Croatian Journal of Food Science and Technology
journal homepage: www.ptfos.unios.hr/cjfst/

Original scientific paper
DOI: 10.17508/CJFST.2018.10.2.07

Microbial safety and polyphenols in cocoa liquor as influenced by pasteurisation and storage time

IVANA LEKO, ĐURĐICA AČKAR*, ANTUN JOZINOVIĆ, JURISLAV BABIĆ, BORISLAV MILIČEVIĆ, DRAGO ŠUBARIĆ

Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek, Franje Kuhača 20, 31000 Osijek, Croatia

ARTICLE INFO
Article history:
Received: February 21, 2018
Accepted: June 15, 2018

Keywords:
cocoa mass,
thermal treatment,
microbial stability,
Folin-Ciocalteu,
vanillin assay

ABSTRACT
The aim of this research was to examine the influence of pasteurisation of cocoa liquor at different temperatures (115, 120, 125 and 140 °C) and 3-week-storage time at 40 °C on microbial safety, peroxide value, polyphenol, and flavan-3-ol content. Peroxide value of the cocoa liquor increased after both, pasteurisation and storage, but not significantly. Although total polyphenol content decreased, flavan-3-ol content was stable during the investigated period. Microbial quality was satisfying, and the most stable mass was the one treated at 140 °C.

Introduction

Along with sugar, cocoa liquor is the main chocolate ingredient. It gives special aroma, texture and colour to chocolate and overall unique sensory perception. The main cocoa liquor characteristics are the result of cocoa bean variety and origin, but growing conditions and after-harvest handling play an important role as well (Beckett et al., 2017; Liu et al., 2017). The detailed review of the influence of all these factors was given by Gutierrez (2017). The first handling step in chocolate factory is cocoa bean roasting, during which the aroma is further developed, through the development of pyrazines, alcohols, esters, pyroles and Maillard products (Hii et al., 2017). However, due to high temperatures (commonly 130–150 °C during 30–45 min, as stated by Zyzelewicz et al., 2016), during this process app. 70% of polyphenols is lost (Zzaman et al., 2014). Considering that polyphenols, esp. epicatechin and procyanidins with up to three subunits, give bitter and astringent taste to chocolate (Watson, Preedy and Zibadi, 2013; Kongor et al., 2016). In sensory terms this is positive effect. However, chocolate polyphenols have positive impact on human health (EFSA, 2006, 2012, 2014) and should be preserved during chocolate processing. In addition to very important role in aroma development, roasting is considered a key step in reduction of microbial load of cocoa (Zyzelewicz et al., 2016). However, *Salmonella* may be heat resistant, depending on initial load, bean moisture and processing conditions (Nascimento et al., 2015). To tackle this problem, as well as to develop aroma further, cocoa liquor may be subjected to further heat treatment in PDAT reactor, Luwa thin-layer evaporator etc. (Minifie, 2012).

The aim of this research was to investigate the influence of thermal treatment at different temperatures (115 °C, 120 °C – for reduced time, 125 °C and 140 °C) on polyphenol and flavan-3-ol content, peroxide value and microbial load of cocoa liquor during 20 minutes, as well as to monitor these parameters during prolonged storage at 40 °C.

Materials and methods

Cocoa bean originated from Ivory Coast and its quality parameters are shown in Tables 1 and 2. It was cleaned, roasted, winnowed and grinded in the industrial scale. After that, the obtained cocoa liquor was thermally treated in the industrial scale during 20 min at 115 °C, 125 °C or 140 °C, after which

*Corresponding author E-mail: dackar@ptfos.hr

batches of 5 kg were kept in laboratory incubator at 40 °C for three weeks with mixing every 4 h. (Additional sample was collected after thermal treatment at 120 °C, but with reduced time of treatment to examine whether combination of higher temperature and reduced time would give the results similar to treatment at 115 °C.). The temperature interval 115–125 °C is commonly used in the industry where the part of research was conducted, and 140 °C was selected as the highest temperature used for the treatment (Gutierrez, 2017). Samples for analyses of peroxide value, microbial count, total polyphenol and flavan-3-ol contents were taken immediately after thermal treatment, after 24 hours, 7, 14 and 21 days of storage.

Peroxide value was determined according to EN ISO 3960:2010. Total polyphenols (Folin-Ciocalteau) and flavan-3-ols (vanillin assay) were determined according to Belščak et al. (2009).

For colour measurement, samples were poured into Petri dishes and cooled down to room temperature. The colour of the samples was determined by chromameter Konica Minolta CR-400, with attachment for solid samples, in CIEL*a*b*. Total colour difference was calculated according to Eq. 1:

$$\Delta E = \sqrt{(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2} \qquad (1)$$

ΔE is the colour difference of the sample on the day of measurement in relation to initial colour of the corresponding sample and L, a, b are values on the day of measurement. L_0, a_0, b_0 are initial values of the corresponding sample.

Colony count was determined according to EN ISO 4833-1, *Enterobacteriaceae* according to EN ISO 21528-2, and yeasts and moulds according to EN ISO 21527-1:2008.

Statistical analysis was performed in Statistica® 10. Experimental data were analyzed by analysis of variance (ANOVA), Fisher's least significant difference (LSD) with significance defined at $p<0.05$, and correlation analysis.

Results and discussion

Cocoa butter content in cocoa beans (Table 2) used for this research was considerably higher when compared to values reported by Torres-Morreno et al. (2014) for beans from Ghana and Ecuador (43.45% and 41.93%), but it is in the average range of cocoa beans according to Dand (2011) and Jahurul et al. (2013). Peroxide value determined in cocoa beans was app. 1.4 mmol O_2/kg, significantly lower than maximum permitted values by the Croatian by-law (NN 41/2012; 70/2013; 141/2013). Polyphenol content in cocoa beans (43.27 ± 2.27 mg GAE/g defatted matter) was lower than reported by Hii et al. (2009) but in accordance with results reported by Belščak et al. (2009). Flavan-3-ol content in cocoa beans (Table 2) was in accordance with previously reported values (Belščak et al., 2009; Wollgast and Anklam, 2000). Microbial quality of the beans (Table 2) was in accordance with FCC Quality Rules (2012).

Table 1. Quality parameters of raw cocoa bean used in the research

Quality parameter	Value	Reference value
Nib (%)	85.42±0.46	75-89.5
Husk (%)	14.44±0.59	10-15
Germ (%)	0.79±0.07	0.5-1.7
Mouldy beans (%)	0	0-2
Rotten beans (trula) (%)	0	0-2
Poorely fermented beans (%)	4±1	0-8
Number of beans in 300 g	310±12	250-320

Table 2. Chemical and microbiological quality parameters of cocoa beans and cocoa nibs

Quality parameter	Cocoa beans	Cocoa nibs
Moisture content (%)	6.13±0.28	1.88
Total fat (% dry matter.)	58.86±1.41	56.64
pH	5.87	5.74
Peroxide value (mmol O_2/kg)	1.38±0.01	2.09±0.02
Polyphenols (mg/kg defatted matter)	43.27±2.27	40.50±0.41
Flavan-3-ols (mg/kg defatted matter)	5.64±0	5.66±0
Aerobic mesophilic bacteria / g	3 000 000	56 000
Enterobacteriaceae /g	<10	<10
Salmonella spp. / 25 g	n.n.	n.n.
Yeasts and moulds / g	7 000	<1000

To obtain cocoa liquor, cocoa beans are roasted and due to high temperatures peroxide value in cocoa nibs was considerably higher than in the beans. However, this value is still below maximum values regulated by the Croatian by-laws (NN 41/2012; 70/2013; 141/2013). Although polyphenol content determined in the nibs (Table 2) was lower than reported by Hii et al. (2017) (58.2 – 69.2 mg GAE/g), it may be that roasting did not significantly influence polyphenol and flavan-3-ol contents. However, coloured compounds are formed during roasting, such as Maillard products (Giacometti et al., 2015), which form already at 70 °C (De Brito et al., 2002) and they may influence the spectrophotometric results. Wollgast and Anklam (2000) and Zyzelewicz et al. (2016) reported reduction of phenolic compound content with roasting. However, this was not always proportional to the time of roasting (Zyzelewicz et al., 2016). Microbial count reduced after the roasting, esp. aerobic mesophilic bacteria (Table 2).

Cocoa liquor is produced by milling cocoa nibs. This phase does not influence significantly peroxide value, polyphenol and flavan-3-ol content. Thermal treatment of cocoa liquor did not significantly influence total polyphenol content either (Table 3, 6). This can also be linked to Maillard reactions and non-enzymatic browning. At the beginning of the storage, peroxide value and polyphenol content did not change significantly (Figure 1, Table 3). However, between 7th and 14th day polyphenol content decreased by more than a half. At the same time, peroxide value increases significantly. These two parameters are in strong correlation (Table 6), showing that at the beginning of the storage polyphenols act as antioxidants, neutralising free radicals (Watson et al., 2013). When polyphenol reserves are exhausted, peroxide value increases. The highest peroxide value at the end of the storage, had, as expected, the cocoa liquor treated at 140 °C, and the lowest was in the raw cocoa liquor. This corresponds to polyphenol content (Table 3), although differences between the samples were not statistically significant.

Flavan-3-ol content was stable both during thermal treatment and during storage (Figure 2) and similar to flavan-3-ol content observed by Ramli et al. (2001). Epicatechin stability during roasting is influenced by its interactions with other compounds. During roasting epicatechin in epimerization reactions converts to catechin (Giacometti et al., 2015), which also may contribute to unchanged flavan-3-ol content during processing and storage.

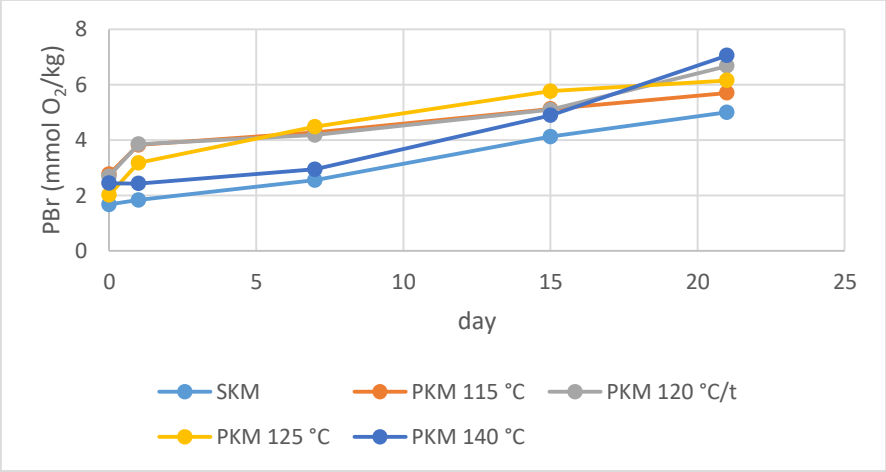

Fig. 1. The stability of cocoa butter in raw (SKM) and thermally treated cocoa liquors (PKM) during storage at 40 °C. Sample 120 °C/t was thermally treated for reduced period of time

Table 3. Total polyphenol content (mg GAE / g defatted mass) in raw (SKM) and thermally treated cocoa liquors (PKM) during 21-day storage at 40 °C

DAY	0	1	7	14	21
SKM	40.82±0.06 $^{a, C}$	39.61±0.35 ^{a,B}	41.51±0.33 ^{d,D}	17.39±0.47 ^{a,A}	17.64±0.11 ^{c,A}
PKM 115 °C	40.90±1.28 ^{a,C}	39.63±0.32 ^{a,B}	40.23±0.04 ^{c,B,C}	17.39±0.25 ^{a,A}	17.56±0.11 ^{b,A}
PKM 120 °C*	41.55±2.55 ^{a,C}	39.93±0.13 ^{a,B,C}	39.53±0.12 ^{a,B}	17.45±0.13 ^{a,A}	17.56±0.09 ^{b,A}
PKM 125 °C	41.68±0.90 ^{a,C}	39.44±0.13 ^{a,B}	39.95±0.04 ^{b,B}	17.66±0.13 ^{a,A}	17.41±0.11 ^{b,A}
PKM 140 °C	39.78±0.29 ^{a,C}	39.85±1.21 ^{a,C}	39.80±0.18 ^{a,b,C}	17.68±0.10 ^{a,B}	16.69±0.13 ^{a,A}
*thermal treatment time reduced; values in the same column with different lower case are statistically different at $p < 0.05$; values in the same row with different upper case are statistically different at $p < 0.05$					

Surface colour of the cocoa liquor after processing and during storage was monitored by tristimulus chromameter in CIEL*a*b* system. Lightness of the cocoa liquor treated at 140 °C decreased during storage (lower L* values, Table 4). It is possible that non-enzymatic browning reactions advanced during storage, which increased the content of the coloured compounds. The surface colour of other samples did not change significantly, with significant proportion of red (a*) and yellow (b*) component in all selected samples (Table 4).

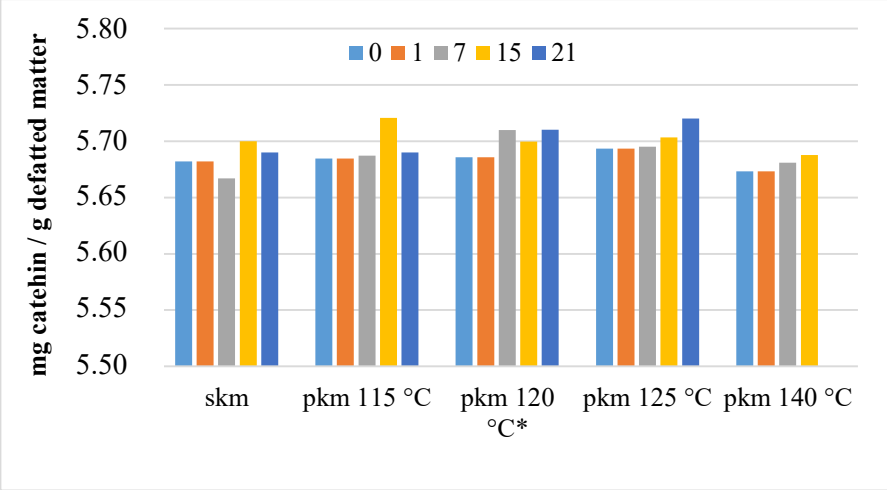

Fig.2. Flavan-3-ol content in raw (SKM) and thermally treated cocoa liquors (PKM) during 21-day storage at 40 °C (*thermal treatment time reduced)

Table 4. The surface colour of raw (SKM) and thermally treated cocoa liquors (PKM) during 21-day storage at 40 °C, measured by chromameter in CIEL*a*b* system. Sample PKM 120 °C was thermally treated for reduced period of time

	L	a	b
Day 0			
SKM	28.35±0.20	9.16±0.13	3.71±0.18
PKM115 °C	28.80±0.18	8.83±0.09	3.06±0.18
PKM120 °C*	28.96±0.26	8.74±0.12	1.99±0.21
PKM125 °C	27.81±0.23	9.00±0.12	2.86±0.20
PKM140 °C	31.68±0.17	7.69±0.14	2.95±0.13
Day 1			
SKM	27.09±0.23	8.59±0.11	2.59±0.05
PKM115 °C	28.75±0.38	8.42±0.20	2.30±0.08
PKM120 °C*	28.79±0.24	8.90±0.23	1.75±0.16
PKM125 °C	27.40±0.14	8.65±0.08	3.35±0.07
PKM140 °C	29.16±0.11	7.98±0.20	2.60±0.08
Day 7			
SKM	26.74±0.18	8.12±0.19	5.07±0.28
PKM115 °C	30.74±0.42	7.91±0.08	1.61±0.09
PKM120 °C*	28.97±0.35	8.37±0.17	3.05±0.14
PKM125 °C	30.74±0.20	8.15±0.03	2.12±0.10
PKM140 °C	27.23±0.08	7.58±0.35	4.55±0.48
Day 14			
SKM	28.92±0.35	8.31±0.32	2.47±0.20
PKM115 °C	28.32±0.33	8.59±0.07	2.26±0.48
PKM120 °C*	28.35±0.23	8.44±0.41	2.67±0.14
PKM125 °C	29.89±0.91	8.67±1.26	2.06±0.59
PKM140 °C	29.53±0.46	7.48±0.49	2.29±0.31
Day 21			
SKM	25.95±0.10	6.84±0.08	2.57±0.08
PKM115 °C	29.44±0.41	6.82±0.11	3.84±0.26
PKM120 °C*	34.04±0.32	7.16±0.07	7.76±0.42
PKM125 °C	27.36±0.16	7.08±0.07	1.64±0.07
PKM140 °C	27.39±0.17	7.16±0.14	2.22±0.14

Table 5. Results of microbial analysis of raw (SKM) and thermally treated cocoa liquors (PKM) during 21-day storage at 40 °C

Sample	Microbial population (CFU)	DAY				
		0	1	7	14	21
SKM	Aerobic mez. bacteria / g	230 000	270 000	220 000	55 000	45 000
	Enterobacteriaceae /g	<10	<10	<10	<10	<10
	Salmonella spp. / 25 g	n.n.	n.n.	n.n.	n.n.	n.n.
	Yeasts and moulds / g	<1000	<1000	<1000	<1000	<1000
PKM 115 °C	Aerobic mez. bacteria / g	38 000	56 000	49 000	50 000	40 000
	Enterobacteriaceae /g	<10	<10	<10	<10	<10
	Salmonella spp. / 25 g	n.n.	n.n.	n.n.	n.n.	n.n.
	Yeasts and moulds / g	<100	<100	<100	<100	<100
PKM 120 °C	Aerobic mez. bacteria / g	51 000	70 000	55 000	61 000	52 500
	Enterobacteriaceae /g	<10	<10	<10	<10	<10
	Salmonella spp. / 25 g	n.n.	n.n.	n.n.	n.n.	n.n.
	Yeasts and moulds / g	<100	<100	<100	<100	<100
PKM 125 °C	Aerobic mez. bacteria / g	8 000	8 900	11 300	4 500	3 000
	Enterobacteriaceae /g	<10	<10	<10	<10	<10
	Salmonella spp. / 25 g	n.n.	n.n.	n.n.	n.n.	n.n.
	Yeasts and moulds / g	<100	<100	<100	<100	<100
PKM 140 °C	Aerobic mez. bacteria / g	<100	<100	<100	<100	<100
	Enterobacteriaceae /g	<10	<10	<10	<10	<10
	Salmonella spp. / 25 g	n.n.	n.n.	n.n.	n.n.	n.n.
	Yeasts and moulds / g	<100	<100	<100	<100	<100

Table 6. Correlation between analysed parameters (marked correlations are significant at $p < 0.05$)

	sample	day	PPO	F-3-OL	colour	PBr
sample		-0.536003	0.522666	-0.538589	-0.555747	-0.536259
day	-0.536003		**-0.989191**	**0.994788**	**0.996614**	**0.994248**
PPO	0.522666	**-0.989191**		**-0.998029**	**-0.996149**	**-0.997305**
F-3-OL	-0.538589	**0.994788**	**-0.998029**		**0.998931**	**0.999638**
ΔE	-0.555747	**0.996614**	**-0.996149**	**0.998931**		**0.998402**
PBr	-0.536259	**0.994248**	**-0.997305**	**0.999638**	**0.998402**	

PPO, total polyphenols (mg/g deffated sample); F-3-OL, flavan-3-ols (mg catehin/ g defatted sample); ΔE, total colour change; PBr, peroxide value (mmol O_2/kg)

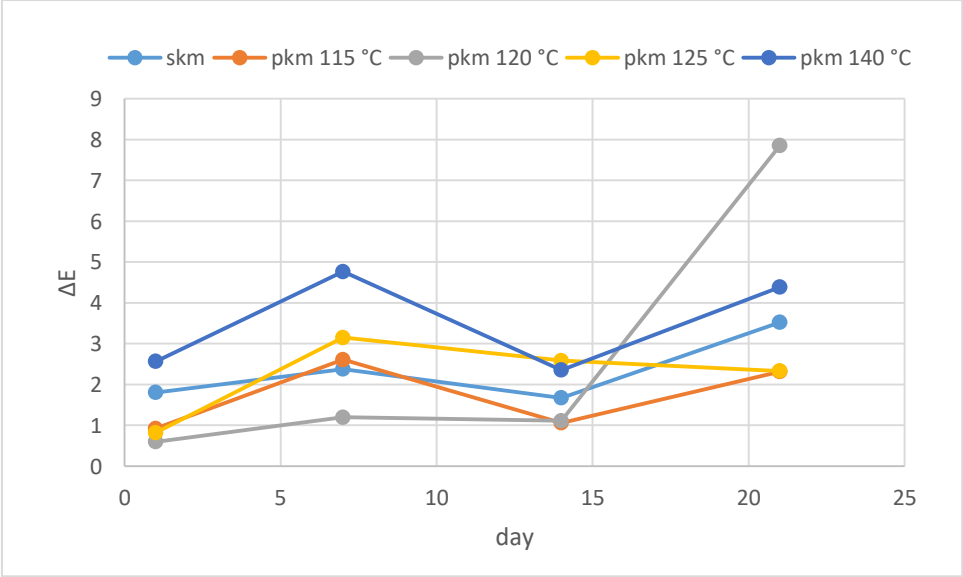

Fig. 3. Total colour change (*ΔE*) of raw (SKM) and thermally treated cocoa liquors (PKM) during 21-day storage at 40 °C calculated from CIEL*a*b* system in relation to colour parameters on day 0. Sample PKM 120 °C was thermally treated for reduced period of time

Total colour difference (*ΔE*) (Figure 3) of cocoa liquor treated at 115 and 125 °C in relation to raw cocoa liquor was under 3, pointing out that only trained sensory panel may perceive the colour change. Colour difference between the sample treated at 140 °C, on the other hand, is visible to average person, and the colour difference sample treated at 120 °C is obvious (*ΔE* value higher than 6) (Ačkar, 2010). The lightness of the latter sample also increased, showing fat bloom due to formation of unstable polymorphs of cocoa butter during the cooling treatment prior to colour measurement (Škrabal et al., 2011).

Yeast, mould, *Enterobacteriaceae* and *Salmonella* count did not change significantly during storage. At 140 °C aerobic mesophilic bacteria (AMB) count decreased below 100, and cocoa liquor is close to sterile. This is why the number of AMB did not increase significantly during the storage. In other samples, initial AMB count after thermal treatment is significantly higher and bacteria continue to grow during the storage, going through the standard cycle. In raw cocoa liquor and the sample treated at 125 °C colonies died off after 14 days of storage, and in samples treated at 115 and 120 °C after 21 days.

Overall, all samples, after both treatment and storage, complied to Croatian the by-laws (NN 74/2008, 156/2008, 89/2010, 153/2011) and the Guidelines on microbiological criteria for food (2011).

Conclusion

Thermal treatment of cocoa liquor results in the reduction of microbial load of the samples. The most stable cocoa liquor during storage is the one treated at 140 °C. Thermal treatment also results in increased peroxide value and decreased polyphenol content. However, flavan-3-ols are stable. Considering all that, cocoa liquor thermal treatment is beneficial tool when cocoa liquor is not directly used in further production, but stored for a period of time.

Acknowledgement

This work was supported in part by Croatian Science Foundation under the project UIP-2017-05-8709.

References

Ačkar, Đ. (2010): Isolation, modification and characterisation of wheat starch. *Doctoral thesis*. Faculty of Food Technology Osijek.

Beckett, S.T., Fowler, M.S., Ziegler, G.R. (2017): Beckett's Industrial Chocolate Manufacture and Use 5th Ed., Wiley Blackwell.

Belščak, A., Komes, D., Horžić, D., Kovačević Ganić, K., Karlović, D (2009): Comparative study of commercially available cocoa products in terms of their bioactive composition. *Food Res. Int.* 42 (5-6), 707-716. https://doi.org/10.1016/j.foodres.2009.02.018

Dand, R. (2011): Quality assessment of cocoa beans for international trade. In: The International Cocoa Trade (R. Dand). Woodhead Publishing Limited, pp. 219-267.

De Brito, E.S., Narain, N., Pezoa Garcia, N.H., Valente, A.L.P., Pini, G.F. (2002): Effect of glucose and glycine addition to cocoa mass before roasting on Maillard precursor consumption and pyrazine formation. *J. Sci. Food Agricult*. 82, 534-537. DOI: 10.1002/jsfa.1076

EN ISO 21528-2:2017 Microbiology of the food chain -- Horizontal method for the detection and enumeration of Enterobacteriaceae -- Part 2: Colony-count technique

EN ISO 3960:2010 Animal and vegetable fats and oils -- Determination of peroxide value -- Iodometric (visual) endpoint determination (ISO 3960:2007, corrected version 2009-05-15; EN ISO 3960:2010)

EN ISO 21527-1:2008 Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of yeasts and moulds -- Part 1: Colony count technique in products with water activity greater than 0,95.

FCC Quality Rules (2012): FCC quality rules (Applicable to contracts concluded on or after 01 March 2012). Federation of Cocoa Commerce Ltd Federation Du Commerce Des Cacaos, London, UK.

Giacommeti, J., Jolić, S., Josić, Đ.: Cocoa processing and Impact on Compositon. In: Processing and Impact on Active Components in Food (Preedy V (ed). Oxford, Academic Press Elsevier, pp. 605-612.

Gutierrez, T.J. (2017): State-of-the-art chocolate manufacture: a review. *Compr Rev Food Sci F*, 16, 1313-1344. doi: 10.1111/1541-4337.12301

Hii, C.L., Law, C.L., Suzannah, S., Misnawi, Cloke, M. (2009): Polyphenols in cocoa (*Theobroma cacao* L.). *Asian Journal of Food and Agro-Industry*, 2 (04), 702-722. http://www.ajofai.info/Abstract/Polyphenols%20in%20cocoa%20(theobroma%20cacao%20l.).pdf

Hii, C.L., menon, A.S., Chiang, C.L., Sharif, S. (2017): Kinetics of hot air roasting of cocoa nibs and product quality. *J. Food Process Eng.* 40 (3), 40:e12467. doi: 10.1111/jfpe.12467

Jahurul, M.H.A., Zaidul, I.S.M., Norulaini, N.A.N., Sahena, F., Jinap, S., Azmir, J., Sharif, K.M., Mohd Omar, A.K. (2013): Cocoa butter fats and possibilities of substitution in food products concerning cocoa varieties, alternative sources, extraction methods, composition, and characteristics. *J. Food Eng.* 117 (4), 467-476. https://doi.org/10.1016/j.jfoodeng.2012.09.024

Kongor, J.E., Hinneh, M., van de Walle, D., Afoakwa, E.O., Boeckx, P., Dewettnick, K. (2016): Factors

influencing quality variation in cocoa (*Theobroma cacao*) bean flavour profile – a review. *Food Res. Int.* 82, 44-52. doi: https://doi.org/10.1016/j.foodres.2016.01.012

Liu, M., Liu, J., He, C., song, H., Liu, Y., Zhang, Y., Wang, Y., Guo, J., Yang, H., Su, X. (2017): Characterization and comparison of key aroma-active compounds of cocoa liquors from five different areas. *Int. J. Food Prop.* 20 (10), 2396-2408. DOI: 10.1080/10942912.2016.1238929

Minifie, B (2012): Chocolate, Cocoa and Confectionery: Science and Technology. Springer Science & Business Media.

Nascimento, M.S., Reolon, E.M., Santos, A.R.B., Moreira, V.E., Silva, N. (2015): *Enterobacteriaceae* contamination in chocolate processing. *Food Control,* 47, 291-297. https://doi.org/10.1016/j.foodcont.2014.07.020

NN 41/2012; 70/2013; 141/2013 Croatian by-law regarding edible oils and fats (Pravilnik o jestivim uljima i mastima).

NN 74/2008, 156/2008, 89/2010, 153/2011 Croatian by-law regarding microbiological criteria for food (Pravilnik o mikrobiološkim kriterijima za hranu).

Ramli, N., Ytim, A.M., said, M., Hok, H.C. (2001): HPLC Determination of methylxanthines and polyphenols levels in cocoa and chocolate products. *Malaysian Journal of Analytical Sciences*, 7 (2), 377-386.

Škrabal, S., Šubarić, D., Miličević, B., Ačkar, Đ., Babić, J., Miličević, R (2011): Utjecaj mlijeka u prahu i čuvanja na stabilnost čokolada. In: 13th Ružička days „Today science-tomorrow industry“ Proceedings, Šubarić, D. (Ed.), Osijek, HR, pp. 418-430.

Torres-Moreno, M., Torrescasana, E., Salas-Salvado, J., Blanch, C (2015): Nutritional composition and fatty acids profile in cocoa beans and chocolates with different geographical origin and processing conditions. *Food Chem*. 166, 125-132. https://doi.org/10.1016/j.foodchem.2014.05.141

Watson, R.R., Preedy, V.R., Zibadi, S. (2013): Chocolate in Health and Nutrition, New York, Heidelberg, Dordrecht, London: Humana Press, 2013.

Wollgast, J., Anklam, E. (2000): Review on polyphenols in *Theobroma cacao*: changes in composition during the manufacture of chocolate and methodology for identification and quantification. *Food Res. Int*. 33, 423-477. https://doi.org/10.1016/S0963-9969(00)00068-5

Zyzelewicz, D., Krysiak, W., Oracz, J., Sosnowska, D., Budryn, G., Nebesny, E. (2016): The influence of the roasting process conditions on the polyphenol content in cocoa beans, nibs and chocolates. *Food Res. Int.* 89 (2), 918-929. doi: 10.1016/j.foodres.2016.03.026

Zzaman, W., Bhat, R., Yang, T.A. (2014): Effect of superheated steam roasting on the phenolic antioxidant properties of cocoa beans. *J. Food Process Preserv.* 38, 1932-1938. doi:10.1111/jfpp.12166