deskriptoren Bitterkeit und Adstringenz sowie dem süßen Geschmack durch typische Flavour-Attribute bestimmter Kakaobohnen-Sorten und ihrer Herkunft bestimmt wurde. Weitere Studien sind notwendig, um diese Hypothese zu überprüfen.

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