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TABLE OF CONTENTS

INNOVATION

NUTRITION, HEALTH AND CONSUMER

SAFETY

SUSTAINABILITY

INDUSTRY 4.0

INNOVATION

Modulating the Swelling Behavior of Casein Microparticles (CMPs) for Delivering Bioactive Compounds

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Abstract- **The aim of the study was to investigate the influence of pH on the stability and swelling behavior of casein microparticles (CMPs) and enhance those properties at a particular pH of interest. Sodium citrate was added as a chelating agent during the preparation of CMPs. The structure of the microparticles was observed under the microscope, stability was measured using a UV-VIS spectrometer, and the swelling process was followed in a microfluidic sieve cell under an inverted microscope.**

The microparticles produced were spherical in shape with a diameter range of 1-30µM. The result from the stability experiment indicated that the CMPs were mainly formed by hydrophobic interactions, as they decomposed entirely upon the addition of SDS. The particle area observed under the microscope swelled about 1.8 fold at pH 8 after two hours while it took only a few minutes at pH 11 and even a few seconds at pH 14 for such an increase in the particle area. The addition of citrate influences the swelling process of CMPs at pH 8. However, no swelling was observed at pH 3, similar to human stomach pH. Due to the characteristic pH-dependent swelling behavior, CMPs could facilitate the microencapsulation of bioactive compounds to protect them from harsh acidic conditions but allow controlled release in an alkaline environment.

Keywords- **casein microparticles, stability, swelling, citrate**

I. INTRODUCTION

Protein-based biopolymers have gained immense interest due to their numerous advantages over synthetic materials such as low immunogenicity, biocompatibility, and biodegradability (Sadiq et al. 2021). Milk proteins retain many structural and physicochemical features, which make them suitable for natural carriers of functionalized compounds. Casein is a collective form of the major milk protein composed of -α_{S1}-, α_{S2}-, β- and κ-casein. The amino acids, phosphorus, and carbohydrates content of those proteins are different. Lack of secondary structure, and a tendency to bind calcium phosphate cause the self-assembly of casein into spherical colloidal structures (de Kruif, 1999). These so-called casein micelles are mainly stabilized by hydrophobic and electrostatic calcium-phosphate bonds (Gebhardt et al. 2011). Having amphiphilic nature, self-assembly and surface active properties, interaction with other polymers, pH-dependent swelling behavior, and ability to take part in hydrophobic and hydrophilic interactions, explain why casein, has been widely studied as carrier material (Livney, 2010; Glab and Boratynski, 2017).

Several methods have been reported for the production of casein microparticles such as gel formation, emulsification, spray drying, heat treatment, coacervation, etc. (Sadiq et al. 2021; Glab and Boratynski, 2017; Marreto et al. 2013,). Instead, we applied a gentle method of microparticle production based on a depletion flocculation reaction (de Kruif and Tuinier, 2001) in a casein-pectin solution at neutral pH. When two casein micelles approach each other under these conditions, the polysaccharides are excluded from the contact areas. The excluded hydrocolloids accumulate outside the interaction region, leading to increased osmotic pressure in the surrounding area. The overall entropy of the system increases, which favors the attraction of casein micelles (Maroziene and de Kruif, 2000). Stable microparticles are formed by film drying followed by enzymatic hydrolysis of pectin (Asaduzzaman et al. 2022). The produced CMPs has suspended again in a buffer solution at pH 6.8. It has been reported that CMPs have a characteristic behavior of tightening in the acidic pH but swelling and dissolving tendency in the alkaline range (Schulte et al. 2020). For instance, the particle area remains stable at pH 3 but increases around twofold at pH 8 within the experimental period of 2 hours. In contrast, it takes only a few minutes at pH 11 or even a few seconds at pH 14 for such an increase in particle area (Schulte et al. 2020). The pH-dependent swelling behavior of casein microparticles indicates that they could be potential biopolymers for microencapsulation through the gastric passage and controlled release in the intestine where pH is slightly alkaline (pH 7-8) (Rouge et al. 1996). Therefore, this study aimed to tailor the swelling behavior of CMPs at a slightly alkaline pH of 8. We applied citrate to alter the charge and water-binding properties of CMPs to enhance their swelling behavior.

II. MATERIAL AND METHODS

A. Preparation of microparticles

The preparation of the CMPs was according to a protocol described by Asaduzzaman (Asaduzzaman et al. 2022). Briefly, the casein solution (7.36%) was prepared in simulated milk ultra-filtrated (SMUF) solution. The SMUF

solution was prepared according to the protocol described by Dumpler (Dumpler et al. 2017). The casein-pectin mix solution was prepared in BisTris buffer (50mM BisTris, 10mM $CaCl_2$, pH 6.8) having the casein and pectin content of 3.0% and 0.3% respectively. For film production, the casein-pectin mix was dried overnight at room temperature for 15 h at a constant RH of 40%. The film produced was then hydrolyzed with pectinase enzyme (37 unit/mL in BisTris buffer) so that the pectin matrix disintegrated and CMPs get released. Finally, the CMPs were separated by centrifugation and suspended again in BisTris buffer. For the sample with an added chelator, the required amount of tri-sodium citrate was added during the preparation of the casein solution (Asaduzzaman et al. 2022),

B. CMPs stability measurement

The CMPs stability was studied according to the method described by Asaduzzaman (Asaduzzaman et al. 2022). Briefly, 1.5 ml CMPs dispersion was filled in a semi-micro cuvette (Eppendorf AG, Germany), and the absorbance was measured at 600 nm for 900 s using a Lambda 365 UV/VIS spectrometer (PerkinElmer, USA). BisTris buffer was used as a reference. Immediately after measurement, 40 µl SDS solution (520 mM in water) was added to the cuvette and gently mixed with the SDS, and measured again. The measurement was repeated two times for each sample.

C. CMPs swelling measurement

The swelling behavior of CMPs was studied according to the protocol developed by Schulte (Schulte et al. 2020). Briefly, the swelling sieve cell was filled with CMPs solution and placed under an inverted microscope (DMIL LED Leica Microsystems, GmbH, Wetzlar, Germany). The dispersion was allowed to stand for approx. 10 min so that CMPs could sediment into the sieve holes. The video recording was collected using a Basler camera (Basler AG, Ahrensburg, Germany) connected to the microscope. A syringe pump (PHD ULTRA™ Harvard Apparatus, MA, USA) was used to flow the exchange medium (at different pH) at 0.05 ml per min. With the activation of the syringe pump, the image of a single microparticle trapped in the sieve holes was started to record (with 2 frames/second for 2 h). Image frames were extracted using PyCharm (version 2021.1.3, JetBrains, Czech) and the area of the CMPs was calculated by a freehand selection of particle outer lines using ImageJ software (NIH, USA). The measurement was repeated two times for each sample.

III. RESULT AND DISCUSSION

A. Particle size and morphology

For particle size analysis, optical images were taken randomly and processed using ImageJ software. Figure 1. shows the particle size distribution and morphology of CMPs. The ize of the microparticles ranged from 1-30µM with an average of ~ 7.5 µm (n=500). The evaluation of the microscopic observation assumed the spherical shape of the produced CMPs, which was proven to be the case in a recent study (Schulte et al. 2021).

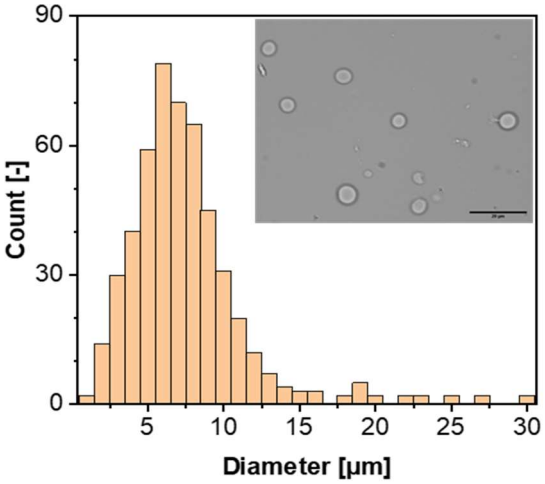

Figure 1. Particle size distribution of CMPs suspended in BisTris buffer extracted from optical images as exemplarily shown as insert.

B. Stability of CMPs

For the stability of the CMPs, the turbidity of the sample was measured with and without sodium dodecyl sulfate (SDS) addition. CMPs sample without SDS (Figure 2. green line), a slight decrease in turbidity over the experimental period of 600 s was observed due to sedimentation (Schulte et al. 2022). Whereas an exponential decrease in turbidity was observed after the SDS addition (Figure 2. black line). Microscopic images show that CMPs remained stable without SDS (Figure 2. top). However, no detectable particle was found after SDS addition (Figure 2. bottom) which indicates that the CMPs might be stabilized by hydrophobic interactions (Asaduzzaman et al. 2022)

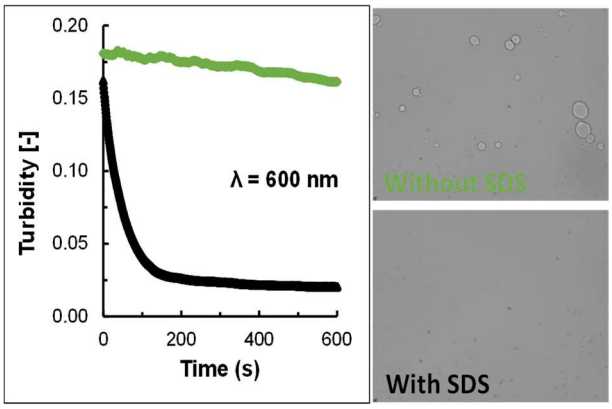

Figure. 2 Turbidity changes of CMPs dispersion over time at pH 6.8 (left) and corresponding microscopic images after measurement (right).

C. *Effect of pH on swelling behavior of CMPs*

The swelling behavior of individual CMPs was studied using a microfluidic chip with a sieve tray designed previously in our lab (Schulte et al. 2020). The sieve cell was filled with the sample solution to trap the CMPs inside the hole of the sieve tray. During the experiment, the exchange buffer was pumped to replace the existing solvent (BisTris buffer). The effect on the swelling behavior of CMPs was studied at pH 3, 8, 11, 12, and 14. The swelling process was investigated by calculating the change in particle area using ImageJ software. Figure 3. shows the swelling of CMPs at different pH media. The swelling was pretty fast at pH 14 where CMPs swelled and decomposed completely within 60 s. Whereas, it takes 720 s and 300 s at pH 11 and 12 respectively. The characteristic swelling factors were > 4.5.

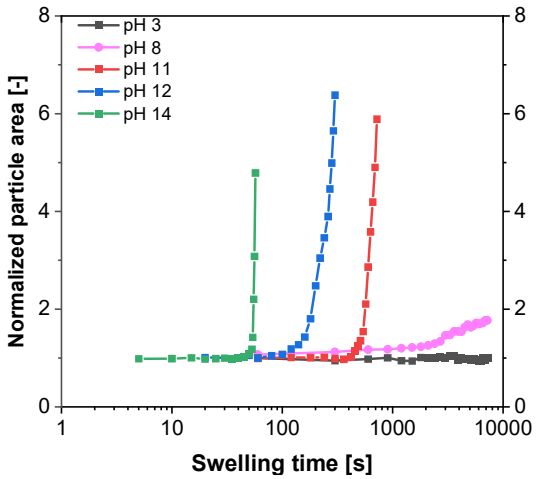

Figure. 3 Change of the particle area (normalized) over time after pH adjustment of the running solution in the microfluidic cell.

At pH 8, the particle area increased only 1.8 times after two hours, and no practical swelling was observed at pH 3. Under the acidic condition, the CMPs were more stable, but the alkaline condition favors the swelling and destabilization of the microparticles (Schulte et al. 2020). The pH-dependent swelling properties of CMPs might facilitate the delivery of bioactive compounds through the gastric passage and release in the small intestine. As the pH of the intestine ranges from 7-8 (Rouge et al. 1996), a further investigation was carried out to enhance the swelling properties using citrate as a calcium chelatant.

D. *Effect of citrate on the swelling behavior of CMPs*

To enhance the swelling of microparticles, the casein dispersion was pre-treated with sodium citrate. Figure 4. shows the swelling behavior of CMPs treated with 5 mM sodium citrate. The initial particle areas were related to the time when no exchange buffer (pH 8) was present in the swelling cell.

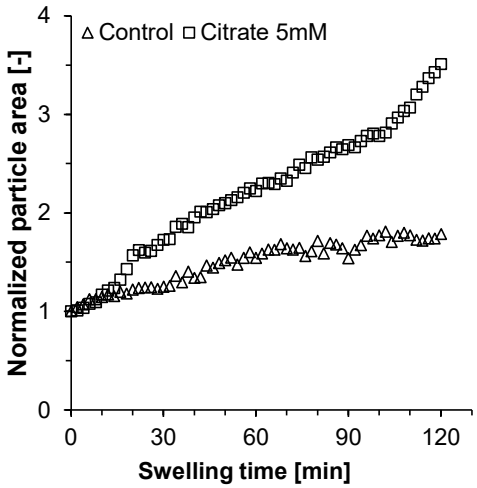

Figure 4. Swelling of CMPs with or without citrate treatment at pH 8.

Changing the buffer solution had little effect on CMPs (Figure 4. control). However, a faster and higher maximum swelling indicated the effect of citrate on the degree of swelling (Figure 4., citrate 5 mM). The particle areas of the CMPs treated with 5 mM citrate increased ~ 3.5 times after 120 min. However, the control samples (CMPs without citrate) did not even double after 120 min. The rate and maximum swelling increased with the concertation of citrate (data not shown) at pH 8 but no swelling was observed at pH 3 (Asaduzzaman et al. 2022). Citrate as a strong chelating agent can chelate Ca^{++} and disrupts the micellar calcium phosphate nanoclusters. Consequently, the charge of the protein increased due to the exposure of the negatively charged phosphoserine groups, which enhances the swelling process (Huppertz et al. 2007).

IV. CONCLUSION

Spherical CMPs produced by a depletion-flocculation reaction process was mainly stabilized by hydrophobic interaction as SDS turbidity measurements indicated. Microparticles were stable at acidic pH but showed swelling and decomposition under alkaline conditions. At the physiologically relevant pH 8 however, they showed greatly reduced swelling behavior. The addition of citrate enhances the swelling of CMPs at this pH, which can also be found in the gastrointestinal tract. Hence, the application of citrate during the production of CMPs could change their swelling behavior and might help in the controlled release of encapsulated compounds at favorable pH conditions.

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Electrospun nanofibres as an emerging delivery system for phenylethanoid glycosides: preparation and *in vitro* evaluation

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Abstract - **Today, the global market is strongly influenced by environmental awareness and a health-promoting lifestyle, underlined by innovative food concepts that offer both nutritional and bioactive value with the aim of preventing various health diseases. With Millennials being the biggest proponents of consuming herbal preparations and phytochemicals, i.e. dietary fibres, vitamins and polyphenols, the functional foods niche has never been more attractive. Polyphenols, as the most studied group of bioactive compounds, possess remarkable biological potential, e.g. free radical scavenging, antimutagenic, anti-inflammatory, antimicrobial etc. Susceptibility to degradation, unpleasant sensory profile, relatively low bioavailability and bioaccessibility are significant drawbacks when it comes to incorporating polyphenols into food, but various encapsulation techniques serve as effective solutions for maintaining functionality, improving their stability and prolonged release. Electrospinning technology represents an innovative technique for enclosing active agents within carbohydrate or/and protein (bio)polymer under a high-voltage electric field to produce nanofibres with advanced structural properties. For the production of polyphenol-loaded nanofibres, defined concentration of pullulan (PUL) and polyethylene oxide (PEO) solutions in concentrated water extract (100 °C, 30 min, 1g:100 mL) of plant species Mountain Germander (*Teucrium montanum*; collected from Varivode, Šibensko- kninska županija) were prepared. The impact of plant extract on conductivity, viscosity and surface tension of solutions was measured and compared with PUL and PEO solutions with distilled water. Encapsulation efficiency was monitored by measuring total phenolic content (TPC) and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, while the predominant phenolic compounds-echinacoside and verbascoside were analyzed using HPLC-DAD. Morphological characterization of obtained nanofibres was studied by scanning electron microscope (SEM). According to the results, nanofibrous mats with 15 % PUL (w/w) were evaluated to have the highest encapsulation efficiency (echinacoside: 92.92%, verbascoside: 49.21 %), homogeneous and bead- free surface morphology. These preliminary results could serve as a basis for further optimization of solutions for the preparation of nanomaterials, enriched with functional agents.**

Keywords - **Electrospun nanofibres, *T. montanum*, polyphenols, *in vitro* digestion**

I. INTRODUCTION

The global increase in the consumption of plant products has led to intensive research on traditional plant species in terms of their bioactive composition, thus promoting the market expansion of polyphenols as the most studied group of bioactive compounds, along with their application in the pharmaceutical and food industries. A major challenge in the incorporation of phenolic compounds into commercial products is primarily the relative instability of polyphenolic components, which is due to the fact that these compounds tend to be oxidized and degraded under the influence of high temperature and pressure, extreme pH, light, during food storage and processing, etc. (Bell, 2001). To overcome the above limitations, encapsulation techniques represent an efficient technological process of "packaging", i.e., protecting active ingredients from various environmental factors by an inert carrier/shell/matrix (Ghosh, 2006).

One of many promising techniques for encapsulating bioactive ingredients is electrospinning-a process driven by an electrohydrodynamic phenomenon in which nanofibres are generated from a polymer solution at high voltage. When a sufficiently large potential difference is established between the polymer solution and the grounded collector, the surface tension of the solution is overcome and "stretching" of the solution occurs, i.e., the formation of the Taylor cone causes the solutions to stretch and form a polymer jet. Under these conditions, the solvent evaporates and continuous fibres can form on the collector. The possibility of obtaining fibres based on biodegradable polymers with high porosity, high surface-to-volume ratio and good thermal stability has generated a great interest in their application in regenerative medicine and bioengineering of tissues, drugs with controlled transfer and release of functional components, production of protective materials for textiles, sensor industry, catalysts, etc. in the last 15 years (Ramakrishna et al., 2006). Among the proteins and polysaccharides used as carriers for active ingredients, gelatine (Wang et al., 2019), amaranth (Blanco-Padilla et al., 2015), whey protein (Drosou et al., 2018), pullulan (Aguliar- Vásquez et al, 2018), chitosan (Qin et al, 2019), hydroxypropyl-β-cyclodextrin (Poudel et al., 2020)

etc., have already been investigated for use. Pullulan is a particularly attractive biopolymer due to its linear structure and non-ionic maltotriose units linked via α 1-6 glyosidic bonds, its stability and solubility over a wide pH range, its GRAS status, and its ability to form stable and flexible electrospinning nanofibres from water solutions (Singh et al., 2019). Since it is an exopolysaccharide derived from starch plants and agricultural waste, it is suitable for sustainable and cost-effective production. Similar advantages in biodegradability, non-toxicity and water solubility are attributed to polyethylene oxide, a polymer of ethylene units with a molecular mass of over 20000 g/mol.

To overcome the challenges in finding a suitable encapsulation technique for phenylethanoid glycosides from *T. montanum* L. as an insufficiently studied plant species, electrospinning technique was chosen for the preparation of a functional delivery systems based on pullulan and polyethylene oxide. First, a water extract was prepared (100°C, 1g:100 mL, 30 min) and the bioactive quality was determined by spectrophotometric methods and HPLC-DAD analysis. Subsequently, PUL and PEO were dissolved in the concentrated extract at defined mass ratios. Physical characterization of the polymeric solutions thus prepared was carried out in terms of conductivity, apparent viscosity and surface tension and compared with the solutions dissolved in water. After the electrospinning process, the encapsulation efficiency (IU %) and morphology of the electrospun nanofibres were evaluated with *in vitro* assessment in simulated gastrointestinal fluids.

II. MATERIALS AND METHODS

A. Materials

Plant material *T.montanum* was collected in Varivode, County of Šibensko- kninska in Dalmatia region. Pullulan was purchased from Biosynth Carbosynth while polyethylene oxide (Mr: 300 000 g/mol) was obtained from Sigma Aldrich. Pepsin from porcine gastric mucosa (474 IU/mg) and pancreatine from porcine pancreas (4*USP) were purchased from Sigma Aldrich. All chemicals used in the experiments were of analytical grade.

B. Bioactive quality of T. montanum aqueous extract

Bioactive potential of plant extract was determined spectrophotometrically on Genesys Technologies 10S UV-VIS spectrophotometer (Thermo Fisher Scientific) using TPC (total phenolic content) method (Singleton and Ross, 1965) and DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method (Brand-Williams et al., 1995). Single phenolic compounds, i.e. echinacoside and verbascoside, were eluted on the HPLC-DAD Agilent 1100/1200 series instrument (Agilent, Santa Clara, USA) using gradient elution with 1% formic acid in water and 1% formic acid in acetonitrile on an Zorbax Extend- C18 column (4.6*250 mm, 5 µm) at a flow rate of: 1 mL/h, injection volume- 5 µl and column temperature- 25 ºC. The UV spectrum of targeted compounds was recorded at 320 nm.

C. Preparation of polymer solutions

First, the aqueous plant extract was prepared by conventional extraction (100 ºC, 1g/100 mL, 30 min) and concentrated in a rotary evaporator (IKA RV 8) to a 10-fold lower volume compared to the initial volume. PUL (15, 12 and 10%) PEO (8 and 5%) and PUL /PEO mixture in 1:1 ratio (w/w) were prepared and mixed in 10-fold concentrated plant extract at room temperature on a magnetic stirrer until a homogeneous solution was obtained.

D. Physical characterization of polymer solutions

Apparent viscosity was measured on rotary viscometer RM 100 plus (Lamy Rheology) at shear rate 10 s^{-1}. Electrical conductivity of solutions was evaluated by conductometer Cond-330i (WTW). Surface tension was determined on tensiometer DCAT with TP 50 control panel and connected software for data analysis (DataPhysics).

E. Preparation of electrospun fibres

Electrospinning of the prepared polymer solutions was performed using an electrospinning device from Spinbox Systems (Bionicia). Each sample was placed in a 10 mL syringe connected to a syringe feeding system and connected by a delivery tube to the single-phase spinning head with the attached needle (22 gauge). The syringe pump delivered the solution at a selected and constant flow rate (0.6-1 mL/h). The positive high-voltage source was connected to the needle via an alligator clip. Under a specific voltage (13.1-21.6 kV), the electrically charged spinning solution formed a Taylor cone at the tip of the needle, from which a polymer jet was formed. With the water evaporating from the jet, continuous fibres were collected on a grounded collector plate covered with aluminium foil, which was located at a certain distance from the needle tip (10-14 cm). The electrospinning process was carried out at a temperature between 25-29 ºC and relative humidity between 32 and 41%. The produced fibres were removed from the aluminium foil and stored in the exicator at room temperature.

F. Determination of encapsulation efficiency for electrospun fibres

Approximately 10 mg of the sample was dissolved in 10 mL of 15% (w/v) sodium citrate. For HPLC analysis, the solution was filtered through a regenerated cellulose microfilter (0.25 µm pore size). The encapsulation yield of the prepared fibres was determined by measuring the TPC and antioxidant capacity using DPPH, and by analysing selected individual phenolic compounds, i.e., echinacoside and verbascoside in the dissolved citrate-fibre solution and their content in the original delivery solution, taking into account the dry matter content in the original polymer solution and in the produced fibres. Values were statistically compared using One way ANOVA with post-hoc test ($p<0.05$) in Statistica 13.3. Software.

G. Morphology of produced nanofibres

The morphology of the fibres was evaluated using scanning electron microscopy (SEM) on TESCAN CAN Mira3 microscope. Fibres were attached to double-sided adhesive tape, adjusted on the specimen holder and then coated with the layer of gold. Scanning was performed with the accelerating beam voltage of 10 kV.

H. In vitro simulation of pullulan fibre digestion

INFOGEST static *in vitro* digestion was conducted according to Minekus et al. (2014) and Brodkorb et al. (2019). Release profile of quantitatively predominate polyphenol-echinacoside from the nanofibres with the highest bioactive quality was monitored in simulated gastric (pH=3, digestion time: 120 min) and intestinal fluid (pH=7, digestion time: 30 by HPLC-DAD methodology.

III. RESULTS AND DISCUSSION

A. Bioactive quality of T. montanum aqueous extract

Table 1. Bioactive characterization of *T. montanum* water extract prepared with conventional extraction

	TPC (mg GAE/L)	DPPH (mmol TroloxE/L	ECH (mg/L)	VERB (mg/L)
TM_E	550.2 ± 30	0.33±6.9	179.9±0.96	51.4±0.12
TM_E;10×	5700.5±50	3.26±5.4	1730.6±1.96	498.9±0.56

TM_E: water extract; TM_E;10×:ten fold concentrated water extract; ECH:echinacoside; VERB:verbascoside

From the results for the individual phenolic compounds, it appears that approximately 32% and 9% of the TPC content can be attributed to the echinacoside and verbascoside content, respectively, in the plant extract. Therefore, water extract of *T. montanum* can be considered as remarkable source of mentioned phenlyethanoid glycosides.

B. Physical characterization of polymer solutions

Table 2. Physical characterization of extract and polymer solutions with water (w) and extract (e). Measurements were conducted on 25 ± 0.5 °C.

Polymer(s) and its mass percent in final solution(%)	Flow rate (mL/h)	Applied voltage (kV)	Tip-to-collector distance (cm)	T(°C)	RH (%)
15_PUL	1	19-21,6	12	26.5	34
12_ PUL	1	19-20,5	12	27.0	38
10_ PUL	1	21,2	10	26.9	42
PUL/PEO (1:1)	0,6	13,1	14	25.9	38

Table 3. Experimental conditions for the preparation of electrospun nanofibres with PUL and PEO dissolved in concentrated plant extract.

Sample	Conductivity (mS/cm)	Surface tension (mN/m)	Apparent viscosity (Pa s)
15_PUL_w	0,121±0,00	60.46±1.12	0,603±0.56
12_PUL_w	0,065±0,00	58.45±0.41	0,229±0.23
10_PUL_w	0,059±0,00	59.22±0.29	0,132±0.05
5_PEO_w	0,331±0,01	58.61±0.22	0,500±0.12
8_PEO_w	0,931±0,00	57.45±0.23	3,353±0.24
PUL/PEO_w	0,110±0,00	58.89±0.14	0,316±0.19
100% extract	3,365±0,01	35.84±1.02	0,027±0.32
15_PUL_e	1,683±0,05	37.79±0.93	0,777±0.05
12_PUL_e	2,160±0,00	38.78±0.48	0,289±0.45
10_PUL_e	2,285±0,01	38.37±0.23	0,147±0.36
5_PEO_e	2,595±0,01	39.26±0.35	0,373±0.14
8_PEO_e	2,225±0,02	39.06±0.09	3,456±0.10
PUL/PEO_e	2,345±0,01	38.88±0.02	0,350±0.79

Polymer solutions were characterized for conductivity, surface tension and apparent viscosity, since these parameters significantly determine overall potential for obtaining adequate polymer jet. From Table 2., it can be seen that plant extract (3,365 mS/cm; 35.83 mN/m) primarily affected on conductivity and surface tension of all polymeric solutions when comparing to the ones dissolved in water. Surface tension for all solutions dissolved in plant extract was relatively unchangeable while the highest conductivity was measured for 5_PEO. Additionally, PEO used in this experiment seems to have higher contribution to the viscosity

than PUL in all polymer solutions. Spin dope solution of 15_PUL, 12_PUL, 10_PUL and PUL/PEO (1:1) were successfully electrospinned, although mixture of PUL/PEO was not able to be adequately removed from the aluminium foil. So, encapsulation efficiency was not calculated for that sample (Table 3.). Changing the molecular weight of PEO could be the good solution for enhancing the physical characteristic of the mixture. Non- optimal conductivity of 5% PEO when comparing to the values of other spin dope solutions may be the reason of impossibility to form stable polymer jet, without breaking up of solution into droplets. Too high apparent viscosity of 8% PEO resulting from too high concentration of polymer seem to have the effect on the impossibility to initiate the continuous jet, due to the strong electrostatic force of the solution. On contrary, PUL in all tested concentration showed good properties for electrospinning.

C. Determination of encapsulation efficiency for electrospun fibres

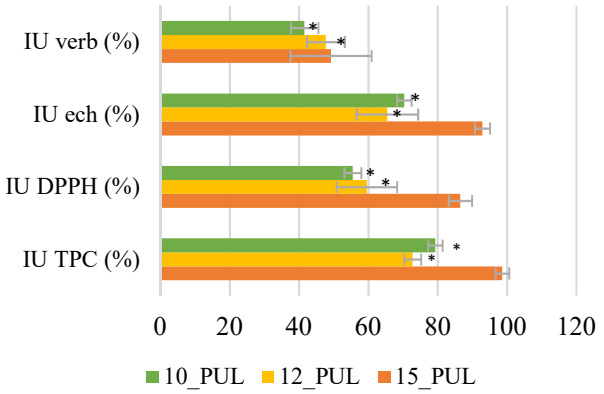

Figure 1. Encapsulation efficiency of TPC, DPPH and individual phenolic compounds- echinacoside (ech) and verbascoside (verb) in prepared electrospun nanofibres with concentrated plant extract. Values marked with * are not statistically significant ($p>0.05$).

According to the results in Figure 1. the encapsulation efficiency for successfully electrospun fibres was high for TPC in all cases (98.65-72.80%). The most efficient antioxidant activity measured by DPPH was obtained for sample 15_ PUL (86.58%). It is also seen that echinacoside was encapsulated more efficiently than verbascoside in all three cases (92.92-65.37 %). Of all the samples, 15_ PUL proved to be the optimal concentration for the phenylethanoid glycosides tested. A good choice for further studies could be mixing pullulan with other polysaccharides or proteins to improve the physical properties and encapsulation efficiency. Blanco-Padilla et al. (2015) successfully incorporated curcumin into an amaranth-pullulan matrix with a minimum content of 50% amaranth.

PUL and PUL /PEO based nanofibres exhibit different quality of morphology according to the SEM analysis (Figure 2.). It can be seen that 15_ PUL and 12_ PUL exhibited a straight and randomly oriented structure with minimal porosity.

D. Morphology of produced nanofibres

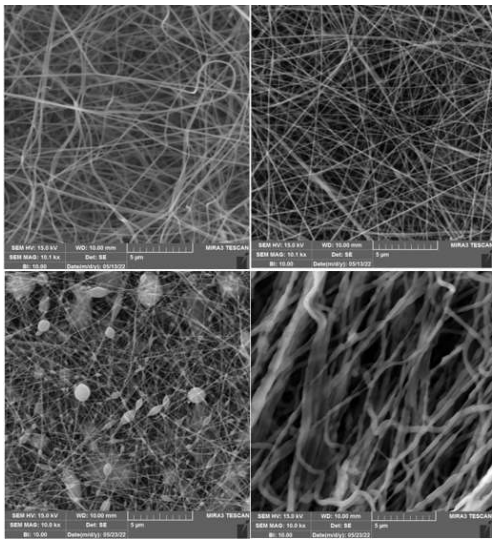

Figure 2. SEM analysis of produced electrospun nanofibres with plant extract: a) 15_PUL, b) 12_PUL, c) 10_PUL and d) PUL/PEO (1:1)

In contrast, 10_ PUL showed a bead-in structure with pronounced porosity of the non-continuous fibres together with the beads. This is the result of the transition from electrospinning to electrospraying and usually occurs when the viscosity, i.e., the concentration of the polymer is too low to produce the fibres. In the case of PUL /PEO nanofibres, the structure is not continuous and the fibres are partially bonded together and relatively torn, resulting in high water absorption and irreversible adhesion of the aluminium foil.

E. In vitro simulation of pullulan fibre digestion

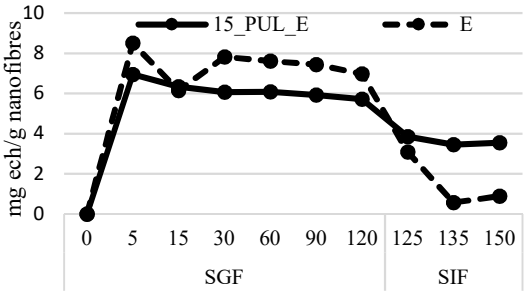

Figure 3. *In vitro* digestion of analyzed echinacoside in incapsulated extract (15_ PUL_E) compared to non- encapsulated extract (E) in simulated gastrointestinal fluid

As can be seen, the release dynamics of echinacoside from the non-encapsulated extract was similar compared to the encapsulated extract (Figure 3.). During the first 5 minutes in SGF (pH=3), the largest amount of echinacoside was released from the nanofibres. During the remainder of gastrointestinal digestion, degradation of the phenolic component occurred, particularly in the SIF (pH=7). This is relatively expected since polyphenols are susceptible to oxidation under alkaline conditions. It can be concluded that the application of a single 15_ PUL as a spin solution does not exhibit adequate gradual gastrointestinal release of the targeted compound. However, other routes of administration may be considered, such as dermal administration, if appropriate. Ibrahim et al. (2022) prepared a gel based on loaded lipid nanocapsules for topical application, achieving a cosmetic anti-ageing agent. Thus, further studies for food use may aim to combine PUL with other carriers, such as carboxymethylcellulose, higher molecular weight PEO, or plant proteins, e.g., low viscosity alginate, various protein isolates, etc., to reduce the high hygroscopicity of PUL itself. For example, Blanco-Padilla et al. (2015) demonstrated gradient release of encapsulated curcumin in an amaranth/PUL matrix throughout gastrointestinal digestion *in vitro*, with antioxidant activity maintained after digestion. In addition, mixing PUL with inorganic salts, such as magnesium or calcium chloride, could improve polymer crosslinking.

IV. CONCLUSIONS

Electrospinning technique was successfully employed in the production of polyphenol-rich nanofibres delivery system. PUL in the concentration range of 15-12% exhibited relatively high encapsulation efficiency, while fibre morphology in terms of the formation and non-porosity was the most adequate for 15_PUL and 12_PUL. Blending the pullulan with other adequate polyssacahrides or proteins may improve the nanofibre structure, enabling more gradual release of functional agents during gastrointestinal digestion.

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Encapsulation of Ground Ivy (*Glechoma hederacea L.*) extract, obtained by natural deep eutectic solvents extraction, in liposomes

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Abstract – **Ground Ivy (*Glechoma hederacea L.*) has been used in traditional medicine for centuries. Its medicinal properties were known even by Galen, who recommended this plant to those suffering from eye diseases. It contains flavonoids (quercetin, apigenin and luteolin), phenolic acids (rosmarinic, caffeic), tannins, sesquiterpenes, bitter substances, a little essential oil, saponin and resin.**
In the present study extraction of ground ivy was performed using different natural deep eutectic solvents mixtures (NDES). For those purposes several different NDESs was used and results show that best eutectic mixture for ground ivy extraction is betain and citric acid in molar ratio 1:1. Extraction was carried at room temperature and yield of rosmarinic and caffeic acid is significantly higher comparing with water extraction at 80°C.
Extracts were encapsulated in liposomes prepared using proliposome and thin film methods, following by sonication to obtain smaller, more stable particles. Encapsulation should preserve actives from external factors such as oxidation, hydrolyzes, temperature degradation etc. The release of ground ivy extract from liposomes was studied using standard Franz diffusion cell. The results show that the release of the extract is slowest from liposomes prepared by the proliposome method, but all systems give satisfactory sustained release.

Keywords – **Natural deep eutectic solvents, Ground ivy, Liposomes, Sustained release**

I. INTRODUCTION

Glechoma hederacea L., known as ground ivy, is one of the plants of *Lamiaceae* family used in folk medicine against headaches, neuralgia and dizziness. As a member of the *Lamiaceae* family, ground ivy has the common characteristic of a high content of rosmarinic acid (Šeremet et al. 2022) which possesses antimicrobial, cardioprotective, antioxidant, hepatoprotective, anti-allergic, anti-inflammatory and antidiabetic activities (Nadeem et al. 2019). Because of all these properties rosmarinic acid is good choice for nutritional supplements or additive to functional food.
The development of new extraction techniques that exclude hazardous solvents and that are sustainable has been the subject of increasing research interest in recent years. Due to the continuous demand of green extraction procedures, Natural Deep Eutectic Solvents (NDESs) have arisen in the last years as new green solvents alternative to the conventional organic ones. Many advantageous features of NADEs such as their sustainability, biodegradability, acceptable pharmaceutical toxicity profiles, and their high solubilization power of both polar and non-polar compounds demonstrate their potential as green solvents for plant extraction. Deep eutectic solvents (DESs) are defined as a mixture of two or more components, which may be solid or liquid and that at a particular composition the melting points of NDESs are significantly lower than the melting points of their individual components. NDESs are a mixture of two or three components that form intramolecular hydrogen bonds; hydrogen bond acceptors usually are organic salts (quaternary ammonium or phosphonium salt), while hydrogen bond donors could be: sugar, alcohol, amino acid, organic acid etc. In some cases, water could be added to the eutectic mixture. Since it is possible to use many different components to obtain the eutectic mixtures, it is possible to obtain a solvent with particular physicochemical properties and selectivity (El Achkar et al. 2019).
Use of rosmarinic acid in the food products could be limited due to its chemical instability, low water solubility and discolouration (Šeremet et al. 2022, Kim et al. 2010), and as one of the solutions, encapsulation has been suggested. Encapsulation in liposomes should preserve rosmarinic acid from external factor, but also could enhance tpical delivery of this active when it is used in pharmaceutical and cosmetic preparations (Subongkot et al. 2021).

II. MATERIALS AND METHODS

A. Materials and chemicals

Ground ivy was harvested in April 2020. on the area of Bilogora (Bjelovar-Bilogora County, Croatia). Aerial parts were separated from roots and air-dried for 3 days to the content of dry matter >90% and afterwards grinded and sieved to obtain fraction (<450 μm) for further applications (Šeremet et al. 2022).
For eutectic mixtures preparation choline chloride (Acros Organics), betaine (Acros Organics), citric acid (Fisher USA), malic acid (Acros Organics), fructose (Acros Organics) and glucose (Fisher USA) were used. For liposomes preparation unsaturated phospholipids were used

namely Emulmetik 930 (Lucas Meyer Cosmetics, IFF) with at least 95% of phosphatidylcholine

B. Methods

Vacuum evaporating method (Dai et al. 2013) was used for preparing natural deep eutectic solvents (NDESs) in certain molar ratios as listed in Table 1.
Briefly: components were dissolved in water and evaporated at 60ºC with a rotatory evaporator (Heidolph Hei-VAP Value, Germany). The liquid obtained was put in a desiccator with silica gel till they reached a constant weight.

Table 1. Composition of prepared NDES

NDES	*Abbreviation*	*Molar ratio*
Choline chloride: Glucose	ChCl:Gl	1: 1
Betaine: Citric acid	Bt:CA	1: 1
Malic acid: Fructose	MA:Fr	1:1

Following extraction procedure was used: 0.1 g of grinded plant material was placed in 50 ml erlenmeyer flask with 10 ml of solvent, covered with aluminum foil and thermostated and mixed using orbital shaker Ika KS 4000 i control (IKA-Werke, Germany) during 30 minutes at 150 rpm. NDESs mixtures for extraction were mixed with demineralized water in 1:1 and 1:3 mass ratio. Extractions with NDES extractions were performed at 25ºC and 40ºC while extraction with demineralized water (W) was at 80ºC.
After finishing the extraction processes samples were centrifuged in an Eppendorf Centrifuge 5430 R (Eppendorf AG, Hamburg, Germany) at 20 ºC and 15,000 rpm for 15 minutes. After centrifugation, the supernatant was separated from the precipitate, filtered through a cellulose acetate syringe filter 0.45 μm (Agilent, Santa Clara, US) and used in the analyzes.
Liposomes were prepared using commercial lipid mixture Emulmetik 930 with ~95% phosphatidylcholine content. Two methods were used for this purpose: the thin film evaporation method and proliposome method (Isailovic et al. 2013). In brief, chloroform solution of Emulmetik 930 (4 mg/ml) was slowly evaporated using the rotary vacuum evaporator (Heidolph Hei-VAP Advantage), under pressure of 120 mbar, temperature of 50°C, during 2 h. The thin film (TF liposomes) was formed and further dried for 15 minutes more to ensure that solvent is completely removed. Subsequently, the obtained lipid film was hydrated with 20 ml of previously prepared ground ivy extract. The emulsion was hand shaken intermittently for 30 minutes with addition of glass beads to suspend the lipid film and improve the formation of liposomes. The final concentration of lipids was 2 mg/ml. On the other hand, proliposome method (PRO liposomes) was conducted as follows: Emulmetik 930, ethanol and water (in the mass ratio 1:1:2) were stirred at 800 rpm for five minutes at 50°C to obtain homogeneous mixture. The mixture was cooled to room temperature and further hydrated using 20 ml of previously prepared ground ivy extract, mixing on the magnetic stirrer. The final concentration of lipids was also 2 mg/ml. Both methods provided multilamellar liposomes.
In order to obtain smaller, unilamellar liposomes with narrow size distribution the additional step of sonication was applied on multilamellar liposomes obtained by previously described methods. For this step ultrasonic processor (Ultrasonic Homogeniser HD 2070.2 Sonopuls) equipped with solid titanium probe (MS 73) was used. 10s on–off cycles were applied during 30 min, at 40% of amplitude and the samples were cooled during whole procedure with ice bath. The obtained liposomes (TF S and PRO S) were used for further analyses.
Relese of extracts from liposomes was studied using Franz diffusion cell (PermeGear, Inc., USA). This cell consists of a donor and a receptor chamber separated with an adequate membrane, chemically inert to the active component. In this study cellulose acetate membrane (pore size of 0.2 μm) was used. An adequate amount (approximately 2 ml) of prepared liposomes was placed in the donor chamber. The receptor chamber, with a volume of 20 ml, was filled with demineralized water. Experiments were performed at room temperature with constant stirring of the receptor solution using a magnetic bead at a speed of 750 rpm. The samples were taken in certain time intervals during 6 h from the receptor chamber and analyzed. After each sample were taken, the same volume of fresh receptor solution was returned to the receptor chamber to keep the volume constant.
Concentrations of rosmarinic and caffeic acid in extracts were determined using HPLC. Quantitative analysis of samples was done by Dionex Ultimate 3000 Thermo Scientific (Waltham, USA) HPLC system and a reverse phase column (PerkinElmer C18, 150 mm × 4.6 mm, 5 μm). Mobile phase was composed of solvent (A) H2O: HCOOH = 100: 0.1 % and solvent (B) MeOH: HCOOH = 100: 0.1 %. It was conducted by gradient elution in a following way: 0-30 min 10 - 35 % B, then 30-30.1 min 35-100 % B, 30.1 - 35 min 100 % B, 35-35.1 min gradient from 100-10 % B and 35.1-45 min 10 % B. A flow rate of 1.0 ml/min was used and column was thermostated at 30 ˚C. Injection volumes of extract and standards (0.5 mM) were 15 and 5 μl, respectively. Detection of compounds was carried out by UV detector at 280 and 310 nm.
The released amount of ground ivy extract from liposomes was determined using UV spectra of the extracts obtained on Shimadzu UV-1800 (Kyoto, Japan). Two absorption peaks around 280 and 320 nm characteristic for chlorogenic acid were measured to estimate the released amount of extracts.

III. RESULTS AND DISCUSION

Concentrations of rosmarinic and caffeic acids in prepared extracts are given in Table 2. It could be seen that highest concentration of rosmarinic acid is obtained with eutectic mixture of betaine and citric acid mixed with water in mass

ratio 1:1 at 40ºC, and it's significantly higher comparing with extracts prepared with other eutectic mixtures or water. When it comes to caffeic acid best yield is obtained with eutectic mixture of choline chloride and glucose mixed with water in mass ratio 1:1. In water extract and extract prepared with eutectic mixture of malic acid and fructose as a solvent caffeic acid is not determined.

Table 2. Concentration of rosmarinic (C_{RA}) and caffeic (C_{CA}) acids in different extracts of ground ivy

Solvent	*Temperature, ºC*	*C_{RA}, mg/ml*	*C_{CA}, mg/ml*
Water	80	0.017	ND
ChCl:Gl/Water (1:3)	25	ND	0.025
ChCl:Gl/Water (1:1)	25	0.029	0.042
MA:Fr/Water (1:3)	25	0.031	ND
MA:Fr/Water (1:1)	25	0.039	ND
Bt:CA/Water (1:3)	25	0.054	0.006
Bt:CA/Water (1:1)	25	0.052	0.003
ChCl:Gl/Water (1:1)	40	0.054	0.011
MA:Fr/Water (1:1)	40	0.051	ND
Bt:CA/Water (1:1)	40	0.071	0.006

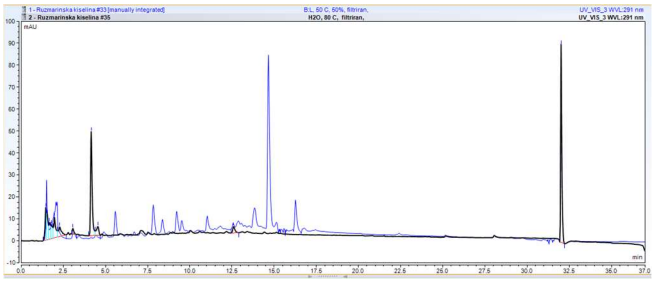

Figure 1. Comparation of chromatograms of water extract (black line) and eutectic mixture (betaine, citric acid) extract (blue line) of ground ivy

Comparasion of chromatograms of water ground ivy extract and extract prepared with eutectic mixture of betaine and citric acid is shown on Figure 1. It can be seen from Figure 1. that not only the concentration of rosmarinic acid is higher, but a much larger number of compounds were extracted using the eutectic mixture (larger number of peaks on the chromatogram).

Table 3. Liposomes mean diameters

	Mean particles diameter, nm			
Sample	1st day	7th day	14th day	21st day
TF	349.6±11.9	286.8±5.8	284.5±11.4	259.0±2.6
PRO	995.7±97.3	839.7±92	442.7±25.2	1828±292
TF S	99.5±0.88	122.7±3.0	134.8±5.6	99.58±0.9
PRO S	241.2±2.5	220.6±4.7	218.3±4.2	281.9±5.3

For encapsulation in liposomes extract of ground ivy were prepared using as a solvent eutectic mixture of betaine and citric acid and water in mass ratio 1:1. As a previously mentioned four different liposome dispersions were prepared and particle size distribution and zeta potential was measured every 7 days during 3 weeks to assess stability. Mean particles diameter are shown in Table 3. It could be seen that sonication give expected results and particle size is significantly lower and do not change much with time which suggest that these liposomes are more stable. Zeta potential for all liposomes was around +11.0 mV.

Extract release from different liposomes is presented on Figure 2. The best sustained release was achieved from liposomes prepared by proliposome method, but these liposomes are not so stable regarding their diameter change trough the time. All liposomes ensure prolonged release, since the maximum released amount of extract is around 40 % during 5 hours.

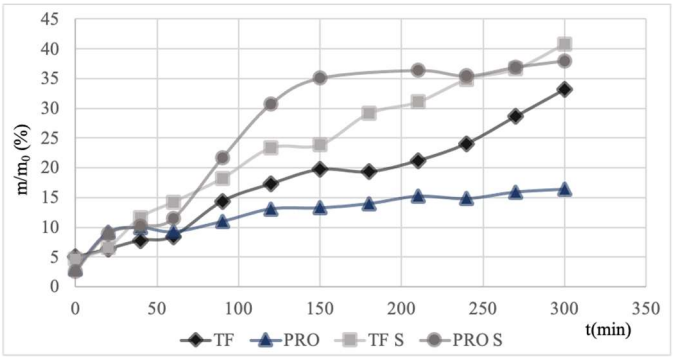

Figure 2. Release of ground ivy extract from different liposomes

IV. CONCLUSIONS

Based on the presented experimental data, the following conclusions can be drawn:

- √ Eutectic mixture of betaine and citric acid is most suitable for extraction of rosmarinic acid from ground ivy.
- √ Eutectic mixture of choline chloride and glucose gives best results for extraction of caffeic acid from ground ivy.
- √ Eutectic mixtures are very viscous and must be mixed with water to get the best results in plants extraction.
- √ The NDES need to be customized to each plant species in order to obtain the best yield of the desired active components.
- √ Extracts of ground ivy could be encapsulated in liposomes to protect actives from external factors.
- √ Sonication of liposomes results in smaller particles, narrower particle size distribution and more stable liposomes.
- √ Liposomes provide sustained release of encapsulated extract

ACKNOWLEDGMENT

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NUTRITION, HEALTH AND CONSUMER

The influence of social factors on the quality of life of celiac *disease patients*

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Abstract - **Celiac disease is an autoimmune disease of a chronic type, which occurs as a result of consuming foods with gluten and its related proteins. It can be found in approximately 1% of the total population of Europe, while in many affected individuals the disease has unclear symptoms and remains unrecognized for a long time. Given that celiac disease is a lifelong disease, with inadequate treatment it can lead to various serious complications, which greatly affect the quality of life of sufferers. Consequently, the only successful way to keep the disease under control the lifelong introduction of gluten-free preparations into the diet.**

To examine how celiac disease and social factors affect the quality of life of patients with celiac disease.

Methods: A quantitative survey was conducted on 48 study participants from the area of northwestern Croatia. A self-made survey questionnaire was used as an instrument for the purposes of the research. The questionnaire was filled out by parents for their underage children with celiac disease, or by adults with celiac disease.

Celiac patients of different age groups rate their quality of life differently and more than half of the participants are members of the Celiac Disease Association. The obtained results show the dissatisfaction of the participants due to the insufficient availability of gluten-free products in stores, the lack of adaptation of school and kindergarten meals for children with celiac disease, as well as the unavailability of gluten-free dishes in most restaurants.

The disease requires more frequent controls, and patients with abdominal disorders who do not adhere to a gluten-free diet have a reduced quality of life. Other studies have also found a negative assessment of the situation for patients with celiac disease which they encounter every day in shops, schools, kindergartens, restaurants and bakeries.

Early detection and treatment of celiac disease improves the quality of life, and the availability of gluten-free products in all aspects of social life positively affects the satisfaction of patients.

Keywords - **Celiac disease, quality of life, social factors, patient.**

I. INTRODUCTION

Celiac disease (CD) represents a chronic autoimmune disease caused by gluten found in wheat, barley and rye (albeit this is not certain for oats), and the disease affects the small intestine. The distribution of gluten is sizeable; it is found in most food products as a basic ingredient or it can be added as a derivative if the food is processed. Gluten is also found in processed foods, many products and medicines. In people with a genetic predisposition, the disease occurs more frequently, and is evidenced by the damage to the proximal part of the mucosa of the small intestine. It can be seen at any age and can come with a plethora of different symptoms. The prevalence of celiac disease is higher in people who have a positive family history of celiac disease. Therefore, possible candidates for testing are first and second cousins of the affected individuals. Celiac disease is also more common in people who already have an autoimmune disease – including type I diabetes, IgA cognitive deficiency, Down syndrome, Williams syndrome, autoimmune liver disease, thyroid disease, Sjögren's syndrome, Addison's disease, and Turner syndrome (Čuković-Čavka et al. 2012, Sapone et al. 2012, Elli et al. 2015, Ludvigsson et al. 2014). The disease itself is often lifelong, and if it is not adequately treated, it can lead to numerous and very serious health complications, thus affecting the quality of life of the sufferers. Given that the diagnosis is difficult to confirm, the disease can be unrecognized for many years. The only successful therapy is the lifelong introduction of gluten-free preparations into the diet (Tack et al. 2010).

II. EPIDEMIOLOGY OF CELIAC DISEASE

Celiac disease was thought to be rare, and the prevalence in America was only 1:6000 inhabitants, while in Europe it was 1:300. Looking at the incidence of celiac disease in European countries, the highest prevalence was recorded in Scandinavian counties, while the lowest was in Germany (Catassi et al. 2014). As serological tests have become more and more available, the prevalence of celiac disease increased due to increased recognition, and ranges from 1:150 to 1:300 of the population. In addition to gluten, genetic predisposition also plays a pivotal role as a cause of disease. This is supported by the data which shows how celiac disease occurs in about 10 to 15% of relatives of the affected in the first generation. The risk of occurrence of the disease in the first generation is 1:10, while in the second generation it is

1:39, which confirms to us that the genetic disposition substantially influences the occurrence of the disease. The possibility of celiac disease in identical twins is about 80%, while in fraternal twins it is 30% (Starčević Čizmarević et al. 2015).

In the Republic of Croatia in 2015, 3,954 patients were diagnosed with K90, which is intestinal malabsorption in general, while 2,324 patients were diagnosed with K90.0, which specifically denotes celiac disease. According to the data found in the Register for Persons with Disabilities, we have 780 patients with a diagnosis of K90, and 518 patients with a diagnosis of K90.0 who claimed their rights to functional impairment (HZJZ, 2021). That the incidence of celiac disease is increasing is further confirmed by the frequency of its occurrence in North Africa and the countries of the Middle East (Catassi et al.2014). A high incidence rate was recorded in Finland and Sweden, approximately 2-3%, while the rate in Germany is only 0.2%. It is also on the rise in Western countries (Mustalahti et al. 2010, Ivarsson et al. 2013).

III. ESTABLISHING A DIAGNOSIS

To diagnose celiac disease, a steadfast knowledge of the nature of the disease is of utmost importance, in order to even have a clinical suspicion. Hence, the first step in to take an adequate medical history and status of the patient to establish such suspicion (Tack et al. 2010). This is followed by serological tests, which are very important not only in diagnosis, but also in monitoring patients. When determining serum antibodies, endomysial IgA antibodies (EMA) and antibodies to tissue transglutaminase (anti-tTG antibodies) are crucial markers. Measuring the titer of IgA anti-gliadin antibodies (AGA) also used to be pursued; however, today this is no longer the case due to the high sensitivity of anti-tTG antibodies. It is important to measure the total IgA in the blood before diagnosing the disease, in order to rule out IgA immunodeficiency and avoid potential false negative results in individuals with IgA immunodeficiency. If we have an established IgA deficit, it is recommended to measure IgG-tTG antibodies. In the diagnosis of this important disease, there are also specific antibodies to gliadin peptides deamidated by tissue transglutaminase (DGP). More specifically, the binding of deamidated peptides to the HLA-molecule is tighter and more definite, thus leading to a strong immune response. These antibodies have a high sensitivity and specificity for celiac disease, just like IgA-tTG antibodies, they are present in patients very early, and are also found in younger children, under two years of age (Gujral et al. 2012, Koning, 2012, Bai et al. 2013, AGA Institute, 2016). Endomysial bodies (EMA) have the highest specificity and sensitivity, which largely depend on the age of the patient. These antibodies can be negative in the first few years of life in children, while in adults they are mostly positive with a specificity of up to 100%, and a sensitivity of 88-100%. In the first years of a child's life, endomysial antibodies are less sensitive than gliadin antibodies; hence, a negative EMA finding does not necessarily exclude the existence of celiac disease in individuals with characteristic presentation. This is precisely why the combination of these two tests is pivotal – to exclude false positive and false negative findings, and to establish the diagnosis correctly and swiftly to prevent manifold complications and reduce mortality (Biagi and Corazza, 2010, Rashid and MacDonald, 2009, Rashtak et al. 2008). The gold standard for diagnosing celiac disease with positive serology is a biopsy of the mucosa of the small intestine; more specifically, two to three samples must be taken from the distal part of the duodenum or the proximal part of the jejunum. The patient must be on a gluten-free diet for at least six weeks before the procedure. It is necessary to take 4-6 biopsies from different places for histological analysis, as sometimes the changes can be localized in one place, with affecting the entire mucosa. In patients with celiac disease, the proximal part of the small intestine is affected, and the degree of damage decreases toward the distal end, and if it is a more severe case, these changes can also extend into the ileum (Ludvigsson et al. 2013, Ciccocioppo et al. 2013). In regard to the clinical picture, the most common symptoms in young children are chronic diarrhea, increased abdominal volume, characteristic copious, shiny, smelly and greasy stools (steatorrhoea), vomiting and weight loss. In adults and older children, extraintestinal symptoms are often present, such as sideropenic anemia, epilepsy, arthritis, muscle and joint pain, irritability, nosebleeds, lack of vitamins and minerals, stunted growth, infertility, behavioral changes, chronic fatigue, depression, headaches, etc. (Iwańczak et al. 2013). Celiac disease is treated with a proper and strict gluten-free diet. Sufferers must eliminate from their diet all products containing wheat, barley and rye, and often also oats due to contamination during storage and processing. Following a gluten-free diet (GFD) leads to the recovery of the intestinal mucosa in patients, with the withdrawal of symptoms within two weeks. The symptoms of the disease often disappear much sooner in children than in adults (Tack et al. 2010, Gujral et al. 2012, Richman, 2012).

IV. SOCIAL AND LEGAL ASPECTS OF CELIAC SUFFERERS

Associations of celiac disease patients are well-organized groups that are available to patients in all major cities of the Republic of Croatia. They aid patients by providing them with advice on nutrition, how to maintain their health, about the type and choice of food, how to properly read declarations on products, but also how to prepare various meals. Associations also organize educational workshops. The main goal of the association is to protect and realize the rights of patients diagnosed with celiac disease, which is also realized with continuous education, providing information about their rights, and by collecting data on products that do not contain gluten and when the presence of gluten is not certain (Rashid et al. 2005). People with celiac disease have the same rights to health care as other insured persons. In 2013, the Law on Food for Special Nutritional Needs (Official Gazette No. 39/2013) was passed, in which the conditions that must be met during production all the way up to placing the food on the market are stated. The Law on Food for Special Needs is aligned with the Commission Regulation (Case, 2005). In Croatia, there is an association called the Croatian Society for Celiac Disease (HDC), which is a non-profit, civil organization under the auspices of the Ministry of Health, the Croatian Institute of Public Health, the Croatian Medical Association, etc. (Rashid et al. 2005).

V. GENERAL STUDY DETAILS

The aim of the study was to examine the quality of life of celiac disease patients in northwestern Croatia, as well as to determine the differences in the quality of life with regard to the time period since the diagnosis of celiac disease. The study also aimed to determine the differences in the quality of life of celiac disease patients with regard to the existence of membership in one of the associations celiac disease patients, to determine the differences in the quality of life of celiac disease patients with regard to the availability of gluten-free products in stores, bakeries, restaurants and educational institutions, as well as to determine the differences in the performance of daily and social activities with regard to the frequency of digestive disorders in patients with celiac disease. The research also aimed to appraise the perception of celiac disease sufferers as to how often healthcare professionals consider the emotional effects of living with celiac disease and to evaluate the frequency of providing psychological and professional support by healthcare professionals to celiac disease sufferers. The hypotheses that were put forward were as follows: "There is no statistically significant difference in the quality of life of celiac disease patients with regard to the time period since the diagnosis of celiac disease". "There is a statistically significant difference in the quality of life of celiac disease patients with regard to their membership in one of the associations for celiac disease patients". "There is a statistically significant difference in the performance of daily and social activities with regard to the frequency of digestive disorders in patients with celiac disease". "Health professionals very often take into account the emotional effects of living with celiac disease." "Health workers very often provide psychological and professional support to patients with celiac disease."

VI. METHODS AND PARTICIPANTS

The methodological approach in this study utilized a quantitative approach and included 49 participants from Northwestern Croatia during August 2021. A smaller convenience sample (n = 49) was used due to the relatively small number of patients with the condition.

To obtain data for the purposes of this research, a survey questionnaire was used that was created independently (informed by the literature). Questionnaires were filled in by parents in instances of minors with the disease, while the adults filled the questionnaire for themselves. The questions in the questionnaires were of a closed type, with only one possible answer out of several offered options. Participation in the research was voluntary and anonymous, and participants could withdraw at any time, which was explained to them as a part of the informed consent process. All questionnaires were filled out online with the use of a Google Form. From the resulting Excel file, they were converted into an SPSS file. Based on the SPSS file, statistical analyzes were performed using the IBM SPSS Statistics for Windows software, Version 25.0 (Armonk, NY: IBM Corp), and graphical representations were made using Microsoft Excel 2010 (Microsoft Corporation, USA) and the aforementioned SPSS program.

The statistical analysis methods used in the study were inferential methods (Kolmogorov-Smirnov distribution normality test, chi-square test, t-test of difference of two arithmetic means and one-way analysis of variance to determine statistical significance of differences between three or more arithmetic means). The study also included multivariate methods (reliability analysis).

VII. RESULTS

The sample of respondents consisted of 10 men (20%) and

39 women (80%). Questionnaires were filled out by individuals mostly aged between 19 and 39 years (51% of them). Based on all chi-square tests, no statistically significant relationship has been found between life satisfaction and independent variables ($p > 0.10$): patient's gender, professional education, age, place of residence, duration of the disease and membership in the Celiac Disease Association (Association in further text). There is a statistically significant difference in the quality of life according to the assessments of men compared to the assessments of the quality of life in women ($p = 0.005$). More specifically, men rate their quality of life as well as the life of their minor male children better in comparison to women for themselves and their minor female children ($2.75 > 2.36$).
There were no statistically significant differences in the assessment of the quality of life of patients living in the city compared to patients living in the countryside ($p = 0.338$). Although these estimates were actually higher in the city than in the countryside ($2.50 > 2.39$), these differences are not statistically significant.
There has been no statistically significant difference in the assessment of the quality of life of patients who lived with celiac disease for a shorter period of time (i.e., up to 5 years) compared to patients who lived with the disease for longer (i.e., over 5 years) ($p = 0.924$).
There were no statistically significant differences in the assessment of the quality of life of patients who were members of the Association compared to patients who were not members of the Association of patients ($p = 0.268$). It should be noted that members of the Association rate their quality of life as better ($2.50 > 2.37$). Celiac sufferers from different educational backgrounds evaluate the quality of their life differently. More specifically, patients with Bachelor and Master degrees assess it as the lowest, and patients with elementary school as the highest. These differences determined by our study are statistically significant ($p = 0.023$). Using the LSD post hoc test, it was determined that there are four statistically significant differences:

a) between primary school and Bachelor degree ($p = 0.031$);
b) between primary school and Master degree ($p = 0.038$):
c) between secondary school and Bachelor degree ($p = 0.023$);
d) between secondary school and Master degree ($p = 0.031$).

Celiac patients of various age groups rate their quality of life differently. More specifically, patients aged 30-49 years rate it the lowest, and juvenile patients (under 18 years) the highest, and such differences were found to be statistically significant ($p = 0.072$). However, by using the LSD post-hoc test, it was determined that there is only one such statistically significant difference, and that is between minors and middle-aged individuals, i.e. 30-49 years old ($p = 0.010$). More interesting is the coefficient of -0.44, which shows that there is a negative, moderate and statistically significant relationship between quality of life and vocational education. This means that patients with lower professional qualifications rate (on average) the quality of their life as better, while patients with higher professional qualifications rate (on average) the quality of their life as worse. The correlation coefficient of -0.24 shows that there is a weaker, negative and statistically significant relationship between satisfaction with the quality of life and the level of professional education of study participants.
Using the t-test, it was determined that there were no statistically significant differences in the quality of life of patients that lived with the disease shorter (i.e., up to 5 years), compared to the quality of life of patients that lived with the disease over a longer time span (i.e., over 5 years) ($p = 0.924$), with confirmed hypothesis. Furthermore, by using the t-test, it was determined that there were no statistically significant differences in the quality of life of patients who are members of an Association, compared to the quality of life of patients who were not members of any association ($p = 0.268$). Therefore, it can be concluded that the postulated hypothesis was not accepted. Based on the obtained results, the other hypotheses were also not confirmed.

VIII. DISCUSSION

In patients with celiac disease, the quality of life is reduced in comparison with the healthy population – primarily due to the chronic nature of the disease itself, as well as complications that the disease can instigate on organ systems (Wagner et al. 2008). In patients, the impact of the disease on social and psychological aspects should definitely be considered. In general, adolescents have the hardest time coping with the disease itself, because they are in a rather sensitive period of life (Wagner et al. 2008, Gray and Papanicolas, 2010, Olsson, 2007). The cooperation of patients with celiac disease is better in those patients who initially had a more difficult and pronounced clinical presentation, who are often monitored and have the support of their family and environment. The key factors that underpins the quality of life of individuals are continuous adherence to a gluten-free diet, counseling and proper education, which leads to the controlled disease, reduction in the symptoms, as well as reduced possibility of unwanted complications (Mearin, 2008). According to research conducted by Cinquetti et al. adolescents suffering from celiac disease have difficulties socializing with friends but have no issue within the family; this is in contrast with the research conducted by Sverker et al. where problems both in socializing with friends and within the family have been

identified (Cinquetti et al. 1997, Sverker et al. 2005, Zarkadas, 2006).In this study, we found that children, adolescents, as well as adults sometimes avoid social gatherings and that most of them are rarely able to adequately fulfill their school or work duties, while in the case of a small number of participants, which is also observed more frequently in smaller number of study participants. According to one Canadian study, early diagnosis of celiac disease leads to a higher quality of life, which can be explained by the fact that later diagnosis of celiac disease leads to greater issues with physical health, a surge in costs for health care provision, as well as more frequent problems in schools and socializing with peers [30]. The same results were found in other studies in which there were no differences between the quality of life in patients with an early diagnosis of celiac disease and the healthy population (Sverker et al. 2005, Cinquetti et al. 1997). In our study, the respondents assessed the quality of life equally, regardless of whether the diagnosis of celiac disease was discovered earlier or later.

IX. CONCLUSION

An adequate amount of knowledge and information about the disease and its symptoms, as well as the implementation of procedures for its diagnosis, greatly contribute to its faster detection – with immense public health significance. Available data indicates that, for every recognized patient with the correct diagnosis, there are seven unrecognized patients with symptoms. This means there are still many question marks about celiac disease. The problem with this disease is that, when we are faced with late diagnosis and poor treatment, it can translate to a graver and cumbersome clinical presentation, and the course of the disease is more serious and difficult. On the other hand, an early diagnosis of the disease means much easier treatment, basically only with diet. Therefore, celiac disease should always be considered, which also means much more frequent screening.

Preventing such chronic diseases and diagnosing them adequately gives us the opportunity to relieve the burden on the health system, reduce the costs of diagnostic procedures, and to start treatment as soon as possible – ensuring in turn a better quality of life for the patients with lower impact on their quotidian lives. We should talk as much as possible about celiac disease and the way the disease affects the life of the sufferer and everyone around her/him. More studies akin to this one are needed in order to increase the level of knowledge and awareness – not only of the individuals with the disease but also of the general and at-risk population, which can contribute to raising their overall quality of life.

A major role is played by nurses with the Master degrees, who can provide education for children in kindergarten, school, and even the elderly with the use of various projects and approaches, while providing psychological support to patients and their family members. Emphasis is placed on public health campaigns and workshops for the purposes of raising awareness and educating the population about the importance of recognizing symptoms and treating celiac disease as early as possible.

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Investigating the addition of red beetroot (*Beta vulgaris* L.) in the improvement of nutritional and sensory properties of tomato paste

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Abstract - **Investigating different components extracted from fruits and vegetables can help to know their specific effect on many applications, like natural food additives. This study aims to investigate the role of *Beta vulgaris* L. in nutritional and sensory properties of tomato paste produced on a laboratory scale. With the purpose of exploring the effects of red beetroot application, were compared raw tomato (Tr) and red beetroot (Br), 100% tomato paste (TP100), red beetroot-tomato paste: 30:70 (TBP30), 50:50 (TBP50) and 100% red beetroot paste (BP100), and a commercial tomato paste product (TPc). Samples were evaluated for nutritional profile, colour values (L*, a*, b*), sensory properties, the total content of betalain, polyphenols, flavonoids, and antioxidant activity with two tests (DPPH and ABTS). Results showed that among protein, fat, carbohydrate, and ash content, no big differences existed. Fresh tomato and beetroot colour values L*, a*, b* resulted respectively, 41.46, 27.06, 23.56 and 25.79, 18.07, 7.82. After processing, an increase on L*, a*, b* values were noted in tomato paste mostly in samples TBP30 and TBP50. The betalain content in beetroot was 313.91 mg/100 g, and a loss of 6 % happened after processing in BP100, whereas in TBP30 and TBP50 its content was preserved in the amount of 25% and 87%. In fresh and processed tomato paste the content of polyphenols and flavonoids was 126 mg gallic acid equivalent/100 g and 64.4 mg catechin equivalent/100 g, and antioxidant activity reached values of 43.02 and 91.6 as % of inhibition. The addition of red beetroot contributed to higher content of polyphenols, flavonoids, and antioxidant activity in tomato paste. The sensory properties, respectively colour and appearance resulted higher in TBP30, while aroma and texture resulted higher in BP100, for overall acceptability of the samples had the highest evaluation according to the trend: TBP30> BP100> TBP50> TP100. In conclusion the addition of red beetroot improved the nutritional and sensory properties of tomato paste, also manifested high stability and antioxidant activity, beside the colour enhancement. These findings suggest the application of red beetroot as a potential plant material in the formulations of tomato paste and as a functional additive beneficial to be used by the food industry.**

Keywords - **betalain, beetroot, nutritional profile, tomato paste, antioxidant potential, sensory properties**.

I. INTRODUCTION

Fruits and vegetables contain many nutrients playing a significant part in the human diet (Choudhary et al. 2019; Esparza et al. 2020; Jiménez-Moreno et al. 2020), and they are rich in bioactive compounds. Tomato (*Lycopersicon esculentum*) is one of the most cultivated crops and its production worldwide exceed 100 million tons/year (Al-Harahsheh et al. 2009), consumed fresh or as processed into processed into tomato sauce, soup, paste, puree, juice, ketchup and salsa.

Beetroot (*Beta vulgaris* L.) has a world-wide distribution and its consumption is increasing steadily due to its recognition as an important source of essential compounds and natural antioxidants. Beetroot is utilized for manufacturing different food products and have been investigated by various researchers and food industries, due to prevailing effect of their color, flavor and nutritional aspect (Chhikara et al. 2018). Beetroot contains highly active pigments, betalains with plenty of health benefits, making them easily compatible with food fortification and supplementation (Zin et al. 2020; Jackman and Smith, 1996).

Betalains (E162), red-violet betacyanins and yellow betaxanthins, are used as natural colorants in food industries. They are water soluble nitrogen-containing vacuolar pigments present the in flowers and fruits of the most families of the Caryophyllales clade (Clement and Mabry, 1996; Herbach et al. 2006). With a high solubility in water, a wider range of pH stability (3-7, exhibiting yellow to red coloration), and the absence of toxicity (Herbach et al. 2006; Stintzing and Carle, 2004; Cai et al. 2003, Aberoumand, 2011). Interest in these molecules has grown since they possess anti-radical and antioxidative activities (Kanner et al. 2001; Pedreno and Escribano, 2000; Stintzing and Carle, 2004; Georgiev et al. 2010; Ninfali 2007; Pavlov et al. 2005; Pyo et al. 2004; Escribano et al. 1998). The aim of this study was to investigate the role of *Beta vulgaris* L. in nutritional and sensory properties of tomato paste produced on a laboratory scale.

II. MATERIALS AND METHODS

The raw tomato (*Lycopersicon esculentum*) and red beet (*Beta* vulgaris L.) were obtained from local farmers in 2022, in Tirana, Albania. The samples after collection were labelled and immediately transported to the laboratory to continue further work with analysis and product processing.

Tomato paste was processed in laboratory scale, following the main steps: selected fully ripped tomatoes, washed and allowed to drain, pulped and screened from the skin and seeds, the pulp was concentrated in a stainless-steel pot (in atmospheric pressure), hot filled (82-88^{o}C) into thermally processed air-tight containers, cooled, stored and stored at refrigeration till further analysis. Raw red beetroots, were separated from stems, washed, sliced and grounded in a blender. For red beetroot paste and their formulation with tomato (30% and 50%) preparation, the same scheme was followed. The samples were coded as follows: Tr (tomato, raw), Br (red beetroot, raw), and tomato paste products as: TP100 (100% tomato), TBP30 (70 % tomato and 30% red beetroot), TBP50 (50% tomato and 50% red beetroot), BP100 (100% red beetroot) and TPc (commercial tomato paste). Samples were evaluated for nutritional profile, colour values (L*, a*, b*), and sensory characteristics. Total content of water, proteins, fat, carbohydrates, total sugars ash, total acidity (TA), were determined according to AOAC (2012), and the results were expressed as g/100 g in fresh weight of sample. pH was determined using a Bench-top pH meter Lab 865 (Xylem Inc., Germany). The colour value CIE (L*, a*, b*) was determined by reflectance on two opposite sides of each sample using a portable colorimeter model NH310 (manufactured by Shenzen ThreeNH Technology Co., Ltd., Shenzhen, China). For extract preparation, the procedure followed was according to Schliemann et al. (1999), with modifications. Samples were extracted with MeOH 80% (solid/solvent mass ratio was 1:10), solvent was slightly acidic, helping in stabilization of betacyanins and preventing the possible oxidation. The mixture was homogenized for 5 min in a vortex, treated for 15 min in ultrasound, and centrifugated (4500 rpm for 15 min). This procedure was repeated three times and supernatants were collected and filtered. The betalain content of methanolic extracts was determined spectrophotometrically (Silva et al. 2020), at a wavelength corresponding to the maximum absorption of each of the betalains, at 538 for betacyanins and 480 nm for betaxanthins, measuring the absorbance with the Libra S22 UV/Vis spectrophotometer (manufactured by Biochrom Ltd., Cambridge, UK). The content of betalains was estimated as the sum of both betacyanin and betaxanthin and the result exspressed as mg per 100 grams of sample in fresh weight (f.w.). The total polyphenols content (TPC) was determined using Folin Ciocalteu′s colorimetric method using gallic acid as a standard, according to Singleton and Rossi's (1965) method with modification. The reaction took 60 min at room temperature in the dark, and the absorbance was measured at a wavelength of 765 nm with a Libra S22 spectrophotometer (Biochrom Ltd., Cambridge, UK). Results were expressed as milligrams of gallic acid equivalents (GAE) per 100 grams f.w. of sample. The total flavonoids content was measured by aluminium chloride colorimetric method according to Chang et al. (2002), with modification and using catechin as a standard. The absorbance was measured against reagent blank at 510 nm. The total flavonoid content was expressed as catechin equivalents (CE) mg/100 g f.w. of sample. The antioxidant activity with two tests, DPPH and ABTS the procedure was followed according to the method of Hoxha & Kongoli (2021), with modifications. A 15-member panel was used to evaluate the sensory characteristics of freshly prepared laboratory processed tomato paste. The panel was staff and students of Faculty of Biotechnology and Food, Agricultural University of Tirana, who were familiar with tomato paste quality. The panelists were asked to rate the tomato paste in terms of colour, aroma, texture, appearance and overall acceptability on a 9-point hedonic scale (1 = I do not like it at all, 2 = I do not like very much, 3 = Dislike moderately, 4 = Dislike a little, 5 = Neither like nor dislike, 6 = Like a little, 7 = Like moderately, 8 = Like a lot, and 9 = I really like it), adapted according to Meilgaard et al. (2007). Data for all parameters were reported as means of 15 judgments.

III. RESULTS AND DISCUSSION

The quality parameters for raw tomato (Tr), red beetroot (Br), 100% tomato paste (TP100), red beetroot-tomato paste: 30:70 (TBP30), 50:50 (TBP50), 100% red beetroot paste (BP100), and a commercial tomato paste product (TPc), are introduced in Table 1. Results showed that comparing Tr with TP100 a decrease in moisture content (~20%) occurred as expected due to evaporation, and consequently an increase in the content of protein (2.7-fold), fat (1.6-fold), carbohydrate and sugars (till 3-fold), ash (4-fold), total acidity (1.4-fold). The commercial tomato paste had different parameters except the fat content compared to TP100 respectively the protein was higher (1.5-fold), carbohydrates and sugars were higher (~ 1.7-fold), ash was lower (5%), total acidity was higher (3-fold) and a lower pH (25%). From comparison of raw tomato and raw red beetroot it was noted that Br had lower moisture content (~5%), higher protein content (2.3-fold), lower fat content (~66%), higher carbohydrate and sugars content (~1.4-fold), higher values of ash (~ 4-fold), a very low total acidity and higher pH (1.4-fold).

Table 1. Nutritional profile of raw tomato, raw red beetroot and their processed products (mean values ± SD, expressed g/100 g f.w. of sample)

Sample	moisture	protein	fat	carbohydrates	total sugars	ash	total acidity	pH
Tr	94.6 ± 1.05	0.46 ± 0.07	0.3 ± 0.01	5 ± 0.1	3.9 ± 0.2	0.44 ± 0.01	0.44 ± 0.1	4.5 ± 0.04
TP100	80 ± 0.9	1.28 ± 0.03	0.5 ± 0.05	16.3 ± 0.19	6.11 ± 0.09	1.97 ± 0.02	0.64 ± 0.01	3.9 ± 0.1
TPc	88.2 ± 1.2	0.83 ± 0.03	0.5 ± 0.05	8.9 ± 0.16	3.5 ± 0.15	2.07 ± 0.03	0.19 ± 0.002	5.2 ± 0.01
Br	90 ± 0.7	1.09 ± 0.02	0.1 ± 0.01	7.13 ± 0.12	6.1 ± 0.3	1.74 ± 0.2	0.05 ± 0.001	6.4 ± 0.02
BP100	78.2 ± 0.6	1.36 ± 0.02	0.1 ± 0.01	19.1 ± 0.42	8.14 ± 0.28	2.03 ± 0.03	0.06 ± 0.001	6.2 ± 0.04
TBP50	78.4 ± 0.9	1.31 ± 0.02	0.15 ± 0.04	18.9 ± 0.2	7.9 ± 0.2	1.99 ± 0.03	0.19 ± 0.01	6.1 ± 0.05
TBP30	79.4 ± 0.75	1.29 ± 0.01	0.39 ± 0.03	17.8 ± 0.1	6.8 ± 0.1	1.98 ± 0.03	0.32 ± 0.2	4.8 ± 0.02

When comparing Br with BP100, as in the case of raw tomato and its paste, due to processing a decrease in moisture content (~15%) happened and an increase in some components happened, such as protein (1.25-fold), carbohydrates and sugars (2.6 and 1.3-fold), ash (1.16-fold), while no differences were noted for total fat, total acidity and pH. Products TP100 and BP100 had slight differences among parameters, and was noted that addition of red beetroot (30% and 50%) caused slight increase in the content values of parameters, except in the total acidity, which resulted in a lower value, probably due to the contribution of red beetroot, which is characterised by a low acidity (0.05 g/ 100 g f.w.).

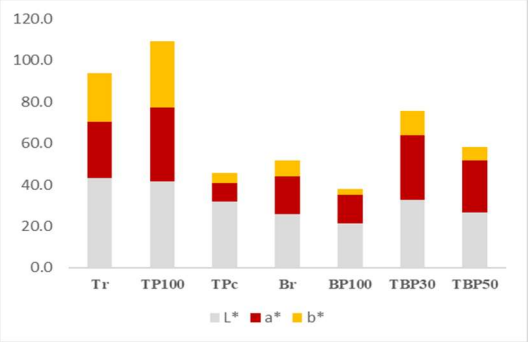

Figure 1. Colour values (L*, a*, b*) of raw tomato and red beetroot and their processed products

The total colour (Figure 1.) of the samples was expressed with CIE parameters L*, a*, b*, which resulted in positive values, respectively 41.46, 27.06, 23.56 (in raw tomato) and 25.79, 18.07, 7.82 (in raw red beetroot). After processing, an increase in L*, a*, b* values were noted in tomato paste, products. The brightness represented with L* values, ranged from 21.3 (BP100) to 43.26 (TP100); a* value, the red colour was higher in laboratory processed tomato paste 35.87 (TP100) and lower in commercial product 8.65 (TPc); b* value, yellow colour was higher in tomato paste 31.82 (TP100) and lower in red beetroot paste 3.04 (BP100), which might indicate the presence of lycopene in Tr, TP100 and TBP30. The differences among laboratory made tomato paste and the commercial products could be due to the influence of the production method, and generally, our results were in good agreement with those reported by Kelebek et al. (2017), Sahlin et al. (2004) and Apuhan (2012).

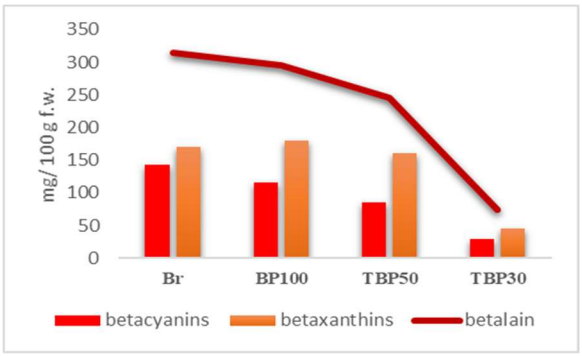

Figure 2. Betalain content in raw tomato and red beetroot and their processed products

The betalain content (Figure 2.), which was calculated as the sum of betaxanthins and betacyanins reached up to 314 mg/100g f.w., and was not detected in Tr, TP100 and TPc samples. The addition of red beetroot in the tomato paste formulations contributed in the red colour enhancement (Figure 1.). Colour is an important quality attribute of food, and today's consumers have a distinct shift towards natural colours (Altinoz & Toptan, 2003), so betalain from red beetroot would be suggested to be utilized as a natural

colorant in the tomato paste. Furthermore, from the results was noted that beside the quantity of red beetroot added in formulations, the pH and temperature could be the factors influencing the stability of betalain during tomato paste processing. Related to the pH influence, in the case of tomato paste which resulted an acidic product (pH=3.9), had a positive effect on betalains stability in the formulations TBP30 and TBP50, as its content was preserved 25-87%.

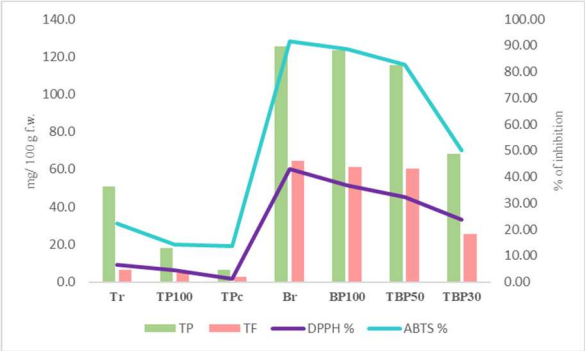

Figure 3. The total polyphenols, flavonoid and total antioxidant activity in raw tomato and red beetroot and their processed products

The application of betalain in food products with a pH below 3,shift towards violet colour and above pH 7, towards blue colour (Wootton-beard et al. 2011). The thermal treatment affected the betalains stability during paste processing, and was noted a 6 % degradation of betalains (in BP100 sample). The raw red beetroot had the content of polyphenols 126 mg GAE/100 g, flavonoids 64.4 mg CE/100 g, and antioxidant activity 43.02% to 91.64 %, and these results we found that agrees with the study of Fidelis et al. (2017). By comparison of raw red beetroot (Br) with raw tomato (Tr) was noted to have higher content of polyphenols (2.47-fold), flavonoids (9.74-fold), and antioxidant activity (6.7-fold with DPPH test and 4.13-fold with ABTS test).

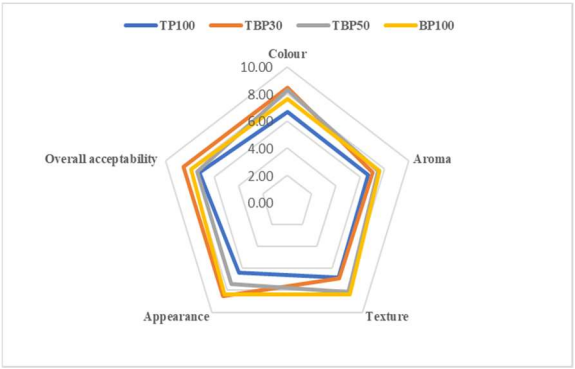

Figure 4. Sensory properties of tomato and red beetroot processed products

In the Figure 4., are presented the sensory properties of proccessed products, respectively colour (6.67-8.47), aroma (6.67-7.60), texture (6.87-8.40), appearence (6.40-8.53) and overall acceptabillity (7.20-8.53). The colour and appearence of tomato paste treated with 30% red beetroot resulted with higher value (8.47 and 8.53), while aroma and texture resulted higher at BP100 (7.60 and 8.40), compared to TP100 and TBP50. In terms of overall acceptability the samples had the highest evaluation according to the trend: TBP30> BP100> TBP50> TP100.

The different amount red beetroot added in the formulations of tomato paste caused differences in the antioxidant activity, which may be attributed to the betalains beside the total polyphenols and flavonoids content. This positive correlation observed confirm the beneficial use of betalains as natural antioxidants. Moreover, by comparison of TP100 with TBP30 and TBP50, would be noted that the addition of red beetroot contributed in higher content of polyphenols (3.7 to 6.4-fold); flavonoids (5.3 to 12.5-fold); and consequently, the antioxidant activity resulted higher (5.25 to 7.14-fold according to DPPH test, and 3.52 to 5.81-fold according to ABTS test). Thermal processing influenced the degradation of polyphenols in 64% and 2 %; of flavonoids 27% and 5%; and antioxidant activity 29% and 14% (DPPH test), 36% and 3% (ABTS test), from comparison of Tr with TP100, and Br with BP100. It was noted that red beetroot is more resistant than tomato, based on the degradation values, and our findings agree with Ceclu and Oana-Viorela (2020) which reported that processed red beetroot manifested high stability and antioxidant activity.

IV. CONCLUSIONS

This study investigated the role of addition of *Beta vulgaris* L. in the quality of tomato paste, produced on a laboratory scale. The results showed clearly that the addition of beetroot in different formulations can improve the overall quality of tomato paste, by causing an increase in the content of nutritional parameters, and influenced in lowering of total acidity. The total colour expressed with CIE parameters L*, a*, b*, resulted in positive values, and increased more after processing into tomato paste. Generally, the laboratory processed tomato paste was brighter, reddish and with the presence of yellow colour. The differences among laboratory processed tomato paste and the commercial product could be due to the influence of the production method, and generally, our results were in good agreement with those reported by literature. Moreover, the addition of red beetroot in the tomato paste formulations had a positive effect on colour enhancement, due to the presence of betalains.Based on this finding, betalain would be suggested to be utilized as a natural colorant in the tomato paste, although more detailed

investigations are needed to evaluate the potential and functions as a natural colourant. Beside the quantity of red beetroot added in formulations, was noted that pH and temperature influence the stability of betalain during tomato paste processing. The laboratory processed tomato paste with a pH=3.9, had a positive effect on betalains stability in the formulations TBP30 and TBP50, by preserving its content >25%. Also, the thermal treatment affected the betalains stability during paste processing, as a degradation ~ 6 % was noted in betalains. Regarding to the total polyphenols, flavonoids and antioxidant activity, raw red beetroot had higher content compared to tomato, and consequently their respective products, while commercial product showed the lowest values among all samples. The addition of red beetroot contributed in higher content of polyphenols up to 6.4-fold, flavonoids up to 12.5-fold, and antioxidant activity up to 7.14-fold according to DPPH test, and up to 5.81-fold according to ABTS test. The different amount red beetroot added in the formulations of tomato paste caused differences in the antioxidant activity, which may be attributed to the betalains. Even though thermal treatment resulted in losses of polyphenols, flavonoids and antioxidant activity, red beetroot is more resistant in their retention compared to tomato. The addition of red beetroot in tomato paste had positively influenced the sensory properties based on scores, respectively for colour up to 8.47 (in TBP30), aroma up to 7.60 (in BP100), texture up to 8.40 (in BP100), appearence up to 8.53 (in TBP30), and regarding the overall acceptabillity the samples had the highest evaluation according to the trend: TBP30> BP100> TBP50> TP100.
The addition of red beetroot improved the nutritional and sensory properties of tomato paste, also manifested high stability and antioxidant activity, beside the colour enhancement. These findings would suggest application of red beetroot as a potential plant material in the formulations of tomato paste and as a functional additive beneficial to be used by the food industry. Further investigation should be done for finding alternative ways in utilization of tomato and red beetroot by-products into value-added food products.

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Influence of vegan product factors on purchase

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Abstract- **Proper nutrition is one of the key prerequisites for health. In order to be able to plan the diet in such a way as to provide the body with all the necessary nutrients, it is important to understand the guidelines of proper nutrition and the function, sources and quantity of the necessary nutrients. As human, we are determined by internal and external factors. External factors are our living environment, the society we belong to, culture, family and other social groups with whom we share the same interests. While internal factors are precisely the motives that move us to action, a complicated set of emotions and personality traits. All of the above defines a lifestyle. Humans by definition are not scales, animals have always been an integral part of the human diet. However, it is precisely under the influence of internal and external factors that people consciously decide on the lifestyle they will follow. Ethical motives, such as concern for the welfare of animals, are the strongest motivators for switching to veganism. The production of vegan products is one of the biggest and fastest growing trends in the food industry in recent years. Considering the lifestyle, the impact of veganism on health, the environment and current trends in the food industry, this work tries to determine which product factors and internal factors such as lifestyle and motivation are important, in terms of their influence on choosing a vegan product. In order to confirm the above, research will be conducted on a deliberate sample of 205 subjects who continuously use vegan products.**

Keywords – ***lifestyle, vegan diet, choice motives, product factors, purchase***

I. INTRODUCTION

There is a well-known saying "You are what you eat." And it is true, the body gets the necessary energy from food for growth and development, for maintaining all metabolic processes in the body. In order to keep our body healthy, it is necessary to supply it with high-value foods that contain all the necessary nutrients, respecting the recommendations on daily energy needs. Healthy foods, a balanced diet, exercise and a positive state of mind are the recipe for a long and healthy life. In order to be able to plan a proper diet, it is first necessary to understand what these nutrients are and from which sources they come, and which nutrients and in what proportions are needed so that the ingested food really provides the body with the necessary fuel. Vegetarianism goes back a long way in history, mainly a small part of the population follows a vegetarian diet. Currently, it is estimated that less than 10% of the human population follows some form of vegetarian diet, but the number is increasing at a rate of 10% per year.

Well-planned and balanced food from plant sources provides the body with all the necessary nutrients. It is considered that the healthiest diet is one with an increased intake of vegetables, whole grains and fruits and a reduced intake of red meat and meat products. Many scientific studies have proven a positive correlation between certain diseases such as type 2 diabetes, cardiovascular disease, high blood pressure, various tumours and excessive consumption of red meat and meat products. Food from plant sources does not contain much fat, has a lower energy value and has a positive effect on reducing body weight, so vegetarians are rarely obese. The modern way of life is conditioned by demographic, economic and social changes. Some of the characteristics of the society are the aging of the population, the increase in the number of small families (1-2 members) and the increasing number of working women. In the desire to better organize and use free time, there have been changes in the traditional way of shopping, preparing food and consuming meals. Since consumers are increasingly aware of the importance of nutrition on health and are informed about the possible causal connection of certain food substances and methods of food preparation with the possible risk of the onset and development of some chronic diseases, dietary rules are becoming increasingly important.

Kotler defines a person's lifestyle as his pattern of living that is expressed through his activities, interests and opinions. It encompasses something more than a mere social stratum or an individual's personality. It portrays the entire pattern of behaviour and interaction of that person with the environment (Koetler:2006:265). Vegetarians as a social group are particularly focused on ethical values, environmental values and health values. Ethical values are one of the most pronounced, and primarily relate to the care of the protection and well-being of animals. Environmental values have become an important social value in developed countries where vegetarians, and especially vegans as a subgroup, are extremely focused on reducing the negative impact of people on the environment. A healthy life, taking

care of nutrition and movement are values that are also important to this social group. The basic motive for switching to a vegetarian diet is precisely the care of animals and the rejection of animal exploitation for human benefit. In the research carried out by the authors of this paper, the majority of respondents (83%) stated that the main motive for switching to vegetarianism was precisely that they did not want to eat animals. The ethical values of vegans as a social subgroup are much stricter than vegetarians. Their lifestyle is determined not only by a vegan diet, but also by rejecting any exploitation of animals for human needs. In developed countries, the health of vegetarians is better compared to the national average and comparable to the health of omnivores with similar origins and lifestyles (Key:2006:39).

Consuming larger amounts of healthy plant-based foods reduces the risk of coronary heart disease. According to the research of A. Satija and associates, conducted in 2017, healthy food of plant origin such as whole grains, fruits, vegetables, nuts, vetch, vegetable oils, tea and coffee reduces the risk of coronary heart disease (Satija et al: 2017: 2). In addition to specific dietary habits, vegetarians tend to adopt other characteristic patterns of behaviour and adopt a health-oriented lifestyle: avoiding smoking, avoiding or moderate alcohol consumption, physical activity, sufficient amount of rest, generally taking care of health and seeking medical help when health problems occur. This kind of behaviour is a possible reason for the reduced mortality in this social group (Dwyer:1988:713).

Vegetarians occasionally have unusual eating habits or other psychological and behavioural disorders that increase the risk of disease. Vegans or vegetarians sometimes self-prescribe therapy with large doses of minerals and vitamins. Such behaviour increases the risk of overdosing the body. Young women who follow a vegan or vegetarian diet sometimes suffer from anorexia or menstrual disorders (Dwyer:1988:714).

II. Materials and methods

The food pyramid emphasizes the importance of consuming a variety of foods from the five main food groups. She also limits the amount of fat, oil and sweets in her diet. Variety, moderation and balance are the most important guidelines for a proper diet. By eating the same food and eating the same foods, the body always gets the same minerals and vitamins. That is why it is important to consume a variety of foods because they provide the body with a wide range of nutrients. There should be foods from different groups on the plate, at least from three groups.

One of the leading motives for switching to a vegetarian diet is the proven positive effect on human health. More and more researches are connecting the frequent consumption of meat, primarily red meat and meat products, with the occurrence of various diseases such as cancer, diseases of the heart and blood vessels, elevated levels of fat in the blood. Compared to omnivores, vegetarians and vegans with a plant-based diet consume large amounts of dietary fiber, antioxidants, and phytochemicals.

Research conducted on a sample of 96,000 members of the Adventist Church in America and Canada, in the period from 2002 to 2007, showed a lower incidence of certain diseases in the vegan population compared to other groups. The incidence of hypertension, some types of tumors, and the general mortality in the vegan population have decreased (Olrich, Fraser: 2014:355S). This type of diet shows a reduced risk of disease from diseases of the heart and blood vessels, colon cancer and an increase in life expectancy (Fraser:2009:1608S).

Positive effects in the prevention of cardiovascular diseases are attributed to the consumption of large amounts of dietary fiber, nuts, soy, plant sterols, flavonoids and other phytochemicals. A lower concentration of total serum cholesterol and a lower concentration of LDL cholesterol among vegetarians is attributed to the intake of a significant amount of dietary fiber, which are also important factors in the prevention of cardiovascular diseases (Krešić:2012:319). People who do not consume meat or consume it to a lesser extent suffer less from some types of cancer. One of the important factors in explaining the lower incidence of cancer among vegetarians is their lower body mass index, since obesity has been proven to increase the incidence of various types of cancer. A diet restricted to meat reduces the incidence of cancer.

Within the vegetarian diet, the protective effect of its components: vitamin C, carotenoids, flavonoids, dietary fibres and other phytochemicals on various cancers has been proven (Krešić:2012:320). The protective effect of a vegetarian diet on the occurrence of osteoporosis is explained by the reduced intake of proteins of animal origin and the frequency of fruit and vegetable consumption. The lower intake of proteins of animal origin compared to omnivores contributes to the reduced excretion of calcium from the bones, since the excretion of calcium is stimulated by the high intake of sulphur amino acids, which are abundant in proteins of animal origin. The high source of potassium and magnesium in fruits and vegetables, in combination with their alkaline ash, has a positive effect on bone remodelling and thus reduces the risk of osteoporosis (Krešić:2012:320). People who follow a vegan diet consume significantly more carbohydrates than omnivores (59% of vegans compared to 45% of omnivores) and significantly more dietary fiber, which leads to a decrease in the stool pH factor in the range of 5.5 to 6.5. These conditions are not favourable for the

development of E. Coli and Enterobacteriaceae bacteria, which prefer a pH higher than 6.5, which is the pH of the stool of omnivores. In addition, the mentioned bacteria prefer to use proteins as a source of energy (Zimmer et al: 2012:58).

Vegans get half as much calcium in their diet compared to other types of diet, 738 mg, compared to 1465 mg for vegetarians, 1470 mg for semi-vegetarians and semi-vegetarians, 1199 mg for omnivores. (Clarys et al: 2014:1324).

In the accelerated rhythm of life, the problems of health and healthy lifestyle are gaining more and more importance. It is necessary to accept the fact that one of the prerequisites for creating a healthier lifestyle is learning and understanding the habits of preserving and developing health and the impact of eating habits on life. A good selection of food products is important for every single person, for their self-satisfaction and for the experience that their own life is filled with meaning and healthy eating. Lifestyle can be defined as a way of living, which means how people spend their time (activities), what they consider important in their environment (interests), what they think about themselves and the world around them (opinions). Lifestyle is influenced by culture, values, subcultures, demography, social class, reference groups, family and individual variables – motives, emotions, personality traits, etc. (Kesić:2006:204).

Only vegan products were included in the research. Since it is clear from the results of previous research that vegan products are chosen on the basis of consumer preferences and indicators of quality of life, a research question was asked: How much are consumers of vegan products aware of the set selection criteria at the time of selection, and how much of the chosen vegan product is an indicator of the standard of living. Empirical research was conducted using the survey method. A highly structured survey questionnaire was used as a measuring instrument. At the beginning of the questionnaire, an elimination question was asked about the use of vegan products. If the questions were answered in the affirmative, the respondent continued to fill out the survey questionnaire to the question about the use of vegan products. If the respondent's answer was affirmative, the respondents could continue with the rest of the research. The questionnaire contained twelve statements that measured the perception of the role and importance of vegan products as a way of eating. Furthermore, the respondents defined their motivations for choosing vegan products shown in table 1 with five statements. The respondents expressed their degree of agreement with the stated statements using a five-point Likert scale, where 1 meant "I do not agree at all" and 5 "in I completely agree". All statements used in the research are listed in table 2, in the paper. Finally, the questionnaire included five questions related to the respondents' demographic characteristics.

III. Results

After the presented theoretical part about nutrients, nutritional needs and the lifestyle and behaviour of consumers from the social group of vegetarians, the sample method and type of research were defined. What is a vegan way of eating, that is, a vegan lifestyle characterized by almost complete avoidance of the use of food and other products of animal origin, including meat, milk, honey, fur, skin. In addition to not consuming meat and dairy products, vegans do not support any kind of animal exploitation, including animal testing and avoid buying and using clothes, shoes and cosmetics that contain animal products, such as leather and fur. Although they share some characteristics, veganism and vegetarianism differ in the consumption of dairy products used in many subtypes of vegetarianism, and in the consumption and purchase of products tested on animals. The research was conducted on a purposive sample of 205 respondents. The goal of this paper is to gain insight into the eating habits and consumer behaviour of vegans. The objectives of the work are defined in accordance with the above:

- determine the differences in consumer behaviour and expectations about the appearance of food of consumers who belong to different categories of vegetarians;
- people become vegan more because of the lifestyle and beliefs regarding ecology, sustainable food cultivation, animal care, than because of the impact on health;
- determine the motives of vegetarian consumers, what motivates them to buy vegetarian and not meat products;
- determine that the habits and expectations regarding food that consumers acquired in childhood or earlier in life, before becoming vegetarians, influence their expectations regarding the appearance and taste of food.

With regard to the set goals of the work, the following hypotheses were defined:
Hypothesis H1: People become vegan more because of the lifestyle and their own beliefs than because of the positive impact on health of a diet with less or no meat.
Hypothesis H2: Due to eating habits acquired in childhood and learned expectations regarding the appearance of food, vegetarians buy products that look similar to standard traditional meat products.
The survey was conducted on 205 respondents. Most respondents are women, 92.57% of them, while only 15 men participated in the research. The most represented age group is in the age group of 26 to 35 years, 34.48% of them. Most of the respondents have university education, 38.42% of them. 38.42% of respondents live in a household of two

people. More than half of the respondents, 55.67% of them, spend between HRK 1,001 and HRK 3,000 a month on food. Most respondents are employed, 74.02% of them. Conventional consumers who consume products of animal origin usually change their lifestyles and styles in adulthood and become vegetarians. The conducted research determined that 68.66% of respondents became vegetarians in adulthood, after the age of eighteen. To the question "When did you become a vegetarian?", the respondents stated that they mostly became vegetarians in adulthood, after the age of 18.

Table no. 1: Results of independent simple t-tests of differences on the components of the attitude scale about choosing a vegan product with different purchase intentions

Dependent variable	Group	M	SD	n	t	df	p	d
Component 1a – A vegan product must not resemble a standard traditional product of animal origin in taste and texture	Always	0,22	1,06	85	2,498	157,1	,014	,359
	Sometimes	-0,13	0,88	117				
Component 2a – I buy vegan food recommended by influencers and other celebrities	Always	0,17	0,88	85	2,056	202,1	,041	,286
	Sometimes	-0,11	1,07	117				
Component 3a – A vegan product should not look like a standard traditional product of animal origin	Always	0,28	0,92	85	3,688	191,5	<,001	,507
	Sometimes	-0,21	1,01	117				
Component 4a – I buy vegan food that resembles the products my mother used to buy and prepare	Always	-0,04	1,00	85	-0,290	176,0	,772	,041
	Sometimes	0,00	0,97	117				
Component 5a – I buy trendy vegan food	Always	0,01	0,97	85	0,280	189,6	,780	,030
	Sometimes	-0,02	1,05	117				

Note: The group refers to the answers to the question "How likely are you to buy plant-based food products in the future?" "Always" refers to participants who answered "I am sure I always will" and "Sometimes" to "I am sure I will sometimes. "

IV. DISCUSSION

The H1 hypothesis claims that people become vegan more because of lifestyle and personal beliefs than because of the positive health effects of a diet with less or no meat. In order to test the H1 hypothesis, the respondents were asked: "Why did you become a vegetarian?". This question was also intended to determine the motives for switching from a conventional diet to a vegetarian diet, and to determine the motives for changing lifestyle. 83.08% of respondents answered that the reason for switching to a vegetarian diet was that they no longer wanted to eat animals. For the majority of respondents, animal welfare is the main motive for switching to a vegetarian diet. In addition to the aforementioned 83.08% of respondents who declared that fact that no animals were killed for its production, it was rated with the highest average rating of 4.72. Variables that are indicators of the importance of motives related to the impact on the environment and ease of application received average ratings of 4.03 and 3.56, respectively. With an average score they do not want to eat animals, an additional 35.82% of respondents want to reduce the consumption of meat and other animal products, 23.88% of respondents declared that their beliefs do not allow them to eat and wear products that come from animals. 25.87% of respondents become vegetarians for health reasons, and 37.81% of respondents stated that environmental protection was the main reason for switching to a vegetarian diet. In order to confirm the H1 hypothesis, the respondents were asked to rate the motivations for buying vegan salami. Of the motives offered in the questionnaire, those that most reflect lifestyle and own beliefs are: the fact that no animals were killed for the purpose of production, positive impact on the environment, ease of consumption, personal preferences regarding taste. The most important motive for buying vegan salami is the of 3, respondents rated the importance of the fact that vegan salami does not contain cholesterol, which is an indicator of a positive impact on health. And with an average score of 3.5, vegan salami is healthier than meat salami. In order to test the hypothesis and define the profile of consumers of vegan

products, the responses of respondents from the survey questionnaire were processed using the SPSS program, which presented the results of descriptive statistics. The exact results of independent t-tests on the components of the attitude scale about choosing a vegan product with different purchase intentions are shown in table number 2.

The respondents were divided into two groups, those who prefer vegan products more (n 85) and those who occasionally prefer vegan products (n 117). The range of obtained results is wide, from a minimum of -0.290 to a maximum of 3.688, with a significance level of 95%. The lowest t-test score of -0.290 is for component 4a – I buy vegan food that resembles the products my mother used to buy and prepare, and for component 5a – I buy vegan food that is trendy, the t-test is 0.280. Variable 3a – a vegan product should not look like a standard traditional product of animal origin, the t-test result is 3.688, and variable 1a – a vegan product should not resemble a standard traditional product of animal origin in taste and texture, the t-test is 2.498, have the highest results. These results show that the vegan consumer profile is closely related to the texture and raw materials of the product, but this consumer profile does not buy trendy food and they do not associate the choice of vegan food with the feeling of childhood and home, and these components are not at all important to vegans. Vegan consumers do not expect vegan food to resemble traditional meat products in taste and appearance. This refutes hypothesis H2.

In order to determine in more detail the influence on consumer purchasing decisions, respondents were asked in the questionnaire to assess the level of influence on choosing to buy vegan food. The aim of this question is to test the H2 hypothesis, which states that "Due to eating habits acquired in childhood and learned expectations regarding the appearance of food, vegetarians buy products that look similar to standard traditional meat products". The variable that the respondents evaluated with the highest average score (2.36) and which has the greatest influence on purchasing decisions is: "I buy vegan food that in form, appearance and taste reminds me of the products I consumed in my childhood". The variable "I buy vegan food that reminds me of the products my mother bought and prepared" was given one of the highest marks, with an average mark of 2.05.

In order to create a detailed profile of vegan consumers, the respondents were divided into clusters according to their preferences and age. In cluster 1 are respondents up to 30 years of age, in cluster 2 are respondents with more than 30 years of age. The results of independent sample t-tests of differences between clusters are shown in table number 3.

Table no. 2: Results of independent sample t-tests of differences between Clusters

Dependent variable	*Cluster*	*M*	*SD*	*n*	*t*	*df*	*p*	*d*
Age	*Cluster 1*	*2,46*	*1,06*	*62*	*2,250*	*129,9*	*,026*	*0,326*
	Cluster 2	*2,16*	*0,75*	*140*				
I became a vegetarian as a teenager, until the age of 18 / I became a vegetarian in adulthood after 18. years	*Cluster 1*	*2,55*	*1,07*	*62*	*-3,528*	*158,3*	*<,001*	*0,496*
	Cluster 2	*3,06*	*0,98*	*140*				
Have you tightened your criteria and become a vegan during your life?	*Cluster 1*	*4,08*	*0,98*	*65*	*3,243*	*165,7*	*<,001*	*0,458*
	Cluster 2	*3,61*	*1,04*	*137*				
Do you pay attention to the manufacturer of the food you buy or is the composition of the product more important to you?	*Cluster 1*	*4,08*	*0,98*	*65*	*2,723*	*167,1*	*,007*	*0,384*
	Cluster 2	*3,69*	*1,06*	*137*				

Would you buy a product that is vegan and looks like a standard traditional meat product?	*Cluster 1*	*2,78*	*1,28*	*65*	*3,128*	*134,5*	*,002*	*0,457*
	Cluster 2	*2,24*	*1,06*	*137*				
Component 1a – When making a vegan product, high production standards were respected	*Cluster 1*	*0,55*	*0,55*	*62*	*8,032*	*216,9*	*<,001*	*1,028*
	Cluster 2	*-0,32*	*1,06*	*140*				
Component 2a – I prefer the vegan product because it is fresher	*Cluster 1*	*0,46*	*0,90*	*62*	*5,748*	*177,9*	*<,001*	*0,793*
	Cluster 2	*-0,27*	*0,96*	*140*				
Component 3a – I buy vegan food that resembles the products my mother used to buy and prepare"	*Cluster 1*	*0,28*	*0,88*	*62*	*3,441*	*190,9*	*,001*	*0,469*
	Cluster 2	*-0,17*	*1,03*	*140*				
Component 4a – I buy vegan food that my friends eat	*Cluster 1*	*-0,04*	*1,09*	*62*	*-0,434*	*152,1*	*,665*	*0,061*
	Cluster 2	*0,02*	*0,95*	*140*				
Component 5a – The composition of the product can be characterized as quality food from which it is made	*Cluster 1*	*0,59*	*0,64*	*62*	*8,350*	*218,8*	*<,001*	*1,094*
	Cluster 2	*-0,34*	*1,02*	*140*				
Component 1b - The production of vegan salami pollutes the environment less	*Cluster 1*	*0,17*	*0,97*	*80*	*1,890*	*170,9*	*,060*	*0,265*
	Cluster 2	*-0,10*	*1,01*	*122*				
Component 2b – No animals were killed for the production of vegan salami	*Cluster 1*	*0,17*	*0,92*	*80*	*1,947*	*181,4*	*,053*	*0,270*
	Cluster 2	*-0,10*	*1,03*	*122*				
Component 2c - Vegan straw has no cholesterol	*Cluster 1*	*0,11*	*1,06*	*80*	*1,206*	*152,9*	*,230*	*0,172*
	Cluster 2	*-0,06*	*0,96*	*122*				

Note: M – mean, SD – standard deviation, n – subgroup size, t – Welch's t-test value, df – degrees of freedom, p –statistical significance, d – Cohen's d effect size.

The obtained results show the profile of consumers of vegan products. People become vegetarians in adulthood. Cluster 2 (people over 30 years old) declared that they became a vegetarian in adulthood, average score 3.06. Through the previous graphs, it was shown that 69% of all respondents became vegetarians in adulthood, and that over

the years they tightened their criteria and became vegans. The range of obtained results of the simple t-test is extremely large, from a minimum of -3.528 to a maximum of 8.350, with a significance level of 98%. The component that is most important to consumers and has the highest t-test result (t-test 8.350) is component 5a – the composition of the product can be characterized as high-quality ingredients from which it is made, and component 1a – high production standards were respected when making the vegan product (t-test 8.032). The age when a person became a vegetarian has no influence on purchasing decisions, the result of the t-test is the lowest here, it is -3.528. Also, one of the unimportant components is number 4a – I buy vegan food that my friends eat, the t-test is -0.434. The obtained results show the profile of vegan consumers for whom the quality of food, high standards in food production and freshness of food are the most important criteria when choosing a vegan product. For this group of consumers, the age and eating habits of friends do not define their own purchasing decisions.

Various scientific studies confirm the justification of ecological motives, because the production of food for the needs of the human population is one of the key polluters, namely a quarter of greenhouse gas emissions are related to the cultivation and production of food. We use half of the world's habitable land for agricultural purposes. Of that, we use 77% of agricultural land to raise livestock for human consumption, which provides us with only 18% of the total caloric value and 37% of protein. In contrast, only 23% of the world's total agricultural area, on which we grow crops exclusively for human consumption, gives us as much as 82% of the total caloric value and 63% of protein. The human population currently stands at 7.8 billion people and is growing by approximately 81 million every year. Predictions are that we will reach 10 billion by 2057 (WorldOMeters,:2020). Due to the increase in the population, the need for food also increases, which puts additional pressure on our Planet and poses a great challenge of how to feed the growing human population and at the same time reduce the negative impact on the environment.

Veganism has been the most significant trend in the food industry for several years. It became especially popular with the advent of plant-based burgers marketed by the companies. Beyond Meat and Impossible Foods. The products of these companies have become a benchmark for producers who are trying to penetrate the growing market of "plant-based" products. The global market for plant-based meat substitutes is 4,282,000 tons, with a market value of 16.29 billion euros. Although it is only 0.22% of the total revenues of the food industry at the world level, it is estimated that by 2024 it will achieve a volume growth of 17%, and according to estimates by Polaris research, by 2027 it will achieve an increase in market value by more than 50%. The demand for meat-free and plant-based foods is growing year by year. In the United Kingdom alone, it grew by 987% in 2017. Such positive market trends and significant growth in demand are an invitation to manufacturers to invest in the development and production of vegan products. The growing offer of plant-based products, new technologies and new recipes allow consumers to choose much more, so vegan products are now being chosen by consumers who do not follow a vegan diet. Today, companies are increasingly addressing flexitarians, people who are trying to live healthier and eat healthier, replacing part of animal foods with plant foods.

Consumers acquire their eating habits in childhood, but over time they adjust them according to their own attitudes and lifestyle. Food manufacturers currently offer them a large selection of products that they can choose from, while trying to satisfy the simplicity of product application and to offer products in familiar forms that will be recognized by other types of consumers.

V. Conclusion

In the narrowest sense of the word, food is fuel for our body. The fuel through which we introduce all the necessary nutrients into the body - macronutrients (proteins, carbohydrates and fats) that we can taste and make us full, and micronutrients (vitamins and minerals) that enable the body to work smoothly. For a long, healthy life and the absence of disease, quality and balanced nutrition, physical exercise, sufficient water intake and a positive state of mind are necessary. When we are hungry, the body signals to us that it needs nutrients, but does not define exactly which ones. This decision is made by each person based on various internal and external factors.

Lifestyle, i.e. personal choices about the way of living, about what is important to us, the activities we spend our time on and what we think about the world around us, are influenced by external factors that define each person, namely his environment, culture, society, family, but also internal factors such as motives that drive us, our emotions and personality traits. Vegetarians, and especially vegans, as a social subgroup much stricter in their attitudes, are particularly focused on ethical values, environmental values, and health values. Concern for the well-being of animals is the first on the list of motives that define the purchase and

life decisions of vegans, because they refuse to exploit animals for their own needs in any way. When looking at the definition of the trend and taking into account the growth of the vegan products market, it can be said that vegan products are the current trend in the food industry. However, when it is taken into account that a vegan diet, when it is optimally balanced and well planned, has positive health effects on the human body and that growing food from plant sources pollutes the environment significantly less, it can be said that veganism is a necessity. Many agencies, scientific research, governments and international institutions such as the United Nations and the World Health Organization recommend reducing the consumption of meat and dairy products to help fight climate change and various diseases.

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Knowing the Albanian "hurma" (*Diospyros kaki Thunb.*) fruit and its drying behaviour based on quality characteristics and bioactive compounds

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Abstract - **The study aims to investigate main physico-chemical parameters, polyphenols and antioxidant potential of Albanian hurma (*Diospyros kaki Thunb.*) in fresh and dried state. The study was conducted during 2020-2021, and samples were collected randomly by trees over 40 years, in full maturity stage, from Tirana, Durres, and Elbasan regions in the central part of Albania, which are recognized traditionally for hurma fruits growing. Drying regimes used in a laboratory scale hot-air dehydrator: temperature 60±0.1°C and air velocity 1±0.1 m/s, relative humidity approximately 40% (at the beginning) and 10% (at the end). The independent variables specified in the experimental design were region of cultivation, and the physico-chemical parameters determined for fresh and dried fruits were: dry matter, total soluble solids (TSS), total acidity, pH, total ash, vitamin C, colour values (L*, a*, b*), water activity (a_w). As well were determined total polyphenols, flavonoids, and antioxidant potential. Drying hurma resulted in a change of all parameters, resulting in an increase of dry matter over 3-fold, total acidity 0.154-0.192 g/100 g increased over 2-fold, pH from 6.6 decreased to 4.6, vitamin C was in a low amount of 7.22-7.74 mg/ 100 g and lost all content after drying, aw 0.929-0.938, was decreased after drying 0.74-0.75. The antioxidant activity in fresh and in dried hurma fruit ranged 37.9-39.3 and 78.2-80.7 ascorbic acid equivalent/100 g, the total polyphenolic ranged 74.2-93.3 and 101.7-125.2 mg gallic acid equivalent/100 g, flavonoid content 21.1-37.8 and 76.7-98.9 mg catechin equivalent/100 g. There is a strong influence of growing region for hurma based on the observed quality parameters for fresh and dried fruits, where those cultivated in Elbasan region had the best quality. This study may serve as basis for selecting hurma varieties, for further development and prospect in fruit growing sector and with potential for diversification by food industry into new products.**

Keywords - **antioxidant potential, drying, persimmon, physico-chemical characteristics**.

I. INTRODUCTION

Persimmon (*Diospyros kaki* Thunb.) is a member of the Ebenaceae family, native of East Asian countries and growing in some Mediterranean countries (Izuchi, 2011). In Albania this fruit is known as "hurma" and exist in a number of cultivars, grown and produced in our country for more than 100 years. Persimmon is a seasonal fruit, which can be purchased from September to early December (Toplu, 2009). It is s mainly eaten fresh, but can be frozen, canned, sometimes used in culinary, also other preservation methods to offer long-term and safe way of storage (de Ancos, 2000). Drying of persimmons has been used traditionally to obtain a product with good sensory attributes as well as storage stability (Park et al. 2006). Persimmon has a quite good nutritional value: it contains many compounds such as different sugars, starch, organic acids and amino acids, proanthocyanidins, flavonoids, carotenoids, triterpenoids and fatty acids (Veberic, et al. 2010; Zhou et al. 2010; da Conceição Santos et al. 2018), proteins, and vitamins A, B_6, B_{12}, C, and D (Kim, et al., 2013). The colour of the fruit is attributed to the carotenoid pigments in the pericarp and the content of lycopene (dark orange) is related with the autumn daylight conditions. Due to its richness in phenolic compounds, vitamin C, and carotenoids, persimmon has a strong antioxidant activity (Jung et al. 2005). In general, microbiological, physicochemical, and sensory properties are considered for determining the shelf-life and quality of foods (MFDS, 2011). Major quality parameters associated with dried foods are the changes in colon, visual appearance, microbial population abundance, texture, nutrients, and water activity (Carcel et al. 2010). However, the quality parameters for determining the shelf-life and quality of dried foods have not been intensively investigated so far in persimmons.

The study aims to investigate main physico-chemical parameters, polyphenols and antioxidant potential of Albanian hurma (*Diospyros kaki* Thunb.), fresh and dried fruits.

II. MATERIALS AND METHODS

Diospyros kaki Thunb. was collected during 2021, picked up randomly by trees over 40 years, in full maturity stage, from Tirana, Durres, and Elbasan regions in the central part of Albania, which are recognised traditionally for hurma fruits growing. Fruits were transported immediately to the laboratory for further analysis and drying. Fruits were pre-

selected with uniform maturity, shape, size, colour, and free from defects. For drying hurma fruits, a laboratory scale hot-air dehydrator was used, and the drying chamber consisted of a centrifugal air blower (horizontally across the ten trays), electrical heating elements, with adjustable temperature here applied 60±0.1°C and air velocity 1±0.1 m/s, relative humidity approximately 40% (at the beginning) and 10% (at the end), (Hoxha and Kongoli, 2016). Hurma fruits were cut in slices of 3 mm and set on thin layer, and the drying process last approximately 6 h. Samples were coded with symbols K: kaki (synonym name of hurma), F:fresh, D: dried, accompanied with their origin region -T: Tirana, -D: Durres and -E: Elbasan, respectively: KF-T, KD-T, KF-E, KD-E, KF-D, and KD-D. Dry matter, water activity (a_w), total ash, total soluble solids (TSS), ratio TSS/TA, total acidity (TA), pH, colour values (L*, a*, b*), as well as vitamin C, total polyphenols, flavonoids, and antioxidant potential were determined according to Hoxha and Kongoli (2021). The fruit extracts prepared based on solid-liquid extraction with 60% aqueous methanol adapted according to method Hoxha et al. (2021). where 1 (±0.001) g of fruits was extracted by using 10 ml of methanol 60 % (solvent/water; v/v), and 1% HCl_{cc}, vortex for 1 min, assisted with ultrasound for 15 min (40 KHz), centrifugated at 3500 rmp for 5 min, filtration, and finally the supernatant collected. All measurements were done in triplicate, and calculated as mean values ± SD.

III. RESULTS

The dry matter ranged between 22.98-76.85g/100g fw. (in fresh weight basis of sample), total ash 0.423-0.883 g/100 g fw., a_w ranged 0.938 to 0.737 (Figure 1.).

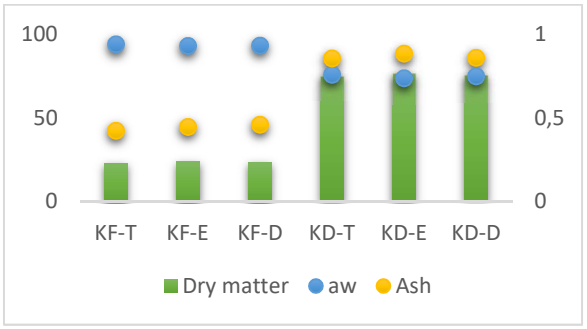

Figure 1. Dry matter (%) and water acitivity in fresh and dried hurma

TSS values resulted 19 to 21°Brix where main component of this fruit is a saccharide comprising glucose and fructose, and the ratio of TSS/TA was 98.95-136.71 (Figure 2.).

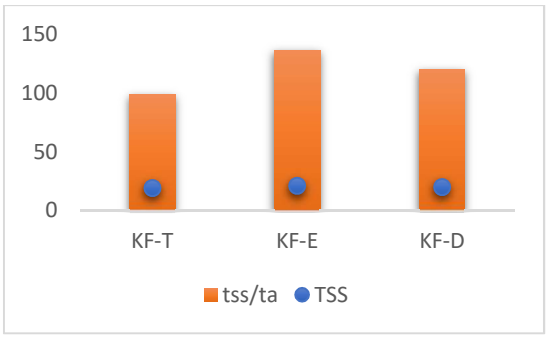

Figure 2. Total suluble solids (TSS) and the ratio of TSS/total acidity in fresh hurma

Total acidity resulted 0.154 to 0.422 g/100 g fw. Where the main organic acids ones in persimmon being malic and citric acids (Ryu et al. 2016); and pH values 4.6 to 6.6 (Figure 3.).

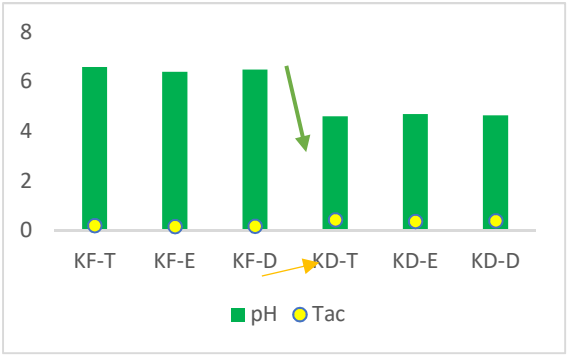

Figure 3. Total acidity and pH values of fresh and dried hurma

Persimmon is a spherical fruit with a colour that ranges from reddish to yellow according to carotene content, the pulp is a viscous orange-red and somewhat rough, depending on the content of tannins. The pulp, regardless of the variety, consists mainly of mucilage and pectin, which is responsible for its characteristic appearance (OMAIAA, 2021). Fresh hurma colour values resulted for L*, a*, b* respectively 65.73-71.82, 10.82-13.63, 35.27-39.81 (Figure 4.).

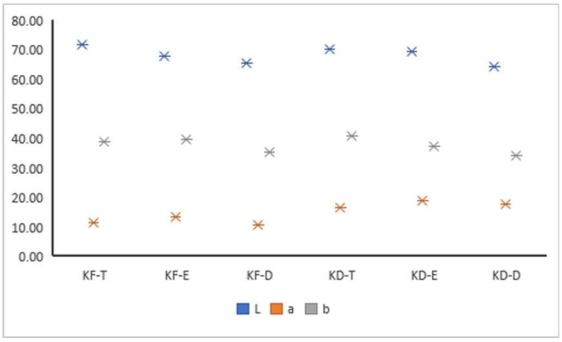

Figure 4. Colour values (L*, a*, b*) of fresh and dried hurma

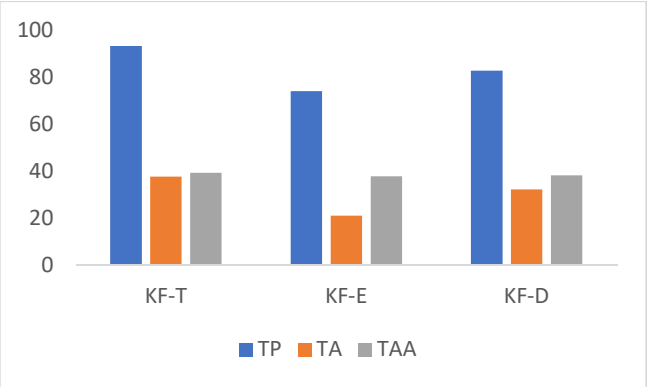

Figure 5. The content of total polyphenols (TP), total flavonoid (TF) and antioxidant activity (TAA) in fresh hurma fruits

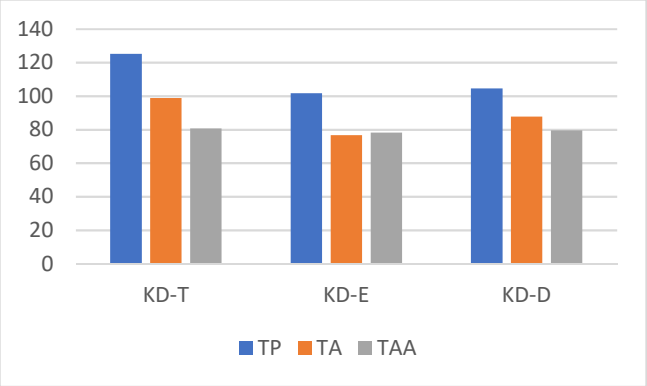

Figure 6. The content of total polyphenols (TP), total flavonoid (TF) and antioxidant activity (TAA) in dried hurma fruits

Total vitamin C was in a low amount of 7.22-7.74 mg/100 g fw., about 2/3 of the total amount is available as L-ascorbic acid (Giordani et al. 2011), and the broad variations of its content can be explained through environmental factors, fruit's inherent qualities, time of harvest, and the general conditions of the crop (Direito et al. 2019). The total polyphenolic ranged 74.2-93.3 and 101.7-125.2 mg gallic acid equivalent/100 g fw., flavonoid content 21.1-37.8 and 76.7-98.9 mg catechin equivalent/100 g fw. (catechins (flavan-3-ol) are the main flavonoids found in persimmons, and the antioxidant activity in fresh and in dried hurma fruit ranged 37.9-39.3 and 78.2-80.7 ascorbic acid equivalent/100 g fw. (Figures 5. and 6.).

IV. DISCUSSION

Fresh hurma fruits showed slight differences in values of parameters, where variety E had highest values for dry matter, total ash, TSS, TSS/TA ratio, pH and Vit C, and lowest for TA, and variety T had the lowest values compared to E and D hurma varieties, whereas variety D showed intermediate values. These values are within the range of other studies (Ozen et al. 2004; Ercisli et al. 2008). Drying hurma resulted in a change of all parameters, resulting in an increase of dry matter over 3-fold, a_w was decreased after drying 0.74-0.75, total ash and total acidity increased over 2-fold, pH from 6.6 decreased to 4.6, vitamin C lost all the content after drying, while bioactive compounds have increased over 1.3 to 3 folds. Hurma fruit colour values resulted for L*, a*, b* after drying no big differences occurred for L* and b*, but a* increased from 16.55 to 18.78. Colour values, water activity, retention of nutrients can be used as factors for determining the shelf-life of dried persimmons, however further studies investigating other quality parameters such as visual appearance, texture, microbial load, and chemical stability to determine the shelf-life of dried foods need to be conducted. Total phenolic content in persimmons as reported by available literature differed extensively (Veberic et al. 2010; Jang et al. 2010) in general resulted that persimmon has higher content of total phenolic compounds when compared to grapes, apple and tomato (Chen et al., 2008). Hurma collected from different locations (Tirana, Durres and Elbasan), shown the influence of growing location on the observed quality parameters for fresh and dried fruits. Hurma cultivated in Elbasan region resulted with best quality, followed by Durres with intermediate quality compared to hurma grown in Tirana.

V. CONCLUSIONS

Albanian hurma fruits beside providing nutrients such as minerals, vitamins, phytochemicals, may serve as good source of natural antioxidants sources, and may be utilised in reformulating and improving food products quality, even as natural colorant, etc. The drying influence of on the quality parameters resulted quite important, as occurred an increase over 3-fold in the dry matter. a_w was decreased after drying 0.74-0.75, total ash and total acidity increased over 2-fold. Furthermore, drying influenced the bioactive compounds content, which were increased over 1.3 to 3 folds. The growing locations identified as independent variable had a strong influence on the quality parameters observed for fresh and dried hurma, where those cultivated in Elbasan region resulted with the best quality, followed by hurma grown in Durres and Tirana region. This study may serve as basis for selecting Albanian hurma varieties, for further development and prospect in fruit growing sector and with potential for diversification by food industry into new products.

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The analysis of dietary habits in acne patients

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Abstract - **Acne is the most common skin disease and ranks eighth in incidence among diseases in the world. 95% of adolescents have acne, and the number of acne in adults, especially women, over the last 30 years has increased. Acne pathology is related to triggers of increased sebum secretion and *Propionibacterium acnes* colonization. Nutrition plays an important role in the etiology of acne and gene expression, and Paleo diet provides promising results for mild and moderately severe forms of acne.**
Subjects and methods: Observational study with a study-specific questionnaire (data on anthropometry, general health status, comorbidities, medications and supplement use, dietary and lifestyle habits, menstrual cycle) was completed by 60 dermatological patients of both gender, aged 15-46 years, from Sarajevo. Compliance between patients' diet and the Paleo diet principles was analysed with a point-based questionnaire. Acne-specific data was collected from medical records. The research aimed to determine the role of dietary and lifestyle habits in acne patients.
Results and discussion: Men have less acne, but a more severe form. 53% of patients had a positive family history for acne. The majority of acne is of unknown etiology (41.7%), followed by bacterial (30.0%), and hormonal (28.3%), which were exclusively present in women. The average compliance with the Paleo diet was 54%, with the lowest consumption of fish, but high consumption of sweets and fast food. The compliance did not differ accoridng to patients age, Body Mass Index, or acne etiology. Also, patients with severe acne in comparison to mild acne, had more meals per day (p=0.046). Dietary supplements are taken daily by 82% of patients; herbal teas (67%), vitamin C (40%), and vitamin D (38%) being the most commonly used.
Conclusion: Patients with hormonal acne are most likely to avoid highly processed foods, i.e. to have diet with higher compliance with the Paleo diet. For the majority of acne patients, the role of diet is unclear. We identified the use of whey proteins as potential triggers of acne in 10% of patients.

Keywords - **acne; etiology of acne; acne severity; observational study; Paleo diet.**

I. INTRODUCTION

Acne is the most common dermatological disease today, affecting primarily adolescents, but can actually affect all population groups. In terms of occurrence, they rank eighth among diseases (Rocha and Bagatin, 2018). About 85% of adolescents will experience acne; in women, most acne occur from 14 to 17 years old, while in the male population from 6 to 19 years old, and they can persist into adulthood (Habeshian and Cohen, 2020). According to the European Academy of Dermatology and Venereology (EADV), adult acne affects 12% of women and 3% of men of 25 years and older. Pathology of acne includes an inflammatory process of the pilosebaceous unit (hair follicle and sebaceous gland), mainly as a consequence of excessive secretion of sebum and colonization by the bacterium *Propionibacterium acnes* (Feldman et al. 2004; EADV Guidelines, 2016). Excessive secretion of sebum can be triggered by internal factors (change in the innate immune system, increased secretion of androgens), change in the composition of the skin microbiota, as well as external factors (López-Estebaranz et al. 2017). In adults, acne is often caused by some endocrine disorder, such as thyroid and adrenal gland disease, type 2 diabetes, and Polycystic Ovary Syndrome (Jabbour, 2003). Other factors, such as diet, stress, physical inactivity, insufficient sleep, and smoking can affect the occurrence of adult female acne (AFA). Stress caused by external factors combined with internal factors and menstrual cycle make women more susceptible to AFA (Albuquerque et al. 2014).
Diet is an important factor in the etiology of acne. Foods with high glycemic index, chocolate, dairy products and processed products, intake of saturated and trans fatty acids, and whey protein supplements are associated with an increased risk of acne and more severe form of acne (Melnik, 2015; Bagatin et al. 2019; Conforti et al. 2022; Meixiong et al. 2022). Generally, the proposed link between foods rich in simple carbohydrates that have high glycemic index, as well as milk and dairy products and acne is through increased postprandial insulin secretion and insulin-like growth factor (IGF-1) (Meixiong et al. 2022). Nowadays, dermatologists are increasingly advising acne patients to avoid refined carbohydrates (Alshammrie et al. 2020). Studies have found that non-Western civilizations (Chinese, Okinawans and Inuit) that eat Paleo diet do not have acne, but once they change their diet to a Western like, cases of acne were reported (Melnik, 2015). A diet rich in omega 3 fatty acids, dietary fiber, antioxidants, vitamins A and C, as well as iodine and zinc has a positive effect on reducing severity and the occurrence of acne (Kucharska, 2016; Conforti et al. 2022). For mild to medium-severe forms of chronic acne, diet can be used a sole treatment, but more severe forms require pharmacological treatment. Yet, diet and pharmacological treatment have a synergistic effect (Matsui, 2019).

The role of diet in acne is yet to be clarified. Recent systematic review on epidemiology of acne found inconsistent data on the role of diet or smoking on the risk and severity of acne (Heng and Chew, 2020).

II. SUBJECTS AND METHODS

A cross-sectional observation study was conducted on 60 dermatological patients who are receiving treatment for acne at one private Dermatology Practice in Sarajevo, Bosnia and Herzegovina. Recruitment was done between June and October 2021.

The EADV classification (2019) was used by the dermatologist for acne diagnosis and severity assessment.

Study-specific questionnaire included questions about general socio-demographic characteristics (age, education, employment etc.), height and current body weight, subjective assessment on body weight fluctuation over the past 3 months, presence of other health issues, medication and supplement use, smoking, coffee and alcohol consumption, number of meals per day, and subjective assessment of physical activity level. Women were asked to provide details about menstrual cycle – menarche, length and regularity of menstrual cycle, amenorrhea occurrence. Along with a study specific questionnaire, patients' medical history was used to collect acne-specific background information (history of treatments, medications etc.).

In order to test compliance with Paleo diet, point-based questionnaire was developed. The questionnaire consists of 15 foods/food groups for which consumption frequency is given. Each consumption frequency corresponds to zero, one or two points, where zero represents the most unfavorable and two the most desired consumption. For example, for sweets, if a patient consumes sweets (chocolates, cookies, cakes, ice cream etc.) every day then he/she gets zero points, while if sweets are consumed rarely two points are allocated. Maximum score is 30 and higher scores correspond with a better compliance to Paleo diet.

Ethical approval for this study was obtained from the Faculty of Food Technology Osijek (Application number: 006-06/21; Class: 003-08/21-01/01). All participants provided signed consent prior to study inclusion.

Parametric statistical tests were used to analyze the data given that data followed normal distribution (tested with Kolmogorov-Smirnov test). The results are given as means and standard deviation (SD), with range where appropriate. Significance level of 0.05 was used. Analysis was performed in Statistica software (version 13.4, StatSoft Inc., USA).

III. RESULTS AND DISCUSSION

Out of 60 patients who participated in the study, 55 (92%) were women and 5 were men (8%). They were between 15 and 46 years old and had Body Mass Index (BMI) between 16.5 to 33.2 kg/m^2 (Table 1.). Positive family history for acne was found in 53% of patients, which is in line with literature data for family history of acne (Rocha and Bagatin, 2018; Heng and Chew, 2020), especially for more severe acne and adult acne (Battaile et al. 2002; Šijak et al. 2019).

Considering aetiology, the majority of acne were of unknown aetiology (41.7%), followed by bacterial (30%) and hormonal (28.3%). People with oily and mixed skin have higher sebum levels, which increases the risk of acne presentation (Bhate and Wiliams, 2013).

Table 1. Selected characteristics of study population (N=60)

	Mean ± SD	Minimum	Maximum
Age (years)	25.0 ± 6.5	15	46
BMI (kg/m^2)	22.4 ± 3.4	16.5	33.2
Meals per day	3.1 ± 1.0	2	5
Paleo diet compliance (points)	16.2 ± 4.7	5	26

SD – Standard Deviation; BMI – Body Mass Index

Mild acne type (*Acne vulgaris* and *Acne comedonica*) were present in 40% and severe (*Acne papulose*, *Acne papulopustose*, *Acne nodulose*) in 60% of patients, and their distribution varied according to gender (Figure 1.). Based on the severity of acne, patients did not differ in age or BMI. Epidemiological studies found that people with higher BMI tend to have more severe acne (Heng and Chew, 2020). Also, mild acne occurred more frequently in women while severe acne occurred more frequently in men (Heng and Chew, 2020)

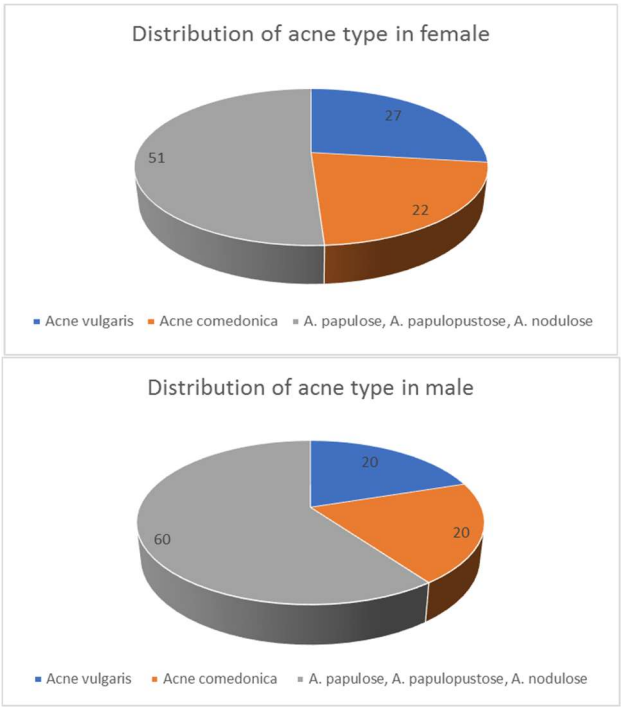

Figure 1. Distribution of acne type between women and men

Courrently, no specific dietary regiment is recommended to accompany acne treatment (EADV, 2016; EADV, 2019). Avoiding milk and dairy, chocolate and high fat diet in general seem to have positive impact on acne occurence and severity (Heng and Chew, 2020).

Patients have 3.1 ± 1.0 meals per day, and patients with severe acne had more meals in comparison to patients with mild acne (3.3. ± 1.2 vs 2.8 ± 0.7, p=0.048). Number of meals per day correlates with energy intake, but both fewer and higher number of meals per day correlate with unfavourable dietary practices, like meal skipping, higher consumption of sweetes and snacks, etc.

The Paleo diet, is a diet modeled after what ancient humans were thought to eat. The modern version we are refeering to suggests eating more lean meats, fish, shellfish, fruits, vegetables, eggs, nuts, and seeds, while limiting grains, legumes, dairy, processed foods, added salt, and refined sugars (Cordain, 2002).

Table 2. Distribution of study population based on their consumption of foods/food groups

Food/food group	Least favourable	Most desired
Satwater fish	51.7	5.0
Olive oil	26.7	38.3
Nuts (almonds, walnuts, hazelnuts)	21.7	45.0
Berries (backberries, raspberries, blueberries)	13.3	35.0
Other friuts (apple, pear, lemon, orange)	3.3	73.3
Tomato (fresh or in a dish)	11.7	76.7
Carrots (fresh or cooked)	18.3	50.0
Dark green leafy vegetables (kale, spinach, swiss chard)	25.0	23.3
Garlic	31.7	30.0
Salt	15.0	8.3
Cereals and bakery products from white flour	31.7	38.3
Juices (carbonated, noncarbonated, syrups)	45.0	35.0
Sweets (chocolate, cookies, biscuits, ice cream)	70.0	15.0
Fast foods (hamburger, pizza, sendwiches, pies)	33.3	21.7
Hard and processed cheese	26.7	33.3

The compliance with Paleo diet can be considered as average. With only 16.2 ± 4.7 points (Table 1.) the average compliance rate ranges from 38.3% to 69.7%. However, when consumption of a particular food/food group is observed, we see very low consumption of foods which were shown to have positive impact on acne (Table 2.). On the other hand, foods rich in simple carbohydrates and saturated fats are consumed often and by the majority of patients (Table 2.). Patients with mild acne type and those with hormonal acne (Table 3.) were more likely to avoid highly processed foods, i.e. have diet which is more in complance with Paleo diet, but no statistical significance was found.

Table 3. Compliance with Paleo diet depending on severity and aethiology of acne (N=60)

		n	Mean ± SD	p
Aethiology	Unknown	25	15.4 ± 4.1	0.126
	Bacterial	18	15.8 ± 4.8	0.727
	Hormonal	17	17.7 ± 5.4	0.300
Severity	Mild	24	16.5 ± 4.8	
	Severe	36	15.9 ± 4.7	0.603

SD – Standard Deviation

Supplementation is used by 82% of patients, mainly various herbal teas (67%), vitamin C (40%) and vitamin D (38%). Combination of vitamin C, D and zinc is used by 11.7% of patients. Market of supplements for acne is diverse, but so far, positive association was found only for zinc and vitamin A supplementation (Burns et al. 2022). Also, low serum levels of vitamin E and D in acne patients suggest that supplementing these vitamins could provide beneficial effect on acne (El-Akawi et al. 2006; Lim et al. 2016).

Protein powder is a known trigger for acne (Heng and Chew, 2020; Melnik, 2015), but 10% of patients use them.

IV. CONCLUSION

Diet of patients with hormonal acne (exclusivelly women) and those with mild acne show better compliance with Paleo diet. Still, for the majority of acne patients, the role of diet is unclear. Additionally, the use of supplements need to be considered more thoroughly, since we found that in 10% of patients, supplements could be the actual triggers of acne, and not something that would alleviate acne problems.

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The quality of breakfast eaten at home *vs.* school among primary school children

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Abstract - **Similar to skipping of breakfast, the consumption of low-quality breakfasts may contribute to the nutritional deficits and poor academic performance among primary school children. The aim of this study was to estimate the breakfast quality of primary school children and whether quality differs between breakfasts consumed at home from ones consumed in the school. The breakfast consumption on weekdays was observed from a 3-day dietary records of 192 children (52.1% of boys) aged 8.9 ± 0.5 years from the City of Zagreb. Breakfast was defined as first meal of the day eaten before 10.30 AM. The energy and nutritional value of breakfasts was calculated using Croatian National Food Composition Tables and food labels of products. Breakfast quality was estimated using the Breakfast Quality Index (BQI) for children. On total, children consumed 422 breakfasts on weekdays, of which 77.9% were consumed at home and 22.0% at school. The average daily energy intake from consumed breakfasts was 287 kcal (211 kcal - 375 kcal). The number of points achieved by BQI was on average 4.0 (4.0 - 5.0) out of a possible 10. The breakfast quality did not differ (p = 0.722) between breakfasts consumed at home (4.0; 4.0 - 5.0) and at school (4.0; 3.0 - 5.0). Most breakfasts were classified as medium quality breakfasts at both consumption locations. According to the BQI components, similar proportion of breakfasts from both locations contained food rich in simple sugars, had lower ratio of monounsaturated and saturated fatty acids than study population's median, provided 200 - 300 mg of calcium and contributed 20 - 25% to daily energy intake. A greater proportion of breakfasts consumed at home contained dairy products and monounsaturated fatty acids rich food. However, a greater proportion of breakfasts consumed at school contained cereals, fruit and/or vegetables, combination of cereals, fruit and dairy products in one meal but also saturated fatty acids rich food. In conclusion, the quality of breakfast should be improved regardless the location of consumption, but also conducting nutritional education and creating an environment that supports desirable eating habits.**

Keywords - **children, breakfast, breakfast quality, school breakfast**

I. INTRODUCTION

It is known that a breakfast is an essential part of adequate nutrition in all populations, especially in children. Several studies have shown that regular consumption of breakfast is associated with higher micronutrient intake and better overall diet quality in school-aged children (Affinita et al., 2013; Gibney et al., 2018; O'Neil et al., 2014; Rampersaud et al., 2005). Breakfast is an ideal opportunity for children to start a day by eating whole grain products, fruit, milk and dairy products. Breakfast also gives them the energy they need for their daily activities. In addition, eating breakfast is associate with better cognitive function and school performance (Affinita et al., 2013; Gibney et al., 2018; O'Neil et al., 2014; Rogers, 1997). Eating breakfast as part of an adequate diet can have a positive impact on children's health and well-being, and parents should be encouraged to offer their children breakfast every day. Despite all the proven positive effects, breakfast is most often skipped, and children who skip breakfast have poorer overall diet quality. In addition, among primary school children the consumption of low-quality breakfasts may also have negative effect as breakfast skipping (Monteagudo et al., 2013; O'Neil et al., 2014; Ramsay et al., 2018).

As there is no definitive consensus on how breakfast is defined, the perception of breakfast depends on the geographical and cultural characteristics of the population and the time at which it is consumed (O'Neil et al., 2014). Therefore, the aim of this study was to assess the quality of the breakfast of primary school children in Zagreb. In addition, the aim was to determine whether there is a difference in quality between breakfasts eaten at home and at school.

II. METHODS

A. Participants and settings

This study was a cross-sectional observational study conducted in 14 primary schools in the City of Zagreb during the school year 2018/2019. The schools and children were selected according to the protocol of the project "Pilot project: school meals and intake of fruits and vegetables in schools with and without gardens", which is part of the European Horizon 2020 project "Strengthening European Food Chain Sustainability by Quality and Procurement"

(Strength2Food, H2020-SFS-2015-2, contract no. 678024) (Quarrie et al., 2021). From the total number of enrolled children (n = 681), only those who completed a 3-day dietary records were included in this study. Therefore, the present study was conducted on 192 children (28.2%) aged 8.8 years (8.6 - 9.2), of whom 52.1% were boys and 47.9% were girls. The research protocol was designed and implemented in accordance with the Helsinki Declaration, and was approved by the Ethics Committee of the School of Medicine, University of Zagreb (380-59-10106-19-11/307). Permissions to conduct the pilot project in primary schools were obtained from the relevant institutions (Ethics Committee of the Institute for Medical Research and Occupational Health: 100-21/16-8; Croatian Ministry of Science and Education and the Education and Teacher Training Agency: 602-01/16-01/00388).
All parents of the children participating in the study were informed about the study protocol and gave their written consent to participate.

B. Dietary intake

The energy and nutritional value of breakfast was estimated using a dietary record for 3 non-consecutive days (2 weekdays and 1 weekend day). For the purposes of this study, only data from dietary records recording food and drink consumption during the weekdays were analysed. All participants and their parents/guardians received detailed written and video instructions on how to correctly complete the dietary records. To measure food intake, the amount of food consumed had to be weighed or reported in household measurements (teaspoon, tablespoon, cup). In addition, the location and time of food and drink consumption were noted. Dietary records were analysed using the "Prehrana" software (Infosistem d.d., Zagreb), which includes the National Food Composition Database (Kaić-Rak & Antonić, 1990), supplemented by the nutrition labels of a food products and the data from the Danish Food Database (National Food Institute, 2019) for the nutrient composition of some foods not included in the Croatian Food Composition Database.
In this study, breakfast is defined as the first meal of the day that children consume before 10:30 AM.

C. Breakfast quality index

The Breakfast Quality Index (BQI) for children and adolescents was used to assess the quality of children's breakfasts (Monteagudo et al., 2013).
The BQI consists of 10 questions, which are shown in Table 1. One point is assigned if the item from the question was included in the breakfast consumed. Therefore, the highest possible score is 10. According to the archived BQI score, the quality of the breakfast was interpreted as low-quality breakfast (≤ 3 points), medium-quality breakfast (4 – 7 points) and high-quality breakfast (≥ 8 points).

Table 1. Included items and scoring of the Breakfast Quality Index (Monteagudo et al., 2013)

Question number	Items included	Yes	No
BQI 1	Cereals and derivatives (bread, breakfast cereals, biscuits, bakery products)	+1	0
BQI 2	Fruit and vegetables (fruit, fruit juice, vegetables)	+1	0
BQI 3	Dairy products (whole and semi-skimmed milk, milk shake, yoghurt, cheese)	+1	0
BQI 4	Foods rich in simple sugars (sugar, jam, honey) <5% of total daily energy	+1	0
BQI 5	MUFA-rich fats (olive oil, vegetable oil)	+1	0
BQI 6	MUFA : SFA > median	+1	0
BQI 7	Compliance with energy intake recommendations (20 - 25% of total daily energy)	+1	0
BQI 8	Cereals + fruit + dairy product in the same meal	+1	0
BQI 9	Calcium (200–300 mg)	+1	0
BQI 10	Absence of SFA and trans-rich fats (butter, margarine)	+1	0

MUFA = monounsaturated fatty acids; SFA = saturated fatty acids

D. Anthropometric assessment

The anthropometric assessment was conducted during the Physical Education and Health classes in schools. The anthropometric assessment included measurements of body weight and height on a combined medical digital scale and stadiometer (Seca, type 877-217, Vogel & Halke Gmbh & Co., Germany) with an accuracy of 0.1 kg and 0.1 cm, respectively. During the measurement, the children wore light sportswear and no shoes. The body mass index (kgm^{-2}) was calculated using the data obtained on body weight and height. In addition, standardised z-scores for body weight, height and body mass index by age and sex were calculated for each child using the AnthroPlus software of the World Health Organisation (Blössner et al., 2009).

E. Statistical methods

Data were analysed using the computer programme SPSS version 23.0 (IBM SPSS Statistics for Windows operating system, 2015, Armonk, NY: IBM Corp.) and graphical data processing was done using Microsoft Office Excel 2016 (Micrsoft, Seattle, WA). All categorical variables were presented as percentages and the continuous variables as median and interquartile range because they were not normally distributed as determined by the Shapiro-Wilk test. The Mann-Whitney U test was used to determine the differences in the BQI of breakfast between meals eaten at home and at school. The Chi-square test or Fisher's exact test was used to determine the difference in the distribution of adequate breakfast between location of breakfast consumption. In addition, the Chi-square test or Fisher's exact test was used to determine the difference in the proportion of breakfasts that scored on each component of the BQI. In all statistical analyses of the results, the significance level was set at $p < 0.05$.

III. RESULTS

The socio-demographic and anthropometric characteristics of the children are shown in the Table 2.

Table 2. General characteristics of respondents[1]

Characteristics	A total of 192 children
Socio-demographic characteristics	
Age	8.8 (8.6 – 9.2)
Sex (% of children)	
Boys	52.1
Girls	47.9
Anthropometric characteristics	
Body height (cm)	135.1 (131.7 – 140.5)
Body height for age z-score	0.7 (0.2 – 1.3)
Body mass (kg)	29.7 (27.2 – 33.5)
Body mass for age z-score	0.4 (-0.1 – 1.2)
Body mass index (kgm^{-2})	16.0 (15.1 - 17.6)
Body mass index for age z-score	0.1 (-0.6 – 0.7)
Eating habits	
Types of school meals (% of children)	
Non-consumers	22.4
Breakfast	30.2
Lunch	5.7
Breakfast and lunch	13.5
Breakfast, lunch and snacks	28.1

[1] All continuous data were expressed as median and interquartile range, and categorical data as percentage.

According to the World Health Organization cut-off values and categories, the children have, on average, an appropriate body weight, height and body mass index. Most children (71.8%) were registered to have breakfast at school.The average daily energy intake from the breakfast consumed was 287 kcal (211 kcal - 375 kcal), which is 17.1% (11.9 - 23.9%) of the average daily energy intake of children. The average BQI score was 4.0 (4.0 - 5.0), suggesting that most breakfasts were of medium-quality (Table 3.). There is no statistically significant difference between breakfasts consumed at home and at school. Less than 2% of the breakfasts eaten at home were of high-quality, while none of the breakfasts eaten at school were of high-quality.

Table 3. The average number of points achieved by the index for assessing the quality of breakfast consumed during the week[1]

Parameter	Total sample	Breakfast at home	Breakfast at school	p value*
Number of breakfasts (n)	422	329	93	-
Breakfast quality index (n)	4.0 (4.0 - 5.0)	4.0 (4.0 - 5.0)	4.0 (3.0 - 5.0)	0.722
Categories of breakfast quality index (% of breakfasts)				
Low-quality breakfast	23.5	22.2	28.0	
Medium-quality breakfast	74.9	76.0	72.0	0.304
High-quality breakfast	1.6	1.8	0.0	

[1] All continuous data were expressed as median and interquartile range, and categorical data as percentage. * The difference between groups of continuous data were tested using the Mann-Whitney U test ($p < 0.05$), and categorical data using the Fisher's exact test ($p < 0.05$).

Figure 1. shows the distribution of breakfasts eaten at home and at school, with points awarded for each question of the BQI. The results suggest that less than a quarter of breakfasts at both locations contributed 20-25% of daily energy intake, less than 30% contained 200-300 mg of calcium, and less than half of breakfasts had a lower ratio of monounsaturated to saturated fatty acids compared to the median of the study population. However, less than 5% of the breakfasts contained foods rich in simple sugars. Significantly more than 13% of breakfasts consumed at home contained dairy products ($p = 0.013$) and more than 7% of breakfasts contained foods rich in monounsaturated fatty acids ($p = 0.021$), compared to breakfasts consumed at school. In

contrast, a greater proportion of the breakfasts consumed at school contained cereals (Δ = 13.5%, p = 0.001), fruit and/or vegetables (Δ = 13.6%, p = 0.001), or a combination of cereals, fruit and dairy products in one meal (Δ = 14%, p < 0.001), but more of them contained foods with saturated fatty acids (Δ = 34.0%, p < 0.001).

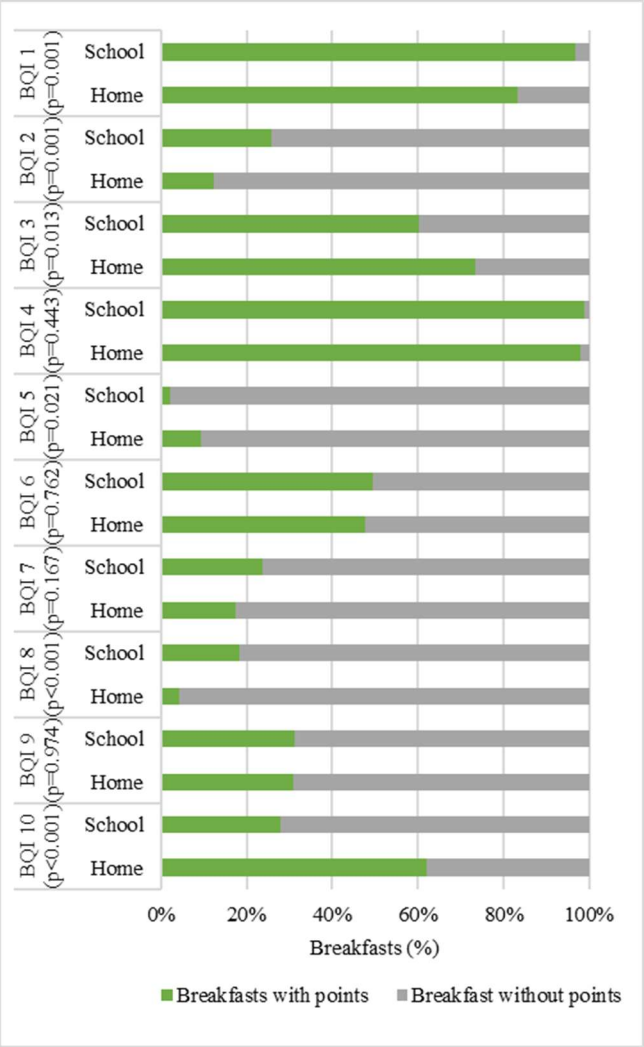

Figure 1. The distribution of breakfasts eaten at home (n=329) and at school (n=93) where points were scored for each question of the breakfast quality index

IV. DISCUSSION

To the authors' knowledge, this is the first study to present the results of assessing the quality of primary school children's breakfast in Zagreb, Croatia, in a comprehensive way and not only in terms of nutritional composition. In addition, the results of this study show the differences in the quality of children's breakfast between home and school. The insights from this study could help in designing nutrition education aimed at changing eating behaviour and maintaining health in primary school children.

In the present study, children had on average of 287 kcal (211 kcal - 375 kcal) per day from breakfast, which was almost 50% less than children (7 - 10 years) had 20 years ago (Colić Barić and Šatalić, 2002). Breakfast contributed to 17.1% of the children's daily energy intake in the present study, which is below the national recommendation (Capak et al., 2013). Breakfast is considered one of the most important meals of the day, and its consumption is associated with more adequate daily energy and nutrient intake, better academic performance, maintenance of adequate body weight and lower cardiometabolic risk in children (Affinita et al., 2013; Gibney et al., 2018; Littlecott et al., 2016; Rampersaud et al., 2005; Ramsay et al., 2018). Nevertheless, review studies suggest that about 8 to 21% of primary school children up to 11 years of age do not eat breakfast every day and that the contribution of breakfast to total daily energy intake is on average 11 to 21% (Almoosawi et al., 2016; Littlecott et al., 2016; Rampersaud et al., 2005). In the present study, 84% of the children ate breakfast on all days recorded in the dietary records.

School food service system in Croatia may offer up to three meals to the children at a subsidised price depending on the class duration and the time of day when classes are held. However, parents decide whether their child attends the school meals and register it one month in advance. Of the total sample, 70% reported eating breakfast at school. However, analysis of dietary records showed that only 22.0% of breakfasts were consumed at school and that children were more likely to eat breakfast at home before school breakfasts. Regardless of where breakfast is consumed, it is important to eat a high-quality breakfast. The results of the present study indicate that majority of breakfasts were of medium-quality and that there was no difference in the quality of breakfast consumed at home and at school. In studies in which the quality of the breakfast eaten was assessed with the BQI or a similar index (Quality Breakfast Score), the results show that the majority of children eat a low- or medium-quality breakfasts (Aranceta et al., 2001; Monteagudo et al., 2013). According to the available literature, eating school breakfast contributes to a higher intake of fruit/vegetables, dairy products, cereals, dietary fibre and calcium (Au et al., 2018; Condon et al., 2009). However, the problem of increased consumption of food rich in sugar and saturated fatty acids through school breakfasts is still present (Condon et al., 2009; Fox et al., 2021). The results of the studies mentioned above cannot be directly related to the present study because the contribution of breakfast to the daily intake of food groups

and nutrients was not observed. However, results indicated that greater proportion of the breakfasts consumed at school contained cereals, fruit and/or vegetables, or a combination of cereals, fruit and dairy products in one meal, but more of them contained foods with saturated fatty acids. In addition, more breakfasts consumed at home contained dairy products and foods rich in monounsaturated fatty acids. Nevertheless, the results suggest that it is necessary to increase the consumption of fruits or vegetables and milk and dairy products among children and to increase the consumption of foods rich in unsaturated fatty acids and to reduce the consumption of foods rich in saturated fatty acids through breakfast in both locations. The above objectives can be achieved through adequate training of staff, the implementation of a high-quality breakfast and the control of school menus (Cummings et al., 2014).

When interpreting the results, it is important to highlight several points. The quality of breakfast was analysed from the dietary records and shows the actual intake of food consumed for breakfast. The quality of breakfast served may differ from that consumed, especially knowing that children most often waste food groups that include fruit, vegetables, whole grains and dairy products. (Ilić et al., 2022; Niaki et al., 2017). The data analysis could not be done at the child level, because sometimes the children ate breakfast at home and sometimes at school. Therefore, the analysis was done at the level of the number of breakfasts. Days over the weekend were not included in the analysis, as children might have different eating habits on weekdays than on weekend days (Dutch et al., 2021).

V. CONCLUSION

The breakfasts eaten at home and at school were on average of medium-quality, with little difference in the foods included. Therefore, it is necessary to improve the quality of breakfasts at both locations, conducting nutritional education and creating an environment that supports desirable eating habits.

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The process of making plant-based milk alternative

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Abstract - **The consumption of plant-based milk alternatives has increased in recent years. Oat products have gained popularity among consumers due to their good nutritional value. They are a good source of dietary fibers, especially β-glucan. Two different oat products, oat protein concentrate and oatmeal, were used in the production of oat drinks. The steps in the production of oat milk were extraction, separation, addition of ingredients, homogenization, pasteurization, and cold storage. The manufacturing process of a plant-based milk alternative, an oat drink, was achieved and successfully documented as a video for educational purposes.**

Keywords - **plant-based milk alternative, oat drink, extraction, processing, educational video**

I. INTRODUCTION

Plant-based milk alternatives are water-soluble extracts of vegetables, oilseeds, legumes, cereals, pseudo cereals and nuts (Silva et al. 2020). They are essential ingredients in many vegan foods, such as plant-based alternatives to dairy products like yogurt, cheese, kefir, butter, and ice cream (Aydar et al. 2020). The consumption of plant-based milk alternatives has increased in recent years for several reasons (Zandona et al. 2020) e.g., lactose intolerance and allergy to cow's milk protein, ethical reasons and lifestyle choices. The above mentioned reasons are common, why consumers may prefer animal milk alternatives.

Oat is the most promising raw material from which functional plant-based drink is produced. Dietary fibers e.g., β-glucan, functional protein, lipid and starch components, as well as phytochemicals in oat grains have health benefits (Sethi et al. 2016). The functionally active component, β-glucan, possesses nutraceutical properties and participates in increasing the viscosity of the solution.

Plant-based milk alternatives are made by extracting the raw material into water and separating the liquid (Silva et al. 2020). Homogenization and heat treatment are necessary to improve the quality and microbiological stability of the final product. Other ingredients e.g., oil, sugar and flavorings can be added to plant-based milks to improve consumers' acceptability of the products. The purpose of this work was to prepare a plant-based milk alternative, oat drink, on a laboratory scale. The main goal was the maximum extraction of starch, proteins, and dietary fibers from the oats to the final product. The process and used methods related to the production of oat drinks are presented.

The work was carried out for the EQVegan (European Qualifications & Competences for the Vegan Food Industry), which is an ERASMUS+ Sector Skills Alliance project (621581-EPP-1-2020-1-PT-EPPKA2-SSA-EQVEGAN).

The material will be used as learning material within the section of plant-based milk alternatives.

II. MATERIALS AND METHODS

A. Materials used

Oat protein concentrate was obtained from Fazer (Finland) and oatmeal from Pirkka (Finland). Rapeseed oil was purchased from K-Menu (Poland).

B. Methods for preparing the oat drinks

Oat drinks were prepared (Figure 1.) by using two different oat products, oat protein concentrate and oatmeal. The desired substances were obtained through extraction with water.

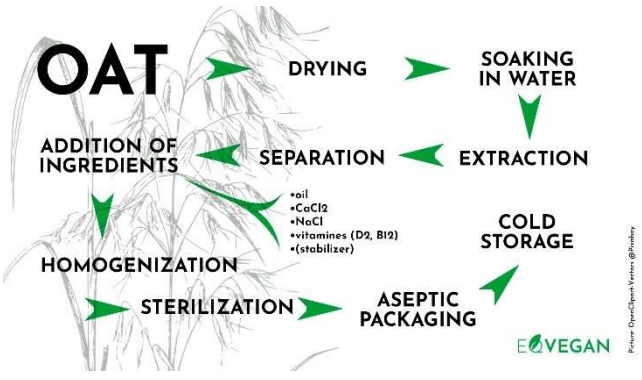

Figure 1. Process chart of oat drink.

The oat protein concentrate was mixed with water in a 57°C hot water bath (Julabo, Germany) using a rotor stator homogenizer (IKA, Germany) at 7000 RPM for 15 minutes. Oat protein concentrate was used at 200 g per 2 kg of water. Centrifugation (Beckman, USA) was used as a suitable separation technique while using fine oat powder. As a result, the precipitate, consisting of starch and excess sedimented oat protein, was separated from the supernatant. The supernatant was manually decanted.

The oatmeal was extracted in an automated malting system (Speidel, Germany) at 57°C for 45 minutes. Oatmeal was used at 2 kg per 10 kg of water, which was supplemented by adding 12 g of $CaCl_2$ and 10 g of NaCl. The separation was done by filtering.

The dry matter content of the mixtures was measured with a moisture analyzer (Precisa, Switzerland) to ensure that the desired levels were obtained. The target dry matter content was 8–10%, as in commercial products.

After the extraction and separation processes, other ingredients (oil, $CaCl_2$, NaCl, vitamins D_2 and B_{12} or stabilizers) can be added. Alternatively, the salts may be added already in the extraction phase. After addition of the rapeseed oil (1 w-%), pre-homogenization of the mixtures was done with a rotor stator homogenizer at 7000 RPM for 5 minutes. After the previous step, homogenization of the mixtures was done with a high-pressure homogenizer (SPX Flow, USA) to stabilize the final product. Two-stage homogenization took place at pressures of 180 and 30 bar for 10 minutes. Eventually, the mixtures were pasteurized with a pasteurization unit (Siemens, Germany) at 85°C for 5 minutes. Aseptic packaging is required to maintain the sterility, and the products were stored under refrigerated conditions, below 4°C.

The oat drink process was recorded. The material was compiled as an educational video with text in English for SeAMK students. For the students at Sedu, food vocational school in Seinäjoki, the material will be translated into Finnish.

III. RESULTS

Oat drinks with different properties were successfully prepared in the food laboratory. The solid content of the final products was 4.4% in oatmeal and 0.5% in oat protein concentrate (Figure 2). The educational video (Kirveslahti et al. 2022) was compiled with all the process steps. These process steps were explained both orally and with text. The finished video will be used as educational material firstly in the Train-the-Trainer programme of the EQVegan project and secondly for students at SeAMK and Sedu.

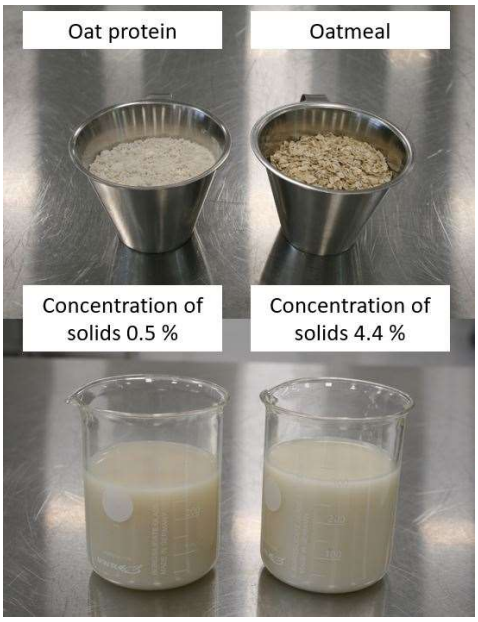

Figure 2. A snapshot from the educational video on oat drink processing.

IV. DISCUSSION

Research in food development responds to the changing needs in the food industry and meets the current demands of consumers by creating new alternatives (Sethi et al. 2016) e.g., vegan analogues to milk, egg, meat and fish products. The food industry is changing, as plant-based products have become more common among consumers (McClements and Grossmann, 2021). This leads to the emergence of plant-based products that are more sustainable and ethical, when compared to animal-based alternatives. The vegan food industry is still developing, and not enough information is available for all players in the industry (Saari et al. 2021).

The video was done as an educational material for students, operators in the industry and industrial management in Finland. The video was prepared in the EQVegan project. It will be distributed through the project for various European Qualifications Framework (EQF) levels leading to European Skills, Competences, Qualifications and Occupations (ESCO) profiles in as many European countries as possible.

Plant-based milk alternatives have different sensory properties, stability, and nutritional composition than animal milks (Silva et al. 2020). Many plant-based milk alternatives face technological problems related to either processing or storage (Sethi et al. 2016). The process parameters (pH, temperature, and extraction time) affect the properties of the final oat drink product.

A. Emulsion stability and sensory acceptability

Particle size, emulsion formation and protein solubility control the stability in plant-based milk alternatives (Sethi et al. 2016). The stability depends on the size of dispersed phase particles, and by reducing the size of the particles, stability can be improved. The use of emulsifiers and stabilizers may also improve the stability.

The addition of flavourings and sugar influences the acceptability of plant-based milk (Sethi et al. 2016). A chalky mouthfeel may be caused by insoluble large particles in the drink (Aydar et al. 2020).

B. Nutritional completeness

The plant source, processing, and fortification affect the nutritional properties of plant-based milk (Silva et al. 2020). Most plant-based milk alternatives lack nutritional balance when compared to animal milk. They often contain added sugars and lack protein content (Aydar et al. 2020). Oat milk can be fortified with calcium, an essential nutrient for growth and development, before being placed in shops and consumed as a milk alternative (Sethi et al. 2016).

However, plant-based milk alternatives contain functionally active components with health-promoting properties, which appeals to certain consumers (Sethi et al. 2016). Plant-based milk alternatives are excellent substitutes for animal milk products, which is due to their content e.g., of phenolic compounds, antioxidants, unsaturated fatty acids, and bioactive compounds e.g., phytosterols and isoflavones (Aydar et al. 2020).

C. Shelf life

Heat treatment is used to extend the shelf life of plant-based milk products by reducing or eliminating the micro-organisms being in the plant-based liquid (Sethi et al. 2016). However, excessive heating can cause cooked flavor, browning, and harmful effects on nutrients e.g., vitamins and amino acids in the drink. Various time-temperature combinations can be used to avoid harmful effects.

The relatively high starch content (55–60%) of oats creates a problem in the heat treatment process of the oat drink and in the production of a stable emulsion (Sethi et al. 2016). The gelatinization of the starch begins when the oat drink is exposed to heat, resulting in a gel-like consistency. The gelatinization temperature range of oat starch is 44.7–73.7°C (Tester and Karkalas, 1996). High viscosity results in lower acceptability of the oat products. Gelatinization during heat treatment can be prevented by starch hydrolysis, thus the liquid maintains its consistency.

V. CONCLUSIONS

The market for plant-based products is growing. Plant-based milk is a valuable alternative for consumers who do not consume animal milk. The main challenge is to provide both palatable plant-based milk alternatives of sufficient nutritional value. These products should also be sensory acceptable. The educational material is needed for students at different levels to enable the food industry to develop vegan foods successfully. Furthermore, fortification of the oat drink is needed to produce nutritionally adequate products. The final characteristics of the oat drink can be optimized during the production by combining various process parameters.

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Our take on sodium intake among Croatian children with celiac disease: sodium content of their gluten-free diets and the contribution of commercial products

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Abstract - **Excess sodium intake in children is related to lower overall diet quality and a greater hypertension risk later in life. Previous research has shown that sodium intake among Croatian adults is more than two times higher than the current World Health Organization (WHO) recommendations. Daily sodium intake in children worldwide often exceeds the recommended intakes as well. However, the data on sodium intake among Croatian children are scarce, especially in those with celiac disease. Many gluten-free products found on the market are highly processed and also high in sodium. The aim of this study was to determine dietary sodium intake as well as exact sodium sources in the diet of children with celiac disease. Sodium intake of eight participants (aged 3-15) following a gluten-free diet was assessed using the duplicate diet method and two-day dietary records in order to obtain both qualitative and quantitative information on all food consumed. A total of 16 samples of whole-day gluten-free meals were analysed using flame atomic absorption spectrometry (AAS) to obtain the sodium content (mg/kg). Average daily sodium intake was 2389 ± 933 mg which equals 6.0 ± 2.3 g of salt intake. Most of the participants' daily intakes exceeded recommendations. On average, 14 % of daily sodium was naturally present in consumed food, while as much as 86 % came from either processed food and food products (27%), commercial gluten-free products (29%) or table salt added during meal preparation (30%). These results indicate that daily sodium intake could be easily manipulated. Educating parents and children to choose products lower in sodium, substituting table salt with different herbs and spices during meal preparation and preparing homemade meals should be a priority.**

Keywords - **sodium, children, celiac disease, gluten-free, salt**

I. INTRODUCTION

Sodium is the most important extracellular cation, necessary for nerve signalling and maintaining osmotic pressure. It also regulates body water volume and distribution through kidney retention or excretion, along with hormones such as renin, aldosterone, and hypothalamic anti-diuretic hormone. The main source of sodium in everyday diet is table salt. It has an important role in industrial food processing due to its ability to regulate fermentation, enhance and intensify flavours, and as a preservative agent. Certain amount of sodium is necessary for optimal metabolic function. However, high sodium intake is directly related to increased systolic blood pressure, and a greater risk for pre-hypertension and hypertension (Yang et al. 2012). This effect was shown to be even stronger when it comes to overweight and obese individuals. Yang et al. (2012) have found that, among USA children and adolescents, each 1000 mg increase in daily sodium intake led to a 74% higher risk for pre-hypertension and hypertension in the overweight and obese group, while that risk was only 6% higher in those with normal weight. Furthermore, one in six children in the USA already have a raised blood pressure, predisposing them for hypertension later in life (CDC, 2018). Croatia follows the worldwide trend, with cardiovascular disease being the leading cause of death at the moment, and obesity being a major risk factor (WHO, 2020). Croatian obesity rates are among the highest in Europe, with 65% of adult population and 35% of children being overweight or obese (Musić Milanović et al. 2021; Eurostat, 2021). Already Ha (2014) advised that a moderate reduction in sodium intake would have major beneficial effects, especially in the realm of cardiovascular disease. Most importantly, it reduces blood pressure in both children and adults, and may lower the risk for hypertension and further cardiovascular complications later in life (He and MacGregor, 2006).

A link has also been found between sodium intake and diet quality in children. High sodium intake is often related to the consumption of processed food, high in calories and rich in sugar and fat, as well as that of sweetened beverages (Gowrishankar et al. 2020). In other words, sodium intake and overall diet quality in children are often inversely associated, and strongly linked with obesity rates.

Celiac disease and total elimination of gluten from the diet can be challenging, especially at a young age. With the gluten-free diet being the only known "cure" for celiac disease, parents and their children are faced with a difficult task – they must ensure a balanced diet that fulfils all the nutritional requirements and prevents malnutrition but at the same time omits all wheat-, rye- and barley-containing

products. Despite the growing presence of gluten-free products on the market, many studies still report the gluten-free diet to be inadequate, with the most common deficiencies being those in fibre content, vitamins D, B_{12} and folate, and minerals such as iron, zinc, calcium, and magnesium (Vici et al. 2016). Because of their availability and practicality, many celiac patients rely on commercial gluten-free products every day. However, some studies highlight their high glycaemic index, as well as a high lipid and salt content (Myhrstad et al. 2021; Tres et al. 2020; Fernandez et al. 2019; Fry et al. 2018).

A clear conclusion regarding dietary sodium in both adults and children was reached by Aburto et al. (2013) – a reduction in sodium intake also reduces blood pressure.

The aim of this study was to determine dietary sodium intake of Croatian children with celiac disease on a gluten-free diet, as well as the contribution of different sodium sources to their diet through commercial products.

II. MATERIALS AND METHODS

A. Participants

A total of 8 children (of which 25% male), aged 3 to 15, were involved in the study, with the assistance of their parents All the participants have been previously diagnosed with celiac disease and are following a strict gluten-free diet. The study was approved by the University of Zagreb, Faculty of Food Technology and Biotechnology Ethics Committee. Each participant provided written consent and had the opportunity to withdraw from the study at any given moment.

B. Dietary intake assessment

Dietary intake was assessed using the duplicate diet method and weighed food records for two random, non-consecutive days of the week. Overall, 16 samples of whole-day meals were collected. Upon reception, each sample was homogenized, divided into smaller portions, and stored frozen until further analysis. Additionally, all the participants kept track of their dietary intake using food diaries. Information was obtained on time and place of meal consumption, meal content, net weight of each ingredient and thermal processing methods. Participants were also required to supply information on food brands and nutrition label of all packaged gluten-free products.

C. Sodium content analysis

Prior to the analysis, all the samples were brought to room temperature. After acid digestion of ~0.2 g of each sample with 7 mL of 60% nitric acid and 1 mL of 30% hydrogen peroxide in the microwave (Ethos Easy, *Milestone*, Italy), all the samples (marked GF1 – GF16) were transferred to measuring flaks (V = 25 mL) and supplemented with water to the mark. Determination of sodium (Na) was performed by flame atomic absorption spectroscopy (200 Series A4 with SPS 4 Autosampler, *Agilent Technologies*, USA) at λ = 589 nm. Salt content of each meal was calculated using the following formula:

$$\text{m (salt), g} = \frac{\text{m (Na), mg} * 2.5}{1000} \quad (1)$$

D. Food record analysis

All the foods consumed were divided into four groups, according to their origin of sodium and degree of processing. Foods such as fruit, vegetables, milk, and milk products were considered to be minimally processed, and sodium in such products naturally present. A distinction was made between commercial products which were industrially processed, i.e. sodium was added during the manufacturing process, but were naturally gluten-free, and those who contained a gluten-free claim. The fourth group was the most variable, as it considered both the salt that was added during cooking and at the table during meal consumption. To sum up, the four groups were:

1. minimally processed food/sodium naturally present (NP),
2. commercial gluten-free products/sodium added during processing (GFP), naturally gluten-free processed food/sodium added during processing (PF), sodium contained in table salt (TS).

In order to calculate the contribution of each food group, Croatian national Food and drink composition tables (Kaić-Rak and Antonić, 1990) were used, in addition to packaging label data, when available. Food records contained no information on the amount of salt added during food preparation or at the table. Therefore, TS was calculated as a difference between the analytical value and the remaining three groups:

$$\text{TS} = \text{daily sodium intake}_{\text{analytical}} - (\text{NP} + \text{GFP} + \text{PF}) \quad (2)$$

III. RESULTS AND DISCUSSION

Table 1. presents exact sodium contents of each sample and daily meal, accordingly. The GF11 sample had the highest sodium concentration, while the GF8 sample had the lowest concentration. However, when meal volumes were considered, participant 3 had the highest sodium intake, whereas the intake of participant 2 was the lowest.

Table 2. Sodium concentration and salt content of each gluten-free sample (GF) and daily meal

Participant	Sample	Sodium concentration [mg/L]	Salt content of the meal [g]
1	GF1	2231.90	8.8
	GF2	1429.82	5.2
2	GF3	1673.45	5.1
	GF4	1205.35	*2.7*
3	GF5	1586.50	*9.5*
	GF6	1186.35	8.0
4	GF7	1163.11	3.7
	GF8	*937.09*	3.4
5	GF9	1895.11	5.3
	GF10	1141.97	3.4
6	GF11	*2459.60*	5.7
	GF12	1730.23	5.9
7	GF13	1344.38	6.5
	GF14	1994.36	9.3
8	GF15	2073.85	9.1
	GF16	1679.49	4.1
average ± SD		1608.29 ± 439	6.0 ± 2.3

The average daily sodium intake of Croatian children following a strict gluten-free diet was 2389 ± 933 mg, which equals 6.0 ± 2.3 g of salt (Table 1.). Among Croatian adults, the average sodium intake estimated using 24-hour sodium excretion method, is equal to 4.5 g/day, equivalent to 11.6 g of salt daily (Jelaković, et al. 2009). According to the World Health Organisation (WHO), the recommended sodium intake is less than 2 g/day – an amount which Croatian adults greatly exceed (WHO, 2012). Sodium intake for children is not defined, but a downward adjustment of 2 g/day is recommended, based on their energy requirements (WHO, 2012). European Food Safety Authority (EFSA), on the other hand, has defined specific dietary reference values for sodium intake in different age groups, both for children and adults. EFSA Dietary reference values for sodium (2019) are also the most recent and were therefore used as a reference. When sodium intakes of participants were compared with EFSA reference values (Figure 1.), major variations were visible. Sodium intake of only one participant (12.5%) was in accordance with the reference values for their age group, while the rest of participants exceeded them during at least one of two days. Overall, both sodium intakes of three participants (37.5%) exceeded the suggested values by 30 to 90%, and a total of four participants (50%) consumed 24 to 84% more sodium than recommended during one of two days. Likewise, Marrero et al. (2014) have shown that 70% of London children and adolescents consume more sodium than recommended for their age.

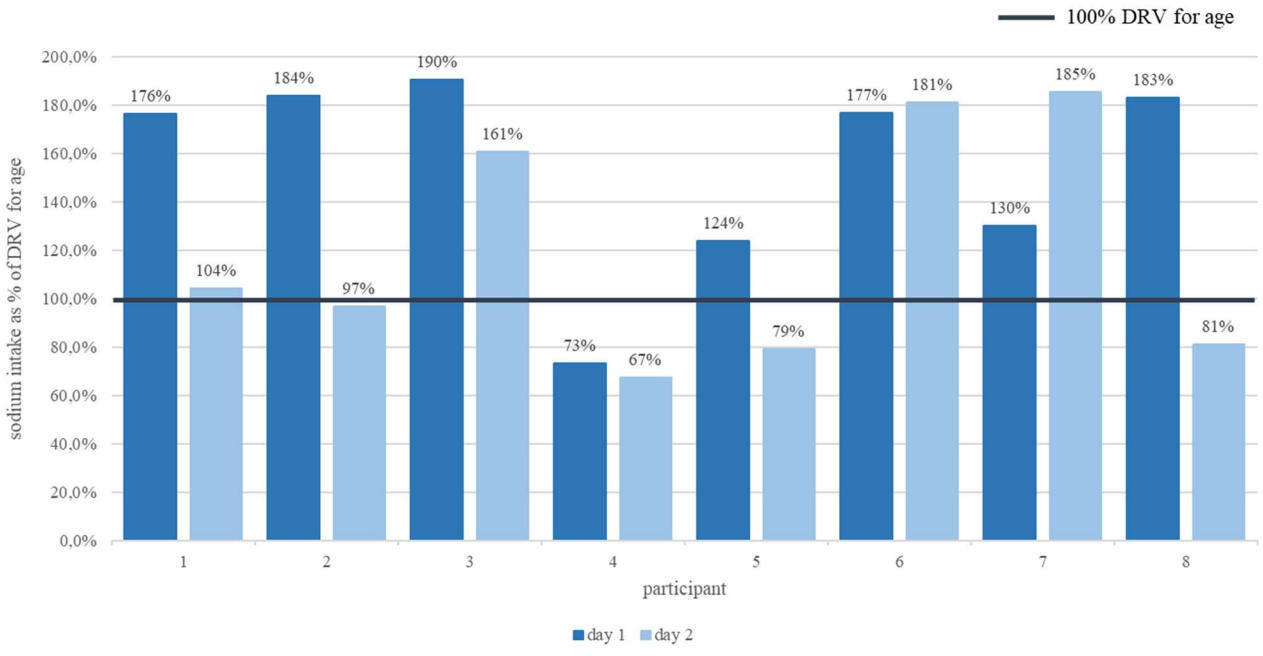

Figure 1. Participants' daily sodium intake compliance with EFSA Dietary reference values (2019)

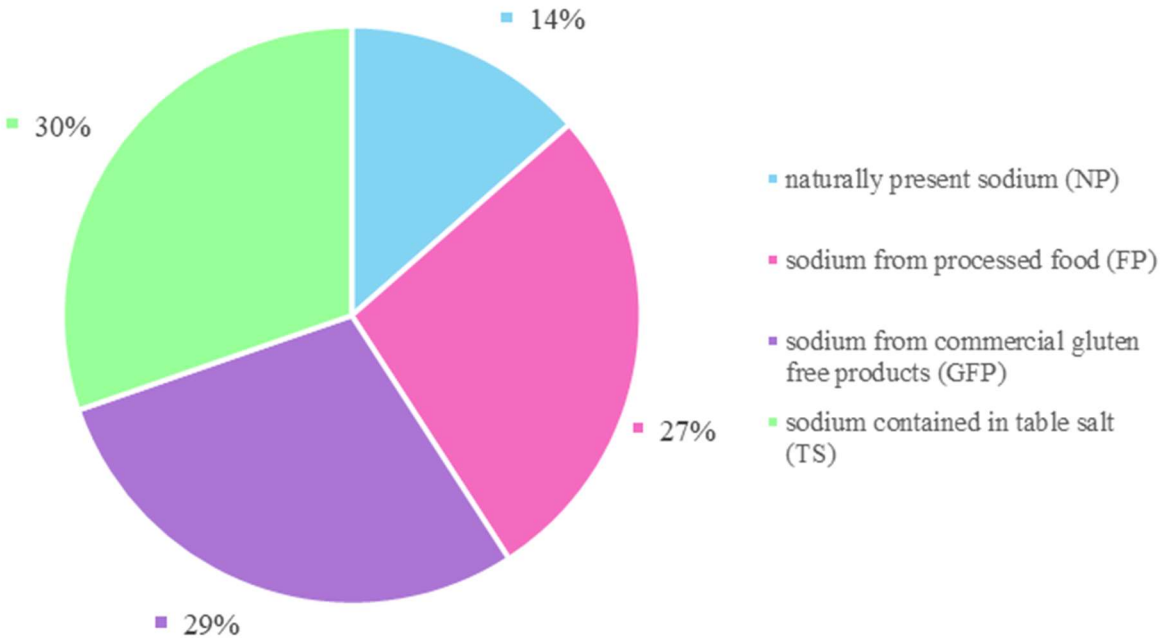

Figure 2. Sodium sources in participants' gluten-free diet and the contribution of each group

Furthermore,food record analysis has shown that food in which sodium does not naturally occur but is instead added during industrial or thermal processing contributed greatly to participants' daily sodium intake. As it is shown in Figure 2., 14% of average daily sodium was naturally present in foods such as fruit, vegetables, and milk products. Commercial gluten-free products (GFP), processed food (PF) and table salt added during cooking or at the table (TS) provided the remaining 86% of daily sodium. However, there was no significant difference among the contribution of each food group. More than 30 years ago, Mattes and Donnelly (1991) have found that the contribution of sodium which is added during processing of foods such as meat and bakery products, but also ready-to-eat meals to the average daily sodium intake is as much as 77%, while 11,3% was added during cooking or at the table, and the rest was naturally present in food. The difference in table salt addition in this study could be contributed to the approximation of TS amounts, or to the fact that due to their condition, celiac disease patients find homemade meals the safest option, meaning that table salt is used more often. Although following a slightly different classification system, other studies have also proved that ultraprocessed and processed food contribute greatly to the daily sodium intake (48% and 34 to 36%, respectively), whereas the contribution of minimally processed food, comparable with NP, is far smaller (17 to 19%) (Cuadrado-Soto et al. 2018; O'Halloran et al. 2016). However, most of these studies have been conducted on a generally healthy population. The data on sodium intake among Croatian children is scarce. Studies regarding children and adolescents with specific health and dietary requirements are generally lacking, especially among those dealing with celiac disease. This is exactly the main strength of this study, as it is one of the first of its kind to bring forward important questions regarding sodium intake and dietary choices of Croatian children following a strict gluten-free diet from an early age. It is, however, not without limitations, the most important being a small sample size and short duration, mostly due to the limitations of the duplicate diet method itself. Not only does it require a high level of engagement on behalf of participants (and in this case, their parents), but it is also financially demanding, knowing that gluten-free products can be up to 113% more expensive than their gluten-containing counterparts (Myhrstad et al. 2021).

IV. CONCLUSIONS

Dietary sodium intake of Croatian children with celiac disease following a strict gluten-free diet exceeds the

recommended values for their age in most (87,5%) cases. Dietary choices have the greatest impact on daily sodium intake, therefore, educating children and their parents on the živ most important sodium sources can have a beneficial effect on their cardiovascular health and should be made a priority Sodium intake can easily be manipulated by choosing gluten-free products lower in sodium, preparing homemade meals from natural ingredients in place of processed food consumption, and substituting table salt with different herbs and spices.

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Are Italian parents willing to accept that their children eat insect-based products? A preliminary study

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Abstract – **The challenge to ensure access to safe and sustainable animal protein has been deeply shifting food systems from intensification of production toward an economy that is increasingly circular, looking at waste prevention, recovery and valorization. Among the strategies put in place at the EU level, insects, approved as novel food with the Regulation (EU) 2015/2283, have been warmly promoted for human nutrition. However, European consumers are still reluctant to accept entomophagy, perceived as a primordial practice. This barrier could be possibly overcome if insects are introduced into the diet during childhood. In this sense, public canteens in kindergartens or elementary schools could introduce insect-based products to make the consumption of these food familiar starting from young age. Due to these premises, our preliminary study aimed at understanding if parents are prone to accept that the canteen service would offer to their children (3-10 years old) a traditional Italian food, namely bread, containing a percentage of flour made with insect (*Tenebrio molitor*). This study uses data collected from an online survey on 206 Italian parents. The questionnaire included a contingent valuation with a *Double bounded dichotomous choice* format. The respondents had to elicit if they are willing to accept the insect-based bread for their pupils in exchange of a given discount on the school lunch fee (10%, 20%, 30%, 40% and 50% discount, allocated randomly to each participant). Additional questions collected information about the children's favorite food offered by the canteens, children's food fussiness, family eating habits, the respondents' degree of neophobia and their health concerns. Results showed that parents, mostly neophobic, are not willing to approve the introduction of insects in children's school lunch, even in exchange for a discount. On the contrary, excessive discounts seem to have been perceived as indicating poor quality of the bread containing insect flour. This rejection seems to be rooted in the fear of giving their children food they are unfamiliar with and about whose safety they have very little information. In fact, only 36.2% are sure that insects have no negative consequences on human health.**

Keywords – **Novel food, *Tenebrio molitor*, Double bounded dichotomous choice, food fussiness**

I. INTRODUCTION

Food production is responsible for up to 30% of anthropogenic greenhouse gas emissions (10-12 Gt CO_2-eq/year) (Smith et al. 2014), about 70% of fresh water consumption (2,663 km^3/year) (World Water Assessment Programme, 2009), and more than a third of all arable land (Aleksandrowicz al. 2016). Alongside these data, the environmental, economic and social concerns deriving from food loss and waste throughout the supply chains are growing (Stenmarck et al. 2016). Furthermore, Western and emerging countries have been suffering the consequences of a poor diet during the last decades, due to the increase in the consumption of calories, proteins of animal origin, saturated fats and ultra-processed products (Ranganathan et al. 2016), together with the change in lifestyle with an increased sedentary lifestyle. In this context, food systems are moving from "intensification" to a production consistent with the principle of circularity, prevention of loss and waste, recovery and enhancement of waste, up to proposing new foods.

Insects seem to represent a concrete solution to reach these goals. Indeed, insects are a source of proteins with a high biological value, and their breeding, based on principles of circular economy (insects can be raised on substrates of various kinds), has been recently proposed a sustainable model due to its reduced impacts (from water consumption to CO_2 emissions) (FAO, 2021). Hence, the consumption of edible insects, called entomophagy, has been recently warmly suggested (Orkusz, 2021; Puzari, 2021). The European Regulation 2015/2283 approved insects as novel food but, to date, only few species have been inserted in the authorized list. *Tenebrio molitor*, a beetle of the Tenebrionidae family, is one of these. Despite the need for alternative and sustainable foods and the recent authorization received in EU, consumers are still reluctant to accept insects as a part of their diet since they tend to invoke a strong negative psychological reaction in subjects, such as disgust (Dobermann et al. 2017). This barrier could be possibly overcome if insects are introduced into the diet during childhood. In this sense, public canteens in kindergartens or elementary schools could introduce insect-based products to make them familiar to consumers, starting from young age. Standing on these premises, our preliminary study aimed at understanding if parents are prone to accept that the canteen service would offer to their children (3-10 years old) a traditional Italian food, namely bread, containing a percentage of flour made with insect (*Tenebrio molitor*).

II. MATERIALS AND METHODS

This study uses data collected from 206 online interviews in Italy in November and December 2021. Respondents were invited to participate in the survey using mailing lists and social networks. The respondents were voluntary, and they did not receive gifts or compensation as a reward for participating in the survey. Experiment participants were parents or responsible for a pupil (3-10 years old) who attended kindergartens and elementary schools and used the schools' canteens. The contingent valuation method (CVM) was utilized to elicit consumers' willingness to accept (WTA) (Nakatani et al. 2014). The bidding procedure applied in the CVM was the double-bounded dichotomous choice model. The respondents had to elicit if they were willing to accept that bread their children eat contained flour made with *Tenebrio molitor* in exchange for a discount on the school lunch fee. The discount was set at one of the following levels: 10%, 20%, 30%, 40%, or 50%. Each respondent faced one of the discount levels assigned randomly. For the respondents who accepted the discount, a follow-up question repeated the offer with the next lower-level discount. Instead, the respondents who refused the discount the same follow-up question offered the next higher-level discount. Bread was appropriate for this experiment because this product is frequently consumed by the Italian families.

Additional questions collected information about the children's favourite food offered by the canteens, children's food fussiness (Smith et al. 2017), families' eating habits, sociodemographic characteristics of respondents and their families, the respondents' degree of neophobia and their health concerns.

The WTA function for bread with insect flour for individual i was:

$$WTA_i = \alpha + \gamma BID_i + \beta' z_i + \varepsilon_i \text{ for } i = 1, .., n \quad (1)$$

where BID is the discount level respondent i faces; ε is an error term that gathers noise and unobservable characteristics; instead, z_i is a vector of observable characteristics of the individual or his/her family (Nakatani et al. 2014). This vector contains age, education, gender of respondent, his or her concern for health, pupil's fussiness, age of pupil, pupil food intolerance, religious prescription, and two variables to control for peers' support in eating insects. Finally, α, β, and λ are the parameters to be estimated.

III. RESULTS

The majority of the respondents were female (86.7%) with an age ranging from 31 and 45 years. Almost 50% of the participants declared an academic degree (bachelor's degree, master degree or master). The percentage of the respondents who would accept the insect-based bread in exchange for a discount on the school lunch fee is displayed in Table 1. The greater acceptability was recorded at a 10% discount (18 subjects out of 46 with this discount would agree to replace traditional bread with bread made with insect flour), while the lowest acceptability was found at the highest proposed discount, i.e., 50%, where only 10 out of 39 subjects would accept the change in the menu. Irrespective of the proposed discounts, only the 29% of the total respondents would accept the insect-based bread.

Table 1. Distribution of response rates to the randomly assigned discount at the first and second bid.

Yes to discount	First bid	Second bid
10% or lower	8.7%	9.7%
20%	4.9%	16.5%
30%	4.9%	18.4%
40%	8.3%	13.6%
50% or upper	4.9%	22.3%
Negative response	68.4%	19.4%

Table 2. shows the items considered to evaluate the effect on the subjects' acceptability towards the offered product. Among the others, only neophobia and age significantly affected ($p<0.05$) the willingness to accept the adoption of insect-based bread in children's canteen.

Considering the results of food fussiness, as many as 170 out of a total of 206 respondents obtained fairly high values on the Food Fussiness Scale, highlighting that the majority of parents considered their child highly selective towards the foods.

Table 2. List of the considered covariates to evaluate what factors would affect the acceptability of subjects towards the offered product.

	Estimate	Standard Error	P-value
Intercept	-2.14	1.66	0.20
Shame on the child	0.05	0.32	0.87
Shame on the parents	0.19	0.30	0.52
Health	0.02	0.02	0.34
Fussiness	-0.02	0.01	0.22
Neophobia	-0.04	0.01	0.00
Age (under 35)	0.67	0.29	0.02
Education	0.05	0.27	0.84
Gender	-0.21	0.44	0.63
School year	-0.41	0.28	0.15
Food intolerance	0.49	0.49	0.32
Religion	-0.28	1.07	0.80

In addition to this, 53.8% of respondents argued that including insect-based products in children's diets would lead them to be negatively judged by friends, relatives, and colleagues in their role as parents. Moreover, from the questionnaire emerged a clear concern for children's health, probably derived from the scarce information about insects. In fact, only 36.2% was sure that insects have no negative consequences on human health, while the remaining 63.8% was divided into people who did not know if the consumption of insects can represent a health hazard (40.5%) and people who thought it is (23.3%). Finally, we remarked that if a general information concerning the nutritional advantages of eating insects and the low environmental footprint was provided to the parents, the percentage of respondents who accepted to include insect-based products in the school canteen was 45.2%.

IV. DISCUSSION

As a rule, the acceptability towards the good or service offered increases while increasing the discount value. On the contrary, in the present case seems that the discount offered discouraged the parents. This phenomenon could be linked to the fact that individuals, usually, when they do not know a product, tend to evaluate it on the basis of extrinsic characteristics such as, for example, the origin or the price. Consequently, a high discount may have been perceived as a signal of product low quality thus leading the subject to reject it. All the control variables were not statistically significant except age (with a positive sign) and neophobia (with a negative sign). This proves that only young and not neophobic respondents were more prone to accept that their children eat insect at school in exchange of a discount on school lunch fees. Past studies found similar results such as Tan et al. (2016) who highlighted that insect-based products are still perceived by the Western population as low-quality products, thus explaining the very low WTA value obtained here irrespective the proposed discount. In line with the study of Lombardi et al. (2019), only age and neophobia significantly affected the WTA.

Recently, a high value of food fussiness was negatively associated to variety of the diet and optimal nutritional intake (Volger et al. 2017), thus leading to consequences such as functional constipation and low weight in early childhood (Viljakainen et al. 2018). This attitude towards food could therefore represent an obstacle to the introduction of insects-based food into the canteen menu.

It is well established that information positively influences willingness to try a novel food. In the specific case of insects, it has been previously noted that informing consumers about sustainability and environmental perspectives might increase insect-based food WTC (Laureati et al. 2016). Present results confirmed this previous evidence; indeed, the percentage of parents who accepted to include insect-based products in the school canteen rose to 45.2% (from 20%) when information about the nutritional value and the environmental benefits of insect consumption was provided.

V. CONCLUSIONS

Considering the challenge to ensure access to safe, nutritious and sustainable foods, it would seem logical to continue to work to inform and educate people in order to direct them towards sustainable insect-based products. Strategies based on dissemination, communication and tasting sessions should be developed to enhance children' and parents' familiarity with insects.

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SAFETY

Knowledge, attitudes, and practices of hand washing in a supermarket chain in Croatia

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Abstract - **The purpose of this study was to determine the level of knowledge about hand washing, attitudes, and practices (KAPs) of food handlers in units with different food safety risks and the impact of demographic parameters on these variables. Questionnaires on hand washing knowledge and self-assessed attitudes and practices were distributed to 117 food handlers in 14 facilities of a supermarket chain in the Republic of Croatia. Employees' knowledge and practices regarding hand washing were further assessed using a questionnaire given to unit leaders and facility managers (N=54). Normality of variables was tested using the Shapiro-Wilk test. ANOVA and post hoc Tukey tests were performed to test for differences in normally distributed variables (knowledge scores). Mann-Whitney and Kruskal-Wallis tests were performed for non-normally distributed variables (attitudes and practices). The Spearman correlation coefficient was used to measure the degree of association between KAPs. The average level of knowledge about hand washing was acceptable and consistent with the other studies. Knowledge about pathogens transmitted by unclean hands should be improved. None of the demographic parameters influenced the knowledge scores (gender, work experience, food safety education, training, unit, or risk level). On the other hand, attitudes and practices differed between units. A positive correlation was calculated between attitudes and practices, demonstrating the importance of raising awareness to ensure food safety. However, employees' self-reported practices did not fully match leaders' perceptions of hand washing, implying that certain actions should be taken to further improve hand washing practices.**

Keywords- **hand washing, supermarkets, knowledge, attitudes, practices**

I.INTRODUCTION

Food contamination mainly occurs due to improper food handling, resulting in numerous foodborne incidents worldwide (Chekol et al., 2019). Supermarket chains are not often associated with foodborne outbreaks and incidents, but they fall into the category of foodservice operators who prepare ready-to-eat foods, which in some cases can be classified as high-risk foods. Foodservice operators include restaurants, school and hospital canteens, catering operations, and some of these establishments were implicated in most outbreaks in the EU in 2017 (EFSA, 2018).

The term "high-risk" refers to a product, operation, or facility. In Croatia, foodservice operators can be officially classified as low, medium, or high risk establishments. De Andrade et al. (2020) have shown that employees of establishments that are more compliant with mandatory programs have higher scores on food safety knowledge and other variables such as organizational commitment and food safety climate than employees of establishments where food safety violations occur.

Supermarkets in Croatia are classified as low or medium risk facilities, but due to their complexity and numerous departments, they usually have units with all three risk levels. When establishing a quality control system, many facilities including supermarkets, opt for stricter requirements to ensure better food safety and quality. In high-risk units, hygiene is critical, so food safety training is more frequent to ensure food safety.

Supermarket chains, as mentioned earlier, are not often associated with food incidents. However, an important limitation of the data presented in any foodborne outbreak report is that information on the causative agents, suspected food vehicle or food business operator type is missing. For example, in 2017, more than 33% of outbreaks reported to EFSA lacked this information (EFSA, 2018). On the other hand, supermarket chains are an ideal system to study the level of food safety knowledge, attitudes, and practices related to different unit risk levels.

The application of the Knowledge, Attitudes and Practices (KAPs) model is a commonly used tool to ensure food safety (Da Cunha, Stedefeldt & De Rosso, 2014). The premise of the KAPs model is that the food handler, provided with knowledge, is able to improve his or her food safety practices. Studies on food safety knowledge or KAPs have rarely been conducted in Croatia. The only published study on food handlers' food safety knowledge was conducted in student canteens in 2019, (Vidaček Filipec et al., 2020), while the application of the KAPs model was not reported.

The aim of this study was to determine the level of knowledge about hand washing, attitudes, and practices of food handlers in units with different food safety risks in the supermarket chain in Croatia, as well as the impact of demographic parameters on these variables. Considering that the COVID-19 pandemic raised hand washing awareness, the study hypothesized that hand washing KAPs are generally appropriate but differ across units with different levels of risk.

II. MATERIALS AND METHODS

Questionnaires on hand washing knowledge and self-assessed attitudes and practices were distributed to 117 food handlers in 14 facilities of a supermarket chain in the Republic of Croatia. In terms of organization within each facility, employees may be assigned to one of 8 units categorized according to risk level as follows: Meat, Fish and Gastro (high risk); Bakery, Fruits and vegetables, Delicatessen (medium risk); Dairy and Packaged products (low risk). One of the food handlers in each unit is the leader responsible for supervising the others. Training sessions are organized at least once a year by the senior management, but at the individual unit level they are organized more frequently and are usually held by the unit leader. The questionnaires were distributed to the quality control manager of each facility, who was responsible for organizing their implementation. For the development of the questionnaire, the instruments from previous studies on KAPs were used, which were then modified to clarify the hand washing issues. The final structure of the questionnaire was validated by three experts. Having completed pilot testing with 16 employees in one facility to verify an understanding of the terminology, the questionnaire structure was finalized. It was approved by the Ethics Committee of the Faculty of Food Technology and Biotechnology, University of Zagreb, Croatia.

The questionnaire consisted of two parts. The first part was used to collect the demographic data of the respondents, while the second part was to assess the KAPs on hand washing. Knowledge of hand washing was expressed as the mean of correct answers. Awareness of the risk of contamination if hands are not washed prior to food preparation or handling represented attitude and was interpreted as positive (i.e., appropriate) if respondents checked that the risk was very high (possible responses were no risk; low risk; 50% risk, high risk; very high risk). Adequacy of practices was assessed by asking how often (never, sometimes, always) food handlers perform proper hand washing procedures.

Employees' knowledge and practices regarding hand washing were assessed using a questionnaire administered to leaders and managers (N=54). They were first asked about the frequency and content of training and hand washing procedures and then to give their opinion on 2 statements ("Food handlers know how and when to wash their hands" and "Food handlers wash their hands regularly and appropriately"). Results were expressed as percentage of those who fully agree, partially agree, or disagree with the statements. The results were analyzed using the SPSS 17.0. program. Normality of variables was tested using the Shapiro-Wilk test. ANOVA and post hoc Tukey tests were performed to test for differences between demographic groups on normally distributed variables (knowledge; later expressed as means). Mann-Whitney and Kruskal-Wallis tests were performed for variables that were not normally distributed (attitudes and practices; later expressed as means, MR). The Spearman correlation coefficient was used to measure the degree of association between KAPs.

III. RESULTS AND DISCUSSION

Knowledge, attitudes, and practices about hand washing are shown in Table 1. 24.8% of food handlers do not know that cotton towels can be a source of pathogens and that they are not recommended for hand drying. The fact that more than 50% of the surveyed employees do not know that

Table 1. Knowledge, attitudes and practices (KAPs) on hand washing (N=117)

Knowledge	**Correct answer (%)**
1. How long should you wash your hands?	30 sec (97,4)
2. Which pathogens may be transmitted by unwashed hands?	*Salmonellae* and Noroviruses (45,3)
3. Mark the incorrect statement about hand washing?	Hands should be wiped with a cotton towel after washing (75,2)
Attitude	**Correct answer (%)**
What is the level of risk for microbiological contamination of food if you do not wash your hands before preparing or handling food?	Very high (73,5)
Practices	**Correct answer (%)**
1. How often do you wash your hands immediately before handling food?	Always (95,7)
2. Do you wash your hands often enough during the working day as stated in the instructions?	Always (89,7)
3. How often do you wash your hands exactly as instructed?	Always (73,5)

Salmonella spp. and noroviruses are both transmitted through unwashed hands proves that there is a lack of education about noroviruses, but also about pathogens in general. In terms of attitude, 26.5% of food handlers do not believe that the risk is very high if they do not wash their hands before preparing or handling food. Despite being educated on hand washing practices, only 73.5% of employees surveyed always wash their hands exactly as instructed. Almost all employees always wash their hands immediately before handling food. Slightly fewer (89.74%) stated that they always wash their hands often enough according to the instructions, for example after touching their face or after going to the toilet.

Almost all leaders and managers, i.e. 98.15%, agree with the statement that employees know when and how to wash their hands, but 24.07% of them believe that they only partially wash their hands regularly and properly (results not shown).

Table 2. shows whether demographic parameters have an impact on KAPs. Of the 117 food handlers, 90 (77%) are women. The literature review shows that demographic characteristics in food service are partially dependent on the type of establishment. Restaurants and bars employ more men (Pichler et al., 2014; Smigic et al., 2016), while female employees make up the majority of food handlers in institutional kitchens or school canteens. Almost all respondents in this study, 99% of them, have a high school degree. In terms of years of work experience in the food sector, food handlers are equally represented in each category.

The mean knowledge score on hand washing was 72.7 ± 23.0, with the highest knowledge score in Fruits and vegetables unit. The calculated knowledge score is in line with the results on general food safety knowledge from other studies conducted in Europe in food service units (Pichler i sur., 2014, Smigic i sur., 2016), and is much higher than the study conducted previously in Croatia in student canteens, where the mean knowledge score was 54.1% (Vidaček Filipec et al., 2020).

There was no statistical difference in knowledge as a function of unit or unit risk level. In addition, none of the demographic parameters had an impact on hand washing knowledge. Education and training are often determinants of knowledge gain (Gruenfeldova et al., 2019), but in this study, all respondents had food safety training, and only two were not trained at the facility. On the other hand, attitudes and practices differed among respondents from different units.

Hand washing awareness was the highest among employees in Fruits and vegetables and Gastro units (all respondents were very aware, i.e., 100% correct responses; results not shown), but with a significant difference from those working in the meat and deli units. Hand washing practices were best in the Gastro unit (92.2%, not shown) and least in the Dairy unit (47.5%).

Table 2. Knowedge, attitudes and practices (KAPs) by demographic characteristics (different letters indicate statistically significant differences in KAPs between categories of demographic characteristics)

		Knowledge score (KS)	**Attitudes**	**Practices**
Total	N	**Mean ± st.dev**	**Min-Max**	**Min-Max**
	117	72,7 ± 23,0	80,0-100,0	40-92,6
Per demographic characteristic	**N**	**Mean**	**Mean Ranks**	**Mean Ranks**
Unit				
Meat	21	73,0	49,4c	57,0ab
Fishery	10	63,3	56,9b	55,0abc
Gastro	23	71,0	74,5ab	85,9a
Bakery	17	78,4	64,1b	72,7ab
Fruits and vegetables	7	81,0	74,5ab	66,6ab
Deli	22	74,2	47,9c	39,2bc
Dairy	4	58,3	59,8b	28,6c
Packed products	13	71,9	52,0bc	38,2abc
Gender				
Female	90	72,2	61,4	58,8
Male	27	74,1	51,1	59,8
Total work experience				
< 2 years	7	81,0	57,8	67,6
2-8 years	35	77,1	62,8	63,4
9-16 years	30	74,4	55,0	58,3
17-25 years	34	67,6	59,0	58,3
> 25 years	11	63,6	58,5	43,6
Work experience in this company				
< 2 years	11	72,7	58,5	57,7
2-8 years	52	78,8	58,8	62,8
9-16 years	30	71,1	60,9	50,1
17-25 years	23	62,3	56,7	62,1
> 25 years	1	33,3	74,5	71,0
Education in food safety				
yes, before starting to work	112	73,5	59,4	58,3
yes, I am previously educated in the field	5	53,3	51,1	75,5
No	0			
Food safety training in this company				
yes, by the colleague	88	75,0	61,9	59,8
yes, by external consultant	27	66,7	52,8	57,1
I did not have training	2	50,0	16,0	50,8
Risk level of the unit				
High	54	70,4	61,5	69,0a
Medium	46	76,8	58,0	55,7b
Low	17	68,6	53,9	35,9c

Table 3. Correlations between KAPs

Level	Spearman rho (N=117)	p
knowledge - attitude	0,126	n.s.
knowledge -practices	-0,188	p<0.05
attitude-practices	0,189	p<0.05

Table 3. shows the correlations between knowledge, attitudes, and practices. A positive significant correlation was calculated between attitudes and practices, implying that employees need to be made aware of food safety risks from unwashed hands and be aware of their role in ensuring appropriate practices. However, knowledge and attitudes were not related, and knowledge and practices were negatively correlated. A number of studies focused on identifying the links between KAPs. Zanin et al. (2017) reviewed 36 scientific papers, in which the relationship between these three variables was compared, and found that knowledge had an influence on attitudes or practices in about 50% of the papers. Our findings contribute to the body of work suggesting that knowledge acquired through training does not transfer to attitudes or appropriate practices.

IV. CONCLUSION

KAPs related to hand washing by food handlers of a supermarket chain were adequate, but the risk level of the unit affected attitudes and practices and not the knowledge score. However, employees' self-reported practices did not fully match leaders' perceptions of hand washing, implying that certain actions should be taken to further improve hand washing practices. Knowledge and attitude were both significantly correlated with practices, but only attitude and practices had a positive correlation, demonstrating the importance of raising awareness to ensure food safety.

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Microbial spoilage in vegan foods

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Abstract - **Microbial spoilage by *Escherichia coli* (*E. coli*) and *Listeria innocua* (*L. innocua*), as a surrogate for *Listeria monocytogenes*, was observed in a vegan snack product and oat drink, both vegan ready-to-eat foodstuffs, to determine a possibly occurring decrease in food safety.**
For this purpose, inocula of non-pathogenic *E. coli*, *L. innocua* or both microbes combined have been injected into the packages of the tested foodstuffs. Storage of contaminated products was performed at different surrounding conditions. The microbial development was determined on several sampling days by cultivation specific agars. Colony counting was performed connected to the subsequent calculation of the cell count. Impact of microbial growth to physico-chemical properties of the foodstuffs was determined with certain devices.
The snack's physico-chemical properties were not affected by the microbes. The oat drinks acidity was reduced by both bacteria. *L. innocua* reduced the particle size and increased the viscosity. The bacteria can grow in both tested products but they were not detected in the non-inoculated foods, contrary to a natural occurring flora. The results indicate supported growth of *E. coli* by *L. innocua*. *E. coli* grew only at 20°C, *L. innocua* at both 9°C and 20°C.
In conclusion, *E. coli* and *Listeria* spp. were not detected in non-inoculated products. Both, oat drinks and vegan snacks can support growth of *Listeria* spp. and *E. coli* after a contamination has occurred, especially when stored at ambient temperature, e.g., 20°C, and in opened packages, leading to a decrease in food safety. It can be expected that the consumption of contaminated products affects the consumers health.

Keywords – ***Escherichia coli*, *Listeria innocua*, food safety, vegan food, ready-to-eat food (RTE)**

I. INTRODUCTION

Since nowadays, a big selection of vegan foodstuffs is available (Leitzmann, 2018) and an increased consumption of ready-to-eat (RTE) foodstuffs can be observed (Kotzekidou, 2016), the food safety regarding these kinds of products gets more and more in the focus of attention. Food safety can be affected, beside others, by biological hazards, including pathogenic bacteria like *Listeria monocytogenes* (*L. monocytogenes*) and the EHEC strain *Escherichia coli* (*E. coli*) O157:H7 (Regulation (EC) No 178/2002, 2002; Lawley et al., 2012; Hunter, 2003; Orsi and Wiedmann, 2016; Bergis et al., 2021; Schlech, 2000; Kotzekidou, 2016; Matissek, 2020; Odonkor and Ampofo, 2012). Therefore, the aim of this work was to investigate the development of pathogenic *E. coli* and *L. monocytogenes* in two vegan RTE foods, oat drink and vegan snacks, using the surrogate bacteria *E. coli* and *Listeria innocua* (*L. innocua*) (Beaufort et al., 2019). The above-mentioned strains were used to observe possible risks, when contaminated vegan products are consumed.

II. MATERIALS AND METHODS

A vegan RTE meat substitute (Atria Suomi Oy, Finland) and oat drinks (Oy Karl Fazer Ab, Finland) have been inoculated with either *E. coli* ATCC® 25922™ (Microbiologics, Inc., St. Cloud, USA) or *L. innocua* (6a) derived from ATCC® 33090™ (Microbiologics, Inc., St. Cloud, USA) or both microbes in combination. *L. innocua* was used as a surrogate for *L. monocytogenes*, a common contaminant of RTE products (Kotzekidou, 2016; Beaufort et al., 2019) and *E. coli* as a surrogate for pathogenic *E. coli* since these bacteria can be transmitted by contaminated drinking water, a main ingredient of both tested products (Odonkor and Ampofo, 2012).
The inocula of *E. coli* and *L. innocua* were prepared according to current instructions and standards and injected into the food in original packages. Different inoculation levels (0, 10^2, 10^3, 10^4 or 10^5 cfu/package) (Figures 1. and 2.) were applied. The incubation was carried out at 9°C or rather 20°C, in closed packages for 1, 3, 6, and 9 days and in opened packages in the time from day 9 to day 13 (vegan snacks) or day 15 (oat drinks) (Figures 1. and 2.).
Additionally, acidity (pH), water activity (a_w), colour changes, changes in particle size, viscosity and gas atmosphere in the packages were measured. Performed measurements depended on the product in original packages. The pH [-], the a_w [-], the gas atmosphere inside the foodstuffs packages [% O_2 / % CO_2 / % N_2] and the viscosity [mPas] have been measured by using benchtop measuring devices. The particle size was assessed bymicroscopic examination.

Colour changes of the food stuff have been evaluated by taking pictures of the foodstuff on each measuring day, followed by the determination of changes by using a software tool. No preparations were needed for the oat drinks. For measuring the snacks pH, a_w, particle size and colour changes, the foodstuff has been mashed prior to the measurements. Additionally, the mash was diluted 1:1 with tap water for the pH measurements.

III. RESULTS

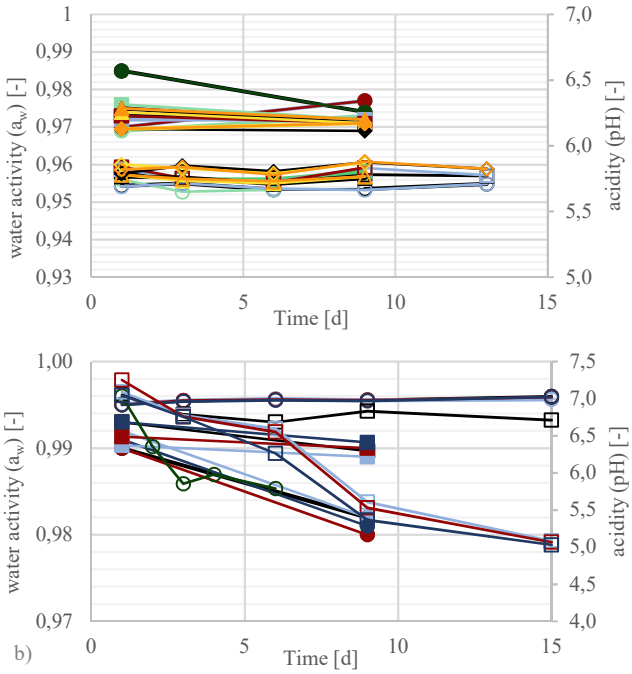

Figure 1. Water activity (a_w) [-] (filled marks) and acidity (pH) [-](unfilled marks) of vegan snacks (a) and oat drinks (b) non-inoculated (black) or inoculated with 10^2 (turquoise), 10^3 (light blue),10^4(red) or 10^5 (dark blue) cfu/package *Escherichia coli* (circle) or *Listeria innocua* (square) or with 10^3 (yellow) or 10^5 (orange) cfu/package *E. coli* together with 10^5 cfu *L. innocua* (triangle) or with 10^3 (yellow) or 10^5 (orange) cfu/package *L. innocua* together with 10^5 cfu *E. coli* (rhombus), stored for up to 13-15 days at 9°C or 20°C (green).

None of the contaminants (*E. coli*, *L. innocua* nor their combination) affected the snack's pH (Figure 1.), a_w (Figure 1.), colour and particle size as well as the gas composition in the packages (data not shown) during the follow-up-study of nine days, regardless of the storage conditions. In the oat drink, both bacteria led to reduced pH-values when proliferation is noticeable (Figure 1.). On the other hand, the a_w is not affected by microbial growth (Figure 1.).

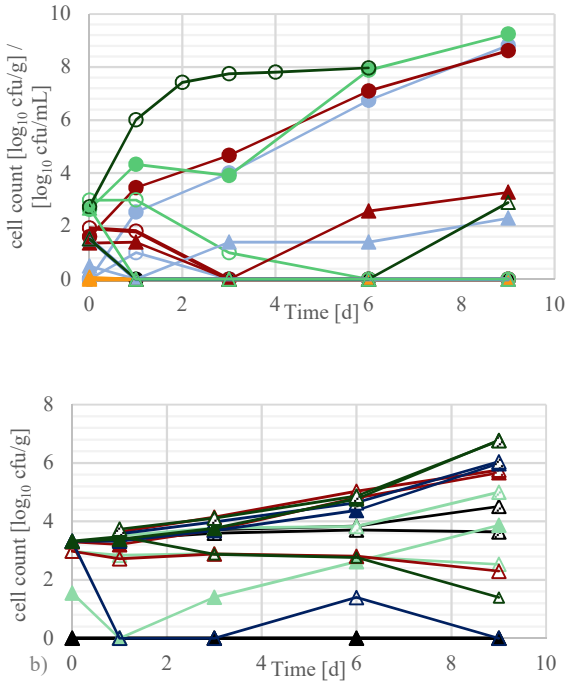

Figure 2. Development of *Escherichia coli* (unfilled marks), *Listeria innocua* (filled marks) and total cell counts ( ) in oat drink [$\log_{10}$ cfu/mL oat drink] (circle) and vegan snacks [$\log_{10}$ cfu/g vegan snack] (triangle), non-inoculated (black) orinoculated with 10^2 (orange), 10^3 (light blue), 10^4 (red), or 10^5 (turquoise) cfu/package of one microorganism (a) or non-inoculated (black) or inoculated with 10^3 (dark blue) or 10^5 (dark green) cfu/package *E. coli* together with 10^5 cfu *L. innocua* or with 10^3 (turquoise) or 10^5 (red) cfu/package *L. innocua* together with 10^5 cfu *E. coli* (b), stored at 9°C or 20°C (dark green) and cultured at one, three, six or nine days.

Contrary to *E. coli*, *L. innocua* reduced the oat drink's particle size (data not shown). Moreover, an increased viscosity, from 50 mPas in non-inoculated drinks to 61 mPas in drinks contaminated with *L. innocua* was observed after 15 days of storage in opened packages (data not shown). An increased viscosity is not detectable in case of a *E. coli* contamination, combined with storage at 9°C. The oat drink's colour was not affected by a microbiological contamination (data not shown). *E. coli* and *Listeria* spp. are not detectable in the non-inoculated tested foodstuffs (Figure 2.). Anyway, a natural occurring microbial flora was detected for vegan snacks (Figure 2.). When *E. coli* and *L. innocua* occur in the tested vegan products, both can proliferate in the foodstuffs, depending on the storage condition (Figure 2.). The trends can be seen in Figure 2. More precisely, *L. innocua*

proliferates in the oat drink independently from the inoculation level ($4x10^0$ - $4x10^2$ cfu/mL) to a cell count higher than 10^8 cfu/mL when stored for 9 days at 9°C (Figure 2.). On the other hand, the *E. coli* count in oat drinks decreases below the detection limit in case of storage conditions of 9°C but it increases when the foodstuff is stored at 20°C (Figure 2.). The same trend is observed in vegan snacks (Figure 2.). In case of contaminated vegan snacks, *E. coli* was detected after 6 days in opened packages and after 9 days in closed packages, both at 20°C, first (Figure 2.). On the other hand, the *L. innocua* cell count in vegan snacks, stored at 9°C, is increasing in case of an inoculation level of 3 cfu/g (10^3 cfu/package) or higher, but after 6 or rather 9 days of incubation first (Figure 2.). Growth was not detected in case of the lower inoculation level (Figure 2.). In contrast to the single application, *E. coli* is detectable on each sampling day, although the cell count is decreasing, when 3.6 x 10^1 cfu/g or 2.0 x 10^3 cfu/g *L. innocua* and 9.6 x 10^2 cfu/g *E. coli* are applied together to the vegan snacks (Figure 2.). In case of a contamination of the vegan snacks with 2.1 x 10^3 cfu/g *L. innocua* and 2.5 x 10^1 cfu/g *E. coli*, *E. coli* is not detectable in each sample or the cell count is decreasing below the detection limit (Figure 2.). When the microbes are applied in combination, the *L. innocua* count and the total cell count are increasing (Figure 2.). The total cell count is increasing in vegan snacks contaminated with only one microbe, too (Figure 2.). Especially noticeable is the strong increase in the total cell count in vegan snacks contaminated with *E. coli* and stored at 20°C (Figure 2.).

VI. DISCUSSION

First, due to the decreases in the *E. coli* count or rather the fact that *E. coli* was not detectable in vegan foodstuffs when stored at 9°C (Figure 2.), it can be stated, that the microbe is not able to proliferate at cooler temperatures and thus it is not able to proliferate in the foodstuffs when stored at proper fridge temperatures. This is strengthened by the observation, that both, oat drink and snacks, enable the proliferation of *E. coli* when stored at 20°C (Figure 2.). Since the storage conditions have been kept at stable storage conditions, an impact by changing storage conditions and temperatures as occurring in daily life to the microbial growth pattern cannot be excluded and can even be expected. On the other hand, *L. innocua* grows at 9°C as well as at 20°C. This is underlined by the fact, that the oat drink's physico-chemical properties are affected by the presence and growth of *L. innocua*. Theobserved reduction in particle size in oat drinks inoculated with *L. innocua* might be explained by a breakdown of nutrients for the microbial metabolism (Bhat et al., 2012; Fritsche, 2016; Madigan et al., 2020). Due to the impact of microbial growth to the viscosity and the product's pH, the determination of these properties might be a rapid way to detect microbial contamination in liquid products. Anyhow, it is noticeable, that the increased viscosity only occurred in opened packages but not in inoculated, closed packages. Therefore, it can be assumed, that the determination of a microbial contamination by measuring a liquid's viscosity is limited by several factors. On the one hand, the contaminant is one limiting factor since *E. coli* growth did not lead to an increased viscosity but *L. innocua* growth did. On the other hand, as indicated above, the presence of oxygen needs to be an elementary factor for changes in the viscosity. The reduced pH-values in oat drinks demonstrate that both, *E. coli* and *L. innocua* can grow in the plant-based drink, depending on the temperature. It can be strongly assumed, that the mixed acid fermentation is performed by *E. coli* when growing in closed packages with low oxygen content (Madigan et al., 2020). Due to this metabolic pathway, different substances are formed (Madigan et al., 2020), leading to a decreased pH in the products as observed. Beside a reduced pH, changes in the gas composition might occur in case of *E. coli* growth, due its metabolism (Odonkor and Ampofo, 2012; Fritsche, 2016; Madigan et al., 2020). Since *E. coli* was not able to grow in the vegan snacks when stored at 9°C (Figure 2.), it is not surprising that the gas composition remained unchanged. The growth behaviour of *L. innocua* and *E. coli* when combined in a foodstuff (Figure 2.) lead to the assumption, that there is a synergistic interaction between the bacteria, more precisely that *L. innocua* supports the growth of *E. coli*. Furthermore, the inoculation level seems to have huge impact on the growth pattern. E.g., *E. coli* was detectable in the vegan snacks for a longer time duration when the foodstuff was contaminated with a higher initial *E. coli* level. Therefore, it might be, that a contamination might occur in an extent, that *E. coli* survives for such a long time, that a health impairment due to the consumption of the contaminated food occurs. Moreover, inhibition of the microbial growth is observed in vegan snacks. Reasons for the inhibition may be the foodstuff's lower pH, the CO_2 content in the snack package or other ingredients in the snack (Bhat et al., 2012; Orsi and Wiedmann, 2016; Juneja et al., 2017). Especially the CO_2 might be the reason for the inhibited growth, since gas is able to prolong the lag-phase (Bhat et al., 2012). That would explain why no increases in the *Listeria* cell count are detectable before day six in case of the vegan snacks inoculated with *L. innocua* only. Additionally, the storage in opened packages supported the growth of both bacteria, most probably due to a better oxygen supply. Furthermore, the inhibited microbial growth explains the unchanged physico-chemical properties in vegan snacks (Odonkor and Ampofo, 2012; Torres, 2010; Madigan et al., 2020; Jemmi and Stephan, 2006).

IV. CONCLUSION

The preventive methods for food safety applied by the producers are effective regarding *E. coli* and *Listeria* spp. since the bacteria were not detected in non-inoculated products. The vegan snacks and especially oat drinks can support growth of *Listeria* spp. and *E. coli* after contamination has occurred, in particular when stored at ambient temperature, e.g., 20°C, and in opened packages. Storage of the vegan foodstuffs in a fridge increases the food safety regarding *E. coli*, but not regarding *Listeria* spp. *Listeria* spp. might support the growth of *E. coli*, even at lower temperatures, at which *E. coli* would not last alone. It can be expected that the consumption of contaminated products affects the consumers health. A contamination cannot necessarily be detected by the investigation of physico-chemical properties.

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Controlling bacterial spoilage in both vegan and meat-based products

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Abstract - **This presentation starts with looking at the European food legislation e.g., (EC) 178/2002, (EC) 852/2004, (EC) 853/2004 and 2006/42/EU, and standards on food quality and safety including the food safety culture. The food quality and safety culture are touched upon in the regulation EU 2021/382. Furthermore, cases on processing of sausage and microbial spoilage of vegan snacks will be reported.**
More details how to solve legislative issues are available in related standards issued by e.g., ISO, EN and BRCGS as well as guidelines issued by e.g., European Hygienic Engineering and Design Group (EHEDG) and the Global Food Safety Initiative (GSFI). The GSFI position paper on the food quality and safety culture, the food safety culture standards, the guidelines on proper hygienic design, and on microbial challenge test instructions provide suggestions how to deal with proper food hygiene as well as food quality and safety culture.
The ANSES challenge test used at Seinäjoki University of Applied Sciences (SeAMK) can be used to check how microbes spoil food. The method was used in testing the surrogate microbes *Escherichia coli* and *Listeria innocua* as contaminants of vegan snacks. The microbial spoilage of vegan snacks is presented in a poster and with a chapter in the proceedings by Carolin Müller and co-workers. Furthermore, at SeAMK there has been process trials performed with both vegan and meat sausages. The sausages were tested for microbial quality after proper and abused cooling. The food safety culture will be used to discuss the process cases.

Keywords – **food safety culture, *Escherichia coli*, *Listeria innocua*, ready-to-eat (RTE) food, vegan snacks, vegan sausage, meat-based sausage**

I. INTRODUCTION

A significant part of the food supply in the developed countries depends on processed food and this means that the food must be produced safely. The following EU regulations in food safety (EC) 178/2002, (EC) 852/2004, (EC) 853/2004, 2006/42/EU and EU 2021/382 deal with e.g., traceability, food hygiene, hygienic design, allergens, and food safety culture in the food process. Related ISO, EN and BRCGS standards as well as guidelines provided by European Hygienic Engineering and Design Group give more information on how to deal with the issues mentioned above (BRCGS, 2022; CEN, 2022; EHEDG, 2022; ISO, 2022). The food safety standards are well-recognised to actors in the food chain (GFSI, 2019). It must be stated that all food producers must include a safety culture in the food handling system, because an increasingly complex food system demands more than reliance on safe food practices (BRCGS, 2022).
The opinion of the Global Food Safety Initiative (GFSI) is that a sustainable food safety system must be based on food safety beyond formal regulations i.e., on a food safety culture in all food processing. The GFSI position paper was designed to help food professionals and promote a positive food safety culture (GFSI, 2019). The rules state facts and the culture should live through experience. Despite this, the European Union (EU) had to issue the regulation EU 2021/382 in spring 2021 on e.g., the food safety culture. GSFI defines the food safety culture as shared values, beliefs, and norms in, across and throughout the organizations (GSFI, 2019).
One of the core themes of the BRCGS Food Safety Issue 9, which was launched in August 2022, is developing the food safety culture on the company's core competencies i.e., creating a culture of excellence in that company (BRCGS, 2022). In our studies, the challenge test method developed by the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) was used to find out how food is spoilt using surrogate microbes (Bergis et al., 2021). The topic of the challenge test is "EURL Lm technical guidance document for conducting shelf-life studies on L. monocytogenes in ready-to-eat foods" and it is based on the examination of potential growth conditions for *Listeria monocytogenes* in food products (Bergis et al., 2021). This test method was applied to study how the surrogate microbes spoil the food tested (Müller et al., 2022).

II. FOOD SAFETY CULTURE TO IMPROVE FOOD QUALITY

The regulation EU 2021/382 states that the food business operators must provide evidence of an appropriate food safety culture. The food safety culture shall be implemented based on nature and size of the food business. The

management and all employees must be committed to both safe food production and food distribution. The management must engage all employees in the food safety practices. All actors must be aware of the importance of food safety hazards including food hygiene issues in the business. The food hygiene must be maintained at a high level when changes are planned/implemented. Sufficient resources in safe and hygienic handling of food must be ensured by all actors including the management in the factory (BRCGS, 2022; EU 2021/382).

The management shall clearly communicate the roles and the responsibilities within each activity in the food business. The communication must be open and clear between all employees within an activity and between consecutive activities. The management must also ensure that the documentation is up to date and is following relevant regulation. And the food safety controls in food manufacturing areas shall suit the production. The process hygiene, the hygienic design of equipment and facilities, the hygiene of personnel including protective clothing etc. must reflect the risks in the process (BRCGS, 2022; GFSI, 2019; 2006/42/EC). The personnel shall get appropriate training and supervision. The food safety of the business must be improved according to both science and technology (BRCGS, 2022; EU 2021/382).

III. CASE 1: MICROBIAL SPOILAGE IN VEGAN FOODS

The consumption of contaminated products affects the consumers health. The preventive methods for food safety applied by the producers are effective regarding both spoilage microbes and pathogens. The study was performed using the challenge test method developed at ANSES (Bergis et al., 2021). This study is reported by Müller et al. (2022) in the poster session. The study showed that the vegan snacks supported growth of both listeria and coliforms after contamination, when stored at ambient temperature. Storage of the vegan foodstuffs in a fridge increases the food safety regarding *Escherichia coli*, but not regarding *Listeria innocua.* The growth of listeria might support growth of *E. coli* at low temperatures in which it would not grow alone. Note that the bacteria studied, *L. innocua* and *E. coli*, were not detected in the non-inoculated products.

IV. CASE 2: TEMPERATURE ABUSE IN THE COOLING OF SAUSAGES

After that the sausages – both meat-based and vegan – had been cooked and smoked according to the receipt the cooling was performed according to instructions. Both types of sausages were showered with cold water inside a cooling cabinet. Showering also prevent wrinkling of the sausage products. The chilling was continued until the internal temperature was approx. 4°C. Cooling and chilling prevent growth of spore-formers which might have survived the cooking process. Both types of sausages – vegan and meat-based - were after cooling kept at both fridge and room temperatures. An abuse in temperature lowered the quality. The preparation instructions for both vegan and meat-based sausages were collected by the co-author Juuso Kumpulainen. It can be stated that nitrite is an antioxidant lowering improper flavours. The growth of spore formers is not a problem under proper cooling and refrigeration. However, the nitrite provides an added level of protection to products kept at abused temperatures (Feng et al., 2013; Ghebremedhin et al., 2022).

V. CONCLUSIONS

The food quality and safety cases described in this presentation show that small failures e.g., dirty surfaces and/or improper cooling in the process can lead to big problems in the food chain. A system with a proper food safety culture is needed to avoid such problems. Well implemented actions enable safe production and through own well-planned actions, the owner/management affects the company's food safety culture. In a mature food safety culture both the vision and the mission of the company have been implemented throughout the whole organisation. The leader purchasing services should select suppliers, who deliver according to the food safety requirements of the company. The owner/management must fully support all employees in their actions and all members of the personnel must be trained and supervised appropriately. However, the food safety culture must be tailored for the company in which it is applied. The maintenance team leader should pay attention to both functionality and food safety performance of the process equipment chosen.

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SUSTAINABILITY

Efficiency of waste water treatment of Slavonski Brod agglomeration

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Abstract - **C-Tech technology (Cyclic activated sludge technology) is being applied to the wastewater treatment plant in Slavonski Brod (WWTP SB). WWTP SB was put into operation in 2014, designed for 80.000 PE and it includes the 3rd stage of processing - removal of C, N and P. Biological wastewater treatment takes place in four SBR reactors comprising three steps: (i) filling / aeration, (ii) sedimentation and (iii) decantation. Each Sequencing Batch Reactor (SBR) contains an anoxic biological selector and an aeration zone, and the anoxic/aerobic zone volume ratio is 2/25. In anoxic biological selectors the concentration of dissolved oxygen is ≤0.5 mgO_2/L, and in the aeration zone DO is 1-2 mgO_2/L. The paper presents the results of composite samples of treated wastewater (effluent), sampled with an automatic sampler for 24 hours.**
The effluent for all parameters defined by the Water Permit at WWTP SB meets the legally defined values as follows: COD <125 mgO_2/L, BOD_5 <25 mgO_2/L, total N <15 mgN/L, total P <2 mgP/L and total suspended solids <35 mgTSS/L, observed during 2019, 2020 and 2021.

Keywords - **WWTP SB, C-Tech technology, effluent, removal of organic and inorganic component, nutrient removal.**

I. INTRODUCTION

Pollutant removal such as nitrogen, phosphorus, and carbon become a vital issue in the wastewater treatment process for its negative effects on the natural water body (Liu et al. 2021). Excess organic matters in the effluents like nitrogen and phosphorus must be removed prior to their discharge into water bodies to prevent eutrophication, oxygen depletion and toxicity (Ghehi et al. 2014). Among economic and sustainable biological nutrients removal technology in municipal wastewater treatment, aerobic biological treatment by SBR system and its modified forms for treating various wastewaters achieve more attention because of its unique advantages, including simple facility and low reactor volume, adequate nutrient removal, and good operational flexibility (Liu et al. 2021). In biological wastewater treatment processes, sufficient nutrients are required for bacterial growth and floc formation (Ghehi et al. 2014). *The Cyclic activated sludge technology* (*C-Tech technology)* (Figure 2.) technology is fundamentally an adaption of the Sequencing Batch Reactor (SBR) process, whereby secondary biological treatment and tertiary settling are combined in a single tank. However, with the *C-Tech*, two or more batch tanks are installed in parallel with their sequences out of phase with each other allowing for a continuous flow through the system. Thus no upstream buffer tank is required, unlike traditional SBR systems. Such a process is known as a cyclic activated sludge process (Hazard et al. 2018).
The C-Tech technology highlights several advantages in application over other processing systems, such as: the use of selectors - which achieves a lower probability of the appearance of filamentous bacteria, control of the aeration speed - which saves on energy, which implies low operating costs, high operational flexibility, and at the same time removal of C, N and P, simultaneous nitrification and denitrification at low DO (Hazard et al., 2018; Gao et al., 2017; Wang et al., 2010; Demoulin et al., 2001; Demoulin et al., 1997) .

II. MATERIALS AND METHODS

A. WWTP Slavonski Brod and C-Tech technology

The public sewage system of the city Slavonski Brod includes area agglomeration of Slavonski Brod which also includes its cadastral district, Brodski Varoš and Brodsko Vinogorje. Vodovod Ltd. manages the total of 215 km of sewage network in Slavonski Brod and 35 km in other area of agglomeration.
Wastewater treatment plant (WWTP) Slavonski Brod is located in the southeastern area of the town of Slavonski Brod, by the Sava river, downstream from the city center (Figure 1.). The recipient of effluent is the Sava river. WWTP SB is designed for a capacity of 80.000 population equivalents (PE) with the possibility of upgrading to 100,000 PE, it includes the 3rd stage of wastewater treatment - removal of nitrogen and phosphorus along with the removal

of organic and suspended solids (NN 26/2020). The maximum amount of wastewater inflow to WWTP SB is 8,400,000 m^3/year, together with collected rainwater. WWTP SB operates in dry weather flow mode, at a concentration of DO in the aeration period of 1.0 to 2.0 mgO_2/L. Exceptionally, during periods of high precipitation intensity, overflow of the increased inflow from the mixed part of the public drainage system into water bodies is allowed through rain overflows, 25 of them.

Figure 1. Location of WWTP SB and recipient river Sava

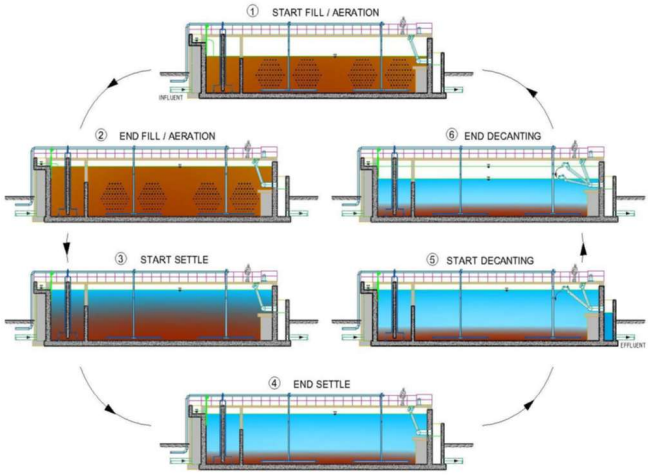

Figure 2. C-Tech process cycle (Hazard and et al. 2018)

C-Tech technology of wastewater treatment - cyclic activated sludge technology (Figure 2.) is applied to a WWTP SB. It is based on the operation of four SBR. The process is broken down into 3 distinct stages that operate on a cycle: (i) fill/aeration, (ii) sedimentation and (iii) decantation (Hazard and et al. 2018).

(i) Fill/aeration : During the fill/aeration stage water enters a single C-Tech basin into the aerated zone via the anaerobic selector. Throughout this fill stage, the aeration zone is continually aerated at a rate controlled by the OUR control system and a portion of the sludge is constantly recycled to the inlet of the selector. The design of this selector and recycle rate combined with the OUR control system allows for the formation of special macroflocs in which simultaneous nitrification/denitrification, BOD_5 removal and bio-P removal occurs.

(ii) Sedimentation: During the settling phase, the inlet to the particular basin is closed, the internal recycle is stopped, and the sludge formed in the previous stage aggregates as a blanket and settles to the base of the reactor tank leaving a top layer of clear treated effluent. In typical wastewater applications, the settled sludge layer has a mean biomass concentration of around 10 g/l.

(iii) Decantation: In the decant phase, the moving weir decanter moves from the top water level to the bottom water level to remove approximately one third of the reactor volume which will be clear treated effluent.

B. Sampling of influent and effluent

Wastewater at the inlet – influent and at the outlet – effluent from WWTP SB is sampled with 2 automatic samplers (Figure 3.), 3 times a week, for 24 hours. The inlet automatic sampler is located after the coarse and fine grid, before the aerated sand pit/grease pit, while the outlet automatic sampler for sampling the purified wastewater is located after the biological purification treatment, before the water flows into the recipient. The average monthly inflow of wastewater to WWTP SB is in the range of 515,500 to 896,000 m^3/month, which largely depends on the amount of rainwater.

Figure 3. Automatic sampler for influent

III. RESULTS

The efficiency removal of organic and inorganic components as an average annual value ± standard deviation for the years 2019, 2020 and 2021 is shown in Table 1., and the quality of treated waste water - effluent, maximum allowed concentrations of selected indicators defined by the Water Permit, and the efficiency of the process expressed as average monthly values for COD in Figure 4., total suspended solids in Figure 5., total N in Figure 6., NH_4-N and NO_3-N in Figure 7. and TP in Figure 8.

Table 1. Effluent characteristics at WWTP SB, expressed as average annual values ± standard deviation, for 2019, 2020 and 2021

	WWTP SB, effluent		
	2019.	**2020.**	**2021.**
Q, m^3/g	561.434±26.186	570.202±60.909	628.208±123.572
COD,mg /L	12,14±2,43	15,34±6,05	11,78±1,41
BOD,mg/L	2,8±1,72	3,73±3,22	2,42±1,35
TSS, mg/L	1,23±0,64	1,84±1,21	1,22±0,56
TN, mg/L	5,2±0,86	5,33±1,36	4,83±0,59
NH_4-N,mg/L	0,57±0,23	2,24±2,43	0,66±0,25
TP, mg/L	1,36±0,26	1,18±0,37	1,18±0,19
pH	7,88±0,13	7,72±0,09	7,36±0,19
T, ºC	9,02±1,44	7,06±1,07	6,78±0,72

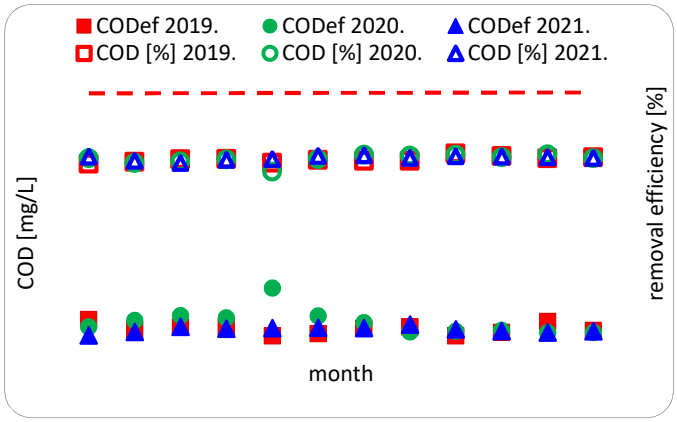

Figure 4. Average monthly COD values in the effluent, maximum allowed COD concentration in purified water and COD removal efficiency at WWTP SB during 2019, 2020 and 2021

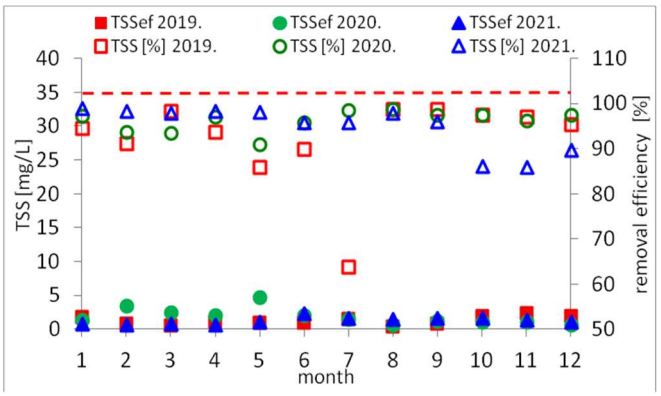

Figure 5. Average monthly TSS values in the effluent, maximum allowed TSS concentration in purified water and TSS removal efficiency at WWTP SB during 2019, 2020 and 2021

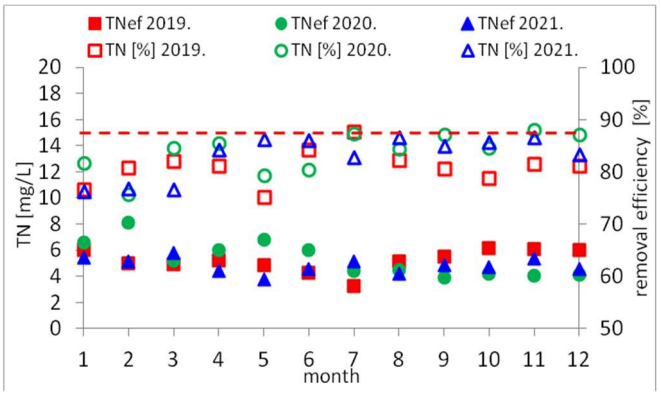

Figure 6. Average monthly TN values in the effluent, maximum allowed TN concentration in purified water and TN removal efficiency at WWTP SB during 2019, 2020 and 2021

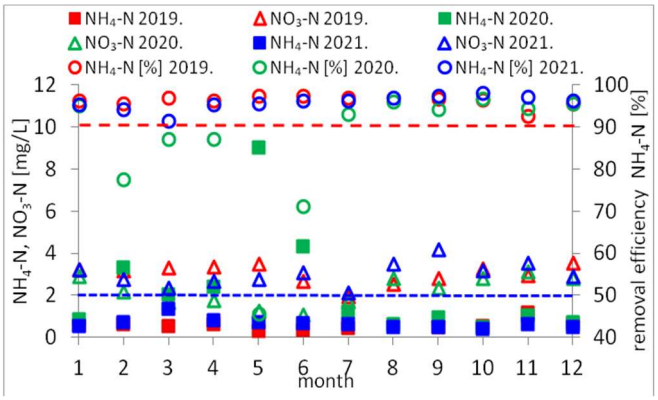

Figure 7. Average monthly NH_4-N i NO_3-N values in the effluent, maximum allowed NH_4-N i NO_3-N concentration in purified water and NH_4-N removal efficiency at WWTP SB during 2019, 2020 and 2021

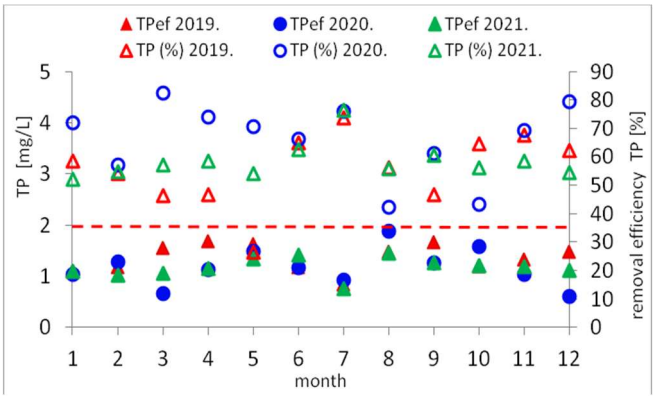

Figure 8. Average monthly TP values in the effluent, maximum allowed TP concentration in purified water and TP removal efficiency at WWTP SB during 2019, 2020 and 2021

IV. CONCLUSIONS

At WWTP SB, the efficiency of C, N and P removal is based on wastewater treatment with C-Tech technology. Biological wastewater treatment takes place in four SBR reactors, which include three steps: (i) fill/aeration, (ii) sedimentation, and (iii) decanting. The effluent for all parameters defined by the Water Permit at WWTP SB meets the legally defined values as follows: COD <125 mgO_2/L, BOD_5 <25 mgO_2/L, total N <15 mgN/L, total P <2 mgP/L and total suspended solids <35 mgTSS/L, observed during 2019, 2020 and 2021.

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Water Permit, Croatian waters (2021)

intelWATT - Intelligent water treatment technologies for water preservation: Case study 3 presentation

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Abstract - **Water is usually the most extensively used raw material for the production of high-value products. Increasing demand of industrial activities result in challenges to integrate an environmental and economic acceptable solution regarding wastewater management. The intelWATT project aims to validate innovative and intelligent water treatment technologies combining freshwater preservation with resource recovery and energy conversion based on the circular economy concept. The case study of simultaneous metal recovery and wastewater treatment in plastic electroplating production will be presented. The treatment process involves state of the art membrane processes, which will result in purified water being directly reused in industrial activity. The status report on the case study will be discussed. The main objective is to reduce the consumption of fresh water by 65%, reuse the electrolytes originating from rinsing baths in electroplating process, avoid the chemical reduction as well as the evaporation of wastewater to preserve fresh water and significantly reduce environmental pollution.**

Keywords - **fresh water preservation, wastewater treatment, metal recovery, electroplating, zero liquid discharge**

I. INTRODUCTION

Water demand is steadily increasing due to the population growth, economic development and changing consumption patterns (Boretti and Rosa, 2019). Therefore, an inadequate discharge of wastewater leads to the pollution of freshwater, especially in developing countries. Increasing industrial activities result in challenges of water waste treatment methods used to remediate contaminants in the water in order to limit its environmental impact (Barbera and Gurnari, 2018). In the last 100 years, global water demand has increased by 600%. Over the next two decades, it will grow significantly not only in industry, but also for domestic and agricultural activities (Boretti and Rosa, 2019). This could lead to water stress or scarcity by 2025 for over 30% of the world's population (Barbera and Gurnari, 2018). New strategies have been proposed and adopted by the European Union throughout directives (European Commission website, 2000; European Commission website, 2010; European Commission website, 2012). However, despite the efforts, 20% to 40% of Europe's water is wasted and water efficiency could be improved by 4% through sustainable water management approaches. This transdisciplinary challenge requires approaches that integrate innovative and energy cost-efficient technologies to reduce water consumption in industry and enhance resource recovery and/or energy generation (European Commission website, 2011).

II. PROJECT INTELWATT

intelWATT is a Horizon 2020 funded project that aims to validate, at TRL7-8, innovative and intelligent water treatment technologies combining fresh water preservation with resource recovery and energy conversion based on the circular economy concept. The decisive role of membrane technology in the overall acceleration of the sustainability transformation was confirmed in many key applications ranging from wastewater treatment, resource fractionation, desalination, membrane distillation, gas separation and protein chromatography (Ezgube and Rathilal, 2020; Lisičar Vukušić et al. 2018; Lisičar et al. 2019; Hosseinipour et al. 2022; Micari et al. 2020; Hammerstein et al. 2022; Esser et al. 2021; Metze et al. 2017; Pastor and Barbe, 2020; Barbe et al. 2001). In this context, the intelWATT project aims to demonstrate improved circularity and significant fresh water savings though three case studies in representative energy and resource intensive industrial activities involving state of the art membrane technology.

A. Cooling tower blow down treatment: water abstractions for production of electricity

The objective of case study 1 is to develop an efficient, cost effective, and smart solutions for water management in a thermoelectric power plant. The aim is to minimize the cooling tower blow down (>99% recovery) by developing a pilot unit of 100 m^3/day treatment capacity installed in the premise of the Greek company Public Power Corporation's unit (natural gas combined circle facility). Cooling towers, an important component of refrigeration systems, are common in industries such as oil and gas, chemical processing and power plants. In a typical cooling tower, water is being pumped from the tower basin (cooling water) and routed through the process. The warm water returns to the top of the cooling tower and trickles downward over the fill material inside the tower. During evaporation, the contained salts concentration rise in the circulating cooling stream, leading to the precipitation of soluble minerals and causing scaling. In order to prevent this effect, a portion of the water is drawn off or blown down for disposal (IntelWatt Project website. Case study 1, 2021).

B. Hybrid process for water recovery and energy harvesting from industrial brines

The objective of the case study 2 is to demonstrate the implementation of maximum water reuses and green energy conversion in a crucial EU and global industrial applications such as mining. The location of the integrated RED/MD (reverse electrodialysis/membrane distillation) pilot plant of this case study is Castellgalí (Spain). This region hosts the intersection point of a brine collector, which picks up the brines from different salt mining industries in the Barcelona's area. The aim is to demonstrate the possibility to valorise this waste brine as renewable energy and water source (IntelWatt Project website. Case study 2, 2021).

C. Simultaneous metal recovery and wastewater treatment in plastic electroplating production

Case study 3 is a typical example of processes with moderate wastewater flow rates but high load of heavy metals that may cause serious environmental problems. Wastewater effluents of this type can be found in electroplating, mineral processing, electric, electronic and chemical industries. In this project, case study 3 focuses on a plastic electroplating process and the main objective is to simultaneously recover resources and purify water in order to reduce fresh water consumption and global environmental impact. This case study is hosted by the company BIA GmbH in Solingen (Germany) (IntelWatt Project website. Case study 3, 2021).

Plastic electroplating is considered to be the most advanced discipline in electroplating. It involves combining the treatment of a non-conductive part with the multilayer electrodeposition process for a decorative and wear-resistant product (Petrinic et al. 2015). It has been identified as one of the most hazardous chemical intensive industries due to the generation of wastewater rich in heavy metals. The circularity of the current wastewater treatment is very limited. It mainly relies on the chemical precipitation of heavy metals and their subsequent mechanical separation. Recent progress achieved in membrane technology offer new opportunities to develop efficient treatment processes with higher circularity (Kamizawa et al. 1978).

Electroplating facilities usually operate different plating lanes. A typical plating lane mainly consists of a series of treatment and rinsing baths (up to 30 in total). Plastic parts to be electroplated hang on flight bars which move and transport a certain amount of liquid containing electrolytes from bath to bath. The contamination of further treatment baths with different metals or additives may have a considerable negative impact on the final product quality and must be avoided. Therefore, the rinsing process (Figure 1.) plays a key role in this process and has to be carefully operated. Rinsing water is extremely polluted and has a content of 10-20% electrolyte concentration when discharged.

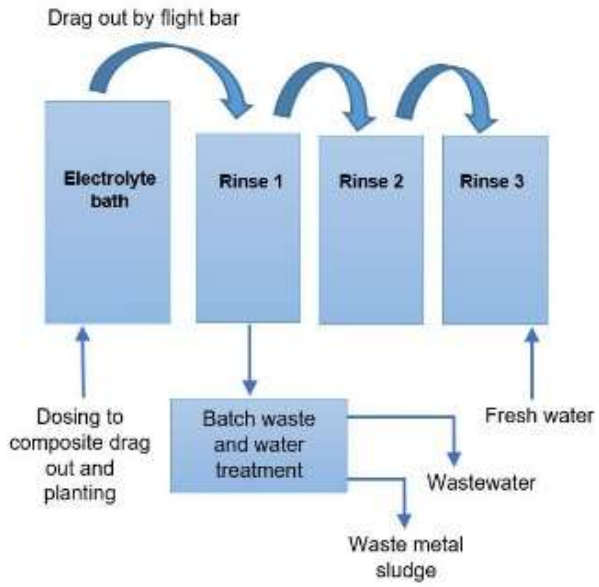

Figure 1. Typical work flow of a plastic electroplating bath series

Currently, these electrolytes cannot be reused, leading to even higher costs of wastewater treatment. Product quality requirements are high in the plastic electroplating industry.

Rinsing is a critical step in this process. The chemical composition of the treatment baths is complex. Beside metal sulphate, they usually contain numerous additives such as total organic compounds and boric acid. The proposed approach for the recycling of metal in plastic electroplating is presented on Figure 2.

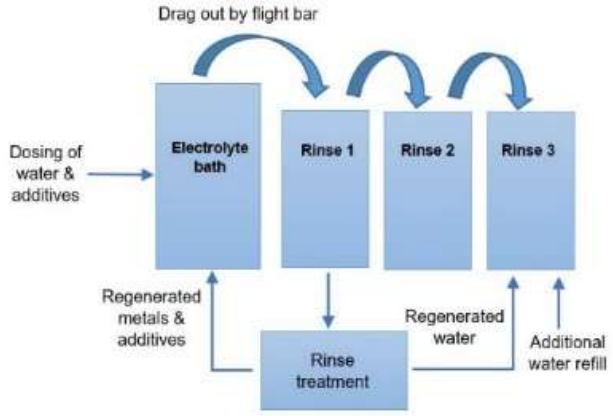

Figure 2. Circular economy approach for the recycling of metal in plastic electroplating

The process shall involve state of the art membrane technology, which is recognized to minimize the limitations of the traditional technologies for metal plating wastewater treatment (Hosseini et al. 2016; Piedra et al. 2015). The purified water is used to refill the rinsing baths. Concentrated electrolyte drag-out is reused to refresh the treatments bath. The main objective is to reduce the consumption of fresh water by 65%, reuse up to 95% (copper and chromium) and 50% (nickel) of the electrolytes contained in the rinsing bath, avoid the chemical reduction and evaporation of wastewater to preserve freshwater and significantly reduce environmental pollution. This study was recently published (Engstler et al. 2022). It explores the feasibility of processing trivalent chromium electroplating wastewater in a straightforward reverse osmosis (RO) treatment to achieve the simultaneous recycling of water and electrolytes. For this purpose, diluted Cr (III) electrolyte bath solution was used as simulated rinse water and feed solution in a RO lab unit. This works aims to demonstrate the applicability of RO technology for zero liquid discharge applications (ZLD).

III. MATERIALS AND METHODS

The water treatment was performed with a reverse osmosis membrane (Filmtec SW30-2540, DuPont) using a diluted Cr(III) electroplating electrolyte as artificial rinse water (0.77 g/L Cr). A series of steady-state RO experiments were conducted at different permeate fluxes (up to 30 L/(m²*h). All RO trials were performed at 25 °C, with a maximum cross-flow velocity of 0.25 m/s and a maximum hydraulic pressure of 80 bar. More information on the membrane specifications is listed in Table 1. The course of the experiment is described in detail in literature (Engstler et al. 2022).

Table 1. Membrane specifications and experimental conditions of the RO experiment (Engstler et al. 2022)

Membrane	Filmtec SW30-2540, DuPont	
Membrane type	Polyamide thin-film composite	
Membrane area	Two sheets, each 80 cm²	
	From data sheet	**Observed in RO experiment**
NaCl rejection	99.4%	93.0%
Maximum pressure	69 bar	80 bar
Temperature	25 °C	25 °C
pH range	2-11	3.8-4.7

IV. RESULTS AND DISCUSSION

Reverse osmosis is known to be used in water treatments to provide high rejection for heavy metal ions in solution. However, the complexity of the feed as well as the high concentrations of the selected electrolyte, make this procedure challenging. Chromium, as the main component for the plating process, was a targeted electrolyte to be recycled. Besides chromium, boric acid was quantified, due to the wanted high concentration near the solubility limit and known relatively low rejection in RO processes. Finally, sulphate was detected because of its high concentration in the electrolyte and divalent anionic character, contrasting the other two compounds. The membrane showed superior rejection for chromium (max. 99.96%) and sulphate (max. 99.63%) even at high feed concentrations and for all fluxes (Figure 3.). The achieved rejection for boric acid was up to 93.8% and the end concentration was 56.86 g/L. In the process the chromium concentration in the retained wastewater increased by a factor of 10.92 (8.40 g/L Cr), the final and maximal achievable concentration level for an operation pressure of 80 bar. The initial concentration of sulphate was increased by a factor of 9.50, leading to a final concentration of 67.71 g/L (Engstler et al. 2022). These results were evaluated and accepted by the electroplating company BIA GmbH.

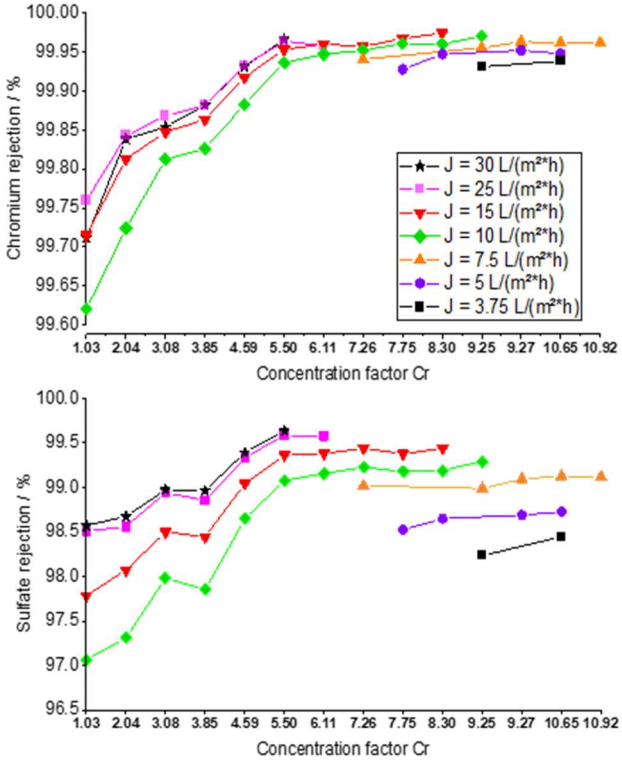

Figure 3. Chromium and sulphate rejection with increasing concentration in the retentate (concentration factor chromium) and permeate flux (Engstler et al. 2022).

V. CONCLUSIONS

The present study demonstrated how a chromium electrolyte containing a variety of compounds can be successfully recovered from electroplating rinse water in a single-step RO process. Furthermore, the water can be reused in the electroplating processes, following a zero liquid discharge approach for fresh water preservation. Due to the complexity of the electrolyte, fouling or scaling of the membrane may occur. Therefore, an effective cleaning procedure has to be developed in order to recover membrane performance. The concept design stage of the RO system, which will be implemented in the electroplating facility, has been completed. The proposed system is very promising and its scale up from lab-scale to pilot scale at the BIA GmbH plant, under the scope of the IntelWATT project, is currently under development. The system will operate at pilot scale (0.5 m³/h) and will play a decisive role for transferring the technology developed at laboratory scale to a full functional pilot plant.

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INDUSTRY 4.0

Production processes and parameters affecting the oil content in potato chips

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Abstract - **Potato chips are a popular snack product on the market, but health-conscious consumers avoid them because of their higher fat content. It is also desirable from an economic point of view and in terms of product shelf life to reduce oil absorption. For this reason, scientists are researching how to reduce the oil content in the product without negatively affecting the sensory properties. The objective of this presentation is to summarize the factors and mechanisms, as well as new methods, for reducing oil content in chips and to provide an overview of the latest published results on this topic. The factors that can contribute to the production of low-fat chips can be divided into several groups: (i) raw material (potato cultivars, agrotechnological methods, and storage conditions); (ii) pre- frying to modify the surface such as pre-drying (osmotic dehydration, infrared vacuum pre-drying, ultrasonic and convective air drying), blanching, pulsed electric field pre-treatment, and ultrasonic treatment, or to modify the slice thickness (use of edible films such as hydrocolloids); (iii) type of frying (e.g. vacuum frying) and frying parameters (temperature and time); (iv) post- frying treatment such as draining under vacuum. For example, potatoes with a higher dry matter absorb less oil, and the altered surface of the fries contributes to a lower oil content. In addition, frying at higher temperatures results in lower oil content in the chips. All of the above factors and their mode of action will be discussed in detail in the presentation. An overview of recent scientific research and the results obtained will also be given.**

Keywords - **potato, chips, oil content, frying**

I. INTRODUCTION

Potato chips is a popular snack all over the world. In 2020, about 1.9 million tons of this product were produced in Europe. Germany, Italy and France have the highest consumption of potato chips in Europe (IndexBox, 2021). During the chips frying, numerous structural changes of proteins, fats, and carbohydrates, gelatinization of starch, and chemical reactions such as Maillard reaction, caramelization, and denaturation of proteins occur, which contribute to the development of the specific color, odor, and taste of chips (Zhang et al., 2018). During frying, a large amount of moisture evaporates from the slices; therefore, chips contains 2% moisture and 35% oil (Pedreschi et al., 2008). Slices absorb most of the oil during frying and after the slices are removed from the oil (Pedreschi et al., 2008). Due to the different absorption mechanism, some scientists have defined three different oil fractions: (i) structural oil (STO) - refers to the oil that the slices absorb during frying, (ii) penetrated surface oil (PSO) - refers to the oil that the slices absorb during cooling after they are removed from the fryer, and (iii) surface oil (SO) - oil that remains on the surface of the slices (Bouchon et al., 2003). Since fried foods, including chips, increase the risk of cardiovascular disease, obesity, hypertension, diabetes, and cancer due to their higher fat content (Zhang et al., 2018), consumers are trying to replace them with healthier snack products, and scientists and manufacturers are trying to find a way to reduce the oil content in the product and keep consumers, reduce costs, and maintain product quality.

The aim of this paper is to summarize the results of previous research that investigated the influence of the parameters and the frying process, as well as the pre- and post- frying treatment on the reduction of oil absorption in chips.

II. PRODUCTION PROCESSES AND PARAMETERS AFFECTING THE OIL CONTENT IN POTATO CHIPS

In the initial phase of frying, the surface of the slices is heated until it reaches the temperature of the oil in which they are fried. After the temperature equalizes, boiling occurs on the surface of the slices and a crispy layer is formed. After boiling, the moisture on the surface of the slices begins to evaporate, creating pores, cracks, and steam bubbles (Arslan et al., 2018). Through the created pores, cracks, and capillaries, the oil penetrates the disks in the places where moisture used to be. Some scientists claim that the amount of moisture evaporated from the slices is equal to the amount of oil absorbed by the slices during frying. After the slices are removed from the oil, the surface of the slices cools due to vapor condensation, the vapor pressure in the slices drops and the oil that was on the surface of the slices penetrates inside the slices through the vacuum effect (Arslan et al., 2018; Liu et al., 2021). Pinthus and Saguy (1994) state in their work that capillary pressure in the pores also contributes significantly to the absorption of oil in the slices. Blumenthal (1991) claims that evaporation of water during frying causes a hydrolysis reaction of cleavage glycerol and fatty acids and leads to the formation of monoglyceride and diglyceride surfactants. All factors affecting the oil content in chips can be divided into several categories, such as: (i) raw material (potato cultivars, agrotechnological methods and storage conditions); (ii) pre-frying treatments such as blanching, pre-

drying, pre-treatment with pulsed electric fields and ultrasound treatment, use of edible films; (iii) type of frying (e.g. vacuum frying) and frying parameters (temperature and time); and (iv) post-frying treatment such as draining under vacuum.

A. Raw material

Raw material characteristics such as shape, size, moisture content, and density affect oil absorption (Zhang et al., 2015). The proportion of dry matter and starch in potatoes, which are one of the cultivar characteristics, have a huge influence on oil absorption in slices. By influencing the dry matter content in potatoes with parameters such as cultivar, tuber maturity, and tuber storage temperature, oil absorption in chips is indirectly affected, which is described in the following subsections. Potato cultivars with lower dry matter and starch content have higher oil absorption (Baumann and Escher, 1995; Ezekiel and Rani, 2006; Abong et al., 2009). Ezekiel and Rani (2006) studied the effect of dry matter on oil absorption in 33 potato cultivars whose dry matter content ranged from 16.3% to 26.2%. Chips prepared from tubers with 26.2% dry matter content had an oil content of 23.1%, while chips prepared from tubers with 16.3% dry matter content had an oil content of 41%. Balbino et al. (2020) investigated the effects of cultivar, anti-browning agent, packaging conditions, and storage time on oil absorption in fried fresh-cut potatoes of Birgit and Lady Claire cultivars. Lady Claire with a slice thickness of 5 mm and a dry matter content of 24.21% absorbed 5.45% oil, while Birgit with a same slice thickness and a dry matter content of 18.45% absorbed 7.51%. These results suggest that oil absorption in chips depends on the dry matter content, i.e. cultivar characteristics.

Abong et al. (2009) studied the effect of potato maturity on oil intake in French fries (chips). According to their results, oil absorption in slices of potato tubers harvested 90 days after planting, before they were mature, was significantly higher than in tubers harvested when they were mature (120 days after planting). The reason for this, in addition to the lower dry matter content, is the undeveloped cell structure of immature potatoes, which is more susceptible to damage during peeling and cutting and contributes to oil absorption.

The storage temperature of potatoes also affects oil absorption during frying. Potatoes stored at 4°C and 8°C tend to absorb more oil during frying than potatoes that were stored at 12, 16 and 20°C. Namely, potatoes stored at higher temperatures have a higher dry matter content due to greater evaporation and respiration and consequently higher weight loss (Kaur et al., 2008).

B. Pre-frying treatments

In addition to slice thickness, there are several pre- frying treatments that can be used to influence the oil content in the chips, such as blanching, osmotic dehydration, pulsed electric field, predrying, ultrasound pretreatment, and edible coatings. Baumann and Escher (1995), who studied oil content in 0.8-1.6 mm thick slices, concluded that as slice thickness increases, absorption of oil during frying decreases. They also found that oil absorption decreased more in slices between 0.8 mm and 1.2 mm than in slices between 1.2 mm and 1.6 mm. These research results also confirm the fact that most of the oil is absorbed at the surface of the slices during deep-frying (Baumann and Escher, 1995)

Blanching potatoes is a common pre-treatment method in the production of chips, which can improve the color and texture of the chips (Pedreschi and Moyano, 2005). Blanching involves immersing food in hot water for a short period of time to inactivate enzymes and microorganisms (Arslan et al., 2018). However, scientific research on blanching and oil absorption in slices is divided. Some researchers claim that blanching decreases the oil content of slices (Aguilar et al., 1997), while others claim that blanching contributes to increase oil absorption in slices. It is possible that this depends on the blanching temperatures and duration (Al-Khusaibi and Niranjan (2011). Aguilar et al. (1997) found that blanching at low temperature (65 °C; 30 min) activates the pectinesterase enzyme, which contributes to the reduction of porosity and thus oil absorption. Moreover, during blanching, gelatinization of starch occurs on the surface of the slices (Pravisani et al., 1985), which could reduce oil absorption in the slices (Al-Khusaibi and Niranjan, 2011). However, Pedreschi and Moyano (2005) studied the oil absorption of blanched slices in hot water (85 °C; 3.5 min) and found that the blanched slices absorbed ~15% more oil than the control (non-blanched) samples after 20 seconds of frying. A similar conclusion was published in the work of Rimac-Brnčić et al. (2004), where dry matter content decreased in all blanched samples due to migration of soluble cellular content into the water compared to the sample before blanching. However, during frying, the samples blanched in water only showed a much greater increase in oil content than the slices blanched in 1% citric acid and 0.5% calcium chloride solutions, which were found to be most effective in reducing oil uptake. This is due to the reaction between calcium and pectin in the potato tissue and the formation of Ca-pectates, which strengthen the primary cell wall and middle lamella, as well as resistance to degradation during frying (Rimac-Brnčić et al., 2004).

Su et al. (2021) studied the effect of osmotic dehydration on oil absorption by immersing slices in 1% -5% sodium chloride and 2.5% monosodium glutamate solutions (MSG) before frying. For slices immersed in 1%-5% sodium chloride, the oil content decreased by 16.6%-31.4%, and for slices immersed in 2.5% MSG, the oil content decreased by 27.8%. They concluded that oil absorption decreases with increasing concentration of solutions. Osmotic dehydration improves the dielectric properties of the slices, which improves heat and moisture transfer and results in smaller, denser pores in the cell structure and less oil penetration after frying.A pulsed electric field (PEF) is another pre-frying treatment used to reduce the amount of oil in the slices, improve texture and crispness, and reduce the amount of

acrylamide formed during frying. After PEF treatment, the slices are smoother. PEF creates pores through which cellular fluid reaches the surface and surrounds the slices, creating a protective layer of water vapor that reduces weight loss and prevents penetrating oil into the slices (Zhang et al., 2021a).
Pre-drying slices by convective air drying before they enter the fryer contributes significantly to reducing oil absorption during frying (Zhang and Fan, 2021). Drying shrinks the surface of the slices, reduces the contact surface between the slices and the oil, and a dry surface on the slices acts as a barrier to prevent oil penetration (Lumanlan et al., 2020). Jia et al. (2018) concluded that reducing the moisture content of potato slices from 80% to 40% during pre-drying treatment reduces oil uptake in the slices from 75% to 40%. In addition to convective air drying, scientists are also exploring infrared vacuum drying. Su et al. (2017) studied pre-drying with infrared energy and found a decrease in oil content from 22.38 to 13.49 g oil/100 g dry weight. This drying method, in fact, affects the surface of the slice and makes it harder and denser, which decrease the penetration of oil into the slice.
In addition, numerous studies have been conducted on the reduction of oil content in slices by ultrasound pretreatment, which achieves even better results in reducing oil absorption compared to convection air drying (Zhang and Fan, 2021; Zhang et al., 2021b; Zhang et al., 2021c). The ultrasonic waves during treatment have a "sponge effect" They create large channels that allow faster mass and moisture transfer during frying. This creates a high vapor pressure in the slices during frying, which reduces the oil absorption rate (Zhang and Fan, 2021).
Edible coatings are already a well-researched method that contributes significantly to reducing oil absorption. The most commonly used edible coatings (hydrocolloids) are: cellulose derivatives, gellan gum, gum arabic, alginate, and carrageenan, which can reduce oil content in chips by 20 to 90% (Hua et al., 2015). These coatings are very resistant and have a high water-binding capacity. Once applied to the slices, they prevent water evaporation and thus oil migration into slices (Hua et al., 2015). Garmakhany et al. (2008) studied the effect of 1% carboxymethylcellulose (CMC), 0.5% xanthan gum, 0.3% guar gum, and 1% xanthan gum coating on oil absorption. Hue et al. (2015) studied the effect of low methoxyl sunflower head pectin on oil content, Su et al. (2021) 25% maltodextrin, 1.0% CMC-Na, and 1.5% chitosan, Tavera-Quiroz et al. (2011) plasticized methylcellulose, and Yu et al. (2021) guar gum with glycerol coating, and all these researchers concluded that edible coatings reduce oil content.

C. Frying

During frying, the evaporation of water from the slices causes the formation of cracks or pores. The size and distribution of the pores greatly affects the oil absorption of the slices. During frying, the number of larger pores with diameters of 100-200 μm increases, contributing to the absorption of oil in the chips while pores with diameters of 0.03-10 μm hinder oil absorption because they increase tortuosity (Zhang et al., 2020; Liu et al., 2021).
The temperature and duration of frying also have a great influence on oil uptake. When frying at higher temperatures, it takes less time to reach the final moisture content, so the oil content in the slices is lower (Baumann and Escher, 1995; Kaur et al., 2008). In the study by Kaur et al. (2008), chips fried at 120°C absorbed 22.1-32.2% oil, while chips fried at 180°C absorbed 19.1-27.6% oil.
Although atmospheric frying is widely used in the food industry, vacuum frying is an often-mentioned technology that contributes to reduce oil absorption during frying. During frying in a vacuum fryer, the chips are heated under negative pressure, which lowers the frying temperature to 120 °C. The vacuum fryer also includes a de-oiling mechanism through a centrifugation system (Moreira et al., 2009). This mechanism removes the oil from the slices before they leave the vacuum. If the de-oiling mechanism did not exist, the total amount of oil on the chips would be easily absorbed into the interior of the slices due to the change in pressure (from vacuum to atmospheric) (Basuny et al., 2012). Numerous researchers have compared the amount of oil in slices fried under atmospheric conditions and in vacuum. Slices fried in a vacuum absorb less oil due to the lower frying temperature, lower moisture and lower pressure in the slices, while during atmospheric frying structural changes in the slices occur contributing to higher oil absorption (Basuny et al., 2012). In the study by Garayo and Moreira (2002), the oil content in atmospherically fried chips was 66% of dry weight and in vacuum fried chips was 37% of dry weight. Oil absorption during vacuum frying is significantly influenced by the frying temperature or time and vacuum pressure. The temperature itself does not affect the oil absorption rate, however, when frying at lower temperatures, the frying time increases, which contributes to an increase in the amount of oil in the slices. The strength of the vacuum affects the moisture content of the slices and the absorption of the oil, depending on how much free water is contained in the slices and how much oil is on the surface of the slices (Garayo and Moreira, 2002). Garayo and Moreira (2002) vacuum fried the samples at oil temperature (118, 132, 144 °C) and vacuum pressure (16.661, 9.888, and 3.115 kPa). In the first 150 seconds of frying, oil absorption was highest at a temperature of 144 °C and vacuum pressure of 3.115 kPa. After the mentioned peak, the oil content of the slices decreases during frying, and at the end of frying, the samples fried under these conditions had the lowest oil content.

D. Post-frying treatment

As mentioned earlier, after frying, the chips absorb the greatest amount of oil during the cooling process. After the chips are removed from the oil, the surface cools, the water vapor condenses, and the pressure inside the slices decreases, causing the oil to penetrate from the surface of the slices into the core. Some scientists claim that the absorption of oil in

the slices after frying also depends on the balance between oil adhesion and drainage (Ahmad Tarmizi and Niranjan, 2010). Reducing oil absorption in the chips during cooling can be influenced in several ways: using superheated steam or hot air to blow the oil away from the surface of the chips, maintaining a high temperature on the chips and slowing down the cooling, by using a vacuum or adsorbent paper that can remove oil from the chips surface (Arslan et al., 2018). Moreira et al. (1997) concluded in their study that 20% of the oil is absorbed by the slices during frying of tortilla chips while 80% of the oil remains on the surface of the chips. 64% of the oil that remains on the surface of the chips, is absorbed into the chips during cooling. Ahmad Tarmizi and Niranjan (2013) concluded that applying a vacuum (1.33 kPa) during the drainage of the chips after frying in the headspace of the fryer reduced the oil content in the chips by 38% compared to chips dried at the atmospheric pressure.

III. CONCLUSIONS

In order to maintain health and improve the quality of life, customers demand products with reduced oil content. The reduction of oil content in the production of potato chips can be influenced by the potato cultivar, but also by a specific pre-frying treatment, or by the frying process including cooling. Cultivars with higher dry matter absorb less oil during frying. It is also important to harvest potatoes when they are ripe and avoid storage temperatures below 4 °C. It is also recommended to avoid a slice thickness of less than 1.2 mm. In addition, different treatments before frying showed promising results, such as osmotic dehydration, convective air drying, infrared vacuum pre-drying, pulsed electric field pretreatment, ultrasonic treatment, and coating with edible films. For reducing oil content, vacuum frying has been found to be a better method than air frying. Since most of the oil is absorbed by the chips during cooling after frying, post-frying treatment is very important, such as draining under vacuum, treatment with superheated steam, or the use of hot air. Considering that oil absorption is the result of different, very complex mechanisms, further research is needed to further reduce the oil content of chips, despite the promising results already obtained.

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