Joanna KOBUS-CISOWSKA[1], Marcin DZIEDZIŃSKI[1] , Oskar SZCZEPANIAK[1], Maria JOKIEL[2], Krystyna SZYMANDERA-BUSZKA[1], Daria SZYMANOWSKA[3,4], Piotr SZULC[5], Aleksandra TELICHOWSKA[6], Elżbieta GORYŃSKA-GOLDMANN[7], Marta LIGAJ[8]
[1] Poznań University of Life Sciences, Department of Gastronomy Sciences and Functional Foods
Wojska Polskiego 28, 60-637, Poznań, Poland
[2] Łukasiewicz Research Network – PORT Polish Center for Technology Development
ul. Stabłowicka 147, 54-066 Wrocław, Poland
[3] Poznań University of Medical Sciences, Department of Pharmacognosy, Poznan, Poland
[4] Poznań University of Life Sciences, Department of Biotechnology and Food Microbiology,
ul. Wojska Polskiego 28, 60-637, Poznań, Poland
[5] Poznań University of Life Sciences, Department of Agronomy, ul. Dojazd 11, 60-637 Poznań, Poland
[6] Foundation for the Education of Innovation and Implementation of Modern Technologies
ul. Widok 11, 62-069 Dabrówka, Poland
[7] Poznan University of Life Science, Department of Economics and Economic Police in Agribusiness, Faculty of Economics and Social Sciences, Poland
[8] Poznań University of Economics And Business, Department of Industrial Products Ecology and Quality, Faculty of Commodity Science, Poland
e-mail: joanna.kobus-cisowska@up.poznan.pl

data przyjęcia: 2020-08-08 ; data akceptacji: 2020-08-28

EVALUATION OF USEFULNESS OF SELECTED FOOD COMPOUNDS AS AN ACTIVE SUBSTANCES WITH MODULATING ABILITY OF BITTER TASTE IN CHOCOLATE

Summary

Numerous compounds with pro-health activity are present in the cocoa bean. Cocoa contains polyphenols such as anthocyanins, catechins, flavan-3-ol proanthocyanidins. The catechins include (-)-epicatechin, (+)-catechin and pigments responsible for cocoa beans color such as cyanidin-3-β-O-galactoside and cyanidin-3-α-L-arabinoside. In addition to polyphenols, cocoa is also rich in alkaloids such as methylxanthine, i.e. caffeine, theobromine and theophylline. The presence of these compounds in dessert chocolate determines its health-promoting properties, but at the same time has a bitter taste, often undesirable by consumers. The aim of the work was to assess the suitability of selected raw materials as a source of active compounds that are modulators of bitter taste in chocolate. Psyllium, eggshells, carrot extract, maltodextrin, vanilla aroma and whey protein concentrate (WPC) were tested. These compounds have not previously been used as taste modulators in dark chocolate, with exception of vanilla aroma and whey protein. Therefore, the results of this study will allow for an evaluation of novel pro-health ingredients as taste modulators. Sensory evaluation in chocolate, allowing the assessment of the impact of the masking effect of the bitter taste, were examined sensitively - using the five-point method. The selected additives were evaluated for cytotoxic activity on the MCF 7 breast cancer cell line. The effectiveness of selected ingredients as modulators of bitter taste has been confirmed in application tests. In chocolate trials, the best effect masking the bitter taste was found for trials with carrot extract and whey protein concentrate in combination with vanilla. It was shown that the proposed additives that modulate the bitter taste such as eggshells are not cytotoxic in relation to breast cancer cells. The addition of plant raw materials, eggshells and WPC proteins may affect the bitter taste of the chocolate and at the same time increase the health-promoting effect.

Key words: *chocolate, health, bitter, taste, MCF 7, modulators*

OCENA PRZYDATNOŚCI WYBRANYCH ZWIĄZKÓW SPOŻYWCZYCH JAKO SUBSTANCJI CZYNNYCH O ZDOLNOŚCI MODULUJĄCEJ GORZKI SMAK CZEKOLADY

Streszczenie

W ziarnie kakaowym obecne są liczne związki o działaniu prozdrowotnym. Kakao zawiera polifenole, takie jak antocyjany, katechiny, flawan-3-ol proantocyjanidyny. Do katechin zalicza się: (-) - epikatechinę, (+) - katechinę oraz barwniki odpowiadające za barwę ziaren kakaowych, takie jak cyjanidyn-3-β-O-galaktozyd i cyjanidyn-3-α-L-arabinozyd. Oprócz polifenoli kakao bogate jest również w alkaloidy, takie jak metyloksantyna, czyli kofeina, teobromina i teofilina. Obecność tych związków w czekoladzie deserowej decyduje o jej prozdrowotnych właściwościach, ale jednocześnie ma gorzki smak, często niepożądany przez konsumentów. Celem pracy była ocena przydatności wybranych surowców jako źródła związków aktywnych będących modulatorami gorzkiego smaku w czekoladzie. Zbadano babkę płesznik, skorupki jaj, ekstrakt z marchwi, maltodekstrynę, aromat waniliowy oraz koncentrat białka serwatkowego (WPC). Związki te nie były wcześniej stosowane jako modulatory smaku w ciemnej czekoladzie, z wyjątkiem aromatu wanilii i białka serwatki. Dlatego wyniki opisanego badania pozwolą na ocenę nowych składników prozdrowotnych jako modulatorów smaku. Oceny sensoryczne w czekoladzie, pozwalające na ocenę wpływu efektu maskującego gorzkiego smaku, zostały zbadane wrażliwie - metodą pięciopunktową. Wybrane dodatki oceniono pod kątem działania cytotoksycznego na linii komórkowej raka piersi MCF 7. Skuteczność wybranych składników jako modulatorów gorzkiego smaku została potwierdzona w badaniach aplikacyjnych. W badaniach z czekoladą najlepszy efekt maskowania gorzkiego smaku uzyskano w próbach z ekstraktem z marchwi i koncentratem białka serwatkowego w połączeniu z wanilią. Wykazano, że proponowane dodatki modulujące gorzki smak, takie jak skorupki jaj, nie są cytotoksyczne w stosunku do komórek raka piersi. Dodatek surowców roślinnych, skorupek jajek i białek WPC może wpłynąć na gorzki smak czekolady, jednocześnie zwiększając prozdrowotny efekt.

Słowa kluczowe*: czekoladowy, zdrowotny, gorzki, smakowy, MCF 7, modulatory*

1. Introduction

Plant-derived food is a main source of compounds acting as antibacterial, antivirus, antioxidative, anti-inflammatory and antiallergic agents. Majority of these bioactive compounds is characterized as tar, unpleasant and bitter in taste by a potential consumer, and taste is the most important stimulus effecting in product choice. Plant alkaloids and other toxic ingredients feature bitter taste, what causes the rejection by the customer, such defensive mechanism helps to avoid the consumption of potentially harmful ingredients i.e. hydrolyzed proteins, alkaloids and rancid fat [1]. Apart of many ingredients affecting negatively the human health, plant materials and products contain a lot of compounds that are beneficial [2]. The latter group include e.g. triterpenes, organic sulfur compounds and phenols [3]. Compounds responsible for bitter taste are highly varied in range of physicochemical properties and chemical structure [4]. These compounds include polyphenols, phenols, amino acids and peptides, esters, lactones and terpenes. Many of the aforementioned compounds provide pro-health effect and is recommended for intake with the diet. Large amount of structurally-different substances can be detected by solely 25 forms of bitter taste receptor [5]. In a response to raising demands of the market, food industry makes an effort to either remove bitter compounds from foods, or applies the masking techniques [1]. To remove these compounds techniques based on adsorption and enzyme hydrolysis are applied. To mask bitter compounds, cyclodextrins are used prevalently. Cyclodextrins immobilize in their structures the flavonoids, this process does not remove flavonoids. Despite of their presence, their biological activity is inhibited. Other methods of the bitter taste reducing are based on adding sugar, salt or fat during culinary treatment [6]. All compounds that can affect the perception of the bitter taste are called ‘blockers’ or ‘bitter tastes regulators’.

Chocolate and cocoa beam are high-calorie products. Energy value of 100 g dark chocolate containing 99% cocoa, estimates 530 kcal, 49 g total fats, 13 g proteins, 8 g carbohydrates, incl. 6 g dietary fibre. The pure 99% dark chocolate has low glycemic index (20) and low glycemic charge (1 per 30 g product). Therefore, it is allowed for consumption by people suffering from diabetes mellitus [7]. Moreover, dark chocolate contains many biologically active compounds [8, 9]. Flavonoids content in chocolate and their antioxidant activity are large compared to other raw materials with antioxidative properties [10]. However, this composition is responsible for bitter taste and therefore dark chocolate is reluctantly consumed by a meaningful number of consumers. Studies show that the intake of 25 g of dark chocolate daily can reduce blood pressure, improve lipid and glucose levels in the blood plasma and inhibit the platelets aggregation. It was shown that flavonoids contained in dark chocolate act as anti-inflammatory and antioxidative agents as well as they play an important role in insulin resistance prevention [11]. Cocoa beans also include anthocyanins (4%), catechins and flavan-3-ols (37%), and proanthocyanidins (58%) [12]. Cocoa in addition to the polyphenols, is rich in alkaloids: methylxanthines e.g. caffeine, theobromine and theophylline. Theobromine occurs in fruits and seeds of cocoa-tree at highest concentration (4%), while caffeine is less concentrated (0,2%), and theophylline amount is scarce [13].

The aim of the work was to assess the suitability of selected raw materials as a source of active compounds that are modulators of bitter taste in chocolate. Psyllium, eggshells, carrot extract, maltodextrin, vanilla aroma and whey protein concentrate (WPC) were tested. These compounds have not previously been used as taste modulators in dark chocolate, with exception of vanilla aroma and whey protein. Therefore, the results of this study will allow for an evaluation of novel pro-health ingredients as taste modulators. For the tested ingredients both antioxidant capacity and modulatory effect on chocolate bitter taste were determined. Among the tested additives, additionally egg shell was proposed. Due to its no common application in foods, the cytotoxicity against MCF-7 cell line was performed.

2. Methods

2.1. Dark chocolate

Chocolate masses and bars were purchased from the semi-industrial scale factory of confectionery products “BARS” Halina Kalemba (Wloszakowice, Poland). The composition information and confirmed data about nutritional value of used chocolate mass is as follow: energy 457 kcal/ 1888 kJ, fat: 35.1 g (saturated fatty acids 21.4 g, monosaturated fatty acids 12.3 g, polysaturated fatty acids 1.4 g, cholesterol: 0.4 mg); carbohydrates: 47.1 g (starch: 3.6 g, sugars: 0.3 g, lactose: 0 g, sucrose: 0.3 g, poliols: 43.3 g); proteins: 5.5 g; digestive fiber: 7.8 g; iron: 13 g; calcium: 37 g.

2.2. Taste modulating ingredients

The materials used to evaluate the bitter taste perception are presented in Table 1.

Table 1. Tast emodulating ingredients
Tab. 1. Składniki modulujące smak

Ingredient	Characteristics
Psyllium fiber	mesh size < 80 μm
carrot extract	an aqueous lyophilisate prepared by maceration with water at 90 °C for 1 hour
deproteined and shredded eggshell	Purchased from EGGNOVO SI (Navarra, Spain), composition of the shell preparation is as follows: 98% calcium carbonate including 38% calcium, 2% soluble protein and 250 ppm strontium, pH 8.34 in water solution, moisture 2.54 (particle size 85%<50 um)
maltodextrin 150	Purchased from Nowamyl (Poland) that contained 0.7% glucose, 2% maltose and 97% polysaccharides
whey protein concentrate (WPC 80)	was purchased from Bartex (Warmian-Masurian Voivodeship, Poland), the composition information and confirmed data about nutrition value of used WPC 80 is as follow: ash ≤5.5%; water content ≤5.0%; fat content≤ 9%; protein content ≥80%
natural vanilla aroma	consisted of invert sugar syrup, glucose syrup, water, vanilla extract, nutrition value was as follow: energy: 280 kcal/1190 kJ, carbohydrate: 70 g (sugar: 48 g)

Source: own study / Źródło: opracowanie własne

2.3. Chocolate preparation

All amount of 100 g dark chocolate was melted using a heating bath. Taste modulating ingredient was added at amount 0.02 g (ms) to 100 g melted chocolate (mr) Then, prepared and properly mixed chocolate was poured into the chocolate mold allowing to divide the chocolate into 5 g cubes. After that point chocolate samples were stored in refrigerator until they solidified.

2.4. Preparation of extracts of bitter taste modulating additives

Each analysed samples of chocolate (5 g) were homogenized and extracted in 70 ml of 60% ethanol for 2 h at room temperature according to Kobus-Cisowska et al. [2]. The extract was filtered through Whatman No. 4 paper and rinsed with 50 ml of ethanol. Extraction of residue was repeated applying the same conditions. The two filtrates of 60% ethanol were combined and evaporated under vacuum at 40°C (ethanol); residual water was removed by freeze drying to produce dry extract. Thus the prepared crude extracts were stored in a dry, dark and cool place until they were analyzed.

2.5. Total content of compounds reacting with Folin-Ciocalteau reagent

Assay was performed for extracts of modulating additives, according to Cheung et al. methodology [14]. The method based on absorbance measurement at wavelength 765 nm (Metertek SP-z830, Taiwan) of color complex obtained as a product of reaction between phenolic group present in tested extract and Folin-Ciocalteau reagent. Assay was performed in triplicate. Results were given in mg quercetin equivalent/g d.w. plant material and mg tyrosine/g d.w. for WPC-80 protein.

2.6. Cytotoxicity determination of egg shell

Cytotoxicity potential was determined against the MCF 7 cell line (ECACC, 86012803). Cells used in experiment were grown in 96 wells microplates (VWR, Poland) at quantity of 5×104 cells per well at temperature 37°C, in atmosphere of 5% CO2 and 95% air, at humidity level 70%. Cell culture was carried out in DMEM (BIOWEST) medium with additions of inactivated bovine serum (10% vol.), sodium pyruvate (1% vol.), Pen-Strep (1% vol.) and mixture of essential amino acids (1% vol.). After the cells confluence estimated 80%, the grow medium was decantated and in its place a mixture of tested extract and DMEM medium enriched in glucose (4.5 g/l), L-gluthamine (4mM), HEPES (25mM) w/o phenyl red (GIBCO) was added. The extracts were mixed with medium at ratio 3:7 (v/v) with final volume of 100 µl. The control sample was prepared, in which cells were grown in a medium containing saline (PBS buffer) in place of tested extract. Each sample (both control and the right test) were put on a microplate in triplicate. Cells were subjected to tested substances by 24 hours. Then, the cytotoxicity assay was performed, using 0.5 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dissolved in extractant (60% ethanol). MTT has tetrasole form, which is turned into formasane by living cells mitochondria. Formasane crystals were dissolved in a mixture of pure (99.8%) ethanol with DMSO (1:1 v/v). Then absorbance measurement at wavelength 570 nm was conducted, with use of SpectraMax i3x (Molecular Devices) microplate reader with SoftMax software. Results were given as a ratio between absorbance of the test sample (Ab) and absorbance of the control (Abc), according to equation 1:

$$(Ab)/(Abc)*100\% \quad (1)$$

2.7. Sensory profiling

Profile assessment of chocolate samples was performed in the sensory laboratory. The sensory profiling was performed by a 16-person panel who had been trained for this purpose, and their sensory sensitivity was confirmed. As a part of the analysis, the following qualitative determinants were marked: color (brown, chocolate color, uniform, homogeneous, off-color), consistency (softness, roughness, surface, desirability), taste (sweet, bitter, sour, chocolate flavor, off-flavour). The intensity of each qualitative mark was determined using a structured 10 cm-long line scale with adequate edge captions (intense/not intense). The obtained results were converted into numerical values and noted in points. Trials were encoded and given to the examiners, guaranteeing the anonymity of the first, by placing them in white, odorless, lockable vessels. All trials were performed at the same temperature (ambient temperature), and the trial quantity was suited for the test so as to guarantee the possibility of testing the product several times. Selected results were based on three individual marks of examiners, done during three sessions.

3. Results

Folin-Ciocalteau method is one of numerous methods often used to determine the total count of bioactive compounds. It can be used for determination of general content of compounds with phenyl groups (polyphenols, amino acids), Analyses results are shown in Table 1. The highest phenolic content was detected for WPC protein – 211.41 mg/g d.w., while the lowest for Psyllium 52.32 mg/g d.w. As a part of the study, the cytotoxicity analysis of deproteined eggshell was performed. Relative cell growth is presented in Table 2.

Table 2. Total phenolic content and cytotoxicity test results
Tab. 2. Wyniki testów całkowitej zawartości fenoli i cytotoksyczności

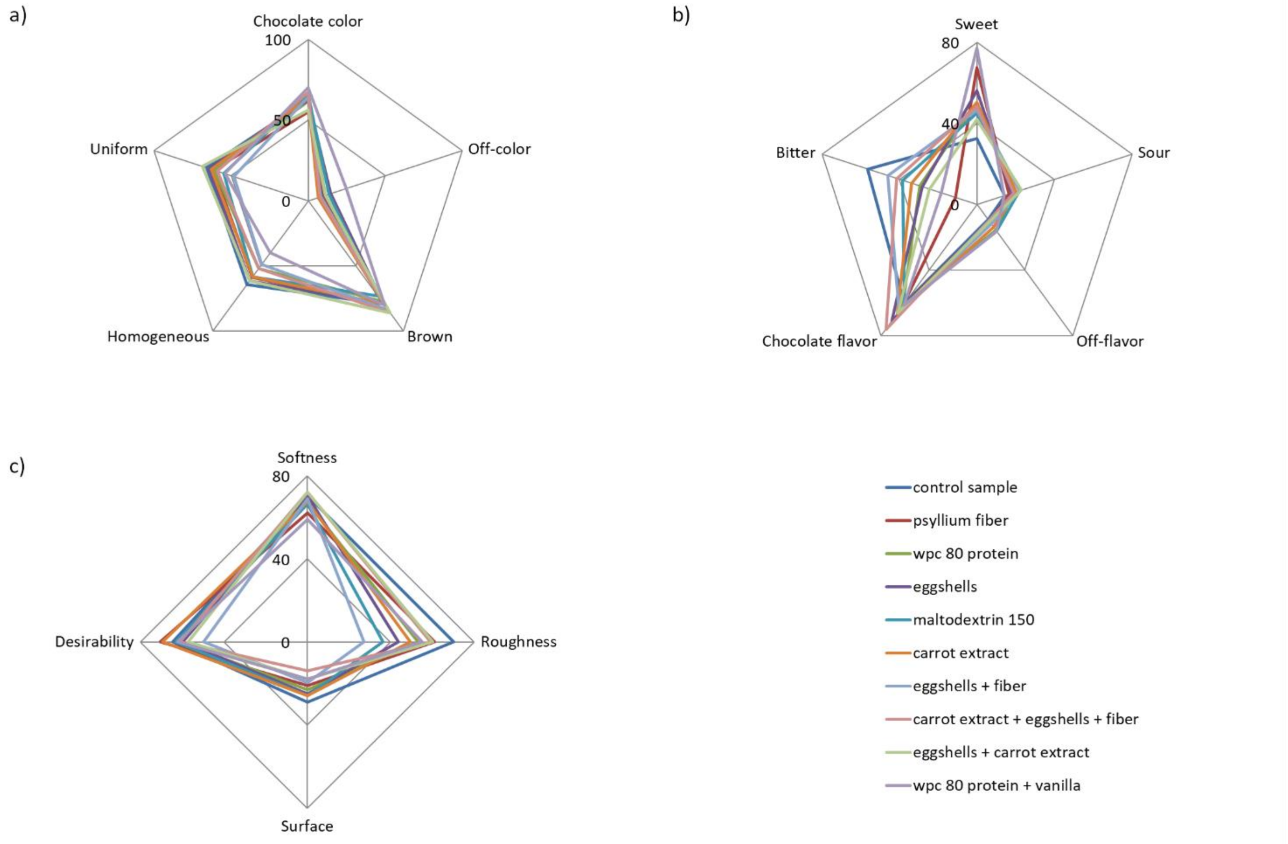
Raw material	Total phenolic content (mg/g d.w.)	Relative cell growth [%]
Eggshell	na	116.00 ± 1.62
Carrot extract	145.96b ± 3.32	na
Psyllium (Plantago ovata)	52.32a ± 0.97	na
WPC 80	211.41c± 4.03	na

Mean values marked with different lowercase letters in the same column are significantly different (p≤0,05); na – not analysed.

Source: own study / Źródło: opracowanie własne

Obtained results indicate that eggshell shows no cytotoxic activity. Neither decrease in cells vitality, nor inhibition of their metabolic activity was detected.

It was shown, that chocolates containing carrot extract WPC 80 protein and WPC 80 with vanilla aroma were perceived as the sweetest and the least bitter ones (Fig. 1).



Source: own study / Źródło: opracowanie własne

Fig. 1. Sensory profiles of tested chocolate samples: a) color, b) taste, c) consistency
Rys. 1. Profile sensoryczne badanych próbek czekolady: a) barw, b) smak, c) konsystencja

Sour taste was perceived at higher intensity in chocolates enriched in eggshell, carrot extract, eggshell and fibre, or eggshell with carrot extract. Off taste was the most intense in chocolate with fibre, eggshell or a mix of WPC 80 and vanilla. Chocolate taste perception was the highest for chocolate enriched in WPC 80 protein, maltodextrin 150 and eggshell with fibre. It was noted that the bitterest was the control sample. The lowest bitterness perception was noted for samples with carrot extract and a mix of WPC 80 protein with vanilla. It was observed that off odor was perceived for chocolate with WPC 80 protein, eggshell and a mix of WPC 80 with vanilla. Chocolate odor was detected at equal level for all tested samples. The analysis showed that the most intense brown color possessed chocolates with carrot extract, eggshell and fibre, and eggshell with carrot extract. The highest uniformity in color was noted for the control sample, incl. WPC 80, eggshell and carrot extract. In the consistency assessment, it was found that the roughest chocolates were in the control test and containing the carrot extract and a combination of egg shells and carrot extract. The softest chocolates were ones enriched in WPC protein, eggshell and fibre combination and a mix of eggshell with carrot extract. It was concluded that the most desirable were the control sample, chocolates with carrot extracts and for chocolates with maltodextrin 150. The chocolates with the most matte surfaces were those that belonging to a control sample and containing egg shell or maltodextrin 150.

4. Discussion

Biological activity of substances potentially being pro-health and functional foods ingredients, has become a subject of a lot of cytotoxicological studies, recently. Large attention is paid to raw materials that have been used for thousands of years in natural medicine, as anti-inflammatory, antidiabetic and anticancer agents. The increasing interest is also characteristic for an eggshell, as a potential source of calcium. The current research methods enable the determination of compounds impact on cancer cells proliferation. Among numerous cell lines applied in an assay of anticancer effect of food ingredients, the usually used ones are gastrointestinal tract cells, directly exposed to consumed substances, e.g. colon epithelial cells, but also breast cancer cells, which best represent the cells of the digestive system. In this study was shown that eggshell has no cytotoxic effect, which is completely new information, as no such test was conducted before.

In our paper, we analyzed the application potential of selected plant materials, as a source of bioactive compounds acting as bitter taste modulators in chocolate. Currently, the growing interest of food industry in the taste masking technologies is being observed. Nowadays, the applied technologies of bitter taste masking are based on limitation or enable of interactions between bitter compounds and receptors placed in taste buds [15]. The main advantage of the latter, more traditional methods is simpler maintaining of positive taste and aroma qualities of food [16]. On the other hand, the former method enables to mask bitter taste in products more effectively. Both methods can be applied simultaneously and decrease food bitterness to the same extent. The state-of-the-art method based on deposition of bitter substances on ion exchange resins, gives as a result a total elimination of unpleasant bitter taste [17]. This method is used in case of processed food products and in potable water, so as to dispose of bitter and tart flavours, completely. In several studies chocolate samples were enriched in selected raw materials. The added ingredients affected positively the inhibition of bitter taste, to a greater or lesser extent. In reference positions there is a comprehensive information about enriching food products in various substances that also affect the bitter taste modulation, e.g. magnesium sulphate, zinc acetate, sodium chloride or sweeteners [18]. Dark chocolate, which contains many valuable for health ingredients, is underestimated by consumers that do not accept its bitterness. Adding substances, feasibly masking the bitter taste, caused that several ingredients, i.e. carrot extract and WPC 80 protein mixed with vanilla, effected in inhibition of bitter flavour in this product. Conducted sensory panel showed high compliance of the respondents for this ingredients. Sensory properties such as taste, color, consistence and odor reflect the quality of the product and are important when choosing a product by the consumer. In this study, the aim to mask bitter taste using the aforementioned ingredients, was fulfilled. Substances that positively affected the bitter taste masking, were carrot extract, sand plantain protein, WPC 80 protein, eggshell and maltodextrin 150.

5. Conclusions

Commonly available food ingredients such as carrot extract, sand plantain protein, WPC 80 protein, eggshell and maltodextrin 150 can be additives that act as modulators of bitter taste, what was confirmed in dark chocolate matrix. The most positive effect in bitter taste masking was observed for samples with carrot extract and a mixture of WPC 80 with vanilla. Bitter taste can be masked using modulators both plant and animal origin. Excellent raw materials for composing blends for masking the bitter taste can be eggshell, carrot extract, maltodextrin 150 and WPC 80 protein in combination with vanilla.

6. References

[1] Drewnowski A., Gomez-Carneros C.: Bitter taste, phytonutrients, and the consumer: A review. American Journal of Clinical Nutrition, 2000, vol. 72, 6. 14241435. doi: 10.1093/ajcn/72.6.1424.

[2] Kobus-Cisowska J. et al.: Composition of polyphenols of asparagus spears (Asparagus officinalis) and their antioxidant potential. Ciência Rural, 2019, vol. 49, 4. doi: 10.1590/0103-8478cr20180863.

[3] Reed D.R. Tanaka T.: McDaniel A.H.: Diverse tastes: Genetics of sweet and bitter perception. Physiol. Behav., 2006, vol. 88, 3, 215-226, doi: 10.1016/j.physbeh.2006.05.033.

[4] Andrade A.C., Martins M.B., Rodrigues J.F., Coelho S.B., Pinheiro A.C.M., Bastos S.C.: Effect of different quantities of miracle fruit on sour and bitter beverages. LWT, 2019, vol. 99, 89-97, doi: 10.1016/j.lwt.2018.09.054.

[5] Rodrigues N. et al.: Application of an electronic tongue as a single-run tool for olive oils' physicochemical and sensory simultaneous assessment. Talanta, 2019, vol. 197, 363-373, doi: 10.1016/j.talanta.2019.01.055.

[6] Jansson T. et al.: Effect of green tea catechins on physical stability and sensory quality of lactose-reduced UHT milk during storage for one year. Int. Dairy J., 2019, vol. 95, 25-34, doi: 10.1016/j.idairyj.2019.03.007.

[7] Ostrowska-Ligęza E. et al.:A comparative study of thermal and textural properties of milk, white and dark chocolates. Thermochim. Acta, 2019, vol. 671, 60–69, doi: 10.1016/j.tca.2018.11.005.

[8] Seem S.A., Yuan Y.V, Tou J. C.: Chocolate and chocolate constituents influence bone health and osteoporosis risk. Nutrition, 2019, vol. 65. Elsevier Inc., 74-84. doi: 10.1016/j.nut.2019.02.011.

[9] Silva A.R.D.A., Bioto A.S., Efraim P., Queiroz G.: Impact of sustainability labeling in the perception of sensory quality and purchase intention of chocolate consumers. J. Clean. Prod., 2017, vol. 141, 11–21, doi: 10.1016/j.jclepro.2016.09.024.

[10] Kobus-Cisowska J. et al.: Enriching novel dark chocolate with Bacillus coagulans as a way to provide beneficial nutrients. Food Funct., 2019, vol. 10, 2, 997-1006. doi: 10.1039/c8fo02099j.

[11] Braca A., Sortino C., Politi M., Morelli I., Mendez J.: Antioxidant activity of flavonoids from Licania licaniaeflora. J. Ethnopharmacol., 2002,vol. 79, 3, 379-381. doi: 10.1016/S0378-8741(01)00413-5.

[12] Rusconi M., Conti A.: Theobroma cacao L., the Food of the Gods: A scientific approach beyond myths and claims. Pharmacological Research, 2010, vol. 61, 1, 5-13. doi: 10.1016/j.phrs.2009.08.008.

[13] Katz D.L., Doughty K., Ali A.: Cocoa and chocolate in human health and disease. Antioxidants and Redox Signaling, vol. 15, no. 10. pp. 2779–2811, 2011, doi: 10.1089/ars.2010.3697.

[14] Cheung S.T.: The effects of chocolates given by patients on the well-being of nurses and their support staff. Nutr. Health, 2003,vol. 17, 1, 65-69. doi: 10.1177/026010600301700108.

[15] Ley J.P.: Masking bitter taste by Molecules. Chemosens. Percept., 2008, vol. 1, 1, 58-77. doi: 10.1007/s12078-008-9008-2.

[16] van Beilen M.: Effects of visual priming on Taste-Odor interaction. PLoS One, 2011, vol. 6, 9, doi: 10.1371/journal.pone.0023857.

[17] Bhalekar M.R., Bidkar S.J., Shete T.K., Madgulkar A.R.: Taste Masking of Cefuroxime Axetil by Ion Exchange Resin Complex. Am. J. Pharm, 2010, vol. 29, 2, 198-204.

[18] Gaudette N.J., Pickering G.J.: The efficacy of bitter blockers on health-relevant bitterants. J. Funct. Foods, 2012, vol. 4, 1, 177-184. doi: 10.1016/j.jff.2011.10.003.

Funding

The work was financed by grant POIR.04.01.02-00-0059/17 from the National Centre for Research and Development in Poland.