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FOOD IN HEALTH AND DISEASE

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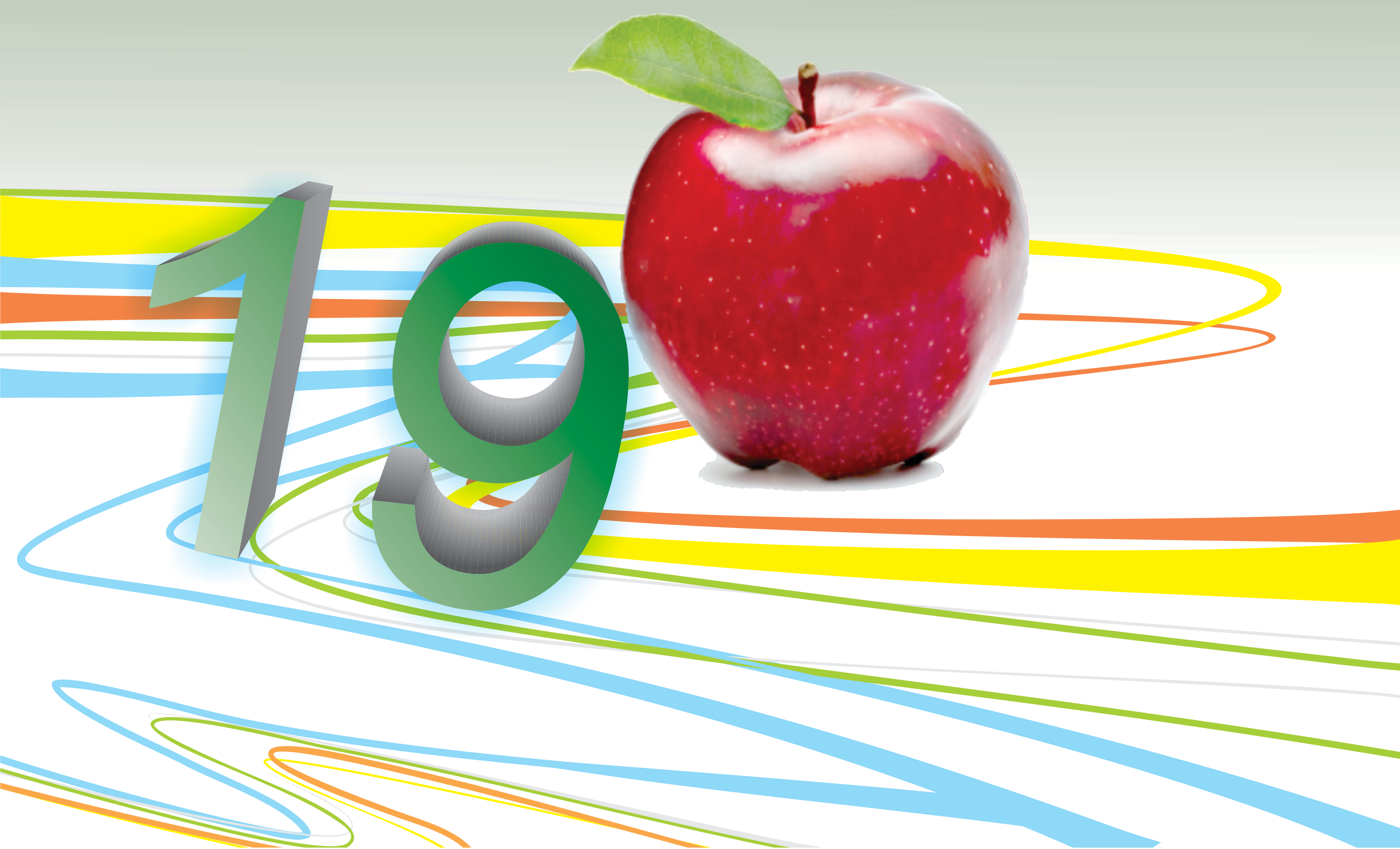

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ASSESSMENT OF CONSUMER HABITS, ATTITUDES AND OPINIONS TOWARDS HONEY

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original scientific paper

Summary

The aim of this research was, through an online questionnaire, to collect data on the habits, attitudes and opinions of consumers towards honey in Croatia, during the spring 2020. The study included 175 respondents, and the results showed that the respondents most often consume honey on a weekly basis (37.1%). Also, 72.0% of sample purchase honey directly from producers, and 73.7% of respondents never purchase honey in a specialised store. Considering the attitudes, the results of the research show that a high share of respondents agrees that they consume honey due to the beneficial effect on health, general quality, and nutritional value (84.0%, 83.4% and 70.3%, respectively). The results of consumer opinions evaluation show that 66.9%, 66.3% and 64.0% of sample agree that the quality of honey is indicated by taste, aroma and texture, respectively, where the highest share (91.4%) of sample agree that honey has a beneficial effect on the immune system, and slightly lower shares agree that honey contains bioactive compounds, has a beneficial effect on the skin, anticancer effect, and a beneficial effect on neurodegenerative changes (80.0%, 69.1%, 56.0% and 49.0%, respectively).

Keywords: honey, consumer opinions, consumer habits, consumer attitudes

Introduction

The properties and chemical composition of honey differ depending on the type of plants from which the bees collect nectar. Honey contains about 200 different ingredients and each has unique nutritional and bioactive properties, but they can also act synergistically (Visweswara Rao et al., 2016). Many consumers are interested in food which bring added value to their health. Increasing trend in healthy lifestyle influences increasing popularity of honey worldwide for its various nutritional benefits (Cosmina et al., 2016; Šedík et al., 2018). Honey makers have to search for new approaches to follow those trends and to learn to understand the numerous factors which influence consumer needs (Ho Chiang Yeow et al., 2013). In performed consumer preferences studies for honey, the data is analysed using qualitative and quantitative methods as well as appropriate econometric models.

There are several factors that affect purchase of honey and they differ among countries. The quality of honey was the main dimension for purchase in many countries: Romania, Hungary, Malaysia, Kingdom of Saudi Arabia, Kenya, Slovakia, Greece (Arvanitoyannis and Krystallis, 2006; Árváné Ványi et al., 2009; Ho Chiang Yeow et al., 2013; Ismaiel et al., 2014; Nabwire Juma et al., 2016; Šedík et al., 2018; Lymperi and Fragkaki, 2020). Further on, price and packaging were one of the most important factors affecting honey purchase for Irish, Hungarian, Czech, Slovenian, Polish and Malaysian consumers (Murphy et al., 2000; Árváné Ványi et al., 2009; Roman et al., 2013; Ho Chiang Yeow et al., 2013; Šánová et al., 2016; Kos Skubic et al., 2017; Kopała et al., 2019). Most of the studies performed indicated the texture, followed by taste and colour, and finally the aroma as the most important honey attributes for consumers in Ireland, Romania, Western Australia, Kingdom of Saudi Arabia, Italy, Czech Republic, Slovakia, Croatia, Poland and Greece (Murphy et al., 2000; Arvanitoyannis and Krystallis, 2006; Batt and Liu, 2010; Ismaiel et al., 2014; Cosmina et al., 2016; Šánová et al., 2016; Bršćić et al., 2017; Šedík et al., 2018; Kopała et al., 2019; Lymperi and Fragkaki, 2020). Labels play a less important role in purchase decision, but significant positive preferences for labelling were recorded for consumers in Kenya (Nabwire Juma et al., 2016). Consumers in Western Australia, Democratic Republic of Congo, Italy, Czech Republic, Kenya, Poland, Greece and Hungary showed strong positive preference for local origin of honey (Batt and Liu, 2010; Gyau et al., 2014; Cosmina et al., 2016; Šánová et al., 2016; Nabwire Juma et al., 2016; Kopała et al., 2019; Lymperi and Fragkaki, 2020; Oravecz et al. 2020). Results of purchasing behavior studies showed that consumers in Hungary, Croatia, Poland and Greece mostly purchase honey directly from the beekeepers (Árváné Ványi et al., 2009; Oravecz et al. 2020; Bršćić et al., 2017; Kopała et al., 2019; Lymperi and Fragkaki, 2020). According to the honey type, most of the consumers in Hungary

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prefer acacia and flower (Árváné Ványi et al., 2009), consumers in Croatia also acacia (Brščić et al., 2017), young generation in Slovakia besides acacia chose linden as well (Šedík et al., 2018), while in Poland multi-flower, lime, acacia, buckwheat, honeydew and rapeseed honey were mainly consumed (Kopała et al., 2019). Medical benefits stand out as the most common reasons for purchasing and consuming honey only for consumers in Romania (Arvanitoyannis and Krystallis, 2006), Kingdom of Saudi Arabia (Ismaiel et al., 2014) and Croatia (Brščić et al., 2017).
The results of consumer preference studies for honey can help beekeepers in their work as well as establish the right tools for their communication with consumers (Oravecz et al., 2020; Šedík et al., 2018). The study about consumer preferences for honey on the Croatian market was conducted by Brščić and coauthors in 2017. Since further increase in the level of consumer awareness increases the importance of information that helps them make decisions, the aim of this research was to investigate the habits, attitudes and opinions of consumers about honey on the Croatian market during spring 2020.

Materials and methods

During this research, an online questionnaire was used to collect data on the habits, attitudes and opinions of consumers towards honey, based on a studies by Arvanitoyannis and Krystallis (2006) on an empirical examination of the determinants of honey consumption in Romania, and Brščić et al. (2017) on an an empirical examination of consumer preferences for honey in Croatia. The questionnaire was conducted on a sample of 175 respondents over the age of 18, from the territory of the Republic of Croatia. It was available for anonymous online completion in May 2020. All completed questionnaires were valid. The research included the demographic characteristics of the respondents, and was focused on consumers from younger age groups, educated and from urban living environment. The questionnaire was divided into sections, and contained groups of questions regarding consumer habits, consumer attitudes, and consumer opinions towards honey. The questions were close-ended types with the possibility of choosing only one of the offered answers, except for the assessment of preferred type of honey.
The collected data were analysed by factor analysis followed by the determination of Cronbach's alpha reliability coefficient (Rajh, 2009). Statistical analysis of the data was performed with the software package Statistica 10 (StatSoft Inc., 2011).

Results and discussion

Socio-demographic characteristics of the sample

The socio-demographic characteristics of the consumers are presented in Table 1. The results of the research showed that a total of 154 respondents were female (88.0%) and male 21 respondents (12.0%). The largest number of the sample members were those aged from 18 to 30 years (80.0%), followed by 19 respondents aged from 30 to 40 years (10.9%), 13 respondents aged from 40 to 60 years (7.4%), while 3 respondents were in the age group over 60 years (1.7%). Also, the largest number of respondents has education at the university level, 137 of them (78.3%), while the rest of the sample members have secondary education (21.1%) and primary education (0.6%). The urban area of residence was chosen by 81.1% of respondents and rural by 18.9% of them (Table 1).

Table 1. Socio-demographic profile of consumers (n = 175)

		Share (%)	n
Gender	Female	88.0	154
	Male	12.0	21
Age (years)	18-30	80.0	140
	31-40	10.9	19
	41-60	7.4	13
	>60	1.7	3
Education level	Primary school	0.6	1
	Secondary school	21.1	37
	University	78.3	137
Area of residence	Urban	81.1	142
	Rural	18.9	33

Consumer habits related to honey

Honey is a frequently consumed product, as indicated by the results of this study according to which a one-fifth of sample members (19.43%) consume honey on a daily basis, and the majority of sample (36.57%) consume honey at least once a week. Almost a one-third of sample members (28.00%) consume honey at least once a month, and only 1.71% never consume honey (Table 2).

Table 2. Consumer (n = 175) habits regarding frequency of honey consumption

I usually consume honey:	n	%
Daily	34	19.43
At least once a week	64	36.57
At least once a month	49	28.00
Less than once a month	25	14.28
Never	3	1.71

Consumer habits regarding the frequency of choosing a particular way of honey purchase were also investigated. The majority of respondents (67.43%) often purchase honey directly from producers, sometimes on the market (36.00%), while the purchase of honey in a specialised store, supermarket or local store is never chosen by 73.71%, 62.28%, and 61.14% of respondents, respectively (Table 3).

Table 3. Consumer (n = 175) habits regarding the way of honey purchase

I buy honey:	often[a]		sometimes		never	
	n	%	n	%	n	%
In local store	16	9.14	52	29.71	107	61.14
In supermarket	15	8.57	51	29.14	109	62.28
In specialised store	9	5.14	37	21.14	129	73.71
On the market	21	12.00	63	36.00	91	52.00
Directly from the producer	118	67.43	33	18.86	24	13.71

[a]Levels of the frequency of honey purchase

Thereby, as the favorite type of honey during purchasing, the largest number of respondents (55) points out floral honey (31.4%), while the second, third and fourth place were taken by acacia honey (26.3%), meadow honey (20.6%) and chestnut honey (8.6%), respectively. The answers were in general very divided and the respondents chose different types of honey as their favorites (Table 4).

Table 4. Consumer (n = 175) habits regarding type of honey which prefere

My favourite type of honey:	n	%
Acacia	46	26.3
Chestnut	15	8.6
Linden	6	3.4
Floral	55	31.4
Meadow	36	20.6
Sage	7	4.0
Honeydew	4	2.3
Lavender	2	1.1
Other types	4	2.3

Further, as can be seen from the Table 3, the traditional way of purchasing honey on the market is somewhat more desirable for the respondents compared to other ways, especially the one directly from the producer. The obtained data indicate the fact that the respondents show traditional behavior, and that the way of honey purchasing is based on experience criteria. The data indicate that a large share of sample does not prefer purchasing a honey in a specialised store, supermarket or local store (Table 3), what additionaly shows the dominance of traditional distribution channels. The results are in accordance with the research of Bršćić et al. (2017), which showed that the most common way of honey purchase on the Croatian market is directly from the producers or beekeepers.

Consumer attitudes towards honey

The next group of questions concerned consumer attitudes towards honey with an attempt to establishing main reasons of honey consumption. Statements regarding consumption of honey were offered to the respondents (n = 175) and assessed by a three-point Likert agreement scale (Table 5).

Table 5. Reasons of honey consumption

I consume honey because of its:	agree[a]		neither agree nor disagree		disagree		Mean (out of 3)
	n	%	n	%	n	%	
Taste	119	68.00	39	22.28	17	9.71	2.58
Nutritive value	123	70.28	33	18.86	19	10.86	2.59
Overall quality	146	83.42	18	10.28	11	6.28	2.77
Method of production	50	28.57	77	44.00	48	27.42	2.01
Safety	64	36.57	82	46.86	29	16.57	2.20
Market availability	45	25.71	70	40.00	60	34.28	1.91
Beneficial effects on health	147	84.00	21	12.00	7	4.00	2.80

[a]*Points of a Likert agreement scale*

As can be seen in Table 5, the majority of sample members (84.00%) agree with the statement that honey has a beneficial effect on health, what is not suprising because data on the positive effect of honey on health due to the variety of compounds it consists of have existed for more than four thousand years (Bogdanov et al., 2008). It can be seen that respondents have confidence in honey given that 83.42% of sample agree with the statement that honey is consumed because of the overall quality (Table 5). Honey has also a significant nutritive value, is a source of various natural macronutrients and micronutrients which, in addition to meeting energy needs and other biological functions, can participate in the prevention of various health problems or diseases (Alvarez-Suarez et al., 2013). High share (70.28%) of respondents agrees with the statement that honey is consumed due to its nutritive value (Table 5). Furthermore, a large share of respondents (68.00%) agrees with the statement that honey is consumed because of its taste. Honey characterises a sweet taste as a result of a high content of various carbohydrates which can contribute to its sweetness. In comparison with other products used as a sweetener, honey is unique due to the variety of compounds (Alvarez-Suarez et al., 2013). Also, because of its taste, honey is a favorite addition to many dishes (Arvanitoyannis and Krystallis, 2006). A small proportion of respondents (36.57%, 28.57% and 25.71%, respectively) agree with the statements that safety, method of production, and market availability are their reasons for honey consumption (Table 5). Thanks to the ingredients that create conditions in which the growth of microorganisms is mostly not possible, honey is a product which also has a long shelf life (Bogdanov et al., 2008), is produced everywhere in the world and its prevalence is very high, which makes it easily accessible and available to all consumers (Arvanitoyannis and Krystallis, 2006).

Table 6. Factor analysis (FA) results of reasons of honey consumption

Factor Loadings (Varimax raw) Extraction: Principal components			
Reasons for consuming honey are		Factor 1	Factor 2
	Nutritive value	0.774	
	Overall quality	0.839	
	Method of production		0.715
	Safety		0.762
	Market availability		0.842
	Beneficial effects on health	0.770	
Statistics			
	Eigenvalue	3.024	1.030
	Variance %	50.396	17.162
	Cumulative variance %	50.396	67.558
	Cronbach alpha	0.750	0.737
	Mean	2.72	2.04

In order to explore the dimensions of consumers' reasons for consuming the honey, FA was conducted with 6 variables related to attitudes important for consumers eating decisions (taste was excluded from the analysis). First two factors emerged with eigenvalues greater than 1 explained 67.56% of total variance. The results shown in Table 6 reveal that factor one involves reasons for consuming such as nutritive value, overall quality and beneficial effects on health and majority of these attributes were highly rated on the consumer importance scale (Table 5). On the second factor the remaining three reasons are listed – method of production, safety and market availability. It can be seen from Table 5 that values of importance for variables market availability, method of production and safety are very low from 1.91 to 2.20. That implies that those reasons for consuming the honey are not highly important. Cronbach alpha implies that selected variables explain very well first (0.750) and second factor (0.737).

Consumer opinions towards honey

The last group of questions concerned consumer opinions towards honey. The first part of the questions from this group referred to selected indicators of honey quality, and the second part to beneficial effects of honey on health (Table 7).

Table 7. Consumer (n = 175) opinions towards quality and beneficial effects of honey

Statement towards honey:	agree[a]		neither agree nor disagree		disagree		Mean (out of 3)
	n	%	n	%	n	%	
The quality of honey is indicated by colour	49	28.00	67	38.28	59	33.71	1.94
The quality of honey is indicated by the texture	112	64.00	41	23.42	22	12.57	2.51
The quality of honey is indicated by aroma	116	66.28	47	26.86	12	6.86	2.59
The quality of honey is indicated by the taste	117	66.86	47	26.86	11	6.28	2.61
The quality of honey is indicated by the data on the declaration or food information	89	50.86	61	34.86	25	14.28	2.37
The quality of honey is indicated by the brand name	18	10.28	63	36.00	94	53.71	1.57
Honey contains bioactive compounds	140	80.00	32	18.28	3	1.71	2.78
Honey contains phenolic compounds	90	51.42	72	41.14	13	7.42	2.44
Honey has a beneficial effect on the immune system	160	91.42	14	8.00	1	0.57	2.91
Honey has a beneficial effect on the skin	121	69.14	51	29.14	3	1.71	2.67
Honey has a beneficial effect on neurodegenerative changes	86	49.14	83	47.42	6	3.42	2.46
Honey has anticancer effect	98	56.00	63	36.00	14	8.00	2.48

[a]*Points of a Likert agreement scale*

Colour of honey is a sensory attribute and thus quality attribute that consumers will notice first. Determination of colour is useful in classification of honey types, and can vary from transparent colourless, yellow, amber, to dark and black, and depends on the age of the honey, its origin and storage conditions (Pavlova et al., 2019). Numerous studies have confirmed the connection between the colour of honey and the content of phenolic compounds, which are also attributed to a beneficial effect on health. A higher content of phenolic compounds results in a darker colour of honey, and in the presence of a lower content of phenolic compounds, honey will be lighter in colour (Alvarez-Suarez et al., 2013). Furthermore, colour is also associated with the mineral content of honey. The mineral content in honey depends on the botanical origin of pollen, climatic conditions and extraction techniques. In the literature, there are research results on the association of higher proportions of minerals and dark honey (Pavlova et al., 2019). Respondents in 28.00% of cases agree that the colour indicates the quality of honey, 38.28% chose the answer "neither agree nor disagree", and 33.71% disagree with the statement (Table 7). The results show a division of respondents' opinions about colour as a parameter of honey quality, which can be related to the previously described numerous physico-chemical factors that can affect the final colour of honey. The texture of honey is another significant sensory property, and primarily depends on the method of production (Official Gazette, 2015). Freshly produced honey is liquid, but crystallises sooner or later during storage. Crystallised honey is repulsive to consumers, and mild heating (32-40 °C) is generally used to return crystallised honey to a liquid state. To avoid unwanted changes in heat-sensitive compounds, it is recommended not to use temperatures above 40 to 50 °C. In addition to high temperatures, the chemical composition of honey can be affected by long-term storage, with the formation of hydroxymethylfurfural and deactivation of the major enzymes present in honey like diastase (amylase), invertase (α-glucosidase), glucose oxidase, along with catalase, acid phosphatase, protease, esterase, β-glucosidase which can also be present (Al-Ghamdi et al., 2019). Considering the texture, the majority of sample members agree that the texture indicates the quality of honey (64.00%), some respondents choose the answer "neither agree nor disagree" (23.42%), and a smaller share of sample (12.57%) disagree with the statement (Table 7). It can be assumed that the results indicate that most consumers consider the texture to be one of the most important parameters of honey quality as they associate it with the crystallisation process due to which crystallised honey has a different, granular texture than the initial viscous one. The next two questions concerned the indication of aroma and taste on the quality of honey. The aroma of honey depends on the composition of the volatile compounds present, and the characteristic sweet taste comes from the most numerous constituents of honey, fructose and glucose. In addition to the sweet taste, honey is also characterised by a sour taste derived from organic acids, however honey is only seemingly sour because a high sugar content masks the acidity of organic acids. Some research results have shown that darker types of honey are often more acidic compared to lighter honey. Storage of honey also increases its acidity and therefore the taste may indicate a reduced quality of honey. Depending on the botanical origin, honey may contain small amounts of bitter substances such as alkaloids, polyphenols, glycosides and terpenoids (Pavlova et al., 2019). Changes in aroma and taste can make honey less attractive to consumers (Castro-Vázquez et al., 2012). The results of this research show that the opinion of the respondents on the indication of aroma and taste on the quality of honey is the same (Table 7). Considering all the statements about the selected indicators of honey quality, the majority of sample members generally agree with the statement that aroma (66.28%) and taste (66.86%) indicate the quality of honey (Table 7). The Official Gazette (2015), document valid in Croatia, prescribes the conditions that honey must meet. Accordingly, the declaration on honey must be in accordance with the Official Gazette (2015), and the data on the declaration may indicate the quality of honey. Half of the sample members (50.86%) agree with the statement that the data on the declaration indicate the quality of honey (Table 7). Furthermore, only a small share of respondents (10.28%) agrees with the statement that the brand name indicates the quality of honey, while a large share (53.71%) disagrees with that statement (Table 7). Compared to the survey conducted in Romania, the respondents included in this research consider brand name much less important (10.28%) as a parameter of honey quality compared to the respondents in Romania (58.0%) (Arvanitoyannis and Krystallis, 2006). Considering all the statements referred to selected indicators of honey quality, it is noticeable that the smallest share of respondents agree that the brand name indicates the quality of honey, a certain share of respondents agree that colour indicates the quality of honey, and a slightly higher share agree that data on the declaration or food information indicate quality of honey. Most of the respondents agree that the texture, aroma and taste of honey indicate the quality of honey. In contrast to this study, the results of a study by Richardson et al. (1994) suggest that consumer assessment regarding to product quality is more oriented to extrinsic characteristics rather than intrinsic ones. Since honey is most often purchased directly from the producer as a non-branded product of non-standard quality, intrinsic visual or sensory characteristics such as colour, texture, taste and aroma can be easily assessed and used as a quality criterion. The obtained results of this research are in line with the results of the research conducted in Romania, with the colour of honey as an exception for which 92.4 % of respondents in Romania agreed to indicate the quality (Arvanitoyannis and Krystallis, 2006).

In the last part of the research, an attempt was made to evaluate consumer opinions towards beneficial effects of honey on health (Table 7). A large share of sample (80.00%) agrees with the statement that honey contains bioactive compounds (Table 7). A slightly lower share (51.42%) agree with the statement that honey contains phenolic compounds, but 41.14% of respondents are not sure and choose the answer "neither agree nor disagree", and 7.42% of respondents disagree with the statement that honey contains phenolic compounds (Table 7). The presence of phenolic compounds in honey has been established in numerous studies (Alvarez-Suarez et al.; 2013; Bogdanov et al., 2008; Cianciosi et al., 2018). Honey exhibits number of physiological effects, which are highly dependent on content of phenolic compounds (Visweswara Rao et al., 2016). The majority of respondents in a high share of 91.42 % agree with the statement that honey has a beneficial effect on the immune system. The answer "neither agree nor disagree" in the case of this statement is chosen by 8.00% of respondents, and only 1 respondent disagrees with that statement (Table 7). The antibacterial, anti-inflammatory and antioxidant properties of honey and its beneficial effect on the immune system are known (Oryan et al., 2016). Honey has a positive effect on the immune system because it increases the amount of important structures in the immune system such as B and T lymphocytes, antibodies, leukocytes and natural killer cells which consequently strengthens an individual's immunity (Timm et al., 2008). Throughout long human history, honey has been used in many cultures to treat burns and heal wounds. It is the oldest biomaterial for wound bending and its effectiveness in wound treatment has been confirmed through numerous studies (Alvarez-Suarez et al., 2013; Henatsch et al., 2018). The botanical and geographical origin, and the chemical composition of honey can contribute to the antibacterial potential of honey, but also be a source of variation in antimicrobial activity between different types of honey (Ghramh et al., 2019). The results of this study show that 69.14% of respondents agree with the statement that honey has a beneficial effect on the skin, 29.14% of respondents in the case of this statement chooses the answer "neither agree nor disagree", and with statement disagree only 1.71% of respondents (Table 7). Recent studies have shown that phenolic compounds present in honey enhance the defense mechanism against oxidative stress and molecular degradation caused by free radicals and therefore have a positive effect in protection against neurodegenerative diseases such as Alzheimer's, Parkinson's or Huntington's disease (Rahmann et al., 2014). 49.14% of respondents, according to the results of this study, agree with the statement that honey has a beneficial effect on neurodegenerative changes, 47.42% of respondents "neither agree nor disagree" with the statement, and 3.42% of respondents disagree with that statement (Table 7). Also, the results of this study show that 56.00% of respondents agree with the statement that honey has an anticancer effect, 36.00% of respondents "neither agree nor disagree", while only 8.00% of respondents disagree with the statement that honey has an anticancer effect. The scientific literature states that phenolic acids and flavonoids in honey represent promising pharmacological agents for the treatment of various types of cancer (Jaganathan and Mandal, 2009). Numerous studies indicate the therapeutic potential of honey, and the positive effects are mostly attributed to the antioxidant, anti-inflammatory and anti-apoptotic effects of the honey compounds (Talebi et al., 2020).

Table 8. Factor analysis (FA) results of consumer opinions towards quality and beneficial effects of honey

Factor Loadings (Varimax raw) Extraction: Principal components					
The quality of honey is indicated by		Factor 1	Factor 2	Factor 3	Factor 4
	Colour		0.558		
	Texture		0.654		
	Aroma		0.827		
	Taste		0.714		
	Data on declaration				0.802
	Brand name				0.639
	Content of bioactive compounds	0.675			
	Content of phenolic compounds	0.782			
Honey has beneficial effect on					
	Immune system			0.703	
	Skin			0.755	
	Neurodegenerative changes	0.669			
	Anticancer effects	0.603			
Statistics					
	Eigenvalue	2.640	1.901	1.294	1.093
	Variance %	22.004	15.842	10.785	9.111
	Cumulative variance %	22.004	37.846	48.631	57.742
	Cronbach alpha	0.683	0.657	0.407	0.331
	Mean	2.54	2.41	2.79	1.97

In order to explore the dimensions of consumers' opinions towards quality and beneficial effects of honey FA was conducted with 12 variables related to opinions important for consumers. First four factors emerged with eigenvalues greater than 1 explained 57.74% of total variance. The results shown in Table 8 reveal that factor one involves opinions about beneficial effects of honey and content of bioactive compounds in it due to which the honey actually has positive effects on human health (Table 7). On the second factor four reasons are listed too, and speak of the sensory properties of honey important for consumers when assessing quality, however, the aroma and taste are more correlated to factor two than the colour and texture. The third factor also corresponding to positive effects of honey to immune system and skin while fourth factor is related to quality of honey given by declaration on the product and brand name (Table 8). Cronbach alpha values for all observed factors were below 0.7. Low Cronbach alpha values could be explained by low number of variables explaining each factor, poor interrelatedness between them and/or their heterogenity (Tavakol and Dennick, 2011).

Conclusions

The results of this study provide useful information for local beekeepers to create marketing strategies. It was found that honey is most commonly consumed on a weekly basis, mostly purchased directly from the producer and almost never in a specialised store. Floral, acacia and meadow were the most preferred honey types. Taste, aroma and texture emerged as the key indicators of honey quality. Most respondents consume honey for its health benefits, general quality and nutritional value and believe that it has a beneficial effect on the immune system and contains bioactive compounds.

The applied measurement scale for measuring consumer reasons for honey consumption was confirmed as a valid instrument. The scale for measuring honey quality and its positive effects on human health should be further improved, indicating a need for additional research in this area in the future.

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NUTRITIONALLY IMPROVED CHOCOLATE SPREADS – A REVIEW

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review paper

Summary

Chocolate spread is one of the favourite breakfast meals and snacks among people of all generations. Increased consumption of these kinds of products with high-energy value leads to obesity and several health problems. Many researchers are trying to produce acceptable products with lower calorie values by reducing fat and sugar contents. Oleogels are one of the most explored solutions for the development of low-fat products and products with lower content of saturated fats. In addition, the fat composition of chocolate spreads can be changed to obtain a better fatty acid profile. Products with a low-sugar content have been proven to have sensory acceptability when sweeteners with low caloric value were used. In addition, researchers proved that chocolate spreads could be enriched with many different bioactive components by using by-products and minimally processed raw materials in the production process.

Keywords: chocolate spread, fat-reduction, bioactive components, sugar, obesity

Introduction

Chocolate spreads are one of the most desirable breakfast meals for children and adults. However, because of their composition, high content of sugar and fat, they are high-energy products. Although high contents of fat and sugar are the main causes of high-calorie values, they are also the main reason for the sensory acceptability of chocolate spreads. The chocolate spread consists of solid particles suspended in the fat network (Manzocco et al., 2014). The fat network is directly linked to sensory characteristics such as mouthfeel, texture and taste (Marangoni et al., 2012).
Nowadays, a great number of people in Western countries are dealing with obesity and weight gain for which the main reason is the frequent and excessive consumption of such products (Fernández-Murga et al., 2011), and this problem puts a new perspective on food producers. The number of obese people is increasing every year, which causes many serious health problems. Because of this problem, the industry is directed to the production of products with reduced fat and sugar contents but at the same time, the food market demands that these products have similar sensory properties as a product with conventional amounts of sugar and fat (Azaïs-Braesco et al., 2009; Acan et al., 2021).
Functional food is a widely used term, but it does not have a universal definition. One of the accepted phrases that are used for defining functional foods is: "Functional food is food in which the concentrations of one or more ingredients have been manipulated or modified to enhance their contribution to a healthful diet" (Thomson et al., 1999).
Chocolate spread is one of the most favourite breakfasts for children and adults. It is composed of high contents of sugar (38 - 42 %) and fat (25 - 40 %), but also contains milk powder, cocoa powder, emulsifier, etc. (Shamsudin, 2013; Popov-Raljić et al., 2013). Chocolate spread contains cheaper vegetable fats, unlike chocolate that contains cocoa butter. In addition, it is required that these products are not solidified at room temperature, because of spreadability, creamy and smooth taste (Lončarević et al., 2016).
This paper gives an overview of low-fat, low-sugar and enriched chocolate spreads. The way a chocolate spread is affected by the change in the composition, sensory acceptability of modified products and their influence on the health of consumers are discussed.

Low-fat chocolate spreads

Low-fat products are becoming increasingly popular among consumers because of their lower energy value, but the fat phase in products like chocolate spreads is very important since it is responsible for their quality and sensory properties (Do et al., 2007). Due to fat's unique properties, the production of low-fat products is challenging, because it is expected that these products will have comparable flavour, taste, and stability as full-fat products (Devereux et al., 2003).

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One of the possibilities for fat replacement is the use of edible oleogels (Almeida and Lannes, 2017). Oleogels are being increasingly used in the development of new food products with lower contents of trans- and saturated fats. Although the main health benefit of oleogels is due to the reduced content of saturated fats, some studies have shown that oleogels have health benefits by reducing the content of lipids in blood after consumption (Hwang, 2020).

Schneider and Souza (2009) produced milk chocolate with the addition of gelatin, which showed an ability to form gels that have a melting point close to body temperature (Karim and Bhat, 2009). After that, Almeida and Lannes (2017) produced chocolate spread with gelatin from chicken by-products. They used 0.3 to 1.2 % of gelatin for the replacement of 15 to 100 % of vegetable fat. The addition of a fat replacer caused the lighter colour of products, higher water activity, and lower consistency. This indicates that chocolate spreads with gelatin could be stored at lower temperatures, and still be spreadable, unlike conventional spreads, which are solid at low temperatures (10 °C). Gelatin is a gelling agent used for centuries in food production. It is a protein extracted from collagen with the ability to form a thermoreversible gel, which is important for food texture (Almeida and Lannes, 2013).

Chocolate spreads with lower fat content can also be developed by a combination of different fats and oils using a mathematical model that can predict food's physical properties (Manzocco et al., 2014). For this purpose, palm-based fats and vegetable oils (sunflower and extra virgin olive oil) were combined. The authors managed to predict which minimal lipid concentration is required to obtain a homogeneous spread with acceptable physical properties. They concluded that in this way, fat can be decreased up to 50% which would also decrease saturated fatty acid content by 90%.

Chocolate spreads with altered fat composition

Chocolate spread quality is based on its composition, mainly on fat types used in the recipe. In the production of chocolate spreads, vegetable fats are mainly used, which give a creamy taste, spreadability, homogeneity, stability, etc. to the product (Lončarević et al., 2016). These solid fats can have a high content of saturated and trans-fatty acids (Pehlivanoğlu et al., 2018). Excessive consumption of saturated fats is linked to an increased risk of cardiovascular diseases. It was shown that the replacement of saturated fat with polyunsaturated fat could affect a decrease of low-density lipoprotein cholesterol in the blood (Siri-Tarino et al., 2010). In the last few years, researchers are trying to replace solid fats in foods like chocolate spreads, but it is still very challenging to obtain products similar to that of conventional composition. This is mainly because of fat's unique effect on the texture and sensorial acceptability (Popov-Raljić et al., 2013).

Jeyarani et al. (2013) produced chocolate spread with soybean oil to increase the content of linolenic acid. Commercial hazelnut spread had a higher content of oleic acid compared to spreads with soybean oil. In addition, linolenic acid was not detected in the commercial spread, unlike in spreads with soybean oil. It is important to state that those spreads, besides nutritionally improved fatty acid content, had acceptable sensory properties. Rapeseed oil can also be used in chocolate paste production when combined with shellac oleogel. This gelling agent enabled a reduction of palm oil and obtaining the stable product (Patel et al., 2014). Fayaz et al. (2017) also used oleogels (monoglyceride, beeswax and propolis wax) for the replacement of palm oil with pomegranate seed oil. This oil is very well known for its high content of polyunsaturated fatty acids (Aruna et al., 2016). Sunflower and olive oil structured with hydrocolloids were also used in the production of chocolate spreads as replacers for coconut fat. Results showed that the combination of these oils and coconut fat in the formulation can result in sensory properties similar to spread produced only with coconut fat (Bascuas et al., 2021).

Low-sugar chocolate spreads

Sugar is one of the most represented raw materials in chocolate spread formulation (38 - 42 %) (Shamsudin, 2013). Because of the increasing obesity in Western countries, the demand for low-calorie products is increasing. Replacement of sugar with lower-calorie sweeteners is one of the best solutions for this problem.

Sugar-free chocolate spreads could be produced with sweeteners derived from disaccharides, which have lower calorie content and would be suitable for diabetics (Shamsudin, 2013). Although this spread was less sweet, it had a pleasant taste and a good spreadability.

Petković et al. (2012) also produced a sugar-free spread with maltitol as a replacer for sucrose. They examined different contents and ratios of maltitol and sucrose. Since maltitol is less sweet than sucrose, these spreads had less pronounced sweetness but were still sensory acceptable. The main conclusion was that with the use of this sweetener, the energetic value of spread could be reduced by 15% (Petković et al., 2012).

Chocolate spreads enriched with bioactive components

Chocolate spread is a chocolate derived, low-cost product that has a lower content of bioactive components than chocolate. In the last few years, many researchers are dealing with the enrichment of chocolates with polyphenols (Barišić et al., 2021) and thus enrichment of chocolate spreads is also becoming more interesting. Acan et al. (2021) used a by-product of the wine and juice industry, grape pomace, as a material for chocolate spread production. Grape pomace is rich in phenolic components and resveratrol, a well-known grape component that has a positive effect on cardiovascular health. The addition of this raw material in chocolate spread significantly increased the total phenolic content, but as the content of grape pomace increased, the digestibility of phenols decreased. The authors suggested that this is because of the high content of proteins in dried grape pomace used in production. Also, the sensory analysis showed that the usage of grape pomace in shares higher than 10% harmed acceptability. This is because grape pomace was used as a sugar and milk powder substitute, which decreased the sweetness of spreads. Also, phenolic components are known to have a slight bitterness, and the increased content of these components in chocolate spread affected sensory acceptability.

Palm oil usually used in chocolate spread production is refined, bleached and deodorised. These processes decrease the content of carotenes and tocols that are naturally present in red palm oil (Gee, 2007). Carotenes present in red palm oil have antioxidative activity and can protect against vitamin A deficiency (Sundram, 2005). Tocotrienols that are also present in red palm oil can improve cardiovascular health (Yew et al., 2007). Because of the great potential of red palm oil, El-Hadad et al. (2011) used it in chocolate spread production. Contents of bioactive components increased in such spreads, but they were less sensory acceptable. In addition, the study showed that the storage at ambient temperature caused the reduction of carotenes, tocopherols and tocotrienols, which means that it would be preferred to store this spread in a refrigerator, which would harm the spreadability of the product.

Chocolate spreads are very often produced with different kinds of nuts, among which, hazelnut is most common. Amevor et al. (2018) used cashew nut as a replacer in the production of spread and examined its effect on the quality of spreads. Cashew nuts are rich in phenolic components, fibres, minerals, proteins, etc. (Chen et al., 2006). Thus, the addition of this raw material in chocolate spread resulted in increased content of minerals, fibres and proteins.

Conclusions

During the last few years, many solutions for the development of nutritionally improved chocolate spreads have been established. One of the most promising solutions is the usage of oleogels for the production of low-fat spreads and usage of low-calorie sweeteners for the production of low-sugar spreads. However, many of the products developed, including the ones with increased contents of bioactive components, have lower sensory acceptability. Further research is needed to solve this problem and find a solution to obtain a product that will not significantly differ from conventional ones.

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AGE-RELATED MACULAR DEGENERATION (AMD), SUPPLEMENTS AND NUTRITION - WHAT DOES THE EVIDENCE TELL US?

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review paper

Summary

Age-related macular degeneration (AMD) is a disease of the macular area and is the most common cause of irreversible vision loss in people over 50 years of age. Many AMD risk factors, including aging, smoking, exposure to UV and blue light, chronic inflammation, and improper diet, can be associated with oxidative stress. In this regard, certain food ingredients and supplements that have antioxidant properties may contribute to the prevention of AMD and it's progression. Polyphenols can be considered as preventive and therapeutic compounds in the prevention of diseases associated with aging due to the antioxidant properties. For example, quercetin, present in onions, improves anti-VEGF therapy, which is the only effective drug for the wet form of AMD, while resveratrol, found in grape skin, red wine, blueberries and mulberries, neutralizes the negative effects of anti-VEGF therapy. Blueberry anthocyanins have a protective effect on induced damage of retina caused by blue light. Carotenoids that are part of the macular pigment; lutein and zeaxanthin, found in leafy green vegetables and eggs, have the ability to filter light ("natural sunglasses"), but also have direct antioxidant properties. Zinc and copper homeostasis in the retina-choroid complex also play a role in retinal health and AMD prevention. Adequate intake of omega-3 fatty acids, which are found in blue fish, nuts and seeds, reduces the risk of progression to the late stage of AMD. Oxidative stress can be exacerbated by improper diet, and thus increase the possibility of the occurrence and progression of AMD, which is why during the eye examination of persons at risk, it is necessary to advise on proper nutrition and appropriate supplementation.

Keywords: Age-related macular degeneration (AMD), oxidative stress, antioxidants, supplements, nutrition

Introduction

Age-related macular degeneration (AMD) is a medical condition of macula and is the most common cause of vision loss in persons over 50 years of age.
There are two types of age-related macular degeneration: dry and wet form.
Dry macular degeneration or “early” stage of AMD is characterized with presence of yellow deposits (called drusen) in macula, hypo/hyperpigmentation of retinal pigment epithelium, which is often found in macula and posterior pole. Wet form of macular degeneration or “late” stage of AMD is more serious condition and includes occurrence of choroidal neovascularization (CNV), retinal and subretinal bleeding, subretinal exudates, periretinal and retinal fibro-glial changes, geographic atrophy in RPE as well as pigment epithelial detachment (PED). Unless treated, wet form of macular degeneration can lead to dramatic decrease of visual acuity within a year (Andrijević Derk, 2015).
Besides classic categorization on dry and wet macular degeneration, there are more detailed classifications. The classification of dry form of AMD relates to number and size of drusen and the changes in RPE. We discern, early stage (Fig. 1a) with soft drusen, 64-124 μm in size and light pigment changes on the posterior pole, and intermediate stage (Fig. 1b) with middle-sized drusen resulting in confluence and big drusen (> 125 μm) accumulated below retina. Late stage of ADM has two subtypes: wet, exudative or neovascular macular degeneration (Fig. 1c) and geographic atrophy or dry degeneration (Fig. 1d) (Žorić et al., 2008).
According to the literature, a dry form of AMD present in 10 - 20 % of patients progresses to wet form, where 40% of patients develop wet form of AMD on both eyes (Andrijević Derk, 2015).
The data from EUREYE study (2006) shows that 3.3% of the population over 65 years have some form of AMD on at least one eye (Andrijević Derk, 2015).
It is believed that in Europe by 2040, the number of people with early AMD will be between 14.9 and 21.5 million and those with late AMD between 3.9 and 4.8 million (Colijn et al., 2017).
AMD is the leading cause of blindness in developed world and among white population of USA and constitutes 9% of blindness on global level. It is estimated that in 2020, number of people suffering from AMD will be 196 million globally and in 2040 that number will increase to 288 million (Chew, 2020). The World Health Organization states that

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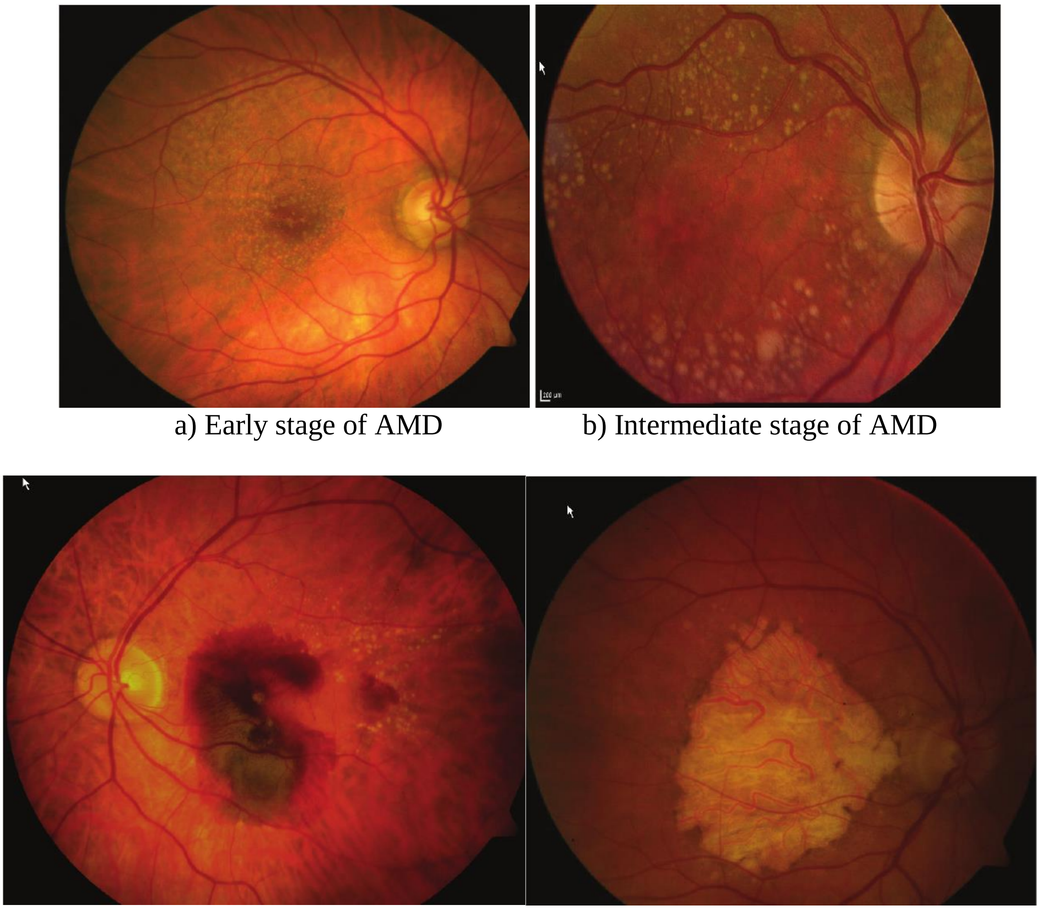
currently there are 14 million people suffering from blindness or heavily damaged vision due to AMD which puts it as a major problem related to vision loss on a global level (Pawlowska et al., 2019).



a) Early stage of AMD b) Intermediate stage of AMD

c) Late stage with neovascularization d) Late stage with geographic atrophy

Fig. 1. AMD stages (Čeklić et al., 2015)

AMD risk factors and pathogenesis

The main factor of AMD occurrence is age-related (50 years and above), then positive family history, smoking, previous operation of cataract, atherosclerosis, obesity followed by increased Body Mass Index (BMI), artery hypertension and cardiovascular diseases, insufficient intake of vitamin A, C and E, omega-3 fatty acids, zinc and lutein in diet, genetic predispositions (polymorphism related to complement H, ARMS2/HTRA1). Possible risk factors are: alcoholism, sun and UV exposure, intake of vitamin B, hormonal status and quantity of vitamin D, C reactive proteins and markers of inflammation (Čeklić et al., 2015).

Smoking as a risk factor is connected with specific genetic factors. High levels of cadmium in urine, occurring in smokers suffering from maculopathy, indicates specificity in its elimination in this group. Smokers who do not suffer from maculopathy have significantly lower levels of this toxic metal (Erie et al., 2007).

The mutation of the complement factor H (CFH) is probably the most noticeable genetic change in patients suffering from AMD (Warwick et al., 2018). Variability of several loci not included in the complement system plays an important role in AMD pathogenesis. Those are ARMS2 gene (Age-Related Maculopathy Sensitivity 2 gene) on chromosome 10 and genes included in angiogenesis (TGFBR1, VEGFA), the high-density cholesterol pathway -HDL (APOE, CETP i LIPC) and immunity regulation (PILRB) (Toomey et al., 2018). Epigenetic regulation of gene expression should also be included in the studies related to AMD pathogenesis, but epigenetic mechanisms in AMD early development are less known than genetic mechanisms. White people and females are most commonly connected to this disease.

However, besides age, family medical history, genetic predisposition and smoking, other risk factors are still controversial and require further examination in order to confirm their participation in AMD pathogenesis (Pawlowska et al., 2019).

AMD is multifactorial disease which is mainly caused by the changes in RPE structure and metabolism as a consequence to changes in Bruch's membrane (BM) caused by aging of the organism. All of these changes start with the fourth decade of life and are expressed more with age, and the result is change in RPE metabolic activity. BM in macula is more affected with metabolic changes than peripheral BM due to higher density of photoreceptors in macula and RPE metabolic activity. Metabolic activity of RPE involves a complex process of removing products of photoreceptors activity (rods and cones), which are at the same time regenerating, removing damaged membrane parts and organelle cytoplasm which due to change in transport through Bruch's membrane, are accumulated in the form of basal laminar deposit (BlamD) and basal linear deposit (BlinD), drusiform changes (D) in the area of cytoplasm of RPE and basal membrane of RPE and Bruch's membrane. Changes within collagenous and elastic layer of Bruch's membrane lead to mineralization of elastic layer as well as thickening of membrane from 2 µm during the first ten years of life up to 4.7 µm in the nineteth decade of life. Moore's and Clover's study shows tenfold decrease in transport through Bruch's membrane between the first and ninth decade of life, with all of the changes being more expressed in macular area in comparison to periphery (Andrijević Derk, 2015).

It is generally accepted that oxidative stress plays an importantrole in pathogenesis of AMD, but the source of oxidative stress in AMD is not generally known. AMD, especially wet form, is linked to several changes in retinal vascularization, which can be attributed to aging or intensive flow of blood in this tissue. These changes along with genetic predisposition of an individual, environmental factors and lifestyle represent major pathogens of AMD (Pawlowskaet al., 2019).

Retina is exposed to sun radiation during the lifetime. It is considered that the blue part of solar spectrum has more significant impact here because cornea and lens absorb the most part of UV radiation (Žorić et al., 2008). Retina has the highest speed of metabolism among tissues in the human body, has a large need for oxygen and is highly susceptible to oxidative stress (Datta et al., 2017). Oxidative stress is the condition leading to disbalance between antioxidants and prooxidants, in favor of prooxidants. In the balanced conditions, free radicals are dissolved by antioxidants of the cells with the help of enzymes (superoxide dismutase, catalase, glutathione peroxidase) or nonenzymatic with the help of glutathione. Long term oxidative stress leads to the damage of biological macromolecules (DNA, lipids, proteins and carbohydrates) causing disbalance of homeostasis in the cell and later in the tissue (Brzović Šarić, 2014). Oxidative damage of lipids - lipid peroxidation has the major role in AMD pathogenesis. Photoreceptor membranes contain a lot of polyunsaturated fatty acids (PUFA), a source of reactive oxygen species (ROS). Chronical exposure to light results in the ROS production. The cells of retinal pigment epithelium are of crucial significance for phototransduction because they phagocyte old tips of photoreceptors outer segments (Sun et al., 2006). Lipofuscin comes as a byproduct of phagocytosis (Delori et al., 2001). Lipofuscin contains complex compound of bis retinoid fluorophore which are causing autofluorescence of the fundus of the eye (Sparrow et al., 2012). The main fluorophore is A2E (N-retinylidene-N-retinylethanolamine), pyridiniumbisretinoid. When A2E is exposed to blue light, it succumbs to photooxidation which results in the ROS production and lipid peroxidation. Oxidative processes in retina, especially those happening during aging of macula can contribute to AMD pathogenesis. Aging, main risk factor of AMD occurrence, is related to ROS over-production in different ways, including lower levels of antioxidants, antioxidative enzymes and accumulation of damages on mitochondrial DNA (mtDNA). Macula concentrates light and shows high metabolic activity and oxygen consumption related to intensive flow of blood. Retina has limited regeneration mechanism and does not possess stem cells which could produce new cells to replace dead ones.

Environmental factors and lifestyle, including exposure to blue light, smoking, and improper diet increase ROS production. However, some nutritive supplements can inhibit oxidative stress related to ROS over-production and alleviate retinal damage. Retinal antioxidant defense can directly inhibit oxidative stress and its byproducts including lipid peroxidation, but is decreasing with age; limited regeneration mechanisms and regeneration of retina also decreases with age, as shown in fig. 2 (Pawlowska et al., 2017).

Many factors that can increase the risk of AMD, including smoking, exposure to UV and blue light, chronic infections and improper diet, could be related to oxidative stress, but it is not known if oxidative stress is a part of occurrence or consequence of the disease or both. In any case, reduction of stress can be extremely important in the prevention and therapy of AMD.

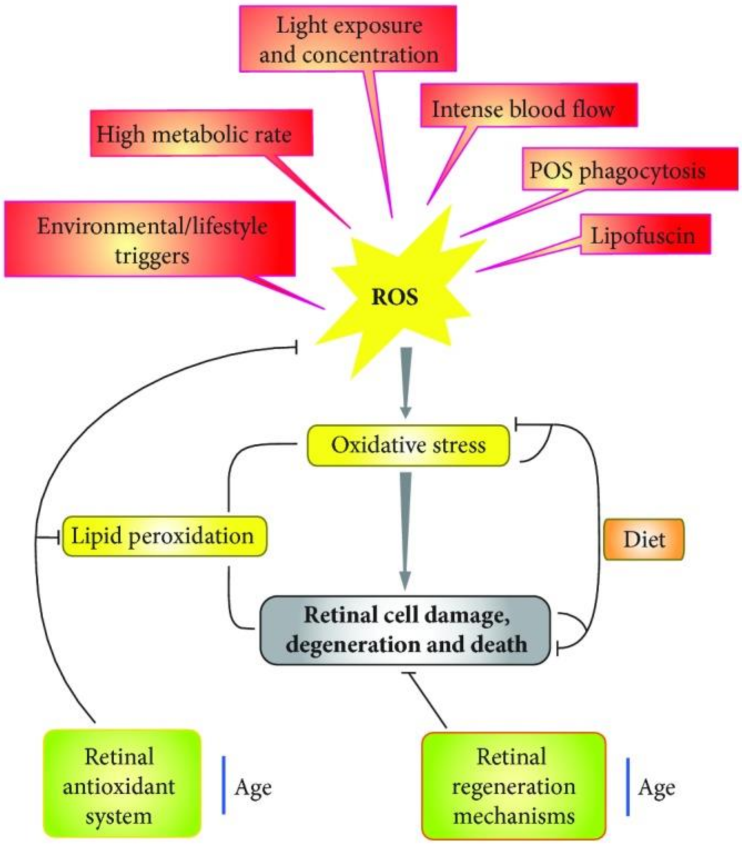


Fig. 2. Risk factors, oxidative stress and the impact of retinal damage in pathogenesis of AMD with representation of defense mechanism; influence of proper diet, retinal antioxidative system and retinal regeneration mechanisms, which decrease with age (Pawlowska et al., 2019)

Antioxidants in retina

Retina has more elements of antioxidant defense; vitamin C and E, carotenoid, lutein and zeaxathin are often considered the most important (Carneiro et al., 2017).

Vitamin C and E are well-known antioxidants.

Both vitamins contribute to the reduction of retinal epoxy adduct as well as the prevention of damages caused by blue light (Sparrow et al., 2003).

Vitam E or α-tocopherol can prevent chain reaction of ROS during the attack on the cell membranes. In order to stabilize ROS, α-tocopherol is turned to α-tocopherol radical which has stable and not reactive form. α-tocopherol radical can be regenerated in its original form with reactions involving vitamin C, glutathione and lipoic acid. Antioxidant capabilities of α-tocopherol depend on the concentration of those compounds which maintain α-tocopherol in reduced state in cases of oxidative stress. It is possible that ROS over-production can cause significant decrease of active vitamin E concentration in tissue. α-tocopherol is discovered in lens, aqueous humour and retina (Penn et al., 1992).

Carotenoid lutein, zeaxathin and meso-zeaxanthin form macular pigment (MP). Their role is to act as antioxidants and protectors of photoreceptors (rods and cones) from blue light (Lawrenson et al., 2019).

The biggest concentration of carotenoid is in the plexiform layer of macula; zeaxathin dominates in the inner part of the macula - fovea, while lutein is spread in the peripheral part of retina (fig. 3). This is the reason why zeaxanthin is more efficient as an antioxidant in the area where risk from oxidation is more likely to happen (Handelman et al., 1988). The concentration of zeaxanthin in fovea is 2.5 times greater than lutein (Widomska et al., 2020).Both carotenoids are present in photoreceptors outer segment: rods and cones which are rich with docosahexaenoic acid (DHA) and are potentially sensitive to lipid oxidation (Carneiro et al., 2015). DHA composes 60% of photoreceptors lipidic membrane and represents a main structural lipid of retina (Souided et al., 2013). DHA can be synthetized from eicosapentaenoic acid (EPA) and its main source comes from diet. Health benefits come from the ability of DHA and EPA to reduce the production of inflammatory eicosanoids, cytokines and ROS (Calder, 2009) and modulation of many gene expression which are involved in inflammation (Bouwens et al., 2009). Anti-inflammatory effects can inhibit the production of new choroidal vessels which can be visible in exudative AMD (Querques et al., 2014). Omega-3 fatty acids (EPA and DHA) are delivered to photoreceptor membranes through RPE. Every disbalance in the lipids of photoreceptors can contribute to the production of drusen in the layers of RPE (Carneiro et al., 2017) which are the beginning of changes related to AMD.

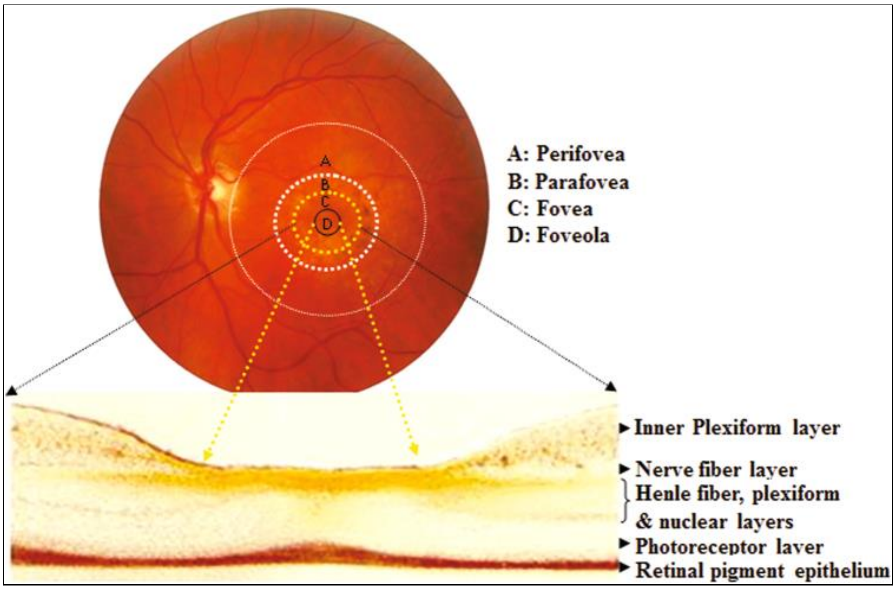


Fig. 3. Topography of macular pigment, schematic view of the distribution of yellow macular pigment on retina: horizontal (up) and vertical section (down) (taken from Arunkumar et al., 2018)

Supplements and AMD

There is evidence from randomized clinical studies that prophylactic intake of antioxidants in the form of vitamins and minerals does not prevent the development of AMD. Five big randomized clinical studies compared the intake of supplements containing vitamin E, beta-carotene, vitamin C or antioxidant combination of vitamins with placebo. The studies randomized more than 75.000 people and tracked their clinical results between 4 and 10 years. It is discovered that people who take the supplements have the similar risk of AMD development as those who do not take the supplements (Ewans et al., 2017).

Many randomized clinical studies also tackled the question of whether or not high dosages of antioxidants in the form of vitamins and minerals can slow down the AMD progression (Ewans et al., 2017). The majority of studies attracted small number of participants and were relatively short in duration, the span from 9 months up to 6 years. However, one big, multicentricrandomized clinical study conducted in the USA, age-related eye disease study -AREDS, randomized 3640 individuals with AMD and had them take supplements to the formulations which contain combination of vitamin C, E, beta-carotenes, zinc or copper or placebo each day. The main conclusion was that daily, long-term supplementation of vitamin C (500 mg), vitamin E (400 international units (IU)), beta-carotene (15 mg), zinc (80 mg, in the form of zinc oxide) and copper (2 mg, in the form of copper oxide) decreases the relative risk of AMD progression to the late stage (Lawrenson et al., 2019). The result achieved with patients who joined the study at later stages of diseases, to be more precise intermediate stage, up to more advanced stages, is 25% progression decrease in 5 years, while the effect on the patients in the early stages is still unknown. Besides AREDS, Report No8 concludes that patients with noncentral geographic atrophy also have the benefits of taking antioxidants and zinc.

Since it is established that smokers have increased incidence of lung carcinoma, the next AREDS2 study showed that removing beta-carotenes or lowering the dosage of zinc in the formula does not have the impact on the progression to the late stage of AMD (ČajkušićMance et al., 2019). Increased incidence of lung carcinoma is recorded with patients who took AREDS formula as opposed to ones who took AREDS2 formula, especially with former smokers due to high dosages of beta-carotenes. Primary AREDS2 analysis showed that adding lutein and zeaxanthin and/or omega-3 fatty acids in the AREDS formula is not linked to significant reduction of risk of late-stage AMD progression in comparison to the original supplement (AREDS formula). Lutein and zeaxanthin are carotenoids which are main components of macular pigment. They have protective role in retina due to their antioxidant properties and abilities to act as filters of blue light. The analysis of AREDS2 suggested that lutein and zeaxanthin can be useful in the reduction of AMD progression if they are taken without beta-carotenes (Lawrenson et al., 2019).

Lutein, zeaxanthin and meso-zeaxanthin form macular pigment (MP). Lutein is precursor of meso-zeaxanthin and recently RPE65 is identified as an isomerase enzyme responsible for conversion of lutein to meso-zeaxanthin in RPE of vertebrate (Shyam et al., 2017). MP's primary function is lowering the dispersion of blue light in inner retina, and dark yellow color and anatomic position of MP are considered to be ideal for protection of foveal region from photo-oxidative damages (Arunkumar et al., 2018). Lutein filters blue light more efficiently than zeaxanthin and meso-zeaxanthin due to its orientation in lipid bilayer (Sujak et al., 1999). Many studies speak in favor of protective antioxidant abilities of carotenoid which are part of MP. Barker et al. (2005) in their study conclude that primates which are, from their birth, fed with food without carotenoid, are more prone to damages resulting from blue light. Photoreceptor cells are more prone to oxidation in comparison to other cells of retina because they contain a lot of polyunsaturated fatty acids in the membrane structure and lean on carotenoid in protection of oxidation stability. Chucair et al. (2007) showed that retinal neurons in rats which were treated with macular carotenoids have greater protection from oxidative stress then untreated group. With identification of lutein and zeaxanthin oxidative metabolites in retina, Khachik et al. (1997) further support the function of macular pigment in oxidative protection of retina.

Previous studies showed that individuals who are using computers or smartphones longer than 6 hours per day had the benefits from supplementation of carotenoids, not only in increased visual efficiency but also in better quality of sleep, reduction of headaches, eye fatigue and photophobia. Thus, supplementation of macular pigment in certain form can be beneficial even to those who are not suffering from AMD (Arunkumar et al., 2018).

The benefits of polyphenols in AMD include other antioxidant mechanisms besides removal of ROS. Dietary polyphenols (phenols) are numerous and heterogeneous groups of chemicals found in plants and beverages. They can be categorized in several groups and according to chemical structure, can be divided into following groups: phenolic acids (benzoic and cinnamic acid), flavonoids (isoflavonoids, neoflavanoids, chalcones, flavones, flavonoids, proanthocyanins, anthocyanidins). Other bioactive polyphenols, also important for the health of humans, are curcumin, resveratrol and ellagic acid (Pawlowska et al., 2019). Polyphenols show effects against aging, and therefore can be considered as preventative compounds in diseases related to aging which are usually induced by chronic oxidative stress and accumulation of their products (Khurana et al., 2013).

Bioavailability of polyphenolic compounds in human eye can be high. In perfusion system of rats in situ, bioavailability of quercetin was 20% of applied dosage (Crespy et al., 2003). High bioavailability of anthocyanins is recorded in ocular tissues after different ways of applications (Kalt et al., 2008). These and other reports suggest that flavonoids, including quercetin and anthocyanins can surpass the blood-retina barrier after oral application, as well as after acute intravenous and intraperitoneal application (Pawlowska et al., 2019).

So far, no therapy has been approved or confirmed for the treatment of geographic atrophy of severest form of "dry" AMD which represents subtype of late-stage AMD, while wet form of AMD is treated moderately successful with inhibitors of vascular endothelial growth factor (anti-VEGF). Multiple studies are currently searching for effective way of treating dry form of AMD.

In connection to that, it is known that quercetin inhibits choroidal neovascularization (CNV) induced by laser radiation in the eyes of rabbits in vivo and in vitro (Zhuang et al., 2011). This flavonoid improved the blood flow of choroidea and reduced migration of umbilical vein endothelial cells during the wound healing. Therefore, it can be considered that quercetin improves anti-VEGF therapy which is the only effective treatment of wet form of AMD (Sulaiman et al., 2014). Patients suffering from wet form of AMD receive a number of intravenous injections against VEGF but there are signals that anti-VEGF treatment can potentially increase the development of geographic atrophy (Gementzi et al., 2017). The most used Bevacizumab (Avastin) has side effects because it neutralizes all VEGF extracted from RPE cells, which leads to disbalance in RPE homeostasis (Ford et al., 2011). Namely, VEGF is necessary for maintenance of retinal structure. Subramani et al. (2017) proved that combined activity of Bevacizumaba and resveratrol only partially neutralized extracted VEGF in ARPE-19 cells in comparison to cells which were treated individually with Bevacizumaba, when it came to neutralization of all VEGF. Resveratrol also showed synergistic protective effects together with zeaxanthin (Pawlowska et al., 2019). Park et al. (2017) state that antiapoptotic effect of curcumin extract (*Curcuma longa L., Zingiberaceae*) and its curcuminoids on cytotoxicity caused by exposure of ARPE-19 cells previously filled A2E to blue light. Similarly, polyphenolic components of *Vaccinium uliginosum* extracts containing flavonoids, anthocyanidins, phenylpropanoids and iridoids decrease ARPE-19 cell death caused by A2E photooxidation (Lee et al., 2016). *Vaccinium uliginosum L., Ericaceae* represent black or bog blueberry. Anthocyanidins in wild blueberries contribute to health of eyes, and cyanidin-3-glucoside in blueberries represent functional food for prevention of diseases related to retina (Wang et al., 2017). Many studies showed the protective effect of polyphenols found in blueberries on the retinal damages induced by light with lipid peroxidation (Ma et al., 2018).

Average level of zinc in the complex RPE - choroidea in patients suffering from AMD is reduced by 24% in comparison to others without AMD. Average level of copper in the complex RPE - choroidea in patients suffering from AMD is reduced by 23% in comparison to others without AMD, even though there was no difference in the levels of zinc or copper in the retina of people which have and don't have AMD. These results in combination with other information that oral supplementation of zinc and copper decreases the risk of AMD progression, suggests that homeostasis of these minerals play a role in AMD and retinal health (Erie et al., 2009).

Diet and AMD

Mediterranean diet, rich with whole grains, fresh season vegetables and fruits, beans, olive oil, nutty fruits, fish, herbs and red wine is tested in randomized controlled clinical research in order to show the beneficial effects in mitigation of cardiovascular diseases. It is discovered that Mediterranean diet is linked to a reduce risk of suffering from AMD. It is known that specific nutritious substances such as lutein and zeaxanthin found in leafy green vegetables, such as kale, are linked to a reduced risk of suffering from AMD. Fish is another important component of Mediterranean diet linked to a reduced risk of suffering from AMD and it is recommended to have fish two times per week (Chew, 2020).

Western diet is linked to a greater prevalence of AMD. Vegetable oils and animal fats containing omega-6 fatty acids, and red and refined meat needs to be consumed minimally in order to reduce the risk of AMD progression. Diet with high glycemic index and alcohol consumption of more than two drinks per day showed connections with AMD (Chapman et al., 2019).

Examination of the fundus which corresponds to the intermediate stage of AMD will in 50% of cases, during the next five years, and in 71% of cases, in the next ten years, advance to the late stage of AMD (Čeklić et al., 2015). Owing to the protective effect of vitamin C, E, carotenoid; lutein and zeaxanthin, polyphenols, zinc and copper, which can be taken with diet and above all Mediterranean diet and/or dietary supplements can reduce the risk of AMD progressing to late stages.

The recommendation regarding Mediterranean diet are intake of food rich with antioxidants and biologically active components, and reduced intake of saturated fatty acids and ingredients with higher glycemic index.

Biologically active components of food are non-nutritive and nutritive ingredients, and they have beneficial effect on health if consumed moderately. These include already mentioned polyphenolic compounds; resveratrol found in the grape skin, red wine, blueberry, raspberry and mulberry, quercetin found in onion and apples, curcumin found in ginger, safflower, curcumin, anthocyanins in blueberry (Jašić, 2010). Their positive effects are especially visible in neovascular AMD whether supporting the effects of anti-VEGF therapy such as quercetin or decreasing side effects caused by anti-VEGF therapy in case of resveratrol (Pawlowska et al., 2019). Besides polyphenolic compounds, under biologically active components we will include carotenoid, beta-carotene found in sweet potato, carrot, leafy green vegetables, apricot, peaches then xantofils; lutein found in goji berries, spinach, kale, eggs; zeaxanthin found in corn and eggs. Omega-3 fatty acids are also included in biologically active components, and their main sources are blue fish (pilchard, mackerel, salmon, cod, tuna), seeds (flex seeds, chia and sesame seeds) and nutty fruits (nuts, hazelnut, almond) (Jašić, 2010).

Oxidative stress which has an important role in pathogenesis of AMD can be increased with improper diet (Pawlowska et al., 2019).

Adherence to Mediterranean diet is connected to reduced risk of drusen growth which can have a beneficial effect in all stages of AMD.Variable factors, such as healthy diet, could play and important role in delaying the progression of early stages of AMD (Merle et al., 2020).

This would suggest that it is never too early or too late to accept healthy diet such as Mediterranean one. It is especially beneficial for geographic atrophy for which we do not have proven therapy and because AREDS2 supplements have beneficial effect on persons with neovascular AMD.

It appears there is a dosage-answer relationship; therefore, the more we adhere, the greater reduction of progression to late stages of AMD (Chew, 2020).

Diet rich with polyphenols has beneficial effects on many factors of risk related to AMD such as obesity, hypertension and hypercholesterolemia and it is proven that healthy diet reduces genetic risk of obesity (Pawlowska et al., 2019).

Choosing Mediterranean diet and physical activity and avoiding smoking and sedentary lifestyle can reduce the prevalence of early AMD, number of individuals developing advanced AMD and consequently reduce the burden on healthcare system which is caused by the treatment of this disease (Carneiro et al., 2017).

Study conducted in Japan proves the effects of diet. It involved 161 subjects with neovascular AMD and 369 subjects pertaining to control group. Subjects filled out a questionnaire about how frequent they consume 58 ingredients in the last month. Analysis of their dietary habits proved many claims about the connection between frequency of consumption of omega-3 fatty acids, vitamin E, zinc, vitamin D, vitamin C and beta-carotenes and neovascular AMD.

They proved reversed connection between consumption of omega-3 fatty acids and neovascular AMD. However, not a single study researched the effect of higher dosage intake of omega-3 fatty acids than those included in typical Japanese diet on neovascular AMD. Blue Mountains Eye Study (Australia) showed the protective effect of omega-3 fatty acids in late AMD, among those with highest quintile intake. Participants in AREDS which reported highest intake of omega-3 fatty acids also had significantly lower probability for neovascular AMD. However, in AREDS2, adding DHA + EPA to original formulation has not additionally reduced the risk of AMD. As already discussed, the cause of that could be inadequate dosage or inadequate length of treatment or both (Aoki et al., 2016).

In Japan, there was a descending trend between zinc intake and neovascular AMD which further supported the claim about protective effects of zinc on neovascular AMD. It should be noted that high dosage of zinc (80 mg zinc oxide), which can only be achieved with supplementation, in AREDS is enough to reduce the risk of neovascular AMD progression.

The same study showed that the vitamin E and vitamin C intake is linked to reduced risk of neovascular AMD. However, it appears that there is a limit and further studies which would involve more subjects in order to determine whether or not vitamin C is enough to reduce the risk of neovascular AMD are needed. AREDS showed that high dosage of vitamin C and E and beta-carotenes is not enough. It is required to add zinc in order to reduce the risk of neovascular AMD and the dosage of 400 IU of vitamin E recommended by AREDS is 13 times higher than the recommended daily allowance -RDA. Such levels of vitamin E can only be reached with supplementation (Aoki et al., 2016).

It is discovered in several studies that vitamin D reduces the risk of early AMD and has anti-inflammatory properties (Pahlet et al., 2013). Numerous genes included in inflammatory response are connected with AMD. Current study showed that vitamin D is linked to neovascular AMD. Fish is a source of vitamin D as well as omega-3 fatty acids (Nakamura et al., 2002), therefore increased intake of fish can be confusing factor because it is not known whether the positive effect comes from vitamin D or omega-3 fatty acids.

Lastly, by using the short questionnaire about the dietary history, they showed that the high intake of omega-3 fatty acids, vitamin E, zinc, vitamin D, vitamin C and beta-carotenes is linked with reduced risk of AMD (Aoki et al., 2016).

Conclusion

AMD is an important cause of vision loss globally. Therefore, it is really important to recognize the risk factors which can be influence on such as smoking, obesity, atherosclerosis and improper diet. Proper diet, above all Mediterranean diet rich with antioxidants, minerals, vitamins and polyphenols has certain beneficial effect on prevention and progression of AMD, which is the reason consultations regarding proper diet and supplementation is needed during eye examination of people at risk.

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COMMONLY USED ARTIFICIAL SWEETENERS IN EUROPE

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review paper

Summary

It has been known for many years that the excessive consumption of sugar (sucrose) has harmful effects on human health. This fact led to a reduction in sugar consumption and the appearance of artificial sweeteners in the 1800s. The first low-cost and low-calorie sugar alternative was saccharin. Since this sweetener gained great popularity, other artificial sweeteners soon followed, including aspartame, acesulfame-K and cyclamates as the most common ones. As the result of a sharp rise in the obesity pandemic in all populations and ethnic groups, a demand for sweeteners with a minimum caloric value has increased dramatically in the last decade as consumers care more about their health. Due to the different regulation of permitted artificial sweeteners in United States (US) and Europe (EU), there are some controversies and suspicions about the relationship between certain sweeteners and a potential health risk. Despite doubts about the safety of artificial sweeteners, many studies have shown the absence of dangers associated with their use (if used in the acceptable daily intake, ADI). Therefore, artificial sweeteners today are considered as safe for consumption by many competent institutions and organisations. Nowadays, artificial sweeteners are fundamental in the food industry and present in many foodstuffs.

Keywords: Acesuflame - K, Aspartame, Cyclamate, Neotame, Saccharine, Sucralose

Introduction

All sweeteners can be classified into nutritive and intensive non-nutritive sweeteners (known as artificial sweeteners) (Fig. 1). Intensive sweeteners have a high sweetening power (much higher than sucrose) so they are used in small amount in order to replace the sweetness of sucrose. They are called non-nutritive sweeteners due to their caloric contribution which is very low or even zero (AL-Ali and AL-Hilifi, 2021; Carocho et al., 2017; Godshall, 2007). More recently, some sweeteners of natural origin have been discovered and classified as intensive non-nutritive sweeteners. Most commonly, they are present as derivatives of various plants, such as steviol glycoside (approved both in USA and EU) and Luo Han Guo fruit extracts-from monk fruit (approved in the USA) (Mooradian et al., 2017; Pearlman et al., 2017; Purohit and Mishra, 2018). Accordingly, intensive sweeteners can be of natural or synthetic origin (Carocho et al., 2017; Schiano et al., 2021).

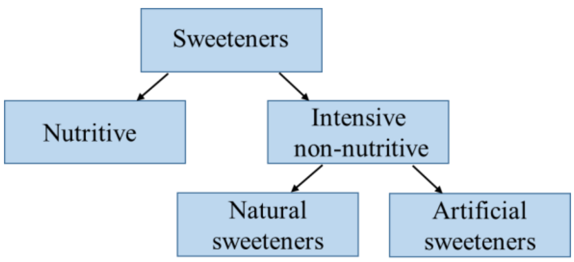

Fig. 1. Classification of sweeteners

(Carocho et al., 2017; Schiano et al., 2021)

Artificial sweeteners became popular during the First World War, due to the agricultural crisis that led to reduction in sugar production. Some types of artificial sweeteners were known even before that time, but were used in much smaller quantities. Later, in the late 1990s, a series of events occurred that increased demand for low-calorie products with reduced or no sugar content. Most artificial sweeteners are synthetic preparations, have nothing in common with the sugar molecule and were discovered by accident. The beginnings of research and production of artificial sweeteners were focused on copying the characteristics of sugar molecules that would stimulate the taste of sweetness, but these experiments were not successful. Therefore, most artificial sweeteners were obtained as a by-product of chemical experiments in some other research unrelated to artificial sweeteners. Artificial sweeteners as we know today have the ability to bind to the same receptors on taste buds as sucrose, thereby triggering and enabling a sense of sweetness. In addition, intense marketing campaigns within the food industry helped in the promotion and revolution of artificial sweeteners (Mooradian et al., 2017; O'Brien-Nabors, 2016; Purohit and Mishra, 2018).

World health organization (WHO) studies have shown that metabolic disorders are so common that they are considered as "epidemic in scale" in industrialized countries, with diseases associated with sugar consumption in the rise (Pradhan, 2007; Rani et al., 2016). Furthermore, today`s lifestyle and market offers have caused changes in diet increasing risk

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factors for metabolic diseases such as diabetes, obesity, hypertension, metabolic syndrome among others (Costa et al., 2019; Scognamiglio et al., 2019). Due to that, prevalence of diseases associated with sugar consumption have increased and sweeteners became widespread in food products (Kim et al., 2017; Mooradian et al., 2017; Philippe et al., 2014).
Since they became available on the market, artificial sweeteners have been considered one of the most significant achievements in the food industry. Despite that, due to different regulations and laws among countries, there are some doubts about the confidentiality of these molecules as foods in human consumption (Basílio et al., 2020). Today, sweeteners have been extensively researched, regarding their sweetening potential, as well as their effect on the health of consumers, economy and society (Mooradian et al., 2017).
Sweeteners have been the subject of controversy for years due to the conflicting opinions. On the one side, there are allegations about the toxicity of some sweeteners to the liver and bladder, about their carcinogenicity, possible influence on fetal malformations, along with other hazards, contrary to the many studies that refuted the relationship between the consumption of artificial sweeteners and the potential diseases (Carocho et al., 2017; Saraiva et al., 2020). Thus, all risk claims were investigated and artificial sweeteners have been defined as safe to use, but there is still some questionable confidence in them as some artificial sweeteners are allowed in the Europe, while in the United States they are banned (Table 1) (Lobach et al., 2019; Farhat et al., 2019; Nichol et al., 2019; Serra-Majem et al., 2018).
In addition to the impact on health as a criterion for choosing the food used, consumers choose food according to sensory properties, so consumers are increasingly resorting to foods that consist of artificial sweeteners because they want (unchanged) sweet taste with lower or even no caloric value. As a result, consumers and food producers are showing increasing interest in food sweeteners that replace sucrose in food, improve the taste of food and at the same time reduce the caloric value of food and the risk of caries (Godshall, 2007; Sorensen et al., 2003; Whitehouse et al., 2008). The criteria for selecting a sweetener are the influence on the aroma of the product and the relative sweetness of the sweetener. In addition, sweeteners should be easy to produce, store and transport and should not be too expensive (O'Brien-Nabors, 2016).
Artificial sweeteners are defined as food additives that give a sweet taste, and known as low-calorie or non-caloric sweeteners (Lohner et al., 2017). Although having similar taste to the sucrose, artificial sweeteners have much higher sweetening power that can vary from a few dozen to a few hundred times sweeter than sucrose, and yet, having negligible caloric value (Bellisle and Drewnowski, 2007; Frank et al., 2008; Hunter et al., 2019; Mueller et al., 2015).

Table 1. Intensive sweeteners approved in the USA and EU (Mooradian et al., 2017; Schiano et al., 2021)

Name	Origin	Number of times sweeter than sucrose	ADI by the US FDA (mg/kg)	ADI by the EU EFSA (mg/kg)
Acesulfame-K	Artificial	200	15	9
Advantame	Artificial	20000	32.8	5
Aspartame	Artificial	200	50	40
Cyclamate	Artificial	30-50	Not approved for consumption	0-11
Neohesperidine DC	Artificial	300-2000	Not approved for consumption	5
Neotame	Artificial	700-1300	0.5	2
Saccharin	Artificial	200-700	15	5
Sucralose	Artificial	600	5	15
Steviol glycosides	Natural	200-400	4	4
Luo Han Guo fruit extracts	Natural	100-250	Not specified	Not approved for consumption

In addition to giving foods a pleasant sweet taste, sweeteners also improve the overall aroma of the product without added sugar and calories, which is important in specific diets (Martyn et al., 2016; Whitehouse et al., 2008). Artificial sweeteners are widely used in the food industry (in candies, beverages, chewing gums, jams, gelatines, bakery and many others foodstuffs). They can be used singularly or in combination with other sweeteners (Grembecka, 2015; Huvaere et al., 2012).
Acesuflame-K, aspartame, cyclamate, neotame, saccharin and sucralose are intensive sweeteners approved in the EU and recognized as the most widely used artificial sweeteners (Carocho et al., 2017; Mortensen, 2006; Whitehouse et al., 2008). This review will provide insight of those artificial sweeteners considering their characteristics,

invention, application, chemical composition and impact on consumer health.

Acesulfame-K

Acesulfame-K is the potassium salt of acesulfame (Fig. 2). As a sweetener, acesulfame-K was found in 1967 when chemist Karl Clauss accidentally noticed the sweet taste of substance from his finger during laboratory work (Clauss and Jensen, 1970). This sweetener is a white crystalline powder that is very soluble in water (Chattopadhyay et al., 2014). Acesulfame-K is over 200 times sweeter than sucrose with clear taste without residual aromas. Due to that, and the fact that it has no caloric value, this sweetener is one of the most commonly used artificial sweeteners. It is used in pastries, candies, frozen desserts, beverages, cough drops and mints (Carocho et al., 2017; Whitehouse et al., 2008).

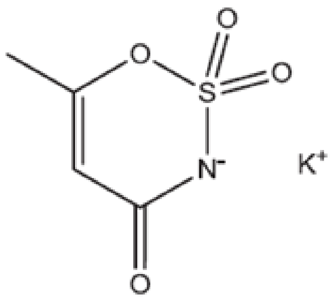

Fig. 2. Chemical structure of acesulfame – K (Carocho et al., 2017)

Acesulfame-K is thermally stable, which makes it suitable for use in baking and cooking (O'Brien-Nabors, 2002). If used alone in sweetening food and drink, it may have a bitter aftertaste wherefore it is often mixed with other sweeteners (usually aspartame or sucralose) (Horne et al., 2002). In the mixture, each sweetener masks the taste of the other and shows synergistic effects making the mixture sweeter than its components. Acesulfame-K cannot be metabolized in the human body and 95% of it excreted unchanged in the urine after its consumption. Therefore, it does not affect the energy and caloric value and potassium intake (despite the potassium content) (Chattopadhyay et al., 2014). In 1988, the use of acesulfame-K was approved in a variety of dry foods and alcoholic beverages.

Although many studies have shown its safety for human health, there have been studies that have indicated some kind of toxicity caused by this sweetener, but these studies have been later refuted (Carocho et al., 2014; Shankar et al., 2013). One degradation product of acesulfame-K called acetoacetamide has potential toxicity if ingested in very large quantities, but human exposure to this compound has been shown to be negligible so FDA has concluded that acesulfame-K is harmless and no further investigation is required (Chattopadhyay et al., 2014; George et al., 2010). Monitoring the effect of acesulfame-K sweetener intake and potential risks was performed mostly by cytogenetic studies in mice. Mukherjee and Chakrabarti (1997) examined certain doses of acesulfame-K on the cytogenetic changes in mice. When applying this sweetener in the dose of ADI there were no genetic changes compared to control mice, while much higher doses of acesulfame-K showed clastogenic and genotoxic properties. In the conclusion, depending on the dose, acesulfame-K may interact with DNA and create genetic damage. According to the recommendation for further research of this problem, later studies confirmed the same conclusion – the negative effect of acesulfame-K is observed only in doses significantly above ADI (Whitehouse et al., 2008). Sylvetsky et al. (2011) reported presence of acesulfame-K in the breast milk of breastfeeding mothers after its consummation, but there are no studies showing the effect on breastfed infants (Sylvetsky et al., 2011). Furthermore, Uebanso et al. (2017) investigated effects of maximum ADI acesulfame-K on the gut microbiome in mice, compared to sucralose intake. Both sweeteners did not show an increased food intake, body weight or organ fat. Furthermore, consumption of acesulfame-K did not change relative amount of faecal microbiomes (Uebanso et al., 2017). Recent studies showed acesulfame-K as a safe substance that is not cytotoxic, carcinogenic or teratogenic (Fowler, 2016; O'Sullivan et al., 2017; Tian et al., 2020).

Aspartame

Aspartame was found in 1965 while studying new ways to treat stomach ulcers using a tetrapeptide normally produced in the stomach. During the synthesis of this tetrapeptide, the intermediate aspartyl-phenylalanine methyl ester is formed. This compound accidentally ended up on pharmacist's taste buds who noticed the sweet taste of the compound (Mazur, 1984). The first approval of aspartame by the FDA was in 1981 when approved as a table-top sweetener, whereupon in 1996 it was approved for general-purpose in all foods and beverages (Whitehouse et al., 2008). Since then, aspartame has been recognized worldwide as it is used by large number of consumers in more than

6000 products (Butchko and Stargel, 2001). Aspartame has a pure sweet taste and its sweetness is 200 times greater than sucrose. It can be found in a wide range of foodstuffs: in sweets, drinks, chewing gums, frozen desserts and yoghurt, gelatines, dessert mixes, puddings and fillings, table-top sweeteners and certain medicines (vitamins and cough drops) (Chattopadhyay et al., 2014; Whitehouse et al., 2008).

Aspartame contains two amino acids, phenylalanine and aspartate (Fig. 3). It can form methanol by hydrolysis in strongly acidic or alkaline conditions. Furthermore, it is easily soluble in water at room temperature with increasing solubility even with lower or higher pH as well as with elevated temperature. Maximum stability of aspartame in aqueous solution is at pH 4.3 (Mazur, 1984). Aspartame is unstable on higher temperatures so it does not tolerate heating and therefore cannot be used in cooking or baking. Also, it is unstable during storage because it decomposes in liquids (Chattopadhyay et al., 2014).

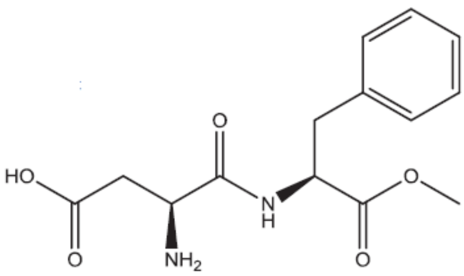

Fig. 3. Chemical structure of aspartame (Carocho et al., 2017)

The metabolism of aspartame can result in formation of formaldehyde, formic acid and diketopiperazine, which makes its safety questionable (George et al., 2010; Kroger et al., 2006). Due to that, aspartame is by far the most controversial artificial sweetener. Although some research demonstrates potential carcinogenic properties of this sweetener, such studies were performed on rats so their association with the human health is not considered relevant. One study (Ferland et al., 2007) examined the influence of aspartame consumption on glucose and insulin levels in male subjects with type 2 diabetes during acute exercise. Results showed that aspartame intake through breakfast simulates the rise in glucose and insulin similar to sucrose intake. According to these results, it is not recommended for diabetics to continue intake of this sweetener as it affects the glucose levels similar to sucrose. Gallus et al. (2007) have been researching the association of artificial sweeteners with cancer risks. Reviewing some case studies, they pointed out the existence of a connection between brain cancer and the use of aspartame. However, the hypothesis of this study has not been confirmed in animal or human studies (Ferland et al., 2007; Gallus et al., 2007; Whitehouse et al., 2008). Furthermore, some published studies showed the correlation between aspartame consumption and migraines. In an experiment with subjects aged 40, 32 and 26 years, migraine occurred as a consequence of using chewing gum with aspartame as a sweetener. This conclusion was reached and confirmed as migraine was alleviated in all subjects when they stopped taking aspartame through chewing gum (Blumenthal, 1997). Headache and/or migraine have been shown to be one of the most common side effects related to the use of aspartame, but it is important to note that this side effect rarely occurs after a single dose of this sweetener (Jacob and Stechschulte, 2008; Lindseth et al., 2014; Lipton et al., 1989; Sun-Edelstein and Mauskop, 2009). The aspartame molecule consists of methanol, aspartic acid and phenylalanine. Since phenylalanine is a controversial substance in people with phenylketonuria, consumption of aspartame is prohibited for such consumers. However, it should be considered that this amino acid is not metabolized equally in rodents and humans, so only the results obtained in studies with primates can be taken into account. Several studies with human subjects showed that despite an increase in phenylalanine due to consumption of a certain dose of aspartame (2-100 mg/kg), there was no visible effect on cognitive-behavioural abilities (Carocho et al., 2017; Lohner et al., 2017; Whitehouse et al., 2008). Many government and advisory organizations proclaimed aspartame safe for human consumption in more than 90 countries (Magnuson et al., 2007). The European Food Safety Authority (EFSA), as the reference institution for food safety, conducted rigorous analyses of various studies on animal and human models on the health effects of aspartame, after which it reached a final conclusion on the use of aspartame and its daily intake. EFSA concluded that aspartame was safe to use if administered up to the prescribed value of ADI, which is 40 mg/kg body weight per day. Experts claim that this intake does not increase the risk of cancer, damage to the nervous system and brain function, nor does it affect the behaviour of adults and children, as indicated by some previous studies (Fitch and Keim, 2012; Kirkland and Gatehouse, 2015; Martyn et al., 2018).

Cyclamate

Although cyclamate was found back in 1937, it was used as a sweetener (in the US) from 1950 to 1969 when US FDA revoked the status of GRAS (Generally Recognized As Safe) for this sweetener, and in 1970 completely banned it. This ban was based on a study linking the metabolism of cyclamate to a toxic compound named cyclohexylamine. Later studies showed that this metabolism is related only to a small population, but this fact was not enough for the FDA to lift the ban. In 1982, one study showed that a mixture of saccharin and cyclamate had caused cancer in laboratory rats, but after reviewing scientific evidence FDA's Cancer Assessment Committee infer that cyclamate has no carcinogenic properties. This finding was confirmed in 1985 by the National Academy of Sciences. Consequently, this sweetener is the best exemplar of legislative differences between the EU and the US since the use of this sweetener in food is permitted in EU, but banned in US (Chattopadhyay et al. 2014; Fitch and Keim, 2012; Renwick et al., 2004).
Cyclamate is a salt of cyclohexyl sulphuric acid (Fig. 4) that can be used in food in two forms, as sodium cyclamate and calcium cyclamate. Both forms of cyclamate show good stability at low and high temperatures. Sodium cyclamate is used as an artificial sweetener and the analogue calcium salt is used mostly in low sodium diets. It is soluble in water, which can be enhanced by preparing sodium or calcium salts (Bopp et al., 1986; Chattopadhyay et al., 2014; Fitch and Keim, 2012).

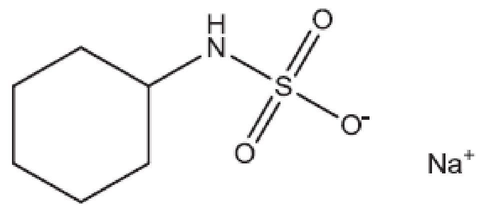

Fig. 4. Chemical structure of Na-cyclamate (Carocho et al., 2017)

The largest producers of this sweetener are China, Indonesia, Taiwan and Spain. Production of this sweetener is the cheapest along with saccharin (O'Brien-Nabors, 2001). In the EU, the recommended daily intake is 11 mg/kg of body weight. Cyclamates are used in baked and processed foods, in many desserts, soft drinks, gelatines, canned fruit, and as a table-top sweetener (Carocho et al., 2014). Cyclamates have no energy value. Although sweetening capacity of cyclamates is 35-50 times greater than sucrose, its disadvantage is mild sour taste. Therefore, it is usually combined with other artificial sweeteners, mostly with saccharin, pointing good sweet synergy. In addition, in such mixtures cyclamate has the property of long-lasting sweetness since mixing with saccharin rejects its sour taste. Thus, a mixture containing 1% saccharin and 99% cyclamate proved to be very suitable for use in human nutrition (Martins et al., 2010; Mitchell, 2006; Renwick et al., 2004; Roberts, 2016).
According to some studies, the compounds that are formed by the breakdown of cyclamate in the intestines under the influence of bacterial flora are cyclohexamines that are carcinogenic, and in some cases they have caused bladder and kidney cancer in the examined rats. Subsequently, further studies did not confirm the association between cyclamate and tumor formation in humans leading to the conclusion that the mechanism of tumor formation due to cyclamate is specific exclusively to animals (Fitch and Keim, 2012). Furthermore, there was a suspicion that cyclamates cause infertility, so a study was conducted that monitored the effect of cyclamates on testicular atrophy in humans, which did not prove any association between infertility and elevated concentrations of cyclamates and cyclohexamine in humans (Serra-Majem et al., 2018). The association of cyclamate with hypertension and tachycardia was tested in study with volunteers. This study showed that the concentration of cyclamate and cyclohexamine did not affect the occurrence of these problems. Consequently, global opinion on cyclamates is that they are not dangerous to human health if consumed in the recommended amounts. This view is not taken only by the competent US institutions, where cyclamates are still banned, but their use has been approved in 55 countries, which speaks volumes about the safety of use of this sweetener (Carocho et al., 2017; Fitch and Keim, 2012).

Neotame

Neotame is the latest artificial sweetener discovered in the 1980s. It is a derivative of aspartame, obtained by reductive alkylation of aspartame. Thus, it has a very similar chemical structure to aspartame (Fig. 5), aspartame and neotame are isomers, respectively. The FDA approved this sweetener in 2002 and it is currently the strongest sweetener available on the market with a sweetening power of 7000 to 13000 times greater than sucrose. In addition, the advantage of neotame is that it has no calories despite its high sweetness. Neotame is approved as a general-purpose sweetener, except in meat and poultry. It is used in pastries, soft drinks, frozen desserts, processed fruits, chewing gum, glazes, jellies, jams, gelatines, puddings, additives and syrups (Mayhew et al., 2003; Whitehouse et al., 2008). Neotame has a pure taste, without sour or metallic taste or after taste. Despite that, neotame is mostly mixed with some other sweeteners (except with acesulfame-K and saccharin). Since neotame does not contain phenylalanine in its composition, it is safe for patients with phenylketonuria and for diabetics (Carocho et al., 2017). The range of uses of neotame is wide; it is added to drinks, lemon tea, sauces, chewing gum, yogurts. Furthermore, it is used as a table-top sweetener and to enhance natural flavours (mostly sour fruit flavours) (Zhu et al., 2016).

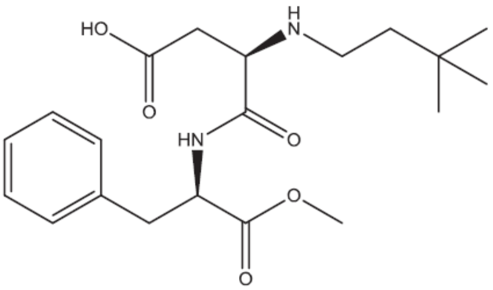

Fig. 5. Chemical structure of neotame (Carocho et al., 2017)

This sweetener is white, odourless crystalline powder that is not hygroscopic so it is stable in dry storage conditions. Regarding metabolism, half of the ingested neotame is excreted in the urine as an esterified neotame, while the other half is not absorbed. Neotame meets basic criteria for the commercial viability of a non-nutritive sweetener (taste, stability, solubility, safety and cost) (O'Brien-Nabors, 2016; Whitehouse et al., 2008).

In terms of safety, neotame, like the rest of the sweetener, has undergone a series of studies that have shown that even doses higher than its ADI are not associated with toxicity or any danger to the consumer. No adverse effects of neotame use have been reported in studies performed in mice and other experimental animals (O'Brien-Nabors, 2001; Nofre and Tinti, 2000; Zhu et al., 2016). Some studies related to neotame reveal changes in body weight in rats. It has been observed that these effects are not caused by neotame toxicity, but because of the tastelessness of the food containing this sweetener consumed by rats. Therefore, rats reduced their daily food intake, resulting in long-term weight loss and less weight gain (Carocho et al., 2017; Whitehouse et al., 2008). Many studies showed no adverse effects of neotame in mice and other experimental animals after physical and pathology examinations (Mitchell, 2006; O'Brien-Nabors, 2001; Nofre and Tinti, 2000; Zhu et al., 2016).

Saccharin

Saccharin was discovered back in 1878, which makes it the first artificial sweetener. It is found at Johns Hopkins University in Baltimore and like most artificial sweeteners, saccharin was discovered accidentally while working in the laboratory on some other issue (Chattopadhyay et al., 2014). Nowadays, this sweetener is produced by a process called *Maumee* and its production reaches industrial proportions. This process is named after the company that evolved this technique (Maumee Chemical Company) (Carocho et al., 2014). As the molecular saccharin is an aromatic organic compound that can be used in the two forms, as a sodium or calcium salt (Fig. 6) (Chattopadhyay et al., 2014). Saccharin is stable at low pH and at high temperatures, making it an ideal sweetener for use in the food production. In addition, it has the advantage of low cost (Charocho et al., 2017; Gupta, 2018; O'Sullivan et al., 2017). It has a sweet taste, but also slightly sour and bitter, so it is used usually in combination with cyclamate and aspartame. It may have 300 times more sweetening power than sucrose, but it has the lowest ADI of all artificial sweeteners (5 mg/kg of body weight) (Charocho et al., 2017; Gupta et al., 2018).

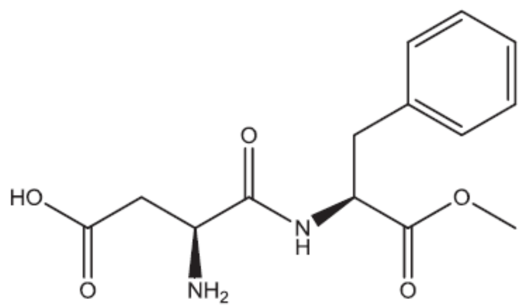

Fig. 6. Molecular structure of saccharin (Carocho et al., 2017)

Saccharin does not metabolize in the body but excretes in the urine after the consumption. Despite that, it is possible for saccharin to pass over the placenta of a pregnant woman and through breast milk, therefore it is not recommended for pregnant or breastfeeding women. Wide use of saccharin is in fruit juices, processed fruits, gelatines, jams, wraps, sauces, desserts, chewing gum, and table-top

sweeteners (Carocho et al., 2017; Chattopadhyay et al., 2014; O'Brien-Nabors, 2001). Saccharin can also be found in cosmetic products (lip-gloss and mouthwash), in vitamins and medications (Whitehouse et al., 2008).
The safety of saccharin consumption has always been questioned, as Canada banned its use in 1977 after some studies on animal showed toxic effect of extremely high doses of saccharin in rats (Chowaniec and Hicks, 1979). Subsequent research has shown that normal amounts of saccharin do not cause cancer in mice, monkeys, and humans (Zurlo and Squire, 1998). All studies that suggested the suspicion of saccharin consumption were based on the formation of tumors in rats, but due to the different anatomy between rodents and humans, the danger to humans is excluded (Carocho et al., 2017; Schiano et al., 2021). One research pointed that saccharin can have effect on the liver increasing the concentrations of liver enzymes. This study was conducted on elderly women with symptoms of chronic fatigue who were taking three medications of which two contained saccharin. Results showed increased liver enzymes during consumption of those medications and its reduction after taking them off (Whitehouse et al., 2008). Today, due to the numerous studies, saccharin is known to be safe for consumption, encouraging increasing use worldwide (Shankar et al., 2013). Also, consumption of saccharin showed very rare presence of side effects (Gupta, 2018). Furthermore, the FDA states that saccharin is not directly linked to cancer in humans and many studies confirmed that saccharin is safe for human consumption (Amin and Al Muzafar, 2015; Andrejić et al., 2013; Azeez et al., 2019; Basílio et al., 2020; Witehouse et al., 2008). Moreover, available data showed common practice at healthcare professionals of usage saccharin in patients with obesity or diabetes in order to reduce weight, as well as in practice of reducing dental cavities (Al Humaid, 2018; Lohner et al., 2020). Today, saccharin is used and approved worldwide (in more than 100 countries) (Schiano et al., 2021).

Sucralose

Sucralose is artificial sweetener that was also discovered accidentally. It was in 1976 when the British sugar company Tate & Lyle investigated many ways to use sucrose as a chemical intermediate. As one of the results of this experiment, halogenated sugars were synthesized and tested. During those tests, the graduate student misunderstood the requirement to "test" chlorinated sugar as a requirement for "tasting". This misunderstanding brought the discovery that many chlorinated sugars are not only sweet, but much sweeter than sucrose (Whitehouse et al., 2008).
Sucralose is obtained by industrial substitution of three hydroxyl groups in sucrose (Fig. 7). This transformation makes this molecule 600 times sweeter than sucrose. Profile of quality and intensity of sweetness of sucralose is very similar to the profile of sucrose. This sweetener has a pleasant sweet taste and modest synergy with other sweeteners (Olivier-Van Stichelen et al., 2019). Sucralose has good water solubility as well as stability over a wide range of temperatures and pH. When stored at high temperature, sucralose releases HCl and creates some sort of colour change (Arora et al., 2009; Chattopadhyay et al., 2014; Roberts, 2016).

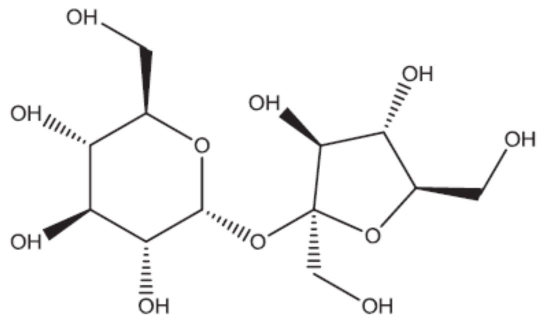

Fig. 7. Chemical structure of sucralose (Carocho et al., 2017)

Although it is made from sucrose, human body does not recognize sucralose as sucrose (sugar). Therefore, sucralose does not metabolize in human body and does not provide energy or calorie. The majority of ingested sucralose does not absorb and is secreted directly in the faeces, while 11 - 27 % is absorbed. Absorbed part is mainly removed by the kidneys from the bloodstream and excreted in the urine. Sucralose is present in many foodstuff; in ice cream, yogurts, canned fruit, caramels, biscuits, soft drinks, dairy products, bakery products, gelatine, jams, chewing gums and many others (Mitchell, 2006; O'Brien-Nabors, 2001).
Sucralose is an organochloride. Although some organochlorides are known to be significantly toxic (Patel et al., 2006), sucralose has been recognized as non-toxic (Olivier-Van Stichelen et al., 2019). Anyway, several studies pointed the potential mutagenicity of high concentrations of sucralose (Berry et al., 2016; de Oliveira et al., 2015; Eisenreich et al., 2020; Grotz, 2008; Sasaki et al., 2002). Furthermore, many preclinical and clinical trials have shown that sucralose has an effect on

levels of glucose and insulin (Pepino et al., 2013). Some studies pointed that sucralose has an effect on the microbial composition of the digestive system by reducing beneficial bacteria (Abou-Donia et al., 2008; Turnbaugh and Gordon, 2009). Recent review by Berry et al. (2016) showed that there is no possible association of sucralose consumption with cancer, even at higher doses. Furthermore, some earlier studies also showed that there is no risk of using this sweetener, and many human studies indicate the general safety of sucralose (Grotz and Munro, 2009). Systematic literature review provided by Fitch and Keim (2012) showed that sucralose does not have an effect on appetite in adults or weight gain in children and adolescents. In addition, this study pointed that, based on limited studies in humans, there is no association between untoward effects and sucralose consumption in the general population (Fitch and Keim, 2012). More than 110 human and animal studies were reviewed by FDA for determining the safety of sucralose. Consequently, FDA characterizes sucralose as safe for human consumption. Only in the case of excessive consumption, sucralose has possible links with migraines, intestinal problems, and colon inhibition (Carocho et al., 2017; Chattopadhyay et al., 2014; Patel et al., 2006).

Conclusion

Artificial sweeteners have long been in vogue and are now an integral part of many processed foods. They are useful substances because they not only provide the sweetness, but increase and enhance the taste of food without added sugar and calories, which is important in specific diets. They are used to control obesity and diabetes, as they provide sweetness in foods without added caloric values. Moreover, an additional advantage of artificial sweeteners over sucrose is that they do not cause caries, as they do not constitute a substrate for the development of bacteria. Although they can reduce calorie intake, their excessive consumption (greater than ADI) can cause certain side effects, so their use should be dosed with caution. Since most artificial sweeteners are not metabolized in the human body, they are considered as safe for consumption. However, there are still some doubts about these compounds due to conflicting data on the consumption of some artificial sweeteners. Nevertheless, both sides certainly agree on one thing - use of artificial sweeteners within recommended daily values is safe for the health of the consumer, therefore should pay the most attention to the amount of sweetener used.

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