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FOOD IN HEALTH AND DISEASE

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CASE STUDY: MOTIVATION, KNOWLEDGE, AND SUPPORT OF THE ENVIRONMENT AS FACTORS OF A SUCCESSFUL WEIGHT LOSS PROGRAM

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Summary

Obesity is one of the biggest public health problems in the world today. The reasons for weight gain in humans are found in the interaction of numerous biological, genetic and psychosocial factors. How to deal with this problem is the subject of numerous studies, and based on the still limited knowledge of weight loss methods, it is concluded that there is no method optimally effective for all individuals. It is considered that the individual approach is one of the best, which is why in this case study the individual approach was first used to monitor the diet and lifestyle of the respondent and then based on the identified irregularities, a weight loss program was developed. The respondent kept a diet diary and a record of life habits for 30 days. 12 critical eating habits and several life habits were identified, which were analyzed in great detail in the first phase of the research. The weight loss program was based on gradual changes in established critical habits, three selected days per week, while on other days the respondent could consume what she wanted. The program lasted six months, had freer access, and was without strict restrictions, which contributed to the high motivation of the respondent for weight loss from the beginning to the end. With the continuous supervision of the program mentor and the support of the environment, the respondent is from the category of obesity III degrees (BMI 40.89 kg/m^2) reduced body weight by 30 kg, and entered the category of overweight persons (BMI 28.49 kg/m^2). A year after the program, she managed to maintain the weight she had achieved. This work confirmed that an individual approach and a gradual change in eating and living habits are effective in the weight loss process.

Keywords: diet, individual approach to weight loss, lifestyle, obesity

Introduction

The problem of obesity today is one of the central problems of humanity, and as things stand for the future too. Nearly two billion people struggle with weight gain, of which 650 million are in the overweight category (WHO, 2020). Obesity is defined as a disease of accumulation of excess adipose tissue to such an extent that it endangers health (Poirier et al., 2011), and its consequences are numerous, from those in the cardiovascular and other systems (Medanić and Pucarin-Cvetković, 2012), to diabetes and various cancers. A consistent association of childhood and adolescent obesity with an increased risk of premature morbidity and mortality, especially cardio-metabolic (Reilly et al., 2010) has been found, the life expectancy of obese people is on average two years shorter (Muennig et al., 2006) and the general health of obese people is usually worse than people of normal weight. In addition, research shows that obese people are very badly affected by public judgment (Puhl and Heuer, 2009) which results in negative feelings towards themselves and the world and creates anxiety, depression, fear and a range of other psychological disorders.

People gain weight for several reasons. It is believed that man today has changed his way of life and in that light the way of eating. It is increasingly easy to accept the choice of ready-made food, bought in a hurry, chooses for a meal something delicious from well-known food chains, and such a diet is rich in energy and weakened by other nutrients (Hall and Kahan, 2018). Less and less is devoted to food choices, meal preparation, family, and without physical activity, the problem becomes the intake of more energy than the body needs and/or is consumed during the day and a person gains weight. The causes of weight gain are multiple and complex and involve the interaction of several biological, genetic, and psychosocial factors (Wyatt et al., 2006), so treatment (weight loss) is equally complex. The problem of weight loss should therefore be approached very carefully because weight loss requires not only a change in diet but also a behavior change. It requires patience, time, physical activity, and knowledge, not only about weight loss methods but also about the fact that the wrong approach to weight loss can lead to complications and problems. For example, a long-term diet high in protein can cause ketoacidosis, kidney and liver disease, and decreased bone density (Štimac and Turk, 2008). A big problem is the well-researched Yo-yo effect in which after losing a diet, you lose weight in a short time, but return quickly over time. The increased urge to eat after losing weight lasts for more than a year is several times stronger than adapting to a new

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condition, and is potentially a major driver of weight gain (Polidori et al., 2016). As a result, only about 20% of obese people preserve and stabilize the weight loss effect in the long run (Wing and Hill, 2001). When losing weight, one should also consider the will factor or motivation of the individual to lose weight, which is initially high, and then usually decreases. This begs the question of whether the willpower to continue limiting food intake weakens or the body physiologically defends genetically based weight? Mann (2018) believes that the answer lies somewhere in between because reduced calorie intake during weight loss leads to changes in hormones, metabolism, and cognitive functions that make it difficult to behave to maintain the weight achieved by the diet. What is certain is that numerous studies confirm the return of kilograms (Wu et al., 2009), more than half recover most of the lost kilograms in the first 12 months.

There are also many misconceptions, beliefs, and prejudices that Jelčić (2014) believes have become so embodied that they have become stereotypes, for example, that obesity is a dietary disease, that obese people eat huge amounts of food, that small changes in energy intake cause big changes body weight over a long period, and this does not happen because there is an adjustment of basal metabolism to changes in body weight. Also, tips and various diets that come from the professional literature, but also websites, advertisements, or weight loss products, cannot be counted and they create even greater confusion in obese people. What to do in the sea of this information? To begin with, it is important to know, and this is what most authors agree, that obesity is a lifestyle disease caused by the interaction of genes and the environment, and that lifestyle changes are possible, but long-lasting because a person changing must get used to new patterns of behavior, new routines, and habits, and the body must get used to the new bodyweight. Also, most authors agree and these claims have their basis in science, that change in the diet is needed, ie reduce excessive energy intake, improve the quality of nutrition and increase energy consumption (Raynor and Champagne, 2016). How, which diet to choose, is the next question?

When choosing a weight loss method, it is necessary to first seek the advice of an expert. It is then necessary to separate medical from therapeutic diets and distinguish them from popular or commercial diets. Know which of the popular diets are accompanied by scientific evidence, and where are they missing? In this context, Freire (2020) classifies a diet into three main categories and provides scientific evidence that they are applicable as dietary strategies for weight loss. The first group are diets based on the manipulation of macronutrient content, they are a diet low in fat, high in protein, and low in carbohydrates, and among them are the popular diet Atkins, Ketogenic, Zone, Ornish, Paleo. The second group are diets based on the restriction of specific foods or food groups (gluten-free, Paleo, vegetarian/vegan, Mediterranean) and the third group is diets based on the manipulation of timing (abstinence from food and drink for some time with modification with a normal diet, ie fasting). Numerous other weight loss methods have been described in the literature such as low-glycemic, balanced, DASH, DPP, Weight Watchers, South Beach, blood type diet (Katz and Meller, 2014), some based on medical, some on an alternative approach, and each of them has its advantages and disadvantages. Medical ones are evidence-based and aim to lose 5 to 10% of body weight over six months while maintaining the achieved body weight for at least two years (Anjali, 2005), alternatives are often not sufficiently explored.

Unfortunately, there is no convincing evidence of which is the best, as there is not enough research to confirm this, but the authors agree that diet should be implemented, not to lose weight, but to achieve good health (Tomiyama et al., 2013; Katz and Meller, 2014) and that there is no optimally effective diet applicable to all individuals. For weight loss, the key to success is a prudent combination of all these approaches in the context of a healthy and balanced diet without serious restrictions or dietary exaggerations and it is best to approach it on an individual basis, with a diet plan based on individual characteristics (Koliaki et al., 2018). Finally, the fundamental thing is to adopt a diet with a negative energy balance, focus on achieving good health with proper nutrition from the guidelines, and with mandatory physical activity, this will ensure long-term success.

Thus, the path to a theoretically designed and practically feasible diet is demanding and complex. Taking this into account, as well as the knowledge about weight loss presented in the introductory part of the paper, this case study presents an example of an individual approach to a weight loss of a 22-year-old girl who has been obese since childhood and was in stage III obesity. The paper aims to emphasize that good preparation for a weight loss program, detailed assessment of improper lifestyle and eating habits, knowledge of improper diet and the consequences of such a lifestyle, and monitoring of a weight loss program are the factors that most strongly influenced the firm decision and emotions to persevere weight loss program as well as maintaining weight after a weight loss program.

Materials and methods

The respondent (Eli) is a 22-year-old girl, a former student of the Department of Food Technology at the

Biotechnical Faculty of the University of Bihać, who has been suffering from obesity since childhood. Interviews with a potential mentor for bachelor's work begin in the spring of 2019 after passing the module Nutrition Science, where she showed extraordinary knowledge and interest in the issues that the module deals with. From April to July 2019, through about 10 consultative meetings to discuss lifestyle and diet, the effects of her obesity on quality of life in general. During the conversation, Eli shows strong motives and desire to lose weight, but she doesn't succeed. She admits that because of her obesity she is very unhappy and insecure. The decision to follow the diet and lifestyle through the development of a bachelor's work and to implement a weight loss program was discussed. It was agreed that Eli would think carefully, study the prepared literature during the summer vacation, and make a decision. At the beginning of September 2019, Eli decided to start the weight loss process with the mentorship of professors and researchers in the field of nutrition.

A plan for making a bachelor paper consisting of three phases was designed. In the first phase, body mass index (BMI), quality of diet, eating habits, and some lifestyle habits were determined and monitored in detail for 30 days (November 1 - November 30, 2019). Preparations lasted 30 days, and the processing of monitoring results took a month and a half. The second phase consisted of the gradual introduction of changes in daily routine and habits, those identified as critical in the first 30 days of the study, lasting six months (15 January - 15 July 2020). The third phase consisted of processing the obtained data, writing and defending the bachelor's thesis, and continuous monitoring of the achieved body weight until the ascertainment of the condition on 15 July 2021 (one year after the weight loss program).

To monitor the selected parameters, a questionnaire was developed combined using the methods proposed by Šatalić (2013), Biro et al. (2002), and Šatalić and Alebić (2008). A food diary was kept in which the time, place, types, and quantities of food and beverages consumed distributed during working days and weekends were entered. Quantification of ingested food was expressed in serving units (small, medium, or large portion) by a combination of the FFQ method and data from the CapNatura (2014) food catalog or labeling from the packaging. Data on daily intake of total energy, energy from proteins, carbohydrates, and fats were calculated using tables on the composition of food and beverages (Kaić - Rak and Antonić, 1990) and other available tables. The number of meals at breakfast, lunch, dinner, and snacks was recorded, and meal times were taken from AHA recommendations (St-Onge et al., 2017). To assess nutritional status, anthropometric measurements (body weight and height, waist circumference, hips, upper arms, forearms, and thighs) were performed with an ordinary house scale and an ordinary tailor's meter, and were measured every five days during the second phase and periodically in the third. In the second phase, the amount of blood sugar (ACCU-CHEK Performa apparatus) and pressure (Boso Medicus uno apparatus) were measured every 10 days. Lifestyle habits were monitored by records of the following parameters: time of getting up, time of first and last meal, time of sleep, daily sitting, all daily activities (housework, moderate and intense physical activity, walking, social life).

Results and discussion

From the diet diary that Eli kept for 30 days, several wrong eating habits were identified (Figure 1). The first is that she consumed a total of 252 meals, which is an average of 8.1 meals on working days and 9.4 meals on weekends (daily range from 4 to 14). This is not a bad habit, as several studies have found that more daily meals reduce the risk of cardiovascular disease (CVD) and diabetes (Cahill et al., 2013), but the distribution of meals during the day was critical because 30% of all meals consumed after 7 p.m. (i.e. at night before bedtime). Eating food late at night, within two hours of going to bed increases the risk of CVD, according to St-Onge et al. (2017) who in addition to investigating how the number of meals and the time of their consumption affects health investigate the impact of skipping breakfast and occasional fasting by reviewing over 120 epidemiological and clinical studies. They mention research with medical workers in the night and day shifts, where it was shown that people who work in the night shift have increased blood sugar, cholesterol, and triglyceride levels, as well as an increased heart rhythm disorder, but they recommend additional research for night work because there is not enough evidence of an adverse effect. They report that a regular breakfast can reduce the risk of adverse effects associated with glucose and insulin metabolism and that there is a link between skipping breakfast and obesity, but breakfast has a limited effect on weight loss, probably because people eat many times a day, and calorie intake and food selection have a greater impact on weight than breakfast alone. It is best to have breakfast an hour after getting up, as this prevents fatigue, the body gets the energy it needs, but it is necessary to avoid foods rich in sugar and gluten like croissants and too many caffeinated beverages, which is the case with Eli. Also, daily and periodic fasting can be effective in losing weight, maintaining weight

after weight loss, lowering triglycerides and blood pressure, but not lowering cholesterol or glucose. Despite insufficient evidence on some topics, St-Onge ultimately concludes that the dietary styles studied may have different effects on cardiometabolic health markers and that recommendations to eat like a king for breakfast, like a queen for lunch, and like a poor man for dinner find their place in science. They add that meals should be taken from the morning from 6.00 a.m. to 6.00 p.m. maximum, noting that it is very difficult to separate when it is time for a meal, due to snacks and the habit of people to constantly "snack on something along the way". According to the AHA recommendations (American Heart Association), breakfast time is from 5 to 10 a.m., lunchtime from 12 to 1 p.m., dinner time from 5 to 7 p.m., while the time of the first, second, and third snacks is calculated from 10 to 12 / 1 to 5 / from 7 p.m. onwards. All other critical eating habits identified in Phase I of this study are presented below:

- High concentration of meals after 7 p.m. until no later than 11:40 p.m., 1-6 meals (average 2.1 working days, 3.3 weekends), 13 / 30 days consume whole meals (appetizer, meal, dessert), 18 / 30 times sweets.
- High concentration of meals at breakfast time, breakfast from 7:30 to 10 a.m., 1-4 meals (average 1.6 working days, 1.0 weekends), breakfast 26 / 30 days, breakfast composition extremely poor, 20 /30 days consuming sweets.
- Extremely a lot of industrial sweets (chocolates, biscuits), 29 / days consumes sweets.
- Extremely a lot of fast food (various pies, hamburgers, hot dogs, french fries), 17 / 30 days.
- Extremely a lot of sugar with Nescafe (2 tablespoons), 23 / 30 days consumed Nescafe.
- Extremely breaded and baked, from 25 meals at home / 19 breaded or baked.
- Add cream, mayonnaise, or ketchup to each dish.
- All side dishes are potatoes, pasta, rice, only once frozen vegetables.
- Extremely few fruits and vegetables, consume bananas and citrus for 8 / 30 days, only one salad.
- Extremely little cooked food, only 6 / 30 days.
- Frequent consumption of snacks and Coca-Cola, 11 / 30 days.
- Little water (average 1.1 L on working days and 1.26 L on weekends)

The results with specific numbers caused fear and concern in Eli, and the tables that visualized irregularities in the colors caused a strong motivating effect. One of the tables (Figure 1) has been adapted for this work, which shows the daily distribution of meals according to the type of food and beverages consumed. Black to gray shades represent various undesirable food groups, the visual texture shows the desired food groups, while the hours when she did not consume anything are white. In the original color chart, when Eli saw how black the blackboard was, she blackened before her eyes and stated to herself, "Well, this is terrible!"

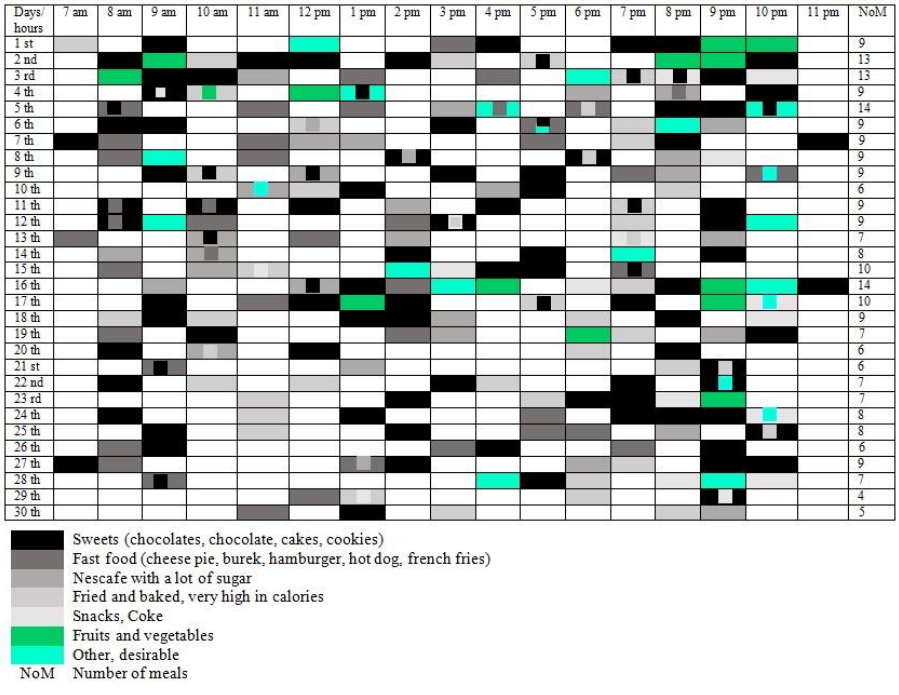

Figure 1. Daily distribution of meals, according to the time and type of food

Of the undesirable lifestyle habits, sitting was the most critical. On working days, Eli was awake for an average of 15.55 hours, of which 73% of the time she sat, on weekends 72%, the longest through hanging out with friends outside. With various screens, she spent an average of 5.05 hours, on weekends 6.58 hours. A comparison with the data on the average daily seating for 63 countries presented by the WHO in 2015 (from 2.12 to 9.47 hours) found that Eli sits more than this range. Given that research shows that long sitting is associated with several health problems (obesity, high blood pressure, high blood sugar, excess body fat around the waist, and abnormal cholesterol levels) and that there is an increased risk of death from CVD and cancer (Mclaughlin et al., 2020) it can be concluded that sitting hours with Eli are one of the riskiest lifestyle factors for her health. In addition, there is no physical activity, except for the average daily walking (3.9 km) and housework (1.10 h), as well as social activities (activism, volunteerism, membership in organizations, no contact with nature).

After the results were determined in the first phase of the research, it was agreed to start phase II from January 15, 2019. Until then, Eli had lived entirely by old habits, studied the literature, and was visibly concerned about her habits. Phase II begins with a gradual change in established critical habits. One day without meat (Monday), a day without sweets (Thursday), and a day of proper nutrition (Saturday) are introduced, and for other days Eli is left with the option to eat as she wishes. The diet diary was kept only for these three selected days. One hour of brisk walking is introduced, at least three times a week. The first evaluation was done after two weeks, and it was found that Eli fully complied with the instructions, but on her own initiative excluded from the diet all types of sweets and all types of fast food. He introduces salads, cooked dishes, and keeps fried chicken dishes into his diet. Reduce the number of meals to 3-4, at the recommended mealtime. In the evening, she introduces green tea with a teaspoon of honey, changes her Nescafe with tea on outings, consumes fruit in 7 / 15 days, an apple that she has never consumed, throws out a banana, previously the most commonly consumed fruit. The result was motivating, a loss of 7.15 kg. There were no changes in the biochemical monitored parameters. However, she was warned to reduce the intensity. In the next two weeks, he loses an additional 2.6 kg and retains the acquired habits. He keeps a diet diary for a full six months, gradually giving up old ones, introducing new habits. An evaluation with a mentor was done every 15 days. The achieved changes are shown in Table 1.

On average, the number of meals was reduced from 252 to 142 per month, all industrial sweets, snacks, Nescafe, and Coca-Cola were excluded. In the evening, 90% of meals are fruits and vegetables and tea with honey, generally reducing the share of evening meals from 30% to 23%. She keeps a high proportion of breaded and roasted meat, because she loves these meals, and it was agreed that the changes be introduced in three selected days so it does not create psychological pressure. Increases water consumption from an average of 1.2 L to 2.0 L.

The results on the average daily energy intake, and the ratio of energy obtained from proteins, carbohydrates, and fats for phase I and phase II, but only for one month (penultimate) are shown in Table 2. About her calculated energy needs (1.893 kcal), Eli consumed on average 22% more energy than needed, and in addition, according to the recommendations to consume 55-75% of energy from carbohydrates, 15-30% from fat, and 10-15% from protein (Šatalić, 2008), obviously ingested excess fat. Later in the weight loss phase, she ingests 47% less energy than needed, reduces her energy intake from fat by 10%, but increases her protein intake more than recommended, as she was warned.

Table 1. Share (%) of intake of main food groups counted as meals by phases

Grocery groups	Phase I (30 days) (252 meals)		Phase II (77 days) (426 meals)	
	NoM*	%	NoM	%
Undesirable				
All sweets, chocolates, chocolate, cakes, cookies	95	37.7	0	0
Fast food (pies, hamburger, hot dog, french fries)	50	19.8	16	3.8
Nescafe with a lot of sugar	32	12.7	0	0
Breaded, baked	19	7.5	33	7.8
Snacks: chips, figs	12	4.8	0	0
Coca Cola	9	3.6	0	0
Desirable				
Fruits and vegetables	11	4.4	171	40.1
Cooked food (beans and soup) and soups	6	2.4	61	14.3
Yogurts, milk, and cereals	2	0.8	30	7.0
Honey	2	0.8	20	4.7
Water, average (L)	1.1-1.3		1.5-2.5	
Total number of main meals	238		331	

**NoM: Number of meals*

Table 2. Comparison of energy intake in the first month of research (phase I) with energy intake in the penultimate month of research (phase II) for selected days

Selected days	Phase I				Phase II			
	Total energy (kcal)	Proteins* (%)	CH* (%)	Fats* (%)	Total energy (kcal)	Proteins* (%)	CH* (%)	Fats* (%)
Saturday	2.576	14.10	42.12	43.77	850	17.20	59.20	23.60
Monday	2.324	14.23	51.61	34.16	1.107	15.29	62.62	22.00
Thursday	2.011	13.46	55.10	31.44	1.072	29.64	37.31	33.72
Mean	2.304	13.93	49.61	36.46	1.010	20.71	53.04	26.44

**Part of energy (%) coming from proteins, carbohydrates (CH), and fats*

Based on body height (163 cm) and age (22 years) in the first phase, using the Harris-Benedict equation (Šatalić, 2008), the ideal body weight (58.3 kg) and BMI of 40.85 kg/m^2 were calculated. Eli was 50 kg overweight and according to BMI entered grade III obesity or morbid obesity. After a six-month weight loss program and a loss of 32.95 kg, the calculated BMI was 28.49 kg/m^2, according to which Eli entered the category of overweight people. Significant changes in lifestyle habits were reflected in the introduction of more physical activities, she increased the average daily walking to 4.9 km on working days and 4.66 km on weekends, acquired a stepper on which she exercised 30 minutes every day, and cycled 45 minutes, three times in a week. In her case, the Covid 19 pandemic had a positive impact on acquiring new habits as Eli spent time with family doing housework and farm chores. The results of monitoring anthropological and biochemical parameters showed that Eli lost the most pounds in the first month (9.75 kg) and that each subsequent month weight loss decreased along with calming the euphoria she had with success in the first month of weight loss. The largest decrease in anthropological parameters was achieved in the waist (24 cm) and hips (23 cm). Biochemical parameters pressure (highest 124 / lowest 70 mmHg) and blood sugar (range 4.7-5.0 mmol/L) were measured every 5 days and were within normal limits at all times.

In the third phase, while Eli was writing her bachelor's work, she continued with her newly acquired eating and living habits. Half of the III phase (15th of January 2021) Eli further reduced her body weight to 70.0 kg, exactly after one year on 15th of July 2021, she further reduced her weight to 69.5 kg.

Finally, as key factors in the success of a weight loss program can be singled out the factor of will or motivation of the respondent, which was high from beginning to end. Researchers claim that the will weakens during dieting, people give up and cannot keep the lost weight because physiological changes occur in the body, which makes it harder for the willpower needed to maintain the achieved weight. It is concluded that the motivation of the respondent Eli was influenced by freer access to change, without strict restrictions, mentoring, and practically daily supervision of the weight loss process. Eli felt safe and listened to the instructions very conscientiously. However, the most important factor, and this is the key difference between this and other known methods for weight loss, emphasizes the obligation of the respondent to learn and acquire knowledge about the principles of weight loss, the principles of proper nutrition, and the effects of obesity on health. As one of the most important elements of the program, the influence of a detailed analysis of habits in the first phase of research and visual graphic representations (table, Figure 1) on the psychological state of the program is highlighted. The knowledge of "blackness" and the dominance of undesirable habits (the tables were mostly black) and of the possible disastrous health consequences for her future life, was a strong motive in this case. Therefore, the authors conclude that the first phase in this research (i.e. the precise identification of undesirable habits) significantly contributed to the weight loss program. This approach of very detailed analysis of negative habits and their visualization is an innovative contribution to all hitherto known methods of weight loss. And finally, this study shows that it is possible to lose weight without excessive denial of certain foods, but it is necessary to change habits with a completely individual approach. The main disadvantage of diets is that they are too restrictive, too demanding, non-individualized, and thus unsustainable. An individual approach to weight loss based on precisely identified irregular lifestyle and eating habits that Torrado et al. (2015) claim are the main causes of negative energy balance and overweight, and the gradual introduction of changes through a combination of recommendations from numerous authors resulted in success in Eli.

Conclusions

In this paper, a strictly individual approach to weight loss and lifestyle change was applied and as such brought excellent results in both diet and behavior. The weight loss method is combined with the recommendations of numerous authors who claim that

lifestyle and poor eating habits are the main causes of weight gain. In a person entering a weight loss program, it is important to make them aware of their undesirable habits and increase their knowledge about the consequences of improper diet and bad habits, and this can be done by a very detailed analysis of these bad habits. Weight loss is indeed a long-term process, requiring great will, motivation, as well as the support of family and the environment, and this paper confirmed that an individual approach and gradual change of eating and living habits are effective methods for weight loss.

References

Anjali, J. (2005): Treating obesity in individuals and populations, *Brit. Med. J.* 331, 1387-90.

Biro, G., Hulshof, K.F.A.M., Ovensen, L., Amorim Cruz, J.A. (2002): Selection of methodology to assess food intake, *Europ. J. Clinic. Nutrit.* 56 (2), 25-32.

Cahill, L.E., Chiuve, S.E., Mekary, R.A., Jensen, M.K., Flint, A.J., Hu, F.B., Rimm, E.B. (2013): Prospective study of breakfast eating and incident coronary heart disease in a cohort of male US health professionals, *Circulation* 128, 337-343.

CapNatura (2014): Food atlas of commonly consumed foods in the Balkan region, Beograd, Srbija, In project: Capacity development Food & nutrition, Central & Eastern Europe/Balkan countries Public health nutrition research.

Freire, R. (2020): Scientific evidence of diets for weight loss: Different macronutrient composition, intermittent fasting, and popular diets, *Nutrit.* 69, 110549.

Hall, K.D. and Kahan, S. (2018): Maintenance of lost weight and long-term management of obesity, *Med. Clin. North. Am.* 102 (1), 183-197.

Jelčić, J. (2014): Debljina, bolest stila života, Algoritam doo, Zagreb.

Kaić – Rak, A. i Antonić, K. (1990) Tablice o sastavu namirnica i pića, Zavod za zaštitu zdravlja Republike Hrvatske, Zagreb.

Katz, D.L. and Meller, S. (2014): Can We Say What Diet Is Best for Health? *Ann. Rev. Pub. Health* 35, 83-103.

Koliaki, C., Spinos, T., Spinou, M., Brinia,M.E., Mitsopoulou, D., Katsilambros, N. (2018): Defining the Optimal Dietary Approach for Safe, Effective and Sustainable Weight Loss in Overweight and Obese Adults, *Healthcare (Basel)* 6 (3), 73.

Mann, T. (2018): Why do dieters regain weight? American Psychological Association. Available at: http://www.apa.org/science/about/psa/2018/05/calorie-deprivation. Accessed: 22.8.2020.

Mclaughlin, M., Atkin, A.J., Starr, L., Hall, A., Wolfenden, L., Sutherland, R., Wiggers, J., Ramirez, A., Hallal, P., Pratt, M., Lynch B.M., Wijndaele, K. (2020): Worldwide surveillance of self-reported sitting time: a scoping review, *Int. J. Behav. Nutr. Phys. Activ*. 17 (111).

Medanić, D., Pucarin-Cvetković J. (2012): Pretilost – javnozdravstveni problem i izazov, *Acta. Med. Croat.* 66, 347-355.

Muennig, P., Lubetkin, E., Jia, H., Franks, P. (2006): Gender and the burden of disease attributable to obesity, *J. Public. Health* 96 (9), 1662-1668.

Poirier, P., Cornier, M.A., Mazzone, T., Stiles, S. Cummings, S., Klein, S., McCullough, P.A., Fielding, C.R., Franklin, B.A. (2011): Bariatricsurgery and cardiovascular risk factors: A scientific statement from The American Heart Association, *Circulation* 123, 1683-1701.

Polidori, D., Sanghvi, A., Seeley, R.J., Hall, K.D. (2016): How strongly does appetite counter weight loss? Quantification of the feedback control of human energy intake, *Obesity* 24, 2289-2295.

Puhl, R.M., Heuer, C.A (2009): The Stigma of Obesity: A Review and Update, *Obesity* 17 (5), 941-964.

Raynor, H.A., Champagne, C.M. (2016): Position of the Academy of Nutrition and Dietetics: Interventions for the Treatment of Overweight and Obesity in Adults, *J. Acad. Nutr. Diet.* 116 (1), 129-147.

Reilly, J., Kelly, J. (2010): Long-term impact of overweight and obesity in childhood and adolescence on morbidity and premature mortality in adulthood: systematic review, *Int. J. Obesity* 35 (7), 891-898.

St-Onge, M.P., Ard, J., Baskin, M.L., Chiuve, S.E., Johnson, H.M., Kris-Etherton, P., Varady, K. (2017): Meal Timing and Frequency: Implications for Cardiovascular Disease Prevention; A Scientific Statement From the American Heart Association, *Circulation* 135, 96-121.

Šatalić, Z. (2013): Kako se mjeri prehrana, U: 100 (i pokoja više) crtica o znanosti o prehrani, Hrvatsko društvo prehrambenih tehnologa, biotehnologa i nutricionista, Zagreb.

Šatalić, Z. (2008): Energetske i nutritivne potrebe, *Medicus* 17 (1), 5-17.

Šatalić, Z., Alebić, I.J. (2008): Dijetetičke metode i planiranje prehrane, *Medicus* 17 (1), 27-36.

Štimac, D., Turk, T. (2008): Debljina i redukcijske dijete, *Medicus* 17 (1), 81-85.

Tomiyama, A.J., Ahlstrom, B., Mann, T. (2013): Long-term Effects of Dieting: Is Weight Loss Related to Health? *Soc. Pers. Psychol.ogy Compass* 7 (12), 861-877.

Torrado, Y.P., Velasco, A.G.V.V., Galiot, A.H., Cambrodón, I.G. (2015): A strategy for weight loss based on healthy dietary habits and control of emotional response to food, *Nutr. Hosp.* 31 (6), 2392-2399.

World Health Organization (2020): Obesity and overweight. Available at: www.who.int. Accessed: 24.8.2020.

Wing R.R., Hill J.O. (2001): Successful weight loss maintenance, *Annu. Rev. Nutr.* 21, 323-341.

Wu, T., Gao, X., Chen, M., van Dam, R.M. (2009): Long-term effectiveness of diet-plus-exercise interventions vs. diet-only interventions for weight loss: a meta-analysis, *Obes. Rev.* 10 (3), 313-323.

Wyatt, S.B., Winters, K.P., Dubbert, P.M. (2006): Overweight and Obesity: Prevalence, Consequences, and Causes of a Growing Public Health Problem, *Am. J. Med. Sci.* 331 (4), 166-174.

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CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF WATER-ETHANOL EXTRACTS OF DANDELION (*TARAXACUM OFFICINALE*)

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Summary

Dandelion or *Taraxacum officinale* is a well - known medical plant that is a source of various nutrients and biologically active substances, and its polyphenolic compounds are considered responsible for the high biological activity of dandelion and its antioxidant, anti - inflammatory and atitumor effects. Aques - ethanol extracts were obtained using various maceration extraction techniques at room and boiling temperature, ultrasonic and Soxhlet extraction. In this study, all parts of the plant (roots, leaves, stems, flowers) previously dried at room temperature were analysed. The content of mineral elements of the plant was determined by AAS, antioxidant activity by DPPH (2,2 - diphenyl - 1 - pycryl hydrazyl) and FRAP (Ferric Reducing Antioxidant Power) method, flavonoids (method with $AlC1_3$), vitamin C, carotenoids and chlorophyll were determined spectrophotometrically. The content of total phenols was determined by the Folin - Ciocalteu method. The content of phenolic compounds is higher in the outer parts of the plant (flowers and leaves) than in the root. The results of chemical analysis show that all its components have special qualities in terms of chemical composition. The best antioxidant activity was shown by the water - ethanol extract obtained using Soxlet extraction.

Keywords: chemical composition, antioxidant activity, *Taraxacum officinale*

Introduction

Dandelion (*Taraxacum officinale*) is a wild plant from the Asteraceae family where recent research shows a pronounced biological potential due to the significant content of bioactive compounds. This plant is rich in polyphenolic compounds dominated by hydroxycinnamic acids (Sun et al., 2014), caffeic acid, flavonoids-apigenin and luteolin-7-glucoside, phytosterols-sitosterol and stigmasterol, carotenoids (Kuštrak, 2005). Also, it is rich in vitamin C and minerals such as potassium, iron, sodium and phosphorus, as well as essential oils, fatty acids (Erhatić et al., 2014) and inulin of variable content. In spring, the root contains only 1-2%, and in autumn up to 40% inulin (Kuštrak, 2005), and in October the root contains the most taraxerin and levulin (Erhatić et al., 2014). A review paper (Di Napoli and Zucchetti, 2021) shows that twelve medicinal properties of *Taraxacum officinale* are often cited in the scientific literature. These properties include diuretic, hepatoprotective, anticholitic, immunoprotective, antiviral, antifungal, antibacterial, antiarthritic, antidiabetic, antihypertensive, antioxidant and anticancer effects. The presence of bioactive compounds in living organisms is of great importance because aerobic organisms in vivo continuously generate free radicals and reactive oxygen species. Free radicals are usually very reactive and in increased concentrations can lead to cell and tissue damage, which can be the cause of a large number of diseases (Nikolić et al., 1998). The body defends itself against free radicals with natural antioxidants that are introduced into the body through food, natural or synthetic substances, and have the ability to resist oxidation or inhibit reactions initiated by reactive species. These compounds differ in chemical structure and have different mechanisms of action. Therefore, various antioxidants are needed to protect the cell from various biomolecules in vivo (Gutteridge and Halliwell, 2010). These are compounds that are present in low concentrations and act as "scavengers" of free radicals, transforming them into stable non-radical products, complexing metal ions, preventing their catalytic function in the processes of lipid peroxide degradation and free radical formation, degrading lipid peroxides, acting as reducing agents, " scavengers " of singlet oxygen, inhibit some enzymes, show synergistic effects, to release hydrogen and chelate metals (Ivanova et al., 2005). Root and young tops are mainly used for medicinal purposes (Grieve, 1931; Rasool and Sharma, 2014; Stewart-Wade et al., 2002). The results of current research show that dandelion root is a valuable source of dietary fiber and natural antioxidants and could be successfully used in foods that can improve digestion and prevent diseases caused by oxidative stress (Petkova et al., 2015). The young leaves of *Taraxacum officinale* are also used as

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food in salads, drinks and vegetable dishes, due to their nutritional value. Studies show that *Taraxacum officinale* leaves contain high concentrations of fiber, minerals, vitamins, and essential fatty acids (Escudero et al., 2003; Di Napoli and Zucchetti, 2021).
The aim of this work is to carry out the extraction of polyphenols from dandelion (*Taraxacum officinale*) with an aqueous solution of ethanol 50% (v/v) as a solvent. The root, stem, leaf and flower were used as samples. Several extraction methods were used: using maceration of samples at room temperature, boiling temperature, ultrasonic extraction and Soxlet extraction. After extraction with samples, antioxidant activity was determined.

Materials and methods

Sampling was performed in 2020. at the end of April and the beginning of May. All parts of the dandelion plant (*Taraxacum officinale*) were sampled. They were then dried to room temperature and stored in paper bags. Samples were ground using an IKA Tube Mill 100 control laboratory grinder and six replicates were taken for each analysis.

The homogenized sample was subjected to maceration extraction techniques at room temperature and boiling temperature, ultrasonic and Soxhlet extraction, with aqueous ethanol solution 50 % (v/v) as solvent. Classic extraction of macerations at room temperature was performed for 240 minutes. After extraction, the liquid extracts were separated from the plant material by vacuum filtration on a Büchner funnel and transferred to vials. Boiling extraction for 30 minutes and ultrasonic extraction (WiseClean WUC) were performed for 30 minutes, the samples were filtered on a Büchner funnel and transferred to vials. Soxhlet extraction was performed in a Soxhlet apparatus with 25 g of plant material each. The extraction lasted 160 minutes. All obtained extracts were stored in a refrigerator at + 4 °C until further analysis.
In the prepared samples, total phenols were determined by the Folin-Ciocalteu method (Dewanto et al., 2002), and the results were calculated from the calibration curve of gallic acid (Figure 1). The concentration of total phenols is calculated according to the equation of direction obtained by Excel, with gallic acid concentrations (mg/L) plotted on the abscissa and absorbance values measured on the photoLlab 6600 UV-VIS WTW spectrophotometer at 765 nm.

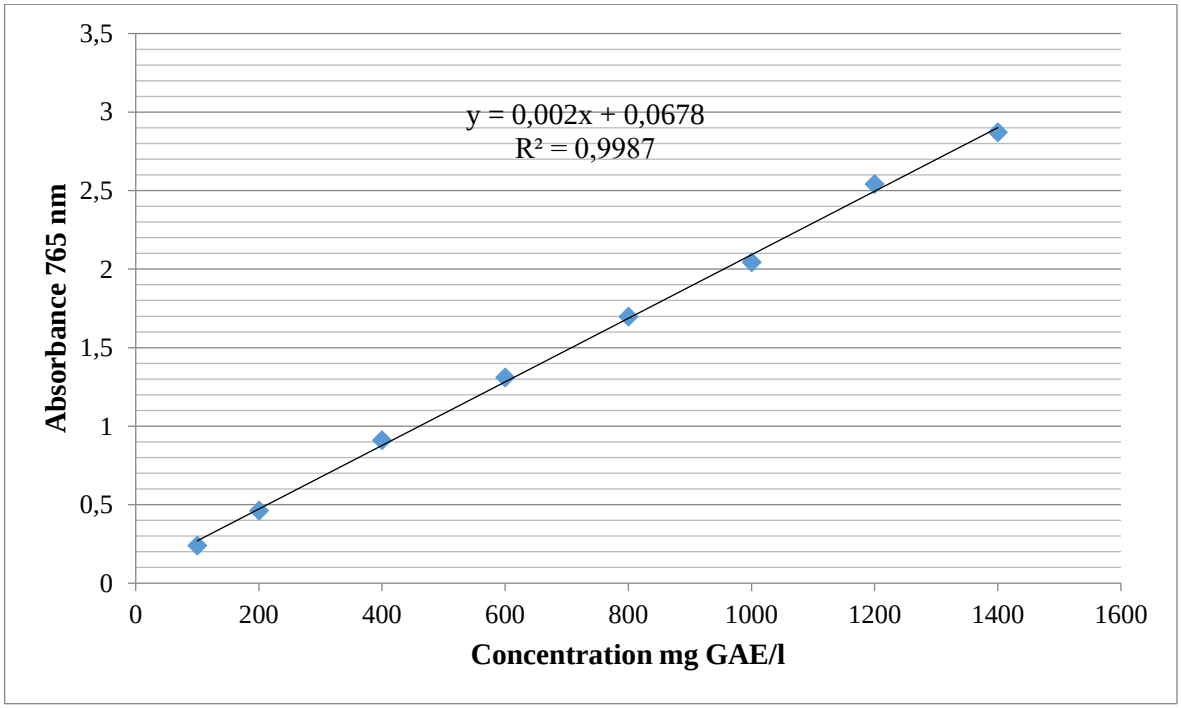

Figure 1. Standard diagram for determination of total phenols

A modified colorimetric method with $AlCl_3$ was used to determine total flavonoids (Khlifi et al., 2011), and a standard quercetin solution was used to construct the calibration direction (Figure 2).

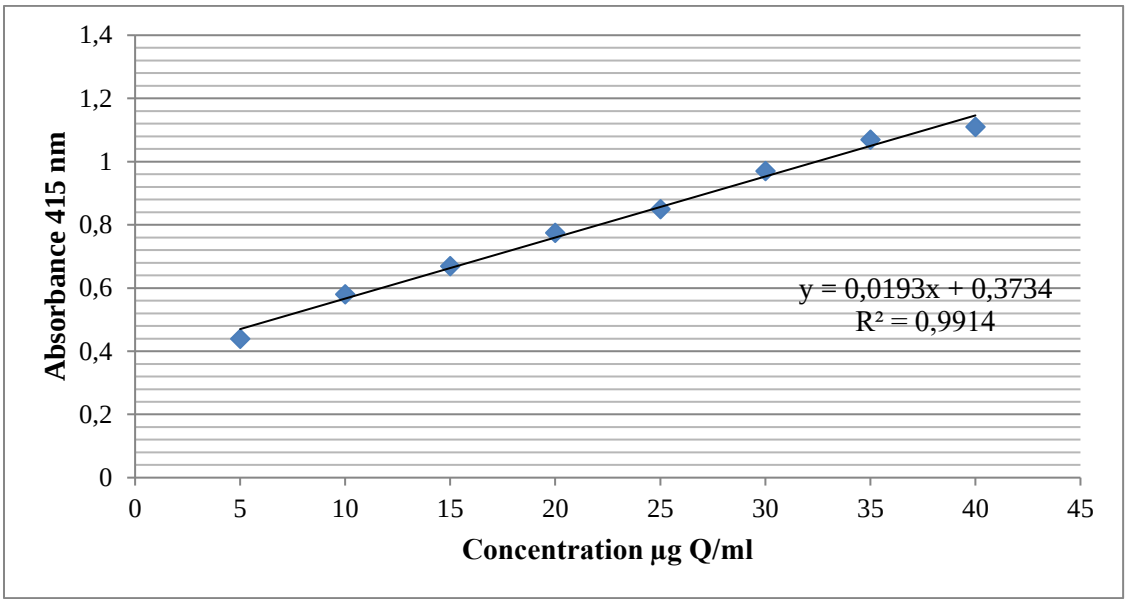

Figure 2. Standard diagram for the determination of flavonoids

The DPPH method was used to determine antioxidant capacity. DPPH (2,2-diphenyl-1-picrylhydrazyl) is one of the most stable organic nitrogen radicals with maximum absorption in UV-VIS at 517 nm. The DPPH radical is a stable nitrogen radical whose solution is dark purple, and with the addition of antioxidants the solution fades as the free radical is reduced to light yellow diphenylpicrylhydrazine, which is monitored spectrophotometrically via a drop in absorbance at 517 nm. The sample was incubated for 15 minutes at 37 °C in the dark. Decreases in absorbance at 517 nm were measured using a spectrophotometer compared to a blank containing methanol. The standard curve was constructed with a Trolox concentration between 0.005 and 1.0 mM. Results are expressed in mM Trolox/g dry weight (Ivanov et al., 2015).

FRAP test: 0.1 ml of test extracts were added to 3 ml of FRAP reagent (0.3 M) acetate buffer, 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ), 20 mM $FeCl_3 \times 6H_2O$ (10: 1: 1, v/v/v) and allowed to stand for 10 min at 37 °C in the dark. Absorbance was measured at 593 nm (Benzie and Strain, 1996). Results from both antioxidant methods were expressed as mM Trolox/g dry weight.

Carotenoids and chlorophylls from dandelion leaves, stems and flowers were extracted from 1 g of fresh sample homogenized with 5 ml of 80% aqueous acetone for 30 min, followed by centrifugation (Hettich centrifuge UNIVERSAL 320) at 4500 rpm for 10 minutes (Guan et al., 2006). The supernatant was diluted 10-fold using 80% aqueous acetone and absorbance was measured at 460, 647 and 664 nm using a photoLab 6600 spectrophotometer.

The obtained absorbance values were included in the Lichtenthaler formula to calculate the pigment concentrations (Lichtenthaler, 1987):

$$\text{Total chlorophyll} = 7.15A664 + 18.71A647 \quad (1)$$

$$\text{Total carotenoids} = (1000A_{460} - 1.82Ca - 85.2Cb)/198 \quad (2)$$

where is: A = absorbance

$$Ca \text{ (chlorophyll a)} = 12{,}25A_{664} - 2{,}79A_{647} \quad (3)$$

$$Cb \text{ (chlorophyll b)} = 21.50A_{647} - 5.10A_{664} \quad (4)$$

The ascorbic acid content was determined according to Bendritter (Bendritter et al., 1998). About 0.5 g of tissue and 10 mL of distilled water were pipetted into the previously weighed and labeled tubes with a screw cap, and then the tubes were centrifuged for 15 minutes at 3000 g at 4 °C. After centrifugation, pipette 300 µL of aqueous extract into 2 mL tubes. To the aqueous extract was added 100 µL of 13.3% trichloroacetic acid, 25 µL of distilled water and 75 µL of DNPH (2,6-Dichlorophenolindophenol Sodium salt Hydrate) reagent. After the addition of the reagent, incubate for one hour in a water bath at 37 °C. Using the same procedure, blank samples were prepared for each sample without the addition of DNPH reagent. At the end of the incubation, DNPH reagent and 500 µL of 65% sulfuric acid were added to all blanks in all samples. All mixtures are mixed once more. Absorbance was measured spectrophotometrically

(photoLab 6600 spectrophotometer) at 520 nm, while ascorbic acid concentration was determined from a calibration curve with known concentrations.
The content of calcium and magnesium mineral elements was performed on an atomic absorption spectrophotometer "Perkin Elmer" AAnalyst -800, whose results are expressed in mg / kg (ppm) "Analytical Methods" FP-3 Analysis of Meat and Meat Products (2000), "Perkin Elmer" AAnalyst -800.

Results and discussion

Previous research shows that the total yield of extracted substances, the content of total phenols, flavonoids and antioxidant activity depend on the applied extraction technique and solvent. According to the literature, methanol is more efficient for the extraction of phenol from plant material, however, we used an aqueous solution of ethanol in this work due to less toxicity.
The results of testing the antioxidant activity of ethanolic extracts of dandelion *(Taraxacum officinale)* indicate that this plant is rich in compounds that have antioxidant activity. In research Nowak et al. (2019), raw dandelion plant material consisted of fresh and dried leaves, flowers and roots. Extracts were prepared for analysis using an ultrasonic bath (extraction time 15, 30 and 60 min) in water and 40% (v/v), 70% (v/v) and 96% (v/v) ethanol mixtures used as solvents. They found that the antioxidant activity of dandelion depended on the type of raw material used, as well as the type of solvent and extraction time. The highest DPPH activity was found to be dried flower extracts prepared in 70% ethanol for 30 min. With the FRAP method, the highest reduction capacity was observed for dried leaf extracts in 40% ethanol 30 minutes.

Table 1. Antioxidant activity of dandelion measured by DPPH and FRAP method, obtained by different extraction techniques

	Sample	DPPH mg Trolox/g dry weight	FRAP mg Trolox/g dry weight
		±SD	
DANDELION	Flower (ST)	118.24±4.02	89.27±2.87
	Flower (TK)	118.55±3.56	88.16±1.59
	Flower (UE)	127.98±3.87	97.25±2.17
	Flower (SE)	130.49±2.18	103.14±2.34
	Leaf (ST)	127.31±1.95	98.23±1.24
	Leaf (TK)	121.18±2.43	99.34±2.05
	Leaf (UE)	129.38±4.65	107.16±2.13
	Leaf (SE)	135.14±3.16	119.27±0.97
	Stem (ST)	99.43±1.99	90.28±1.84
	Stem (TK)	98.27±1.65	92.13±1.63
	Stem (UE)	104.53±2.53	103.55±0.99
	Stem (SE)	109.28±3.17	108.14±1.82
	Root (ST)	52.75±0.65	45.34±0.73
	Root (TK)	50.89±0.88	46.17±0.44
	Root (UE)	56.48±1.09	49.18±1.25
	Root (SE)	61.36±2.01	53.54±1.42

Results from both antioxidant methods were expressed as mM Trolox/g dry weight, SD = Mean of 6 measurements, ST = sample maceration extractions at room temperature, TK = boiling temperature, UE = ultrasonic extraction, SE = Soxlet extraction.

The influence of solvents on the extraction of phenolic compounds, from dandelion leaves, was studied in the research of Petkova et al. (2015). *Taraxacum officinale* subsequent extracts of aqueous root showed the highest antioxidant activity DPPH test 83.1 ± 3.2 mg TE/g dry matter; FRAP test 46.9 ± 1.3 mg TE/g dry matter, while aqueous extracts showed highly defined activity only by FRAP test 52.9 ± 0.3 mg TE/g dry matter.
According to research performed by Ivanov (2015), the results indicated that total phenolics, chicoric acid concentration, DPPH, FRAP and CuPRAC values were higher in 50% ethanol extract of *Taraxacum officinale* leaves: 33.90 ± 0.57 mg GAE/g dry matter, 3.1 g/100g dry matter, 136.3 mM TE/g dry matter (DPPH method), 131.5 mM TE/g dry matter (FRAP method) and 407.8 mM TE/g dry matter (CuPRAC method). Based on the values obtained in Table 1, it can be seen that dandelion leaves (*Taraxacum officinale*) extracts obtained by Soxlet extraction with aqueous ethanol 50% (v/v) as solvent, compared to the above authors, show approximately equal values of antioxidant activity DPPH method, while other values are much lower. Extraction is the most important phase in polyphenol isolation. The solubility of polyphenols depends on the degree of polymerization, interaction with other components and the polarity of the solvent Mujica et al. (2009), where the lipophilicity/hydrophilicity of the compounds in plant

material should be taken considered. The choice of extraction conditions, primarily the type of extraction, duration and temperature significantly affect the antioxidant activity. According to our research, it was found that in the case of classical extraction maceration at room temperature and with increasing temperature, the value of antioxidant activity did not change significantly, the assumption is due to the duration of extraction. Also, thermal processing conditions might result in the loss of natural antioxidants because heat may accelerate their oxidation and other degenerative reactions.

Kenny et al. (2014) found maximum quantities the ethyl acetate crude extract (*Taraxacum officinale* - root) demonstrated the highest antioxidant activity for both the DPPH (227.728 ± 11.849 mg TE/g) and FRAP (463.066 ± 3.942 mg TE/g) assays. This extract also contained the highest phenolic content (228.723 ± 2.392 mg GAE/g), which is significantly higher than the value of our results (Table 1).

Table 2. Total phenol and flavonoid content in dandelion flowers, leaves, stems and roots (sample maceration extractions at room temperature)

	Sample	Total phenols (mg GAE/g dry matter)	Flavonoids (mg quercetin/g dry matter)
		±SD	
DANDELION	Flower	26.08±0.67	4.98±0.51
	Leaf	30.05±0.89	2.26±0.31
	Stem	23.89±0.75	-
	Root	4.23±0.43	-

Milek and Legath (2015) isolated phenolic compounds from dandelion flower and leaves by ultrasonic extraction. Methanol, ethanol and acetone were used as solvents in a concentration of 70%. In the isolation of phenolic compounds from the leaves, the highest yields were given by extraction with acetone, followed by methanol and ethanol. They found 362.14 ± 6.76 μM quantities of total phenolics in the extracts of *Taraxacum officinale*.

According to research by Ghaima et al. (2013), the content of total phenols in dandelion leaves extracted with ethyl acetate was determined to be 10.2 mg GAE/g dry matter, which is much lower than our results (Table 2). Much higher values are reported by Stylianon et al. (2014) who extracted phenolic components by maceration in dandelion leaf and root. For the content of total phenols in the leaf, they state the values of 52.29 ± 0.0178 mg GAE/g, and in the root 9.61 ± 0.1397 mg GAE/g. Ivanov (2015) found maximum quantities of total phenolics in 50% ethanol extract of *Taraxacum officinale* leaves: 33.90 ± 0.57 mg GAE/g dry weight.

The influence of solvents on the extraction of phenolic compounds from dandelion leaves was also studied in the research of Petkova et al. (2015). Values of total phenols ranged from 4.5 to 9.2 mg GAE/g dry matter. The highest total content of polyphenols and total flavonoids was registered by subsequent extraction of water (9.2 ± 0.3 mg GAE/g and 1.7 mg EQ/g dry matter, which is significantly less than the value of our results. Khan et al. (2019) also determined the content of total phenols. They found varied from 41.47 mg/g to 691.6 mg/g quantities of total phenolics in the extracts of *Taraxacum officinale*. The maximum phenolic contents were found in hydro-alcoholic extract (691.6 mg/g GAE) in comparison with aqueous extract.

The results of current investigation demonstrate that dandelion is a valuable source of natural antioxidants and could be successfully used in foods with the potential to improve digestion and prevent from oxidative stress related diseases.

Table 3. Amount of vitamin C, chlorophyll and carotenoids in dandelion flowers, leaves, stems and roots (sample maceration extractions at room temperature)

	Sample	**Vitamin C** [mg/kg]	Total chlorophyll [mg/kg]	Total carotenoids [mg/kg]
		±SD		
DANDELION	Flower	58.54±0.85	-	43.56±0.44
	Leaf	106.49±1.27	427.18±3.87	198.29±1.56
	Stem	96.89±1.13	47.21±0.48	24.87±0.66
	Roof	18.02±0.56	-	-

The research by Pădureț et al. (2016) found that the highest content of vitamin C in dandelion leaves is 121,862 mg/kg. These values are slightly higher compared to our results (Table 3). This research shows the results of Pădureț et al. (2016), which is, that the leaf contains a higher proportion of vitamin C than in other analyzed parts of the dandelion plant.

The chlorophyll value of 87.62 mg/kg was determined according to the results of Pădureț et al. (2016). Our results showed lower values compared to the above results. The chlorophyll content in the stem was 47.21 mg/kg.

Table 4. Average values of calcium and magnesium mineral elements (sample maceration extractions at room temperature)

	Sample	**Ca** [mg/kg]	Mg [mg/kg]
		±SD	
DANDELION	Flower	328.33±8.16	5.19±0.35
	Leaf	448.26±9.23	6.31±0.49
	Stem	366.28±8.08	4.23±0.38
	Roof	408.21±10.12	2.17±0.27

The leaves of dandelion had magnesium and calcium levels of 6.31±0.49 and 448.26±9.23 mg/kg, respectively. According to research by Biel et al. (2017), dandelion leaves contain an average of 0.24 g magnesium and 0.67 g calcium/ 100 g dry matter. According to the available data in literature (Harrington et al., 2006), dandelion leaves contain an average of 0.353 g Mg and 0.96 g Ca/100 g dry matter. This discrepancy may result from the genetic, climatic, and soil differences. According to Šelih et al. (2014), the elemental composition in plants depends on internal factors, the application of fertilizers, and local fertility conditions because the elements are absorbed from the soil.

According to Escudero et al., (2003) 100 g of dandelion leaves contain of vitamin C 121 mg/kg, chlorophyll 450.784 mg/kg, carotene 206.429 mg/kg and minerals 499.7 mg/kg Ca and 6.299 mg/kg Mg. These values are slightly higher compared to our results (Tables 3 and 4). *Taraxacum officinale* leaves are rich in iron, calcium, magnesium, phosphorus, vitamins A and C, the B vitamins (thiamine and riboflavin). This plant is one of richest vegetable source of beta-carotene 0.84 mg/g compared to 0.61 mg/g of carrots tissue Gail (1994).

Conclusions

Based on the obtained results, it can be concluded that the dandelion or *Taraxacum officinale* plant contains more phenolic compounds in the outer parts of the plant. The choice of extraction conditions, primarily the type of extraction and temperature affect the antioxidant activity. Based on the obtained results, it can be concluded that the applied extraction technique had an impact on the values of antioxidant activity. In our research, the highest antioxidant capacity was achieved using Soxlet extraction. According to the literature, methanol is more efficient for the extraction of phenol from plant material, however, we used an aqueous solution of ethanol in this work due to less toxicity.

References

„Analytical Methods“ Atomic Absorpcion Spectrometry FP-3 Analysis of Meat and Meat Product, Perkin Elmer Instruments LLC,Part No. 0303-0152, USA, Publication, August 2000.

Bendritter M., Maupil, V., Vergely, C., Dalloz, F., Briot, F., Rochette, L. (1998): *Fund. Clin. Pharmacol.* 12, 510-516.

Benzie, F., Srain, J. (1996): Ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: The FRAP assay., *Anal. Biochem*, 239, 70-76.

Biel, W., Jaroszewska, A., Łysoń, E., and Telesiński, A. (2016): The chemical composition and antioxidant properties of common dandelion leaves compared with sea buckthorn, *Canadian Journal of Plant Science* 97(6), 1165-1174.

Dewanto, V., Wu, X., Adom, K.K., Lui, R.H. (2002): Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity, *J. Agric. Food Chem.* 50., 3010-3014.

Di Napoli, A., Zucchetti, P. A. (2021): Comprehensive review of the benefits of *Taraxacum officinale* on human health, *Bull Natl Res Cent* 45 (110), 1-7.

Gail, P.A. (1994): The Dandelion Celebration: A Guide to Unexpected Cuisine. Cleveland, Ohio: Goosefoot Acres Pr, 1994.

Ghaima, K. K., Hashim, N.M., Ali, S. A. (2013): Antibacterial and antioxidant activities of ethyl acetate extract of nettle (*Urtica dioica*) and dandelion (*Taraxacum officinale*), *Journal of Applied Pharmaceutical Science* 3 (5), 96-99.

Grieve, M. (1931): A Modern Herbal. New York: Dover Publications.

Guan, Y. T., Cenkowski, S., Hydamaka, A. (2006): Effect of drying on the nutraceutical quality of sea buckthorn (*Hippophae rhamnoides L. ssp. sinensis*) leaves, *Journal of Food Science* 70 (9), 514-518.

Gutteridge, J.M.C., Halliwell, B. (2010): *Biochemical and Biophysical Research Communications* 393 (4), 561-564.

Erhatić, R., Vukobratović, M., Dudaš, S., Mužić, M. (2014): "Kemijske karakteristike populacija maslačka s križevačkog i riječkog područja", *Agronomski glasnik* 76 (2014), 127-136.

Escudero, N.L., DE Arellano, M.L., Fernández, S., Albarracín, G., Mucciarelli, S. (2003): *Taraxacum officinale* as a food source, *Plant Foods for Human Nutrition* 58, 1–10.

Harrington, K.C., Thatcher, A., Kemp, P.D. (2006): Mineral composition and nutritive value of some common pasture weeds. *N.Z. Plant Prot.* 59, 261–265.

Ivanov, I. (2015): Polyphenols Content and Antioxidant Activities of *Taraxacum officinale* F.H. Wigg (Dandelion) Leaves, *International Journal of Pharmacognosy and Phytochemical Research* 6(4), 889-893.

Ivanova, D., Gerova, D., Chervenkov, T., Yankova, T. (2005): Polyphenols and antioxidant capacity of Bulgarian medicinal plants, *J. Ethnopharm.* 96, 145-150.

Kenny, O., Smyth, T. J., Hewage, C. M., Brunton, N. P. (2014): Antioxidant properties and quantitative UPLC-MS/MS analysis of phenolic compounds in dandelion (*Taraxacum officinale*) root extracts, *Free Radicals and Antioxidants* 4 (1), 55–61.

Khlifi, D., Hamdi, M., El Hayouni, A., Cazaux, S., Souchard, J.P., Coudere, F, Bouajila, J. (2011): Global chemical composition and antioxidant and anti-tuberculosis activities of various extracts of Globularia alypum L. (Globulariaceae) leaves, *Molecules* 16, 10592-10603.

Kuštrak, D. (2005): Farmakognozija: fitofarmacija, Zagreb: Golden marketing-Tehnička knjiga, ISBN: 953-212-202-8.

Lichtenthaler, K.H. (1987): Chlorolphylls and carotenoids: pigments of photosynthetic biomembranes, *Methods in Enzymology* 148, 350-382.

Miłek1, M., Legáth, J. (2015): Total Phenolic Content and Antioxidant Properties of Taraxacum officinale Extracts Obtained with Different Solvents, *Res J. Chem. Environ. Sci.* 3, 59-63.

Montefusco, A., Semitaio, G., Marrese, P. P., Iurlaro, A., De Caroli, M., Piro, G. Lenucci, M. S. (2015): Antioxidants in varieties of chicory (*Cichorium intybus* L.) and wild poppy (*Papaver rhoeas* L.) of Southern Italy, *Journal of Chemistry*, 1–8.

Mujica, M.V., Granito M., Soto, N. (2009): Importance of the extraction method in the quantification of total phenolic compounds in Phaseolus vulgaris L, *Interciencia* 34, 650–654.

Nikolić, G., Nikolić, S., Milić, B., Čanadanović- Brunet, J. (1998): Primena metode elektronske spinske rezonance za proučavanje antioksidantnih svojstava prirodnih fenolnih jedinjenja, *Acta Fac. Med. Naiss* 15(4), 183-188.

Nowak, A., Duchnik, W., Zielonka-Brzezicka, J., Muzykiewicz, A., Florkowska, K., Klimowicz, A., Kucharski, Ł., Wysocka, D., Dziedzic, A. (2019): The antioxidant activity of ethanolic and aqueous extracts of dandelion (*Taraxacum officinale L.)*, *Pomeranian J. Life Sci.* 64, 83–88.

Pădureţ, S., Amariei, S., Gutt, G., Piscuc, B. (2016): The Evaluation of Dandelion (*Taraxacum officinale*) Properties as a Valuable Food Ingredient, *Romanian Biotechnological Letters* 21 (3), 11569-11575.

Petkova, N., Ivanov, I., Topchiev S., Denev P., Pavlov, A. (2015): Biologically active substances and in vitro antioxidant activity of different extracts from dandelion (*Taraxacum officinale*) roots, *Scientific Bulletin. Series F. Biotechnologies.* 19, 190–197.

Rasool, S., Sharma, B. (2014): *Taraxacum officinale*: a high value less known medicinal plant. *Ann. Plant. Sci.* 3(12), 908–915.

Stewart-Wade, S.M., Neumann, S., Collins, L.L., Boland, G.J. (2002): The biology of Canadian weeds. 117. *Taraxacum officinale* G. H. Weber ex Wiggers., *Can J Plant Sci.* 82(4), 825–853.

Stylianou, N., Gekas, V., Istudor, V., Ioniţă, C. (2014): Research regarding *Taraxacum officinale* (L.) Weber with the intention of therapeutic exploring. Note I. Studies of phenolcarboxylic acids, *Farmacia* 62(2), 358-365.

Sun, Z., Su, R., Qiao, J., Zhao, Z., Wang, X. (2014): Flavonoids extraction from *Taraxacum officinale* (Dandelion): Optimisation using response surface methodology and antioxidant activity, *J. Chem.*, 1-7.

Šelih, V.S., Šala, M., Drgan, V. (2014): Multi-element analysis of wines by ICP-MS and ICP-OES and their classification according to geographical origin in Slovenia, *Food Chem.* 153, 414–423.

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NEAR INFRARED SPECTROSCOPY (NIRS) AS FOOD QUALITY AND FOOD FRAUD DETECTOR – APPLIED ON FISH STICKS

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original scientific paper

Summary

The aim of this study was to present the professional application of near infrared spectroscopy (NIRs) in the detection of food quality on purchased fish sticks. The measured NIR spectra of samples prepared from fish or squid were related to nutritional labels such as the amount of certain macronutrient and the proportion of the starting material (fish/squid). In the standard procedure, NIRs coupled with chemometric tools such as principal component analysis (PCA) and partial least square regression (PLSR) was used to investigate the qualitative and/or quantitative capabilities in determining food quality. Excellent qualitative differentiation was achieved with PCA, with biplots showing how the explanation of variation increased from 80.12 % when only nutritional information was used in the observation to 96.89% when nutritional information was coupled with the corresponding NIR spectrum. Since higher levels of animal protein in food are associated with an increase in price, the detection probabilities of different protein sources (fish/meat) were tested using PLSR, with 100% of the samples successfully detected. PLSR was also used to detect the correlation of the NIR spectra to the macronutrient contents and the strongest correlation was determined for proteins (R^2 = 0.99). The results confirmed the feasibility of using NIRs in the qualitative evaluation of samples where it is possible to determine the predominance of fish or squid, and also to estimate the expected protein content. The protein content is related to the price of the product, since all products containing animal proteins have higher prices that grow proportionally to their share. NIRs is not a qualitative method, but it can help in the selection of products, whose exact composition and possible adulteration can be confirmed by additional laboratory analysis.

Keywords: NIR spectra, fish sticks, chemometrics, qualitative analysis, proteins

Introduction

The European Environment Agency presented the data of fish and seafood supply with the average of 22.6 kg/capita/year as the EU-28 average. Croatia as a Mediterranean country lies under the average with 19.7 kg/Capita/year (EEA, 2016). The overview for the world food supply quantity is presented in Figure 1.

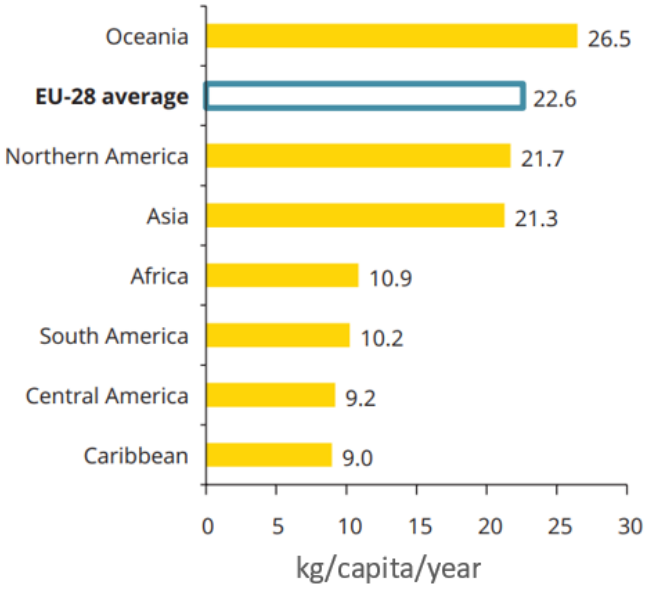

Figure 1. (FAO, 2016, FAO Food Balance Sheets: Food Supply Quantity)

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Based on the FAO data, (EEA, 2016; FAO, 2016), since the 1990s, fish supply derived from aquaculture increased since the 1990s from 7 to 44 %. China has played a major part in achieving this increase and currently represents over 60% of world aquaculture production (FAO, 2016; STECF, 2014). It is anticipated that by 2030, over 60% of fish supply for human utilization will be provided by aquaculture (FAO FishStat, 2016). With the increase of food demand, the food system becomes more important. The HLPE (2014) defined a food system as 'all the elements (environment, people, inputs, processes, infrastructures, institutions, etc.) and activities that relate to the production, processing, distribution, preparation and consumption of food, and the outputs of these activities, including socio-economic and environmental outcomes'. Food security is a core purpose of the food system (Maggio et al., 2015), and is seen from the perspective of access to food and its nutritional value, which moves away from the previous paradigm of focusing on the production of food (Ericksen, 2007; Gustafson et al., 2016). Unfortunately, in food access or production, forgery (adulteration) or quality manipulation may occur. Based on the Food Monthly Reports of the European Commission (2021), food quality and its safety must be monitored since cases of unapproved treatment of products regularly occur. Food fraud is also considered a problem, with and as the most common olive oil, milk, honey, saffron, orange juice, apple juice, grape wine, vanilla extract and fish being the most adulterated foods (EC, 2022). Some of the cases from the EC Report from June/2021, related to fish are: (i) Illegal unapproved treatments which include carbon monoxide and injection of nitrates and nitrites, in order to preserve the appearance of freshness given by the red colour, (ii) discovered mislabelled fishes (with the mislabelling rate of 17-36 %) as well as threatened species or (iii) sizing of tons of sea foods without documentation or product traceability requirements, especially red tuna (EC, 2021, p1). Therefore, different organisations are working with the goal of developing trustable methods for food analysis and determination of potential frauds. Such methods have already been developed for several foods (honey, wine, olive oil). For olive oil quality/fraud control, a spectrophotometric method (IOV, 2019) focussing on the quality of fats is used. Fats are characterised by the CH and OH bound and one of the methods for their analysis is investigating absorption of electromagnetic radiation at wavelengths in the near infrared range 780–2500 nm. Furthermore, NIR spectroscopy is a method used in order to investigate foods which comprise of broad bands arising from overlapping absorptions that correspond mainly to overtones and combinations of vibrational modes involving CH, OH and NH chemical bonds (Bøknæs et al., 2002; Valinger et al., 2011).

The aim of this paper was to present a professional aspect of NIR spectroscopy application on available fish sticks made by different producers. The aim was to relate the measured NIR spectra with nutritive labelling and to identify the ability of NIR spectroscopy usage in qualitative differentiation of samples which are prepared from fish or squid.

Materials and methods

Different fish stick products (n=5) were purchased in a local grocery store. Data of the basic nutritional composition as well as the content of fish or seafood (Table 1) were derived from the product labels.

Table 1. Nutritional value per 100 g of product with declared fish and squid content

Fish sticks (abbreviation)	**E** (kcal/kJ)	**Proteins** (g)	**Carbohydrates** (g)	**Fats** (g)	**Fish (squid) share (%)**
Product 1 (P1)	236/987	7	34	8	22 (0)
Product 2 (P2)	198/829	10.4	16.7	9.8	55 (0)
Product 3 (P3)	280/1172	11	30	13	22 (0)
Product 4 (P4)	228.5/956	12.9	21	10.3	0 (61)
Product 5 (P5)	206/866	12.8	16.8	9.8	0 (65)

Fish sticks were left at room temperature for 10 minutes after which they were scanned with a probe connected to the NIR device. The probe was placed on a part of the sample (i) coated for frying (with bread crumbs) and (ii) a cross-section of the stick with no coating. The procedure was repeated 3 times.

NIR spectroscopy was conducted with the NIR spectrometer (NIR – 128 –1.7-USB/6.25/50 mm Control Development Inc., South Bend, USA) with software Spec32. Spectra were recorded in absorbance mode in the wavelength range from 904-1699 nm. For the chemometric analysis raw NIR spectra without any pre-processing were used.

Data Analysis by use of chemometrics

Principal component analysis (PCA) was applied in order to determine the qualitative similarities or differences of the fish sticks samples. The partial least squares regression (PLSR) was applied to investigate the potential of quantitative prediction abilities for all observed parameters given on the product label (Table 1). In order to compare the model results, the coefficient of correlation (R_c), mean square error (MSE), root mean square error (RMSE), Ratio of standard error of performance to standard deviation (RPD) as well as the residual predictive deviation (RPD) were calculated (Fearn, 2002). All calculations were conducted using MS Excel and its *XLStat 2014* add on.

Results and discussion

Environmental conditions at which the samples were recorded (temperature and humidity) were measured using a data logger (Datalogger LOG 32T, Dostmann electronic GmbH, Germany) and average values were 22 °C with 31.6% RH and ± 0.1% deviation.
Measured NIR spectra of fish sticks products are presented in Figure 2. Only one sample, fish stick P2 partially stands out, while other spectra almost overlap and have the same trend. To give better insight where in the spectrum similarities/differences related to the chemical composition of the observed samples can be expected, the basic bands (Okparanma, et al., 2018) are also shown.

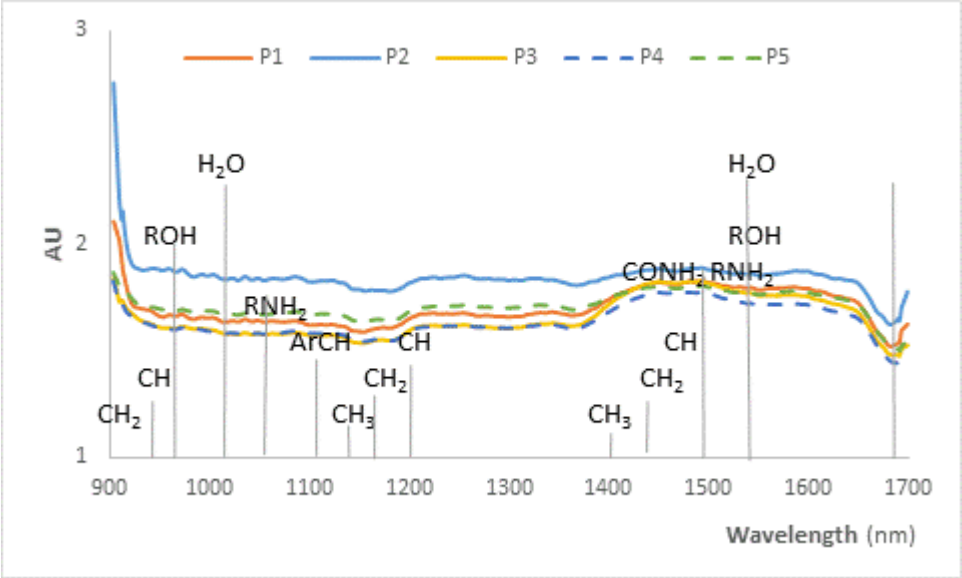

Figure 2. Near infrared scan of fish sticks samples (P1-P3 contain fish, P4 and P5, dashed lines, contain squids)

Due to invisible significant differences in certain parts of the raw NIR spectra, it was necessary to apply chemometric techniques, most commonly PCA and PLSR (Yu, 2021).
To identify qualitative similarities/differences in the observed fish sticks samples, PCA was applied and the obtained results are presented in Figure 3, in a form of biplots. The percentage of the explained variance based on the nutritional composition (Figure 3A) was 80.12%, while the percentage of explained variations in the observed data set increased to 96.89% when the NIR scans were associated with the nutritional composition (Figure 3B). Based on the Biplot in Fig. 3B, samples of fish fingers positioned in the upper part of the score plot, in the 1st & 2nd quadrant (P1 and P2). For those samples content of carbohydrates (CHO) is higher than the content of proteins and fats. The products P4 and P5, which contain squid are positioned in the same quadrant as variable "% squid", in the fourth quadrant.

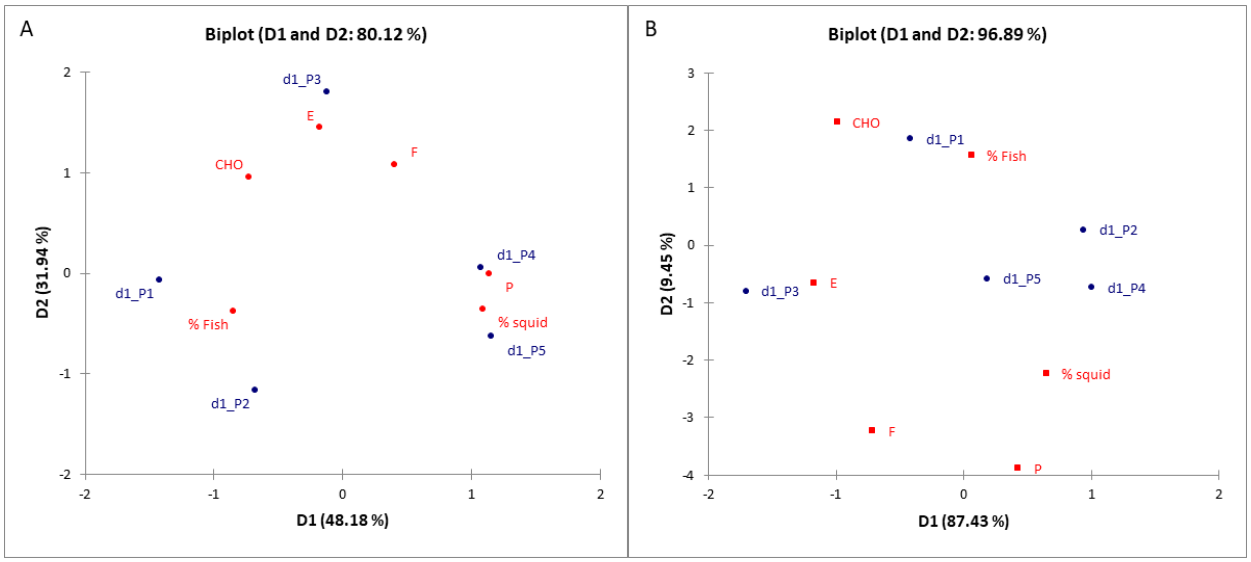

Figure 3. Distribution of the fish stick samples based on nutritional composition (A) and the NIR scan related to the nutritional composition (B)

The qualitative differentiation is confirmed to be successful when the NIR scan was included in the PCA observation (Figure 3B vs Figure 3A). These findings created the predispositions for the next step, which is the quantitative assessment, i.e. the possibility of potential prediction of the nutritional composition of fish sticks based on NIR spectra (Cozzolino et al., 2005; Nilsen and Esaiassen, 2005). To implement this, NIR spectra were used as input data related to currently available nutritional data from the packaging (as outcome variables). The relationship of the mentioned input/output data was presented by following parameters: R^2, SD, MSE, RMSE, RER and the RPD (Birkić et al., 2022). The trends that are desirable are the lowest errors with as little scatter as possible and the highest possible values of the correlation coefficient, RER and RPD (Balbino et al., 2022). The ratio between the standard deviation of the population and the standard error of cross-validation, RPD value > 3 is considered as a value and such models are recommended for screenings, while RPD > 5 is considered as a good value and such models are suitable for quality control. Also, it is expected that the range error ratio (RER) is larger than the RPD values (Fearn, 2002). The strongest correlation between the NIR spectra information and the information from the product label was determined for proteins ($R^2 \approx 0.99$) and all RER values were, as expected, higher that the RPD values. It can be seen from Table 2 that almost all parameters could be screened by NIRs. Content of proteins and fish share in the products are even suitable to be screened using NIRs. The reason for this are the vibrations of certain molecule bands of the illuminated sample which absorbed light selectively and generated specific NIR spectra (Downey, 1996; Folkestad et al., 2008; Mathiassen et al., 2011). In this case, the NIR spectra shows the overtone and combination bands of N-H, O-H, and C-H groups (Balbino et al., 2022, Yu, 2021).

Table 2. Relation of nutritive information and NIR spectra calculated by partial linear regression

Parameter	R^2	SD	MSE	RMSE	RER	RPD
Fish (yes/no)	0.917	0.315	0.020	0.141	7.088	3.882
Energy (kcal)	0.825	26.940	145.153	12.048	6.806	2.670
Proteins (g)	0.988	0.537	0.058	0.240	24.553	9.989
CHO (g)	0.906	4.833	4.672	2.161	8.006	3.659
Fat (g)	0.925	0.990	0.196	0.443	11.289	4.072
Fish (%)	0.948	10.300	21.150	4.600	11.957	4.901
Squid (%)	0.894	22.540	101.630	10.080	6.448	3.426

The proteins are related to the N-H band vibrations (Pérez-Marín et al., 2006) which correspond to the vibration in the range of 1400-1600 nm (second overtone region) as well as in the range of 900-1100 nm (third overtone region) (Eldin, 2011) which is in the range of the used device, 900-1700 nm.

Conclusions

The results confirmed the possibility of applying NIRs in the qualitative assessment of fish sticks composition for which it is possible to determine whether it has dominant share of fish or squid. NIR is also capable of protein content estimation, which can prove to be important in determining the price of the products. Namely, all products with animal protein content have higher prices, which grow in proportion to the share of proteins they contain. Consequently, NIR screening can provide an insight whether the price of the product is justified based on the protein content. Although NIRs is not a qualitative method, it can help in the screening of food product composition, with the condition that this composition is later confirmed by additional analytical methods.

References

Balbino, S., Vincek, D., Trtanj, I., Egređija, D., Gajdoš-Kljusurić, J., Kraljić, K., Obranović, M., Škevin, D. (2022): Assessment of Pumpkin Seed Oil Adulteration Supported by Multivariate Analysis: Comparison of GC-MS, Colourimetry and NIR Spectroscopy Data, *Foods* 11(6), 835.

Birkić, A., Valinger, D., Jurinjak Tušek, A., Jurina, T., Gajdoš Kljusurić, J., Benković, M. (2022): Evaluation of the Adsorption and Desorption Dynamics of Beet Juice Red Food Colour on Alginate Microbeads, *Gels* 8(1), 13.

Bøknæs, N., Jensen, K.N., Andersen, C.M., Martens, H. (2002): Freshness Assessment of Thawed and Chilled Cod Fillets Packed in Modified Atmosphere Using Near-infrared Spectroscopy, *LWT - Food Sci. Tech.* 35(7), 628-634.

Cozzolino, D., Murray, I., Chree, A., Scaife, J.R. (2005): Scaife Multivariate determination of free fatty acids and moisture in fish oils by partial least-squares regression and near-infrared spectroscopy, *LWT - Food Sci. Tech.* 38(8), 821-828.

Downey G. (1996) Non-invasive and non-destructive percutaneous analysis of farmed salmon flesh by near infra-red spectroscopy, *Food Chem* 55(3), 305-311.

EC (2021): Monthly Summary of Articles on Food Fraud and Adulteration, Food and Fraud Cases. 2021. https://knowledge4policy.ec.europa.eu/node/47003_hr

EC (2022): Supporting policy with scientific evidence: Food Fraud. https://knowledge4policy.ec.europa.eu/node/221_hr

EEA Report (2016): Seafood in Europe, A Food system approach for sustainability. Report No 25/2016.

Eldin AIB (2011): Near Infra-Red Spectroscopy. In: Wide Spectra of Quality Control (ed. Akyar, I). Intech.

Ericksen, P.J. (2007): 'Conceptualizing food systems for global environmental change research', *Global Environmental Change* 18(1), 234–245.

FAO (2016): The state of world fisheries and aquaculture. Contributing to food security and nutrition for all, Food and Agriculture Organization of the United Nations, Rome.

FAO FishStat (2016): 'Marine fish + crustaceans' (http://faostat3.fao.org/home/E) accessed 8 August 2016.

Fearn, T. (2002): Assessing Calibrations: SEP, RPD, RER and R2, *NIR News* 13, 12.

Folkestad, A., Wold, J.P., Rørvik, K-A., Tschudi, J., Haugholt, K.H., Kolstad, K., Mørkøre, T. (2008): Rapid and non-invasive measurements of fat and pigment concentrations in live and slaughtered Atlantic salmon (Salmo salar L.), *Aquaculture* 280(1-4), 129-135.

Gustafson, D., Gutman, A., Leet, W., Drewnowski, A., Fanzo, J. and Ingram, J. (2016): 'Seven food system metrics of sustainable nutrition security', *Sustainability* 8, 196.

HLPE (2014): Food losses and waste in the context of sustainable food systems, High Level Panel of Experts on Food Security and Nutrition of the Committee on World Food Security, Rome.

IOV, international olive council (2019): Method of analysis – Spectrophotometric investigation in the ultraviolet. https://www.internationaloliveoil.org/wp-content/uploads/2019/11/Method-COI-T.20-Doc.-No-19-Rev.-5-2019-2.pdf

Lin, M., Cavinato, A.G., Huang, Y., Rasco, B.A. (2003): Predicting sodium chloride content in commercial king (Oncorhynchus tshawytscha) and chum (O. keta) hot smoked salmon fillet portions by short-wavelength near-infrared (SW-NIR) spectroscopy, *Food Res. Int.* 36(8), 761-766.

Maggio, A., Van Criekinge, T., and Malingreau, J. P. (2015): Global food security 2030: Assessing trends with a view to guiding future EU policies, JRC Science and Policy Reports — Foresight series, European Commission, Joint Research Centre, Publications Office of the European Union, Luxembourg.

Mathiassen, J.R. Misimi, E., Bondø, M., Veliyulin, E., Østvik, S.O. (2011): Trends in application of imaging technologies to inspection of fish and fish products, *Trends Food Sci. Tech.* 22(6), 257-275.

Nilsen, H., Esaiassen M. (2005): Predicting sensory score of cod (Gadus morhua) from visible spectroscopy, *LWT - Food Sci. Tech.* 38(1), 95-99.

Okparanma, R. N., Araka, P. P., Ayotamuno, J. M., & Mouazen, A. (2018): Towards enhancing sustainable reuse of pre-treated drill cuttings for construction purposes by near-infrared analysis : a review, *JCECT* 9(3), 19–39.

Pérez-Marín, D., Fearn, T., Guerrero, J.E., Garrido-Varo, A. (2009): A methodology based on NIR-microscopy for the detection of animal protein by-products, *Talanta* 80(1), 48-53.

STECF (2014): The economic performance of the EU aquaculture sector (STECF-14-18), JRC Science and Policy Reports, European Commission; Scientific, Technical and Economic Committee for Fisheries, Publications Office of the European Union, Luxembourg, EUR 27033 EN, JRC 93169, 451.

Uddin, M., Okazaki, E., Ahmad, M.U., Fukada, Y.,Tanaka, M. (2006): NIR spectroscopy: A non-destructive fast technique to verify heat treatment of fish-meat gel, *Food Control* 17(8), 660-664. https://doi.org/10.1016/j.foodcont.2005.04.009

Valinger, D., Gajdoš Kljusurić, J., Kovačević, D., Kurtanjek, Ž. (2011): Application of NIR spectroscopy for monitoring quality of surimi. Proceedings of 13th Ružička days, Today Science – Tomorrow industry, Vukovar, 16-17. September 2011. (ed. D. Šubarić), pp. 431– 438.

Yu, W. (2021): Applications of near infrared spectroscopy for fish and fish products quality: a review, *IOP Conference Series: Earth and Environmental Science* 657, 012115.

Hrana u zdravlju i bolesti, znanstveno-stručni časopis za nutricionizam i dijetetiku (2022) 11 (1) 21-28
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DIETARY SUPPLEMENTS AND HERBAL PREPARATIONS AS A SUPPORT FOR PATIENTS WITH SOME TYPE OF ARTHRITIS

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review paper

Summary

Introduction: It is estimated that today 54 million people in the world live with some type of arthritis. The three most common forms of arthritis are infectious, rheumatoid and osteoarthritis. Although disease occurs most commonly in the old age, rheumatoid arthritis can affect people in the most productive period of life, between ages of 20 and 40. First line of medical treatment for this disease are anti-inflammatory drugs, but corticosteroids and antibiotics are also used very commonly, along with various measures of physical medicine. Dietary supplements and herbal preparations are used only sporadically, despite accumulated evidence of their beneficial effect on arthritis symptoms.
The aim of paper: The aim of the paper was to collect and analyse literature data on dietary supplements and herbal preparations in support of arthritis treatment.
Results: Herbal preparations based on willow, red pepper and most recently, turmeric show potential in the treatment of arthritis. Dietary supplements as antioxidants (vitamins C and E, minerals zinc and selenium), chondroitin, glucosamine, collagen and hyaluronic acid also show positive effects. In addition, numerous food ingredients poses anti-inflammatory effects, such as acetylsalicylic acid, omega-3 fatty acids, polyphenols and flavonoids. These research findings suggest that a proper food selection and menu planning, combined with some of the aforementioned could affect the symptoms and progression of the disease.
Conclusion: Although medical treatment is still the first choice for people with arthritis, accumulating evidence supports the use of various dietary supplements and herbal preparations to alleviate symptoms of arthritis. Additionally, given that many foods contain ingredients with potent anti-inflammatory characteristics, proper food selection could add the additive effect and improve quality of life of people with arthritis.

Keywords: arthritis, herbal preparations, dietary supplements

Introduction

The word arthritis comes from the Greek word „arthros“, which means joint and from the word „itis“ which means inflammation. Arthritis is often used as a term for any disorder that causes joint damage or inflammation. Symptoms most commonly include join pain and stiffness (NIAMS, 2014). Other symptoms may include redness, heat and swelling and the decreased range of motion of arthritic joints (CDC, 2016). In some types of arthritis can be affected and other organs. There are over 100 types of arthritis described in the literature, and the most common forms that occur are osteoarthritis and rheumatoid arthritis (NIAMS, 2014).
Osteoarthritis is a degenerative joint disease and usually occurs with age, affecting the fingers, knees and hips, while rheumatoid arthritis is an autoimmune disease and most commonly affects hands and feet (NIAMS, 2014). Except these two most common types, it often occurs as septic arthritis, which is also known as infectious arthritis, and is usually caused by bacteria. It can also be caused by a virus or fungus. This type of arthritis is an inflammation of the joints caused by a microorganism. Other common type of arthritis include gout, systemic lupus, fibromyalgia and others (CDC, 2015). Today with the increase in life expectancy, the prevalence of osteoarthritis, which is also the most common type of arthritis, has increased (Pradeep, 2019). Although osteoarthritis is not necessarily a consequence of aging, there is a strong relationship between age and the increasing incidence of this disease. The connection could be correlated with the aging of cartilage *in vivo* chondrocytes, which in turn causes an age-related decline in the ability of cells to maintain articular cartilage. That means, increasing age increases the risk of osteoarthritis because chondrocytes lose the ability to replace their extracellular matrix (Martin et al., 2002). The body cartilage located on the joints erodes during osteoarthritis which in turn causes pain and can lead to the loss of joint function in affected individuals. The pathophysiology of osteoarthritis is still insufficiently studied and there are currently no treatments that modify the disease. Today, joint replacement remains as the only final treatment. Basic and clinical research are now focused on

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understanding the aging process of cartilage and its role in osteoarthritis (Pradeep, 2019).

Rheumatoid arthritis is an autoimmune disease whose outcome is difficult to predict (Combe, 2009). Early diagnosis is crucial for optimal therapeutic effect, especially the patients with well-characterized risk factors for poor outcomes, such as: high disease activity, the presence of antibodies and early joint damage (Josef et al., 2016). Although it most commonly occurs in the elderly, rheumatoid arthritis affects people in the most reproductive period of life, between the ages of 20 and 40. In most patients, disease progression occurs with bone erosion and cartilage fracture resulting in joint destruction, functional impairment and increased mortality. The goal of treating patients should be to achieve clinical remission to prevent structural damage and long-term disability. The early use of effective anti-rheumatic drugs that modify the disease is a key point in patients at risk of developing persistent and erosive arthritis. Intensive treatment such as combined DMARDs (disease modifying anti-rheumatic drugs) with steroids or biological therapies can induce high remission rates and control radiological progression. In most cases, it provides better outcomes than the DMARD monotherapy in the early detection of rheumatoid arthritis, so it is desirable to consider this therapy very early in high-risk patients. Careful monitoring of disease activity and radiographic progression is mandatory to adjust DMARD therapy and strategy (Combe, 2009).

Infectious arthritis is a disabling and potentially life-threatening condition that requires fast diagnosis and treatment (Learch, 2003). It can be caused by bacteria, viruses, fungi or parasites. It can occur in the context of another systemic infectious disease when microorganisms spread through the bloodstream from the primary focus to the joint, and less often, it is caused by direct injury to the joint during trauma, surgery or puncture of the joint. The main pathogen in adults is *Staphylococcus aureus* and therefore the initial treatment therapy is focused on this microorganism. Early recognition and detection of infectious arthritis can prevent a poor outcome, especially in elderly patients or those with underlying joint disease (Smith, 1990). Detection of emerging arthrogenic viruses has altered the ethology of infection-related arthritis. The role of viruses in the pathogenesis of chronic inflammatory diseases such as rheumatoid arthritis is increasingly recognized (Ashish etal., 2014).

Herbal preparations and dietary supplements to support arthritis treatment

Chronic inflammatory joint disorders such as osteoarthritis and rheumatoid arthritis lead to an increase in inflammation and oxidative stress resulting in progressive histological changes and disabling symptoms. Conventional drugs are powerful, but at the same time, they are often associated with serious and sometimes life-threatening side effects. Although medical treatment with conventional drugs is still the first choice of arthritis sufferers, various herbal preparations and dietary supplements can help primary medical therapy. They can reduce the number of the symptoms and affect disease progression. This can improve and control further course of the disease, but it is important to include them with the right choice of foods.

Herbal preparations

Willlow (Salix alba)

Willow extract has been used for thousands of years as an anti-inflammatory, analgesic and antipyretic drug. However, a small number of clinical studies have supported the use of willow bark extract in chronic lower back pain, joint pain and osteoarthritis pain (Mohd et al., 2015). However, the effectiveness of willow bark extract in treating painful movement disorders, such as back pain and arthritis pain, has been attributed to the content of salicin and its salicylate prodrug derivatives (Nahrstedt et al., 2007). It is already known that osteoarthritis is a disorder associated with aging, and that it can be caused by the accumulation of advanced glycation products (AGE). Excessive degradation of collagen and aggregate type II by matrix metalloproteinase (MMPs) disintegrants, and AGE-induced thrombosporin-type metalloproteinase (ADAMTS) is a key event in the pathogenesis of arthritis (Gao et al., 2019). Activation of the nuclear kappa B factor pathway (NF-kB) induces the expression of a cascade of pro-inflammatory cytokines such as interleukin (IL)-1β and tumour necrosis factor α (TNF-α). The study examined the effects of salicin on AGE induced by articular extracellular matrix degradation in human chondrocytes (SW1353). The study found a new beneficial role of salicin in saving the breakdown of collagen and type II aggregates, reducing oxidative stress, reducing pro-inflammatory cytokine expression and inhibiting NF-kB pro-inflammatory signalling pathway activation in AGE-stimulated chondrocytes. Therefore, salicin may have great potential as safe and effective therapy against development and progression of osteoarthritis (Gao et al., 2019). In recent years, various *in vitro* studies have shown that the anti-inflammatory action of willow bark extract is associated with a decrease in

the regulation of inflammatory mediators TRF-α and NF-kB. Although willow extracts are mostly standardized to salicin, other salicylates are also contained as well as polyphenols and flavonoids that may also play an important role in therapeutic terms. Side effects are minimal compared to nonsteroidal anti-inflammatory drugs including aspirin. The reason to concern due to use may relate to allergic actions in persons sensitive to salicylate (Mohd et al., 2015).

Red pepper (Capsicum annuum)

Capsaicin is a hot alkaloid of red pepper and has been extensively studied for its biological effects, which are of great pharmacological importance. It has many effects but its potential clinical value for pain relief, cancer prevention and weigh loss is of great importance. Capsaicinoids have been shown to be potential capsaicin receptor agonists (TRPV1). Therefore, they could act in a receptor-dependent way, but also in a receptor-independent way. The involvement of neuropeptides, substance P, serotonin and somatostatin in the pharmacological activities of capsaicin was intensively investigated during the study of their action. It has been proven that topical application of capsaicin relieves pain in arthritis, postoperative neuralgia, diabetic neuropathy, etc. The anti-inflammatory and antioxidant properties of capsaicin have been established by numerous studies (Srinivasan, 2016). Long-term use of capsaicin reversibly depletes stores of substance P and possibly other neurotransmitters from sensitive nerve endings. This reduces or eliminates the transmission of painful stimulation from peripheral nerve fibres to higher centres. In clinical studies of patients with post-hepatic neuralgia, diabetic neuropathy or osteoarthritis, adjuvant topical capsaicin therapy achieved greater relief in most studies. Topical capsicin is not associated with serious systemic side effects, but many patients report burning and stinging, especially during the first week of use. Therefore, because of its effects, local capsaicin deserves consideration as adjunctive therapy in chronic pain conditions, including osteoarthritis, which is difficult to treat (Rains et al., 1995). There is also interest in the use of capsaicin in injections to treat conditions such as focal pain, arthritis and other musculoskeletal disorders. Recently, capsaicin injection has shown therapeutic efficacy in a patient with Morton's neuroma, a painful condition of the foot associated with compression of one of the digital nerves. Pain relief is associated with changes in tactile sensitivity. Although injection causes short-term pain, short systemic exposure and the potential to establish long-term analgesia without other sensory changes create an attractive clinical profile. Short-term and long-term effect result from functional and structural changes in nociceptive regions (Chung et al., 2016).

Turmeric (Curcuma longa)

Curcumin is a polyphenolic compound derived from turmeric. It has various pharmacological effects including anti-inflammatory, antioxidant, antiproliferative and antiangiogenic activity. Numerous studies have examined its effects. In a first phase clinical trial, curcumin was shown to be safe even at high doses of 12g/day in humans, with poor bioavailability. The main reason contributing to low plasma and tissue concentrations of curcumin are the consequences of poor absorption, rapid metabolism and rapid systemic elimination. To improve the bioavailability of curcumin, a number of approaches have been taken, from the use of piperine that interferes with glucuronidation, the use of liposomal curcumin, the production of curcumin nanoparticles, and the use of curcumin phospholipid complexes and the use of structural curcumin analogues (Anand et al., 2007). A study was conducted in which curcumin was derived from ordinary spicy turmeric and formulated into biodegradable nanoparticles with the aim of improving oral bioavailability. *In vivo* pharmacokinetics found that nanoparticles show at least a 9-fold increase in oral bioavailability compared to curcumin given with piperine as an adsorption enhancer indicating the potential of this method (Shaikh et al., 2009). However, despite low bioavailability the therapeutic efficacy of curcumin in various diseases including cancer, cardiovascular disease, diabetes, arthritis, neurological disease and Crohn's disease has been documented (Anand et al., 2007). Numerous clinical studies of curcumin supplementation on a variety of disease including osteoarthritis have been conducted in recent decades. The mechanism of acting of curcumin including modulation of the eicosanoid pathway toward the anti-inflammatory pathway and modulation of serum lipid levels was investigated, and minimal or no side effects were observed (Yung et al., 2019). In one study, the highly available form of curcumin in the all-natural matrix of turmeric was evaluated for its ability to improve the clinical symptoms of rheumatoid arthritis. A randomized, double-blind, placebo-controlled parallel study was conducted to evaluate comparative efficacy of two different doses of curcumin with that of placebo in patients with active rheumatoid arthritis. Twelve patients in each group received placebo, 250 or 500 mg of curcumin

product twice daily for 90 days. Patient responses were assessed using the American College of Rheumatology (ACR) responses, visual analogue scale (VAS), C-reactive protein (CRP), disease activity assessment (DAS28), erythrocyte sedimentation rate (ESR) and rheumatoid value factor (RF). Patients with rheumatoid arthritis who received low and high doses of curcumin reported statistically significant changes in clinical symptoms at the end of the study. These results were confirmed by significant changes in ESR, CRP and RF values in patients receiving the study product compared to initial values and placebo. The results suggest that curcumin in the turmeric matrix acts as an analgesic and anti-inflammatory agent for the treatment of rheumatoid arthritis (Amalraj et al., 2017).

Vitamin and mineral based dietary supplements

A functional immune system is essential for a healthy life. This is achieved by coordinated activation and interaction of different immune cells. Yet, activating the immune response is just as important as deactivating it when pathogens are out of the organism, otherwise host tissue damage can occur, where damage can reach life-threatening levels. Autoimmune diseases (AD) affect five to eight percent of the world's population and are the result of attacks by one's own immune cells on other tissues and organs. In recent years, the incidence has been steadily rising, reaching alarmingly high numbers especially for: type 1 diabetes mellitus, Crohn's disease, rheumatoid arthritis, Sjogren's syndrome and multiple sclerosis. It is important to concentrate on the importance of diet for balancing immune function (Wassels et al., 2020).

Rheumatoid arthritis as an autoimmune and inflammatory disease can cause joint damage. Among the risk factors, diet plays an important role as it can make it worse or reduce inflammation. Selenium is considered an essential trace element that is structural component of antioxidant enzymes, but its concentration can be affected by diet, medication and genetic polymorphism. Studies have shown that patients with rheumatoid arthritis have a deficit of nutrients from some groups, which is related to the parameters of disease activity. There has been shown to be a change in serum selenium levels in this population. Experimental studies examining the effects of new selenium nanoparticles in rheumatoid arthritis-induced models have shown promising results in restoring antioxidant enzyme levels. Glutathione peroxidase (GPx), which is an important protein for selenium and may have a modulating effects on inflammatory processes in rheumatoid arthritis, plays a special role (Turrubiates-Hernandez et al., 2020).

Zinc is a nutritionally essential mineral and plays a role in arthritis as an effector of the immune system, inflammation and metabolism. Patients with rheumatoid arthritis have decreased levels of zinc in their blood. It is unknown if this change is just manifestation of the acute phase of the inflammatory response or relates to altered zinc availability in tissues, and therefore requires zinc-related dietary changes. Zinc is a cofactor in over 3.000 human proteins and as a signal ion affects many pathways relevant to arthritis as a disease. How will the zinc affect the disease depends not only on zinc status, but also on many proteins that maintain cellular zinc homeostasis. The conclusion is that this metal ion in arthritis should be routinely monitored and that it has untapped potential for treatment (Frangos et al., 2020).

Vitamin D affects physiological systems that extend far beyond its established functions in calcium and bone homeostasis. Among them were the strong immunomodulatory effects of the active form of vitamin D, 1,25-dihydroxyvitamin D3 (1,25 (OH) 2D3) highlighted. The nuclear vitamin D receptor (VDR) for 1,25-(OH) 2D 3 is expressed by many cells within the immune system. Vitamin D deficiency has been associated with a variety of autoimmune disorders including rheumatoid arthritis (Harrison et al., 2020). The positive effects of vitamin D on the immune system include an increase in the microbicide capacity of monocytes/ macrophages and a decrease in inflammatory cytokines produced by lymphocytes. Despite some controversy, most research supports the idea that lower vitamin D levels correlate with more severe clinical manifestations in rheumatoid arthritis and other rheumatic diseases. Therefore, vitamin D supplementation is important to establish normal serum vitamin D levels. The effect of vitamin D is associated with proteoglycan (PG) which is a specific cartilage antigen (Ishikawa et al., 2017).

Chondroitin and glucosamine

Chondroitin sulphate is a sulphated glucosaminoglycan composed of alternating saccharide chains, more precisely from N-acetilgalactosamine and glucuronic acid. It is usually attached to proteins as part of proteoglycans. It is an important structural component of cartilage and allows it to withstand greater pressure (Baeurle et al., 2009). For the production of chondroitin sulphate (CS) the industry uses sources that originate from animal tissue, both terrestrial and aquatic animal

species. Chondroitin sulphate possesses a heterogeneous structure, and the physicochemical profile in different species and tissues is responsible for the different and specialized functions of these macromolecules (Volpi, 2019).

Glucosamine is an amino sugar in which the hydroxyl group of glucose is replaced by an amino group. It is an important part of the polysaccharides chitin and chitosan and is very hydrophilic. It represents an important compound required for the formation of cartilage cells and represents one of the elementary units of the cartilaginous matrix (Ma et al., 2019).

Chondroitin sulphate an glucosamine sulphate have beneficial effects on the metabolism of *in vitro* cell models derived from synovial joints: chondrocytes, synoviocytes and subchondral bone cells involved in osteoarthritis. They increase the synthesis of collagen and proteoglycans type I in human joint chondrocytes and are able to reduce the production of some anti-inflammatory mediators and proteases, reduce the process of cell death and improve the anabolic/catabolic balance of the extracellular cartilage matrix (ECM). The structure-altering effects of these compounds have been reported and analysed in recent meta-analysis. Results from the osteoarthritis of the knee show a small but significant narrowing of the joint space (Henrotin et al., 2014). Therefore, glucosamine sulfate and chondroitin sulfate are often used to prevent further joint degeneration in osteoarthritis.

Methylsulfonylmethane (MSM) is a compound that contains organic sulphur and has also been shown to slow the anatomical progression of joints in knee osteoarthritis. MSM is often combined with glucosamine and chondroitin sulphate. A study was conducted to compare the clinical outcome of glucosamine-chondroitin sulphate (GC), glucosamine-chondroitin sulphate-methylsulfonylmethane (GCM) and placebo among patients with knee osteoarthritis. 147 patients participated in this double-blind randomized controlled clinical study (Lubis et al., 2017). They were rated by the WOMAC score, which represents the arthritis index of the University of Western Ontario and MaMaster and is widely used in the evaluation of osteoarthritis of the hip and knee. It is formulated in the form of a questionnaire that is conducted independently and consists of 24 items that are divided into three subscales: pain, stiffness and physical function (ACOR, 2013). Patients were divided into three groups: GC (n=49), GCM (n=50) and placebo (n=48). The GC group received 1500 mg of glucosamine + 12000 mg of chondroitin sulphate + 500 mg of saccharumlactis. The GCM group received 1500 mg glucosamine sulphate + 1200 mg chondroitin sulphate + 500 mg MSM, while the third group received placebo. The drugs were given once a day for three consecutive months. After twelve weeks, significant differences were found between the three treated groups according to the WOMAC score (p=0.03) and according to the VAS score (p=0,004). The results showed that the combination of glucosamine-chondroitin sulfate-methylsulfonylmethane showed clinical benefit for patients with osteoarthritis of the knee compared with GC and placebo (Lubis et al., 2017).

Collagen

Articular cartilage is a connective tissue consisting of a specialized extracellular matrix (ECM) that predominantly dominates. Collagen and type II aggregate are the major ECM proteins in cartilage. The group of minor collagens includes collagen types IV, VI, IX, X, XI, XII and XIII. Although they make up only a small part of the mature matrix, smaller collagens play important structural roles in the mechanical properties, organization and shape of articular cartilage and fulfil certain biological functions. Progressive destruction of the cartilage includes the breakdown of matrix components including small collagen (Luo et al., 2017). Undenatured collagen type II (UC-II) is used as a dietary supplement and is obtained from chicken thoracic cartilage. A study was conducted to evaluate the effectiveness and tolerability of UC-II on knee arthritis pain and associated symptoms compared with placebo and glucosamine hydrochloride plus chondroitin sulphate. A randomizes study conducted with volunteers divided in three groups for 180 days. The results showed that in the group of volunteers who used UC-II there was a significant decrease in the total WOMAC score compared to placebo (p=0,002) and GC (p=0,04). Supplementation with UC-II also resulted in significant changes for all three WOMAC subscales: pain (p=0.0003 versus placebo, p=0,016 versus GC), stiffness (p=0,004 versus placebo, p=0,044 in relation to GC) and physical function (p=0,007 in relation to placebo). The study concluded that UC-II improved knee joint symptoms in subjects with osteoarthritis of the knee and was well-tolerated (Lugo et al., 2016).

Hyaluronic acid

Hyaluronic acid is polyanionic natural polymer that occurs as a linear polysaccharide composed of glucuronic acid and N-acetyl glucosamine that is repeated via a β-1,4 bond. It has a wide range of

applications due to its excellent physiochemical properties such as biodegradability, biocompatibility, non-toxicity and no immunogenicity. It serves as an excellent tool in the following biomedical applications: osteoarthritis surgery, ophthalmic surgery, plastic surgery, tissue engineering and drug delivery. It is a powerful antioxidant and one of the most famous properties is that it binds water to tissue (Sudha et al., 2014). It is synthesized by the bioactivity of hyaluronan synthase (HAS) which is said to have three isoforms (HAS 1, HAS 2 and HAS 3) in humans (Itano et al., 1999). Hyaluronic acid is widespread but is mainly localized in the extracellular matrix and body fluid, where it contributes to the high elasticity of fluid and the elasticity of connective tissue absorbing mechanical stress, for example between cartilage and cartilage surface (Neustadt et al., 2007). Regarding pain suppression, hyaluronic acid alleviates prostaglandin or bradykinin-induced pain in animal experiments (Gotoh et al., 1993). Hyaluronic acid binds to CD44 signalling receptor and mediated mobility receptor (RHAMM) molecules. Since CD44 is the primary receptor for hyaluronic acid and has been found to bind to other matrix components including collagen, chondroitin sulphate and osteopontin (Taylor et al., 2006). In the treatment of arthritis it can be administered in the form of injections where it is administered intraarticularly and in tablets form where it is administered orally. Intraarticular application of hyaluronic acid is widespread in the treatment of osteoarthritis (Simon et al., 2007). Hyaluronic acid injection provides transient pain relief but its mechanism of action has not yet been fully elucidated. According to a recent recommendation by the International Society for Osteoarthritis Research (OARSI) (Zhang et al., 2008) in the treatment of osteoarthritis of the hip and knee, the strength of the recommendation (SOR) for intraarticular hyaluronic acid is relatively low (64%), whereas in nonsteroidal anti-inflammatory drug (NSAID) for oral administration as an analgesic it is 92-93 %. The effect of hyaluronic acid in osteoarthritis is often attributed to its potential use in viscosity supplementation (Neustadt et al., 2007). A direct effect of hyaluronic acid against inflammation and cartilage degradation in osteoarthritis has been proposed. In addition to osteoarthritis, intraarticular injection of hyaluronic acid has also been used in patients with inflammatory arthritis such as rheumatoid arthritis (Matsuno et al., 1999).

Omega 3-fatty acids

Osteoarthritis and rheumatoid arthritis are characterized by abnormal lipid metabolism that manifests as an altered fatty acid profile of synovial fluid and tissue. The way in which dietary supplements can affect the symptoms of rheumatoid arthritis in particular is being investigated. In addition to classical eicosanoids, the potential role of polyunsaturated fatty acids has become the focus of more intensive research (Mustonen et al., 2021). Eicosaoentaenoic acid (EPA) and decosahexaenoic acid (DHA) are omega 3 (n-3) fatty acids found in fatty fish and various oils. These fatty acids can partially inhibit many aspects of inflammation, including leukocyte chemotaxis, adhesion molecule expression and leukocyte-endothelial interactions, production of eicosanoids such as prostaglandins and leukotrienes from n-6 fatty acid, arachidonic acid, production of inflammatory cytokines and reactivity of helper T lymphocytes 1. EPA induces eicosanoids, which often have lower biological potency than those produced from arachidonic acid, and EPA and DHA cause anti-inflammatory mediators that address inflammation, called resolvins, protecins and marexins. Animal experiments show the benefit of marine n-3 fatty acids in rheumatoid arthritis models (Calder, 2015). The Western diet is deficient in omega-3 fatty acids, but in contrast, it has excessive amounts of omega-6 fatty acids compared to the diet on which human beings evolved and on which their genetic patterns were determined. Excessive amounts of omega-6 polyunsaturated fatty acids (PUFAs) and the very high omega-6/omega-3 ratio found in today's Western diet promote the pathogenesis of many diseases including: cardiovascular diseases, cancer, inflammatory and autoimmune diseases, whereas increased omega-3 levels PUFA (lower omega-6/omega-3 ratio) has suppressive effects. A ratio of 2-3/1suppresses inflammation in patients with rheumatoid arthritis. A lower ratio of omega-6 and omega-3 fatty acids is more desirable to reduce the risk of many chronic diseases of high prevalence in Western societies as well as in developing countries (Simopoulos, 2008). Because n-3 PUFA and γ-linolenic acid (GLA) are well-known anti-inflammatory agents that can help treat inflammatory disorders, a study was performed in patients with rheumatoid arthritis. The method used was randomized, prospective study lasting 12 weeks. Sixty patients with active rheumatoid arthritis participated in the study. They are supplemented with fish oil (group I), fish oil with night primrose oil (group II) and without additives (group III). The results showed a score of disease activity 28, the number of painful joints and the result on a visually analogous scale were significantly reduced after supplementation in groups I and II (Veselinovic et al., 2017).

Polyphenols and flavonids

Polyphenols are compounds that have been extensively tested for their anti-inflammatory,

antioxidant and immunomodulatory properties in many chronic inflammatory conditions. Among the polyphenols: epigallocatechin gallate, carnosol, hydroxytyrosol, curcumin, resveratrol, kaempferol and genistein are the most studied in arthritis. The most important results of the study show that polyphenolic compounds are able to inhibit the expression and release numerous anti-inflammatory mediators and proteolytic enzymes, the activity of various transcription factors and the production of reactive oxygen species *in vitro*. Studies in animal models of rheumatoid arthritis, osteoarthritis and gout show results in terms of tissue damage reduction, restored cartilage homeostasis and reduced uric acid levels (Oliviero et al., 2018). Fruits such as pomegranate and berries represent a rich selection of various bioactive compounds in the diet, especially polyphenolic flavonoids that are associated with antioxidant, anti-inflammatory and analgesic effects. Recent research shows the protective role of fruits and their polyphenols in preclinical, clinical and epidemiological studies of osteoarthritis and rheumatoid arthritis. Fruits such as blueberries, raspberries, strawberries and pomegranate have shown promising results in reducing pain and inflammation in experimental models and clinical studies of arthritis in humans. There are also some evidence of a role for specific fruit polyphenols such as quertcetin and citrus flavonoids in alleviating the symptoms of rheumatoid arthritis (Basu et al., 2018). There is growing evidence that food polyphenols can show therapeutic efficacy in rheumatoid arthritis through their: antioxidant, anti-inflammatory, apopoptic and immunosuppressive effects and modulation tumour necrosis factor-α (TNF-α), interleukin (IL)-6, mitogen-activated protein kinase (MAPK). IL-1β and others (Behl et al., 2021).

Conclusion

Although medical treatment is still the first choice for people with arthritis, various herbal preparations and dietary supplements with the right choice of food could help primary medical therapy and reduce the number of symptoms and affect the progression of arthritis.

References

Amalraj, A., Varma, K., Jacob, J., Divya, C., Kunnumakkara, A.B., Stohs, S.J., Gopi, S. (2017): A novel highly bioavailable curcumin formulation improves symptoms and diagnostic indicators in rheumatoid arthritis patients: A randomized, double-blind, placebo-controlled, two-dose, three-arm, and parallel–group study, *J Med Food.* 20(10), 1022-1030.

American College of Rheumatology. Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC). Accessed 12 July 2013.

Anand, P., Kunnumakkara, A.B., Newman, R.A., Aggarwal, B.A. (2007): Bioavailability of curcumin: problems and promises, *Mol pharm.* 4(6), 807-18.

Ashish, J.M., Vinod, R. (2014): Infections and arthritis, *Best Pract Rest Clin Rheumatol.* 28(6), 935-59.

Baeurle, S.A., Kiselev, M.G., Makarova, E.S., Nogovitsin, E.A. (2009): Effects of the counterion behaviour on the frictional-compressive properties of chondroitin sulfate solutions, *Polymer.* 50(7), 1805-1813.

Basu, A., Schell, J., Scofield, R.H. (2018): Dietary fruits and arthritis, *Food Funct.* 24;9(1), 70-77.

Behl, T., Mehta, K., Sehgal, A., Singh, S., Sharma, N., Ahmadi, A., Arora, S., Bungau S. (2021): Exploring the role of polyphenols in rheumatoid arthritis, *Crit Rev Food Sci Nutr.* 17, 1-22.

Calder, P.C. (2015): Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and clinical relevance, *Biochim Biophys Acta.*1851(4), 469-84.

CDC. „Arthritis“ July 22, 2015. Achived from the original on 22 September 2016.

CDC. „Arthritis Types“ June 22, 2016. Achived from the original on 14 September 2016. Retrieved 14 September 2016.

Chung, M.K., Campbell, J.N. (2016): Use of capsaicin to treat pain: Mechanistic and therapeutic considerations, *Pharmaceuticals (Basel)* 1;9(4), 66.

Combe, B. (2009): Progression in early rheumatoid arthritis, *Best pract Res Clin Rheumatl*. 23(1), 59-69.

Frangos, T., Maret, W. (2020): Zinc and cadmium in the aetiology and pathogenesis of osteoarthritis and rheumatoid arthritis, *Nutrients.* 26;13(1), 53.

Gao, F., Zhang, S. (2019): Salicin Inhibits AGE-induced degradation of type II collagen and aggrecan in human SW1335 chondrocytes: therapeutic potential in osteoarthritis, *Artif Cells Nanomed Biotechnolog.* 47(1), 1043-1049.

Gotoh, S., Onaya, J., Abe, M., et al. (1993): Effects of the molecular weight of hyaluronic acid and its action mechanisms on experimental joint pain in rats, *Ann Rheum Dis.* 52(11), 817–822.

Harrison, S.R., Li, D., Jeffery, L.E., Raza, K., Hewison, M. (2020): Vitamin D, autoimmune disease and rheumatoid arthritis, *Calcif Tissue Int.* 106(1), 58-75.

Henrotin, Y., Marty, M., Mobasheri, A. (2014): What is the current status of chondroitin sulfat and glucosamine for the treatment of knee osteoarthritis, *Maturitas.* 78(3), 184-7.

Ishikawa, L.L.W., Colavite, P.M., Silva, T.F. de, C.F., Mimura, L.A.N., Franca, T.G.D., Zorzella-Pezavento, S.F.G., Chiuso-Minicucci, F., Marcolino, L.D., Penitenti, M., Ikoma, M.R.V., Sartori, A. (2017): Vitamin D deficiency and rheumatoid arthritis, *Clin Rev Allergy Immunol.* 52(3), 373-388.

Itano, N., Sawai, T., Yoshida, M., et al. (1999): Three isoforms of mammalian hyaluronan synthases have distinct enzymatic properties, *J Biol Chem.* 274(35), 25085–25092.

Josef, S.S., Aletaha, D., Mclnnes, I.B. (2016): Rheumatoid arthritis, *Lancet.* 22;388(10055), 2023-2038.

Learch, J.T. (2003): Imaging of infectious arthritis, *Semin Musculoskelet Radiol*. 7(2), 137-42.

Lubis, A.M.T., Siagian, C., Wonggokusuma, E., Marsetyo, A.F., Setyohadi, B. (2017): Comparison og Glucosamine-Ghondroitin Sulfate with and without Methylsulfonylmethane in Grade I-II Knee Osteoarthritis: A double Blind randomized controlled trial, *Acta Med Indones*. 49(2), 105-111.

Lugo, J.P., Saiyed, Z.M., Lane, N.E. (2016): Efficacy and tolerability of an undenatured type II collagen supplement in modulating knee osteoarthritis symptoms: a multicenter randomized, double-blind, placebo-controlled stady, *Nutr J.* 29;15, 14.

Luo, Y.Y., Sinkeviciute, D., He, Y., Karsdal, M., Henrotin, Y., Mobasheri, A., Onnerfjord, P., Bay-Jensen, A. (2017): The minor collagens in articular cartilage, *Protein Cell*. 8(8), 560-572.

Ma, Q., Gao, X. (2019): Categories and biomanufacturing methods of glucosamine, *Appl Microbiol Biotechnol.* 103(19), 7883-7889.

Martin, J.A., Buckwalter, J.A. (2002): Human chondrocyte senescence and osteoarthritis, *Biorheology.* 39(1-2), 145-52.

Matsuno, H., Yudoh, K., Kondo, M., Goto, M., Kimura, T. (1999): Biochemical effect of intra-articular injections of high molecular weight hyaluronate in rheumatoid arthritis patients, *Inflamm Res.* 48(3), 154–159.

Mohd, S., Sydney, J.S. (2015): Efficacy and safety od white willow bark (Salix alba) extracts, *Phytoter*. 29(8), 112-6.

Mustonen, A.-M., Nieminen, P. (2021): Fatty acid and oxylipins in osteoarthritis and rheumatoid arthritis –a complex field with significant potential for future treatments, *Curr Rheumatol Rep.* 28;23(6), 41.

Nahrstedt, A., Schmidt, M., Jaggi, R., Matz, J., Khayal, M.T. (2007): Willow bark extract: The contribution od pholyphenols to the overall effect, *Wien Med Wochwnschr*. 157(13-14), 348-51.

National Institute of Arthritis and Musculoskeletal and Skin Diseases. Arthritis and Rheumatic Disease. October 2014, retrieved from original 31 Mart 2021.

Neustadt, D., Altman, R.D. Intra-articular therapy. In: Moskowitz RW, Altman RD, Buckwalter JA, Goldberg VM, Hochberg MC, editors. (2007): Osteoarthritis. Philadelphia, PA: Lippincott Williams and Willkins, pp. 287–301.

Oliviero, F., Scanu, A., Zamudio-Cuevas, Y., Punzi, L., Spinella, P. (2018): Anti-inflamatorry effects of polyphenols inarthritis, *J Sci Food Agric*. 98(5), 1653-1659.

Pradeep, K.S. (2019): Ageing and Osteoarthritis, *Subcell Biochem.* 91, 123-159.

Rains, C., Bryson, H.M. (1995): Topical capsaicin. A review of its pharmacological properties and therapeutic potential in post-herpetic neuralgia, diabetic neuropathy and osteoarthritis, *Drugs Aging.* 7(4), 317-28.

Shaikh, J., Ankola, D.D., Beniwal, V., Shing, D., Ravi Kumar, M.N.V. (2009): Nanoparticle encapsulation improves oral bioavailability of curcumin by at least 9-fold when compared of curcumin administered with piperine as absorption enhancer, *Eur J Pharm Sci.* 28;37(3-4), 223-30.

Simon, L.S., Strand, V. (2007): The pharmacologic treatment of osteoarthritis. In: Moskowitz RW, Altman RD, Buckwalter JA, Goldberg VM, Hochberg MC, editors. Osteoarthritis. Philadelphia, PA: Lippincott Williams and Willkins, pp. 267–286.

Simopoulos, A.P. (2008): The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic disease, *Exp Biol Mad (Maywood*). 233(6), 674-88.

Smith, J.W. (1990): Infectious arthritis, *Infect Dis Clin North Am*. 4(3), 523-38.

Srinivasan, K. (2016): Biological activities of red papper (Capsicun Annuum) and its pungent principle capsaicin: a Review, *Crit Rev Food sci. Nutr.* 3;56(9), 1488-500.

Sudha, P.N., Rose, M.H. (2014): Beneficial effects od hyaluronic acid, *Adv Food Nutr Res.* 72, 137.176.

Taylor, K.R., Gallo, R.L. (2006): Glycosaminoglycans and their proteoglycans: host-associated molecular patterns for initiation and modulation of inflammation, *FASEB J.* 20(1), 9–22.

Turrubiates-Hernandez, F.J., Marquez-Sandoval, Y.F., Gonzales-Estevez,, G., Reyes-Castillo Z., Munoz-Valle, J.F. (2020): The relevance of selenium status in rheumatoid arthritis, *Nutrients.* 30;12(10), 3007.

Vaselinovic, M., Vasiljevic, D., Vucic, V., Arsic, A., Petrovic, S., Tomic-Lucic, A., Savic, M., Zivanovic, S., Stojic, V., Jakovljevic, V. (2017): Clinical Benefits of n-3 PUFA and γ-Linolenic Acid in Patients with rheumatoid Arthritis, *Nutrients.* 25;9(4), 325.

Volpi, N. (2019): Chondroitin sulfate safety and qulity, *Molecules* 12;24(8), 1447.

Wessels, I., Rink, L. (2020): Micronutrients in autoimmune diseases: possible therapeutics benefits of zink and vitamin D, *Nutr. Biochem.* 77, 108240.

Yang, M., Akbar, U., Mohan, C. (2019): Curcumin in autoimmune and rheumatic diseases, *Nutrients* 2;11 (5), 1004.

Zhang, W., Moskowitz, R.W., Nuki, G., et al. (2008): OARSI recommendations for the management of hip and knee osteoarthritis, Part II: OARSI evidence-based, expert consensus guidelines, *Osteoarthritis Cartilage* 16(2), 137–162.

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THE EFFECT OF IODINE INTAKE FREQUENCY FROM SALT AND SALT-CONTAINING PRODUCTS ON THYROID DYSFUNCTION

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Summary

The human body needs small quantities of iodine per day. In 1990, the World Health Organization (WHO) Assembly adopted the universal salt iodization as the method of choice for eliminating iodine deficiency. In order to examine the influence of the frequency of iodine intake from salt and salt-containing products on thyroid dysfunction, a questionnaire was created and its answers were analyzed. The results of the study showed that there are significant statistical differences between subjects with thyroid dysfunction who consume less salt in food and have a reduced frequency of consumption of salt-containing products compared to subjects in the control group.

Keywords: iodine, salt, thyroid dysfunction

Introduction

A major factor in iodine deficiency is low concentration of iodine in the consumed food (Benoist et al., 2004). The recommended daily intake of iodine for infants is 50 μg/day, children (1-6 years) 90 μg/day, children (7-12 years) 120 μg/day, adults and children (over 12 years) 150 μg/day, and for pregnant and lactating women 200 μg/day (WHO, 1996). In order to ensure the intake of 150 μg of iodine per day through iodized salt, the World Health Organization and the United Nations International Children's Fund (UNICEF) in 1996. recommended that the iodine content in salt is 20-40 mgI/kg (ZZJZ, 2006). In 1990, the World Health Organization Assembly adopted the universal method of iodization of salt as the method of choice for the elimination of iodine deficiency disorders (IDD). This strategy is based on the fact that: (a) salt is one of the few goods consumed by the entire population; (b) salt consumption is fairly stable throughout the year; (c) salt production is usually in the hands of several producers; (d) salt iodization technology is easily feasible and available at a reasonable cost (Benoist et al., 2004).

This recommendation was based on data indicating iodine loss from the place of production to its delivery the household, and that 20% of iodine is lost by cooking before use. Also, iodized salt does not cause side effects nor does iodine from iodized salt carry risks for people who already have enough iodine in their bodies (Ranganathan and Reddy, 1995).

Sources of salt intake in the human body through diet can be roughly classified into two groups. As a spice, kitchen salt is added in small amounts to enhance the flavor of dishes (Yee et al., 2011). Another source of salt intake is in the form of preservatives. Significant sources of hidden salt are found in semi-ready and ready foods such as: cured meats, pâtés, hard cheeses, cheese spreads, snacks (chips, chopsticks, peanuts and pistachios), ready-made sauces, mustard, mayonnaise, hamburgers, bagged soups, corn and cereal flakes. Baked goods, especially bread, are a major source of kitchen salt and account for 25-30% of the daily salt intake (Ugarčić-Hardi et al., 2010) (Table 1). Iodized salt from processed foods is an important source of iodine, especially for adults. The use of iodized salt by the food industry should be enforced along with population monitoring to ensure sustainability of optimal iodine intake (Chotivichien et al., 2021). It is concerning that the use of kitchen salt in the preparation of food as a spice but also as a preservative in various products greatly exceeds the daily needs of the human body, which are small, only 5 g/day of salt is needed (WHO, 2006).

According to a study conducted in Croatia, the intake of kitchen salt in both sexes is over twice the recommended amount (as recommended by the WHO). The highest daily intake was 29.9 g for men and 19.4 g for women. A statistically significant correlation was observed between body mass index and kitchen salt intake in the whole group of respondents (Đurić et al., 2011). Reducing dietary salt intake from the current values of 9-12 g/day to the recommended level of less than 5-6 g/day will have major positive effects on cardiovascular health. The World Health Organization and the Food and Agriculture Organization (FAO) recommend consuming less than 5 g of salt per day (Sung Kyu Ha, 2014). During a study that was conducted on the Italian population, researchers have come to the conclusion that whit a daily consumption of 5 g of salt iodized at 30 mg/kg, the estimated daily iodine intake

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resulted within the range of optimal iodine intake in all age groups. In children the recommended iodine intake is achieved with a daily consumption of 3 g of iodized salt (Pastorelli et al., 2015).

Table 1. Iodine content in various dietary products (Pennington and Spungen, 2010)

Product	Quantity of product	Amount of iodine (µg)
Cod	85 g	99
Corn grits	1 cup	68
Milk, 2% fat	240 ml (1 cup)	56
Milk, fat free	240 ml (1 cup)	51
White bread	50 g (2 pieces)	46
Beef liver, cooked	85 g	36
Beans, cooked	90 g (1/2 cup)	35
Potatoes, baked	110 g	34
Whole wheat bread	50 g	32
Egg, boiled	50 g	24
Oatmeal, cooked	1 cup	16

Potassium iodide (KI) and potassium iodate (KIO_3) are substances mainly used for salt iodization. In a mixture with salts KI is less stable compared to KIO_3. KI can be used for iodization of highly purified salt, but that salt has be stored in dry conditions and should be consumed a few months after production. Otherwise, iodine stabilizers need to be added, such as Sodium Thiosulfate or Calcium Hydroxide, 1 g of stabilizer per 1 kg of salt iodinated with KI. KIO_3, recommended by the World Health Organization for salt iodization, is generally stable and resistant to evaporation. KIO_3 should be used for iodization of salt that is less purified and if it's assumed that the salt will be exposed to heat or humidity, and will be stored or transported for a long time (Tahirović, 2004).

To date, the available results are conflicting, depending on the country (Table 2.), salt iodization policy, as well as time frame of data collection. However, ensuring an optimal iodine supply by salt fortification, without exceeding the current recommendation by the World Health Organization for salt intake, seems to be an achievable goal (Nista et al., 2022). Iodine fortification of kitchen salt has recently been increased in Switzerland from 20-25 mg/kg to ensure iodine sufficiency in the population, whereas in Germany fortification still remains at a mean level of 20 mg/kg implying a growing risk of increasing iodine deficiency rates if general salt intake drops (Esche et al., 2020).

The human body contains 15-20 mg of iodine, 70-80 % of which is concentrated in the thyroid. Of the total amount of iodine ingested, 15% goes to the thyroid within 24 hours. Excess iodine is excreted through urine (Ristić-Medić et al., 2009). The thyroid is an endocrine gland located in the front lower part of the neck, and its hormones help the body use energy, enable the proper function of the heart, brain, muscles and other organs (ATA, 2014).

The thyroid secretes Triiodothyronine (T_3), Thyroxin (T_4) and Calcitonin. The biosynthesis of thyroid hormones depends on the intake of exogenous iodine through food and water. Iodine is absorbed through the small intestine and transferred through plasma to the thyroid where it is concentrated, oxidized, and then incorporated into Thyroglobulin (Tg) to form Monoiodotyrosine (MIT) and Diiodotyrosine (DIT), later on T_4 and T_3. Thyroglobulin undergoes proteolysis, and the released hormones are secreted into the circulation, where specific binding proteins transfer them to target tissues (Rousset et al., 2015).

Control of thyroid hormone secretion is achieved by a '*negative feedback mechanism*'. The Hypothalamus secretes the Thyrotropin-releasing hormone (TRH), which affects the adenohypophysis by stimulating the secretion of thyroid-stimulating hormone (TSH), which has a role in all of the stages of thyroid hormone synthesis (Corvilain et al., 1988).

Disorders of the thyroid gland occur due to certain irregularities in its performance or as a result of enlargement of the gland (Khan et al., 2002). Women are more likely to get sick than men, and the symptoms of the disease sometimes go unrecognized (Ashwell et al., 1999). People at increased risk for thyroid dysfunction are those with a family history of such disorders, gland dysfunction after childbirth or surgery, women aged 55 and older, and people from regions with endemic iodine deficiency (Stockigt, 2003). The most common thyroid dysfunction are Hypothyroidism and Hyperthyroidism. Hypothyroidism refers to decreased production of thyroid hormones leading to clinical manifestations of thyroid insufficiency. Hyperthyroidism is excessive production and / or secretion of thyroid hormones (Persani, 2012). A study conducted in China did not show a significant association between salt consumption and the current high prevalence of goiter in that country during 2011-2013 (Liang et al., 2017).

Table 2. Average iodine intake in daily salt intake in some countries of the region

Country	Iodine limit in kg of salt (mgI/kg)	Mean value of iodine in kg of salt (mgI/kg)	Recommended average daily salt intake (g)	Average daily iodine intake (µg)	Average amount of daily iodine intake in 6 g salt/day (µg)
Serbia	12-18	15	6	72-108	90
Slovenia	20-30	25	6	120-180	150
B&H	20-30	25	6	120-180	150

Source: Rulebook on quality and other requirements for salt intended for human consumption and food production, Official Gazette of Serbia and Montenegro, 31/2005; Rulebook on the quality of salt, Official Gazette of the Republic of Slovenia, No. 70/03, 31/04, 45/08 - ZKme-1 and 46/18; Rulebook on salt for human consumption, Official Gazette of B&H number: 39/09

Materials and methods

This study consisted of a questionnaire on eating habits and lifestyle to determine whether the frequency of iodine intake from salt has an impact on thyroid dysfunction. The questionnaire was standardized and developed by using general recommendations and guides on proper nutrition as well as recommendations of associations of patients with thyroid disorder (WHO, 2006; ATA, 2017). The questions in the questionnaire were formulated using the Likert scale for offered questions (Wuensch, 2009). This scale enabled better processing and quantification of the offered answers, as well as the calculation of statistical significance and correlation factors.

The subjects were people aged 28-73 years with thyroid dysfunction and a group of healthy people. A pilot group of 20 respondents aged 30 to 60 years was used to validate the questionnaire. The validation of the questionnaire examined metric characteristics, and whether the questions used in the questionnaire were appropriate and showed the correlation we were looking for. Verbal expressions offered in response were used as quantifiers of the frequency of consumption of individual foods.

The questionnaire on the eating habits of the respondents and their lifestyle looked like this:

Name and surname _____ Age _____ years

Weight: _____ Height: _____ BMI: _____

Disease (round): Hypothyroidism Hyperthyroidism

Circle the answers to the following questions.

1. Food I eat: a) I don't add extra salt b) I try and then add salt c) I add salt first and then I eat

2. In your opinion, you consume:

a) Foods with a very low salt intake b) moderately salty foods c) very salty foods

3. Do I consume cured meat products in my diet?

a) Daily b) 3-5 times a week c) 2-3 times a week e) once a week e) never

4. How much bread do you eat per day?

a) More than half a loaf of bread b) half a loaf of bread c) more than 5 slices

d) 3-5 slices e) two slices

5. Do you consume pies and other pastry dishes in your diet?

a) Daily b) 3-5 times a week c) 2-3 times a week e) once a week e) never

Subjects were divided into two groups of 100: test (I1) and control (K1). Subjects from group (I1) were persons with Hypothyroidism, and subjects from group K1 were persons with good thyroid function.
Statistical analysis was performed using SPSS software version 23.0 (SPSS, Inc., Chicago, Illinois). Continuous variables with normal distribution were expressed as mean ± standard deviation, and continuous variables whose distribution is not normal were expressed as median and interquartile range (IQR). Normal data distribution was evaluated by Kolmogorov-Smirnov and Shapiro-Wilk tests. Categorical variables were expressed as a number (percentage). The T-test was used to compare the two independent groups of continuous variables following the normal distribution, and the Mann-Whitney U test was used for the continuous independent variables following the normal distribution. The Wilcoxon signed rank test was used to compare two dependent continuous variables that do not follow the normal distribution. A chi-square test or Fisher's exact test was used to compare categorical variables if the number of variables in any cell of the contingency table was less than 5. A P-value <0.05 was considered statistically significant.

Results and discussion

One hundred respondents were included in the examined and control group at the beginning of the research and the same number of them completed the questionnaire on eating habits. The initial characteristics of the respondents are shown in Table 3. The mean age of the respondents in the examined group was 57 years (IQR, 46-65), and the majority of the respondents were women (90/100, 90%). The mean age of the subjects in the control group was 51 years (IQR, 41–58), and the majority of the subjects were women (66/100, 66%). The average body mass index (BMI) was 28.11 for the subjects of the examined group and 27.17 for the subjects of the control group. Decreased thyroid activity is thought to lead to obesity and thus to an increase in BMI, probably as a result of decreased metabolism (Åsvold et al., 2009). According to the results in this study there are no significant differences in BMI between the two groups which can be explained by the fact that in the control group consisted of a higher number of men whose BMI is higher than women, which brought the mean value closer to the BMI of the respondents in the examined group. Significant differences in height were detected between the subjects of the examined and control group, which was to be expected since the control group consisted of more men who are usually slightly taller than women. This data did not affect the course of the research.

Table 3. Initial characteristics of the respondents in the study

Data on respondents	Examined group (n= 100)	Control group (n = 100)	p value
Age (median,Q1 - Q3)	57 (46-65)	51 (41-58)	<0.001
Sex	10 Male/90 Female	34 Male/66 Female	<0.001
Weight (kg) (mean value ± standard deviation)	76±13	79±15	0.133
Height (m) (mean value ± standard deviation	1.64±0.08	1.69±0.1	0.007
BMI (median,Q1 - Q3)	28.11 (25.14-30.83)	27.17 (24.83-29.73)	0.327

Statistically significant at the level of p<0.05

To determine the impact of the frequency of intake of iodine from salt and salt-containing products, the answers to the questions from the questionnaire were compared between control and examined group of respondents. Their statistical processing showed that there are significant differences in the frequency of consumption of salt and salt-containing products between the subjects of the examined and controlled groups (Table 4).

Table 4. Estimation of frequency of salt and salt-containing foods intake for examined and control groups

Questions	Examined group (n = 100)	Control group (n = 100)	p value
The food I consume: (median, Q1 - Q3)	1 (1-2)	2 (2-3)	<0.001
In your opinion you are consuming: (median, Q1 - Q3)	1 (1-2)	2 (2-2)	<0.001
I consume cured meat products in my diet: (median, Q1 - Q3)	2 (1-2)	3 (3-4)	<0.001
How much bread do you eat per day (median, Q1 - Q3)	3 (2-3.25)	4 (3-5)	<0.001
Do you eat pies and other doughy dishes? (median, Q1 - Q3)	2 (2-3)	3 (3-4)	<0.001

Statistically significant at the level of p <0.05

It was estimated that in the pediatric population, approximately 65–73 % of the total iodine intake was derived from food and 27–35 % from iodized salt and that iodized salt actually only contributed to 20% of

the total salt intake (Iacone et al., 2021). Iodine in iodized salt does not carry risks for people who already have enough iodine. Salt iodization at the current level of enrichment (15–30 ppm iodine) does maintain intake in a safe daily range for all populations, regardless of their iodine status (Ranganathan and Reddy, 1995). In a cross-sectional study conducted by Yu et al (2021), researchers did not provide information on side effects using iodized salt nor did they specifically consider adverse effects, and we can be sure that adverse effects are small or almost non-existent. In order to examine the side effects of iodized salt it would require a large study of at least two years, not focusing on the rate of goiter and urinary iodine excretion, and paying special attention to the mental and physical development of children and mortality (Clar et al., 2002). In a study conducted between the years 1990 and 1999. involving 26.010 respondents, Huang et al. (2001) concluded that iodized salt intake and the incidence of hyperthyroidism had a significant correlation. However, the typical model of the change of disease patterns incidence needs further study. The high incidence of thyroid diseases caused by high iodine intake has been contentious. Zhao et al. (2014) investigated the relationship between iodine intake and thyroid diseases through the comparison of urine iodine concentration (UIC) of patients with thyroid diseases and healthy volunteers and through assessment of iodine intake among the residents. The susceptibility of thyroid diseases among subjects was significantly associated with the female sex (odds ratio (OR) = 3.3), older age (OR = 2.1), and high iodine intake (OR = 1.3). In conclusion, high iodine intake was likely to lead to the occurrence of thyroid diseases, such as Hashimoto thyroiditis, nodular goiter, and hyperthyroidism, through a long-term mechanism.

Subjects in the focus group usually do not add additional salt to the food they consume. There is a statistically significant difference between the examined and control groups regarding the frequency of salt intake ($p<0.001$) but we also need to take into account the addition of salt to food during cooking, this fact is unknown to us and we cannot say with certainty that the subjects in the focus group consume less iodine without adding salt to their food. The method of food preparation could also affect the iodine content in the final product. The loss of iodine depends on the cooking method and the frequency of adding salt during cooking. Minimum losses were found during shallow frying where cooking time of salt was 1 minutes and 15 s and maximum during pressure cooking where cooking time of salt was 26 minutes (Rana and Raghuvanshi, 2013).

Furthermore, according to the respondents, they tend to use food with a low salt intake, while most of the respondents in the control group stated that they consume moderately salty food ($p<0.001$).

At the top of the list of foods as sources of iodine in the diet are cured meat products as well as whole grain bread and other pastry dishes (Pennington and Spungen, 2009). Baked goods are a major source of salt and account for 25-30% of the daily salt intake (Ugarčić-Hardi et al., 2010). Bread is widely considered to be the food that provides the most dietary salt. As such, it is one of the key public health targets for a salt reduction policy (Quilez and Salas-Salvado, 2012). Research has shown that commercially prepared foods (including bread and pizza) are decreasing the amount of iodized salt (Ugarčić-Hardi et al., 2010). Purchasing data suggests that bread and cereal products are responsible for approximately 18% of normal daily unrestricted sodium intake; this percentage may be substantially higher in subjects attempting to follow a reduced sodium diet by avoiding added salt and salty sauces and meats (Daugirdas, 2013).

There is a statistically significant difference between the examined and control groups in the frequency of intake of cured meat products ($p <0.001$) as well as the daily consumption of bread ($p<0.001$). We do not know the quantities of the consumed portion so some of the subjects may have a higher intake of salt from cured meats in one consumption, than the other who has consumed three or more portions. Therefore, the obtained results of the questionnaire must be observed with caution.

Alternative substances and processing technologies for reducing or replacing sodium in meat products are being actively researched with the goal of the industrial development and manufacture of low-salt meat products. Studies have shown that sodium chloride cannot be replaced or reduced by over 50% using a single process (Kim et al., 2021).

Respondents of the examined group consume more than 5 slices of bread per day, while in the control group there very varying answers (some consume more than 5 slices per day, others consume ½ loaf of bread and more than half a loaf of bread per day). If one considers the cuisine of the region, it is to be expected that pies and doughy dishes are consumed several times during the week. There are certain statistical differences in the responses to this question. While the examined group consumed pie and doughy dishes mostly once a week, and a couple of responses said 2-3 times a week, most of the responses of the control group stated that baked goods were consumed 2-3 times a week. In a study conducted in Croatia, salt content varied widely between bakeries, with an

average content of 2.30±0.22 g per 100 g of bread, which is almost twice the threshold content (1.4%) defined by the Croatian National Regulation on Cereals and Cereal Products (Delaš Aždajić et al., 2019). According to the obtained results we can conclude that the respondents of the examined group consume more salt by consuming larger amounts of bread and pies than the respondents of the study group.

Conclusion

Based on the research, obtained results and discussion, it can be concluded that there is a statistically significant difference in the answers given by the subjects of the examined and control group, regarding the frequency of salt intake and other foods labeled as sources of salt in the diet. Subjects of the control group consume larger amounts of salt and salt-containing food in their diet, thus ensuring the daily needs for iodine. Subjects in the examined group, despite suffering from thyroid dysfunction (hypothyroidism) which additionally signals iodine intake, have lower salt intake and salty foods resulting in reduced iodine intake and prolonged disease.

References

American Thyroid Association. ATA. Iodine Deficiency 2014. Available: https://www.thyroid.org/iodine-deficiency/ Accessed Jan.17, 2017.

American Thyroid Association. ATA. General Information/Press Room. Prevalence and Impact of Thyroid Disease 2017. Available: https://www.thyroid.org/media-main/press-room/ Accessed Jan.20, 2018.

Ashwell M, Bussell G, Clasen L, Egginton J, Gibson S, Govindji A et al. (1999): Reader's digest guide to vitamins, minerals and supplements. Reader's Digest Association Inc., Pleasantville, New York, USA, pp. 253-254

Åsvold, BO, Bjøro, T, Vatten, LJ (2009): Association of serum TSH with high body mass differs between smokers and never-smokers, *Journal of Clinical Endocrinology & Metabolism* 94, 5023–5027.

Benoist, B, Andersson, M, Egli, I, Takkouche, B, Allen, H (2004): Iodine status worldwide. Iodine deficiency disorders: a public health problem, WHO Global Database on Iodine Deficiency; Department of Nutrition for Health and Development World Health Organization Geneva; ISBN 92 4 159200 1

Chotivichien, S, Chongchaithet, N, Aksornchu, P, Boonmongkol, N, Duangmusik, P, Knowles, J, Sinawat, S (2021): Assessment of the contribution of industrially processed foods to salt and iodine intake in Thailand, *Plos One* 6;16(7), e0253590.

Clar, C , Wu, T, Liu, G, Li, P (2002): Iodized salt for iodine deficiency disorders. A systematic review, *Endocrinology & Metabolism Clinics od North America* 31(3), 681-98

Corvilain, B, Van Sande, J, Dumont, JE (1988): Inhibition by iodide of iodide binding to proteins: the "Wolff-Chaikoff" effect is caused by inhibition of H2O2 generation, *Biochemical and Biophysical Research Communications* 154(3), 1287-1292.

Daugirdas, JT (2013): Potential importance of low-sodium bread and breakfast cereal to a reduced sodium diet, *Journal of Renal Nutrition* 23(1), 1-3.

Delaš Aždajić, M, Delaš, I, Aždajić, S, Štimac Grbić, D, Vahčić, N (2019): A cross sectional study of salt content in bakery bread in Zagreb, Croatia, *Archives of Industrial Hygiene and Toxicology* 70(3), 219-223

Đurić,, J, Vitale, K, Paradinović, S, Jelaković, B (2011): Unos kuhinjske soli i arterijski tlak u općoj populaciji, *Hrvatki časopis za prehrambenu tehnologiju, biotehnologiju i nutricionizam* 6 (3-4), 141-147. Available: https://www.hah.hr/pdf/brosura-manje-soli-2014.pdf. Accessed May.20, 2017.

Esche, J, Thamm, M, Remer, T (2020): Contribution of iodized salt to total iodine and total salt intake in Germany, *European Journal of Nutrition* 59(7), 3163-3169.

Huang, Q, Jin, R, Zou, D (2001): Study on the effects of increased iodized salt intake on the incidence of thyroid diseased, *National Library of Medicine* 22(6), 455-8.

Iacone, R, Iaccarino Idelson, P, Campanozzi, A, Rutigliano, I, Russo O, Formisano, P, Galeone, D, Emdio Macchia, P, Strazzullo, P, The Minisal-Girsci Study Group (2021): Relationship between salt consumption and iodine intake in a pediatric population, *European Journal of Nutrition* 60, 2193–2202.

Kim, TK, Yong, HI, Jung, S, Kim, HW, Choi, YS (2021): Technologies of the production of meat products with a low sodium chloride content and improved quality characteristics- a review. *Foods* 10(5), 957.

Khan, A, Ali Khan, MM, Akhtar, S (2002): Thyroid Disorders, Etiology and Prevalence. *Journal of Medical Sciences* 2, 89-94.

Liang, Z, Xu, C, Luo, YJ (2017): Association od iodized salt with goiter prevalence in Chinese populations: a continuity analysis over time, *Military Medical Research* 21;4, 8.

Nista, F, Bagnasco, M, Gatto, F, Albertelli, M, Vera, L, M Boschetti, M, Musso, N, Ferone, D (2022): The effect of sodium restriction on iodine prophylaxis: a review, *Journal Endocrinological Investigation* Online ahead of print.

Pastorelli, AA, Stacchini, P, Olivieri, A (2015): Daily iodine intake and the impact of salt reduction on iodine prophylaxis in the Italian population, *European Journal of Clinical Nutrition* 69(2), 211-5.

Pennington, JAT, Spungen, J (2010): Bowes and Church's Food Values of Portions Commonly Used: Macronutrients: Recommended Dietary Allowances (RDAs), Adequate Intakes (AIs), and Estimated Average Requirements (EARs), 19th ed. Library of Congress Cataloging in Publication Data, pp.185.

Persani, L (2012): Clinical review: Central hypothyroidism: pathogenic, diagnostic, and therapeutic challenges, *The Journal of Clinical Endocrinology & Metabolism* 97, 3068-3078.

Quilez, J, Salas-Salvado, J (2012): Salt in bread in Europe: potential benefits of reduction, *Nutrition Rewievs* 70(11), 666-78.

Rana, R, Raghuvanshi, RS (2013): Effect of different cooking methods on iodine losses, *Journal of Food Science and Technology* 50(6), 1212-6.

Ranganathan, S , Reddy, V (1995): Human requirements of iodine & safe use of iodised salt, *Indian J Med Res* 102, 227-32.

Ristić-Medić, D, Piskasckova, Z, Hooper, L, Ruprich, J, Casgrain, A, Ashton, K et.al. (2009): Methods of assessment of iodine status in humans: a systematic review. American Society for Nutrition, *American Journal of Clinical Nutrition* 89 (6), 2052S-2069S.

Rousset, B, Dupuy, C, Miot, F, Dumont, J (2015): Thyroid Hormone Synthesis And Secretion Chapter 2. *Endotext*.

Stockigt, J (2003): Assessment of Thyroid Function: Towards an Integrated Laboratory - Clinical Approach, *Clinical Biochemist Reviews* 24(4), 109–122.

Sung Kyu, Ha (2014): Dietary Salt Intake and Hypertension, *JL Electrolyte Blood Press* 12(1), 7–18.

Tahirović, H (2004): Jodirana so. Akademija nauka i umjetnosti Bosne i Hercegovine, 37, 1-19. Sarajevo.

Ugarčić-Hardi, Ž, Dumančić, G, Koceva Komlenić, D, Jukić, M (2010): Količina soli u hrvatskim pekarskim proizvodima, *Hrvatski časopis za javno zdravstvo* 6-21. Available: http://www.hcjz.hr/index.php/hcjz/article/view/799 Accessed Apr.17, 2017.

World Health Organization. WHO. Nutrition Unit. Recommended iodine levels in salt and guidelines for monitoring their adequacy and effectiveness 1996. Available: https://apps.who.int/iris/handle/10665/63322 Accessed Jan.25, 2016.

World Health Organization. WHO. Reducing salt intake in populations: Report of a WHO forum and Technical meeting Paris, Francuska 2006. Available: http://www.who.int/dietphysicalactivity/reducingsalt/en/index3. Accessed Apr.20, 2017.

Wuensch, KL (2009) "What is a Likert Scale? And How Do You Pronounce 'Likert?"'. East Carolina University. Available: http://core.ecu.edu/psyc/wuenschk/StatHelp/Likert.html Accessed May.16, 2019.

Yee, KK, Sukumaran, SK, Kotha, R, Gilbertson, TA and Margolskee, RF (2011): Glucose transporters and ATP-gated K+ (KATP) metabolic sensors are present in type 1 taste receptor 3 (T1r3)-expressing taste cells. PNAS: 5431–5436 Available: http://www.pnas.org/content/108/13/5431.short. Accessed Apr.15, 2017.

Yu, Z, Yu, Y, Wan, Y, Fan, J, Meng, H, Li, S, Wang, Y, Wang, T, Ling, R (2021): Iodine intake level and incidence of thyroid disease in adults in Shaanxi province: a cross-sectional study, *Annals of Translational Medicine* 9(20), 1567.

Zavod za javno zdravstvo Federacije Bosne i Hercegovine. ZZJZ. Strategija za prevenciju jod deficitarnih poremećaja u Bosni i Hercegovini (nacrt) Sarajevo 2006. Available: http://www.zzjzfbih.ba/wpcontent/uploads/2009/02/strategija_komplet1.doc Accessed Dec.25, 2016.

Zhao, H, Tian, Y, Liu, Z, Li, X, Feng, M, Huang, T (2014): Correlation between iodine intake and thyroid disorders: a cross-sectional study from the South of China, *Biological Trace Element Research* 162(1-3), 87-94.

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Conference proceedings:

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