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Distinct capability of some fats on unsaturated fatty acid and antioxidant enrichment of foods for ketogenic diet purpose

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2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (PubChem CID: 5815211)
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ABSTRACT

Ketogenic diet is a dietetic approach for the treatment of refractory childhood epilepsy. In this experiment we aimed to assess the total lipid content enrichment capability of different however, less studied fats, as well as the possibilities to transfer unsaturated fatty acids and antioxidants into different foods, as these substances proved to be relevant in the mechanism of the ketogenic diet. We also determined the stability of different fats during food preparation and short-term storage. French fries, meatloaf and fried zucchini were prepared with sunflower oil, olive oil, coconut oil, rape oil and lard. The total lipid contents, iodine values, rancidities and antioxidant-activities of the samples were ascertained immediately after the preparation and after 3-day-storage on 4 °C. Our results indicate that the different foods are distinct in their lipid uptake capabilities, while the fats cannot be distinguished by their penetrance abilities into the foods. Sunflower and rape oils proved to be the best for unsaturated fatty acid enrichment but as rape oil proved to be sensitive to both heat and storage time, sunflower oil seems to be the best option. Anitoxidant-acitvity did not show any tendencies.

1. Introduction

Ketogenic diet (KD) is a high-fat, adequate-protein, low-carbohydrate diet, which was first used for the treatment of childhood epilepsy in the first years of 1900s [1]. Later on the field of application was extended and now it is under clinical tests for many diseases and special conditions (e.g. cancers [2], or even for sport nutrition [3]). The results of these experiments are still contradictory [2,4], This may be due to the lack of knowledge of the exact mode of action of the KD. Since the exact patho-biochemical pathways were unknown, many studies have investigated the possible biological target points of the altered macronutrient intake [5,6]. As a result our frontiers of knowledge extended and moreover there are many identified biochemical process that are affected by the KD, especially in epilepsy [7].

One of the most important pathways is the reduced glucose level, which leads to the decreased speed of glycolysis. The other key phenomena is the alteration of neuron surface expressed potassium ion channels, hence the modification of membrane potential and excitability as well [5]. Regarding food science the most interesting discovery is the relevance of the fatty acid quality. During the early years of the application of KD only the ratio of fat:

Abbrevations: KD, Ketogenic diet; ROS, reactive oxygen species; UFA, unsaturated fatty acid; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid; TBA, thiobarbituric acid; MDA, malondialdehyde; TEAC, trolox equivalent antioxidant capacity; ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid.

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carbonhydrate had believed relevant nevertheless new research insights refuted this point of view. New results highlight the role of polyunsaturated fatty acids (PUFA) [8] and omega-3 fatty acids [9] in seizure control. Studies indicated that the occurrence of the KD evoked elevated PUFA concentration in the brain is crucial for seizure control. It boosts the activity of brain-specific uncoupling proteins in the mitochondria, thus reduces the reactive oxygen species (ROS) generation as well [10], which would otherwise damage the brain. It is also well known that PUFAs stimulate the mitochondrial biogenesis, hence increase the ATP production capacity and enhance energy reserves, leading to stabilized synaptic function and improved seizure control[11]. The elevated PUFA concentration in the brain also increases the expression of proliferator-activated receptor-α (PPARα) [12], which leads to the decrease of pro-inflammatory IL-1β cytokine that has got an important role in hyperexcitability and seizure generation [7].

The relevance of these results is huge, because as theoretically, the enrichment of different foods is possible with many types of fats. Each has its unique fatty acid composition, hence we have a chance to control not just the quantity but also the quality of circulating fatty acids heading to the brain.

The commonly used sunflower oil contains 46.2 g/100 g monounsaturated fatty acids (MUFA) of, 36.4 g/100 g PUFAand 132 iodine value. In contrast, other fats and oils can be easily distinguished from sunflower oil, as olive oil (MUFA: 72.9 g/100 g; PUFA: 10.5 g/100 g; iodine value: 84), coconut oil (MUFA:5.8 g/100 g; PUFA: 1.8 g/100 g; iodine value: 8), rape oil (MUFA:63.2 g/100 g; PUFA: 28.1 g/100 g; iodine value: 50) and lard (MUFA:45.1 g/100 g; PUFA: 11.2 g/100 g; iodine value: 8) havedifferent values [13,14].

This distinct fatty acid characteristics of the different fats also affect their stability, hence the applicability during the food preparing technology. Zhang et al. investigated the stability of some PUFA's, and found that linoleic acid, linolenic acid, arachidonic acid are relatively stable compared to docosahexaenoic acid and conjugated linoleic acids which have got fast oxidation rate [15]. The picture is further complicated by the fact that fats are the mixtures of fatty acids and other lipids so the oxidative stability of the characteristic fatty acid is not enough to describe the stability of the whole fats during heath treatment. The smoke point is often used as an indicator of heat tolerance of fats. This parameter gives the temperature needed for a fat at which it gives off smoke. From the aforementioned fats, sunflower oil has got the highest smoke point of 227 °C, coconut oil (204 °C), rape oil (204 °C) and lard (192 °C) all possess lower smoke point. The olive oil is the most sensitive with its smoke point value of 160 °C [16].

In addition to PUFAs, plant origin oils may also contain other health benefit substances remained from the raw plant materials that can improve the free radical scavenger capacity of the human body [17,18]. It is relevant because lately the relative oxygen species (ROS) were coupled with the epileptic seizures as well [19]. Taking everything into consideration the well-chosen fats in the diet can act on at least two ways: give proper quantity and quality of fatty acid intake as well as provide adequate antioxidant supply.

Owing to the above mentioned reasons we aimed to investigate the applicability of sunflower oil, olive oil, coconut fat, rape oil and lard to enrich french fries, meatloaf and fried zucchini with lipid mass, UFAs, and antioxidants. The stability of these oils during the food preparation, and short-term storage at 4 °C were also assessed

2. Material and methods

2.1. Sample preparation

To investigate the total lipid content, UFA, and antioxidant enrichment capabilities of the different fats, refined sunflower oil, dry refined coconut oil, refined rape oil, extra virgin olive oil and lard were tested. All of the fats were purchased from commercial trade.

From the three selected model foods, french fries were prepared by chopping the peeled potatoes to approximately 4 cm height × 1 cm wide × 1 cm long pieces and then were deep fried in the different oils for 15 min.

Meatloaf samples were prepared as described in the followings. 500 g of swine minced meat was mixed well with 2 eggs, 2 milk-soaked rolls and 1 roasted onion. This mixture was fried for 20 min in the different fats.

To prepare fried zucchini, round shaped 1 cm thick pieces were sliced from the peeled zucchini then the slices were breaded, which means turning into flour, egg, and breadcrumbs, and fried for 10 min in the different fats.

Each food was let to soak in the hot fats for further 5 min following the cooking time. In the case of sunflower oil, foods were prepared without soaking and these samples were used as controls.

Samples were measured directly after preparation and after three days, stored in a household fridge, adjusted to 4 °C.

2.2. Laboratory measurements

The total fat content was estimated gravimetrically following apolar extraction[20]. Briefly: 2 g of the sample was weighed then 10 cm^3 5% sulfuric acid containing ethanol was added and the sample was kept on 70 °C for 10 min. After this destruction step, 50 cm^3 petroleum-ether was added and the sample was shaken for 30 min. Following the extraction time, 30 cm^3 distilled water was added and the separation of the polar and apolar layers were enabled for 5 min. 10 cm^3 of the apolar layer was evaporated at 105 °C then the remaining material was weighed, and the total lipid content was calculated.

The lipid uptake values were calculated by comparing the samples to the sunflower oil unsoaked control sample's total lipid content values measured in the same time.

Unsaturated Fatty Acid content was estimated by the measurement of Iodine value, based on ISO 3961:2013 [21].

In order to measure rancidity, TBA assay was applied [22]. 10 g of the sample was weighed and 10 cm^3 of TBA reagent (containing 20% trichloroacetic acid, 0,5% thiobarbituric acid and 2,5 M HCl) was added. The sample was kept in a boiling water bath then it was spined on 200g for 10 min. The polar layer was used for the determination at λ = 532 nm against malondialdehyde (MDA) standard curve.

The measurement of antioxidant-activity was performed with the TEAC assay [23]. To determinate the samples' antioxidant-activity 50% ethnaloic extraction was prepared by shaking the homogenized samples on 40 °C for 20 min at 180RPM on a laboratory shaker. After centrifugation the supernatant was used for measurements. The reaction mixture contained 10 μl of the sample; 0.07 mg myoglobin in final volume isolated from horse hearth, dissolved in 50 mM, pH 7.4, 9% NaCl and 1% glucose containing potassium-phosphate buffer; 0.015 mg ABTS(2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) and 0.025 g H_2O_2 in final volume, dissolved in 0,1 M pH5 citrate buffer. This mixture was shaken for 5 min at 37 °C then alkaline stop solution was added, and measured at λ = 405 nm against trolox(6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) calibration curve.

Each of the measured parameters was performed in triplicate.

2.3. Statistical analysis

To compare the different parameter values by the applied fats and measurement time points (fresh and stored samples) in