Doctoral (Ph.D.) Thesis

Investigation of changes in the fatty acid composition of certain plant based cooking oils on exposure to heat, with the possible use of polyunsaturated fatty acids in coronavirus disease

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Introduction

Lipids are a group of a very diverse compounds that can present many different and complex biological functions in the human body. The different types of fatty acids, phospholipids, triglycerides and cholesterol, are having particular importance in the view of human nutritional biology. In the last century, the role of fatty acids in complex regulatory mechanisms have become a better understanding as the body of scientific knowledge has grown. Thus, dietary fatty acids are not only cellular constituents or components involved in digestion or nutrients involved in energy production, rather they are also related to human metabolism in such important parts like cell survival, differentiation, intra- and extracellular signalling processes which take a central part in the survival and differentiation of the cell. For certain hormonal systems, fatty acids can be both starting and intermediate metabolites. Last but not least, different fatty acid metabolites are actively involved in the regulation of inflammatory processes in our body.

Beyond the purely scientific importance of the metabolism of fatty acids, lay people have the opportunity to regularly encounter these dietary components in their meals. The use of different dietary fats requires proper awareness, as they are the most energy-dense sources of all macronutrients. Due to food heat preparing processes dietary fats are transformed, so the quality and quantity of fatty acids have the ability to vary at different stages of the food use cycle. Beyond the standardised, well-controlled use in the industry, the quality and quantity of fatty acids in foods is not constant due to the changing processes in the kitchen.

The present thesis examines the complex mechanisms of action of fatty acids from theory to practice. We have carried out experiments to find out what is happening with certain fatty acids when they are exposed to a series of heat processing. On the other hand, we have investigated their role in inflammatory processes as a regulatory mechanism. In this context, we have expected that fatty acid intake may have an effect onto processes which are involved in both acute and chronic inflammation.

Objective

What type of fatty acid changing does the selected vegetable oils (sunflower oil, palm oil, rapeseed oil, extra virgin olive oil, soybean oil) undergo when they are heated by household conditions?

Plant based cooking oils contain high levels of polyunsaturated fatty acids, including essential fatty acids. The quantity and quality of these fatty acids can also change significantly when they are heated. It is not clearly understood what kind of changes could occur in the direction of repeated heat processing of plant based cooking oils with its fatty acids.

To study the effects of heat treatment periods, the fatty acid changes of selected plant based cooking oils were investigated by modelling specifically kitchen conditions.

Most of the available studies report on changes in oils heated for long periods of time, days or even weeks, sometimes on an industrial scale in tanks of several hundred litres. The heating numbers and temperatures in our model system were based specifically on the cooking habits of the population and on the recommendations of international and national organisations.

Based on our own heat processing protocol, we have analysed how the fatty acid composition of vegetable oils changes at maximum allowable heating temperature (180oC) and the maximum number of repetitions (10).

Unfortunately, no data are available already on changes in the levels of physiologically important fatty acids at maximum temperature and number of replicates under household conditions. The focus of our study was not on the oxidation by-products, rather on usage of the fatty acid composition and their changes in plant based cooking oils after heating processes.

What changes can occur in the nutritional biological effects of plant based cooking oils as a result of heating processes?

Those fatty acid changes which occur during heating processes can have affect the biological effects of plant based cooking oils on the human body. To assess this, nutritional indices were calculated (n3/n6 PUFA ratio, unsaturation index, atherogenicity index, thrombogenicity index, hypocholesterolemic/hypercholesterolemic index) in order to get a preliminary data of the changes in the nutritional biological effects of plant based cooking oils during monitoring fatty acid changes.

Materials and methods for chemical analysis and nutritional indices

We selected the plant based cooking oils that are widespread and available in Hungary for the sample selection of our study, accesed at we took into account the oils listed in the United Nations Food and Agriculture Organization's Table of Quantities and Ratios of Vegetable Oils, global oil and fat consumption data. Our heat treatment protocol was based on the recommendations for the public from the European Food Information Council (EUFIC). The choice of heating temperature and frying frequency for edible oils was based on the recommendations of good hygiene practice part in the National Food Chain Safety Agency's document on heating. Only freely available vegetable oils from retail stores were used after selection.

According to the selection criteria, a total of 5 types of vegetable oils were chosen to our study.

The location of our experiment was the practice kitchen of the Faculty of Health Sciences at University of Pécs (Rét street 4., Pécs). Between 2017 and 2018, the fatty acid measurements were performed in the Chromatography Laboratory of the Paediatric Clinic part of the Clinical Center at University of Pécs (Pécs József Attila utca 7).

Data collection and methods

Oils purchased from retail outlets were stored in a cool, dark place until its used. For frying, we purchased yellow potatoes available from a local commercial supermarket. The main criteria for choosing potatoes was traceability, so we bought pre-packaged goods that contained clear information at least about the variety and the origin of the potatoes. Edible potatoes were purchased in 2.5 kg packs. The denomination of the variety of potatoes was 'Musica. '

Heating protocol

The skins were removed from the potatoes and cut into slips about 1x1 cm wide (length followed the shape of the potato, typically varying between 4-6 cm). 100 g ± 1 g of potatoes were weighed and used for each heating period. The oils were mixed according to the type of oil on their packaging (did not mix the different type of oils). We measured 2000 ml from the oils for the first frying cycle.

Preparation and sampling

The first sample was taken from oils has been already mixed. During the frying process, the oils were heated up to 180°C in ordinary steel pans on a gas cooker. The temperature of the oils

was monitored by a long stem mercury thermometers. When the oil reached 180°C we fried the potatoes for 5 minutes, making sure to maintain the temperature at 180°C. After the 5th minute, the fried potatoes were removed by metal spatulas. The oils were filtered after each frying cycle using a plastic net filter to remove visible impurities. We let the heated oil to cool down until 70 C in open air. Then the 2nd sample was taken out after the first heat treatment. The described cooking process was performed 10 times in a row by repeating the same steps. Additional sampling was performed after the 5th and 10th cooking periods. The samples were stored at -20°C for one day and then at -80°C until the chemical analysis. Before the analysis, the previously samples were stored at -80°C were we thawed and let them warm until 37°C.

Sample preparation process

For sample preparation, we used the modified method of Beermann et al. The samples were analysed by gas chromatography using an Agilent 6890N gas chromatograph with flame ionisation detection (FID) and with direct injection into the capillary column (COC).

In our study, for a sample from a given heating cycle of an oil 3 parallel measurements were taken and repeated twice, thus its fatty acid content data were obtained at 6 measured points from one time point of a sample and the resulting values were averaged in the end.

Calculation of nutritional indices

Among the nutritional indices, the atherogenicity index (IA), the thrombogenicity index (IT), the hypocholesterolemic/hypercholesterolemic index (HH) and the health promotion index (HPI) were calculated based on the study by Chen and Liu.

Statistical analysis

Chromatogram data were integrated using Chromeleon 7.1. For our statistical analysis we used IBM SPSS version 27, to compare means of the groups one-way ANOVA test was performed followed by a Bonferroni test.

Conclusions and discussion from our chemical analysis and calculation of nutritional indices

Significant differences were recorded at several points during the heating processes. The most significant changes were found in the TFA content of plant based cooking oils. All raw plant based cooking oil contained TFA, and the TFA content of all plant based cooking oils increased continuously and significantly during the heating periods.

Essential fatty acids were significantly reduced in plant based cooking oils. Rapeseed and soybean oils with higher ALA content, the ALA content of these oils decreased significantly after each frying period. Significant differences were also consistently measured for the other oils, and all oils showed a decrease in ALA content compared to baseline. A clear decrease in LA content was also recorded in all oils. The sunflower oil with higher LA content showed a significant decrease after each heating period and a similar phenomenon was observed for rapeseed oil with lower LA content and extra virgin olive oil.

Our results confirm the changes in IA and IT previously described in the literature, as these indices increased for all oils at the end of the heating periods. Our results show that heating induces fatty acid changes which enhanced the atherogenic and thrombogenic properties of plant based cooking oils. Oleic acid and polyunsaturated fatty acids have hypocholesterolaemic, while saturated fatty acids are hypercholesterolaemic properties. Thus a decrease in the HH index can indicate potentially adverse cardiovascular effects. In our research, the HH index decreased in all plant based cooking oils, so edible oils which were undergone at adverse changes may negatively affect cardiovascular health during household conditions.

Previous studies have only recorded changes in up to one or two nutritional indices, whereas in the present study we calculated five nutritional indices for each oil at each time. Our results show that heating changed the indicies into a negative direction int the case of all plant based cooking oils. According to the assessment of the nutritional indices, the most stable oil in terms of its fatty acid composition was extra virgin olive oil that showed the smallest difference, while the most sensitive oils were sunflower and rapeseed oils, which had the largest differences in nutritional indices. Based on our results, we suppose that the ideal frying medium would be a mixture of several oils or fats with complementary properties.

Our results show that heating changes in plant based edible oils do not positively affect the fatty acid composition of these oils. In summary, the amount of saturated fatty acids increased while the amount of essential fatty acids decreased as a result of repeated heat treatment periods. In

particular, all plant based edible oils showed an increase in TFA after each heating period. Decreases in essential fatty acids were also observed in our study both in case of ALA and LA fatty acids. The extra virgin olive oil contained a relatively small amount of ALA, and this small amount of ALA was reduced by more than ten percent after the tenth heating treatment, and most of the ALA was lost from the rapeseed oil.

Overall, there are still several gaps in our understanding of the explanation of fatty acid changes we have descriebed. There is no data describing precisely what physico-chemical changes occur in the household conditions we have tried to imitate/reproduce that could explain the fatty acid changes what described.

In terms of the health effects of adverse fatty acid changes, we should highlight the described increase in TFA fatty acids. According to our results, if we take into consideration the recommendations of the manufacturers and the international organisation and reheat the oils for frying at maximum ten times in a row, despite of teh changes it seem unlikely that we can approach the maximum TFA intake levels described before. Similar conclusions can be drawn for a steady decline in essential fatty acids. Neither ALA nor LA fatty acids were disappear in that amounts which would be a health concern. In order to achieve an appropriate fatty acid ratio for a healthy diet and to reduce the amount of saturated fat intake, it is reasonable to avoid the deep frying techniques we have tried to remodel. Athough the fatty acid changes during repeated heating of plant based oils alone do not affect their essential fatty acid content to an extent that would put at risk to the essential fatty acid intake of consumers.

Brief overview of the 2019 coronavirus pandemic

The coronavirus outbreak that started in the Chinese city of Wuhan at the end of 2019 and was quickly classified as a pandemic did not come as a surprise to experts. SARS-CoV-2 is the 7th coronavirus with the potential to infect humans and cause severe illness. The genetic make-up of SARS-CoV-2 is single-stranded RNA with a genome of 29 903 nucleotides, making it the second largest known RNA-genome virus. A huge effort has been made recently for a better and thorough understanding the disease (COVID-19) caused by SARS-CoV-2. The clinical presentation of COVID-19 disease varies widely, ranging from disease without clinical symptoms, through moderate flu-like symptoms to life-threatening hospitalization with symptoms requiring life-saving interventions.

At the start of the pandemic, scientific research focused on understanding the disease and treating its different manifestations. As time has gone by, more and more studies have reported on the preventive and supportive role of vitamin D, and other preventive nutritional factors in COVID-19.

A serious complication of COVID-19 disease is the development of the cytokine storm (also known as hypercytokinemia, cytokine release syndrome, or macrophage overactivation syndrome) due to an overreaction of the immune sístem of the body. The cytokine storm develops rapidly and significantly which increases the risk of mortality in patients who become critically ill. Cytokine storm is characterised by disruption of cytokine networks, which plays an important role in the development of clinical symptoms and severe disease outcome. In COVID-19 disease, the regulation of cytokine and chemokine production can be out of balance/altered, which can cause different reactions in the patient's body (production of some cytokines tends to be reduced while others become more intense). Among the proinflammatory cytokines that are perturbed, IL-6 and IL-1β appear to be key players. Even before the advent of coronavirus infection, it was known that the production of these cytokines was dependent on the body's supply of fatty acids. N-6 fatty acids (especially AA) are precursors of eicosanoids, whereas n-3 fatty acids primarily propagate the synthesis of less inflammatory eicosanoids.

Objectives of the literature research

Based on available scientific data, we investigate whether dietary factors such as long-chain polyunsaturated fatty acids intake have an effect on cytokine storm as a high-risk complication of COVID-19 disease.

The precursor compounds of the enzymes involved in the production of cytokines are LC-PUFA lipids such as DHA and EPA. The intake of these fatty acids affects the body's production of interleukin and cytokine. Therefore, based on the available scientific literature, we asked whether EPA and DHA supplementation could have any effect on interleukin and cytokine production?

Furthermore, based on the available scientific data, how does the intake of EPA and DHA affect the levels of interleukins, which are the central mediators of the cytokine storm?

In addition to IL-6 and IL-1ß, which play a central role in the cytokine storm, other proinflammatory cytokines can also significantly increase the inflammatory processes in the body. We were interested to see what further inflammatory cytokine changes might be expected with dietary supplementation of EPA and DHA.

Material and methodology of the scientific literature research

In our study, we conducted an informative, non-systematic literature research because we did not have the necessary resources for a systematic review. However, even in this limited form, our research method was based on the hierarchical pyramid system of evidence-based medicine. We lokked for the on pubmed database for pre-specified intermediates (Il-6, IL-1ß, TNF- α) related to the cytokine storm.

When reviewing publications, we felt it was important to find studies that published more than 15 years ago. Our literature research was based on in vitro studies that investigated these intermediates in the context of n-3 LC-PUFA (EPA and DHA) supplementation. The available experimental results were also reviewed in the light of clinical studies, where the search criteria were the same as for in vitro searches. From the non-systematically reviewed literature, we selected 10 in vitro and 10 randomised clinical trials, which we assessed in more detail, and then summarised the results of the 3 in vitro and 5 randomised clinical trials that we considered most relevant. This preliminary literature search will be complemented by a systematic literature review.

We were looking for answers to the practical question of what doses and forms of EPA and DHA supplementation are effective and safe.

In the first wave of the pandemic, we committed to publishing our results quickly, as there was little clinical knowledge available at the time to control the unfolding epidemic. The acceptability of this unusual approach in the circumstances was confirmed in hindsight by the publication of our literature review in a high-impact journal in the first year of the pandemic.

Conclusions and discussion of the scientific literature research

In our study, we sought the answer to the question of what metabolic changes can occur in EPA and DHA supplementation in the concentrations of cytokines that are involved and central regulators in the cytokine storm as the life-threatening complication of COVID-19. The available scientific data suggests that levels of the cytokines IL-6 and IL-1β can be reduced by supplementing EPA and DHA at different doses. We also found that the polymerase (ADP-ribose) enzyme, which also has anti-inflammatory effects and whose expression can also be influenced by EPA and DHA supplementation, can optimise the inflammatory cascade process in the event of a cytokine storm.

The effects were demonstrated in in vitro tests, animal studies and randomised clinical trials before the COVID-19 pandemic. The described reduction in IL-6, Il-1β occurred consistently in all experimental designs in response to EPA and DHA supplementation.

EPA and DHA supplementation may also improve the condition of COVID-19 patients through other metabolic factors. Reduced plasma triglyceride levels are a well-known effect of EPA and DHA supplementation, which also appears to be a significant preventive factor against serious complications of COVID-19.

When taken in the right doses, EPA and DHA supplementation is a safe, mass-applied treatment with a low side-effect profile that can improve many aspects of patient well-being and can also help prevent COVID-19 disease.

The results of our literature research may provide a basis and scientific support for further research. Our hypothesis now seems to be confirmed by the results of a small, randomised clinical trial.

New findings from our studies

The fatty acid composition of plant based cooking oils commonly used in households are significantly altered when heated at lower temperatures and for shorter periods of time, simulating household conditions.

All plant based cooking oils used in the study (sunflower cooking oil, extra virgin olive oil, rapeseed cooking oil, soybean cooking oil, palm oil) contained trans-fatty acids in unheated, raw form.

For all plant based cooking oils in the study, the trans fatty acid content increased continuously and significantly as a result of the heating procedure to the greatest extent until the 10th heating.

Significant decreases in essential fatty acids, both alpha-linolenic acid and linoleic acid, were recorded in all plant based oils during the heating processes.

All five calculated nutritional indices showed unfavourable nutritional biological changes when plant based cooking oils were heated.

EPA and DHA supplementation appear to be theoretically justified in the prevention and treatment of a severe, sudden cytokine storm that poses a significant life-threatening risk to patients with new coronavirus disease.

Practical application of our results

By modeling household conditions, following the guidelines of the frying process of edible oils recommended by the manufacturers, significant changes in fatty acids can occur during heating plant based cooking oils once and several times. The decrease in essential fatty acids with positive physiological effects, the increase in trans-fatty acids with negative physiological effects, and the development of nutritional indices further reinforce the general recommendation those kitchen technology methods that require high fat and multiple heat treatments are not recommended.

In spite of the unfavorable fatty acid changes modelling under household conditions, there were no changes in fatty acids occur that would affect consumers' adequate essential fatty acid or harmful trans fatty acids intake following the recommendations of manufacturers, international and govermental organizations for heating edible fats.

The persistence of the COVID-19 pandemic today calls for the development of effective prevention strategies. Dietary supplementation of EPA and DHA may theoretically affect the body's cytokine homeostasis, thereby optimizing the activation of IL-6, IL-1β and other inflammatory cytokines. These mechanisms may reduce or even significantly mitigate the development of the cytokine storm.

Publications

Articles related to the thesis

1. Szabó Z, Marosvölgyi T, Szabó E, Koczka V, Verzár Z, Figler M, Decsi T. Effects of Repeated Heating on Fatty Acid Composition of Plant-Based Cooking Oils. *Foods*. 2022; 11(2):192. https://doi.org/10.3390/foods11020192

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2. Szabó, Z., Marosvölgyi, T., Szabó, É., Bai, P., Figler, M., & Verzár, Z. (2020). The Potential Beneficial Effect of EPA and DHA Supplementation Managing Cytokine Storm in Coronavirus Disease. *FRONTIERS IN PHYSIOLOGY, 11*. http://doi.org/10.3389/fphys.2020.00752

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Abstracts related to the thesis

1. Koczka, V., Marosvölgyi, T., Szabó, Z., Figler, M., Varga, V., Kedves, A., & Szabó, É. (2020). Többszöri hevítés hatására bekövetkező zsírsavváltozások növényi étkezési olajokban. In *Táplálkozéstudományi Kutatások X. PhD online konferencia programja és az előadások összefoglalói* (pp. 13–13).

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