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Moderate but not high daily intake of chili pepper sauce improves serum glucose and cholesterol levels

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ABSTRACT

Chili and its main active ingredient capsaicin have been shown to induce several favorable biological activities. In the present study we have tested the effect of three chili sauces mimicking popular commercially available chili sauces with an increasing amount of capsaicinoids on a broad range of serum biochemical markers in thirty healthy subjects. We show that in the region where daily use of chili is traditionally low, moderate consumption of 4.4 mg of capsaicinoids per day has beneficial health effects, such as decrease in glucose level, LDL cholesterol and C-reactive protein. However, when a product with higher daily dose of capsaicinoids (16.7 mg/day) was consumed, those effects were lost. Considering that biochemical marker levels returned to the initial levels after a week-long wash-out period, we suggest that regular intake of moderate amounts of chili product would be most profitable.

1. Introduction

Chili peppers (*Capsicum* sp.), although originally South American, are used in culinary cultures worldwide and their production has in the last decade been increasing (Faostat, 2017; www.fao.org/faostat/). Chilies contain non-caloric bioactive ingredients, which have lately gained considerable interest as a potential anti-obesity dietary intervention. Studies investigating how chili peppers affect energy balance, metabolism and other health related parameters are focused mainly on the activation of transient receptor potential vanilloid-1 (TRPV) receptors by capsaicin, their most abundant bioactive compound, also responsible for its pungency.

In animal models, several benefits of either single capsaicin containing meal or regular capsaicin intake were described. These include accelerated thermogenesis, an increase in lipid oxidation (Kawabata et al., 2009), inhibited adipogenesis and therefore reduced deposition of lipids in liver and as visceral fat (Li et al., 2012). Improved glucose tolerance was also found consistently (Lee et al., 2013; Song et al., 2017). Moreover, some studies found that capsaicin has anti-inflammatory effects. Mice fed a high-fat diet supplemented with capsaicin exhibited lower levels of metabolic endotoxemia and chronic low grade inflammation with lower body weight gain (Kang et al., 2010). In addition, injection of capsaicin in obese mice exerted an anti-inflammatory effect on adiposse tissue and decreased macrophage infiltration (Kang et al., 2010). Capsaicin could also induce vasodilatation by stimulating NO production, which makes it useful to reduce hypertension and delay the onset of a stroke (Yang et al., 2010).

Studies in humans gave less conclusive results. While capsaicin was able to increase thermogenesis and energy expenditure and change substrate oxidation according to most studies, there are also reports where the same parameters were not affected. This may be due to different concentrations of capsaicin used, length of intervention, body composition or previous habits of subjects' chili consumption (reviewed in Fattori, Hohmann, Rossaneis, Pinho-Ribeiro, & Verri, 2016). Moreover, many studies evaluating influence of capsaicin or its analogs on concrete serum markers are limited to particular subject groups. In women with gestational diabetes (Yuan et al., 2016) capsaicin decreased postprandial hyperglycemia, reduced insulin resistance and the ratio between insulin and glucagon, reduced fasting triglyceride and total cholesterol levels. Further, an increase in fasting serum HDL cholesterol was reported for subject whose initial HDL cholesterol level was too low (Qin et al., 2017). Capsaicin could also reduce blood pressure in hypertensive, but not in normotensive subjects (Harada and Okajima, 2009). In addition to studies focusing on capsaicin, several population-based studies confirmed the association between self-reported intake of spicy food with lower serum LDL, an inverse

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association with total mortality and mortality due to particular diseases but also an association with increased serum triglycerides (Xue et al., 2017).

Fresh chili peppers and their products are for an average consumer more likely the source of capsaicin than capsaicin capsules. The advantage of those is that in addition to capsaicin, chili products contain several others bioactive compounds that have health beneficial effect, such as vitamins C and E, carotenoids, phenols and flavonoids (Wahyuni, Ballester, Sudarmonowati, Bino, & Bovy, 2013). On today's market, a consumer can access chili products with varying content of capsaicin and varying level of pungency, measured in Scoville heat units (SHU). Compared to most widely used jalapeno peppers, scoring 2500–5000 SHU, some chili sauces can reach and exceed 1 million SHU.

The degree of heat level of chili peppers is regulated by genotype and environmental factors, including weather and growing conditions (Zewdie & Bosland, 2000; Gurung, Techawongstien, Suriharn, & Techawongstien, 2011; Bosland & Votava, 2012). It has been noted that environmental stress, such as water deficit (Ruiz-Lau et al., 2011; Phimchan & Techawongstien, 2012), high temperature (Gurung et al., 2011) and soil nutrient imbalances (Montforte-Gonzalez, Guzmán-Antonio, Uuh-Chim, & Vázquez-Flota, 2010), leads to increase in the capsaicinoid levels in different chili pepper cultivars. Chili pepper cultivation is widespread in most tropical, Mediterranean and temperate regions, resulting in the high genetic diversity of local chili pepper cultivars (Nicolaï, Cantet, Lefebvre, Sage-Palloix, & Palloix, 2013), and consequently in greater heterogeneity of capsaicinoids content (Gurung et al., 2011). For successful cultivation of local ecotypes and stable amount of capsaicinoids, the investigation of chili pepper genetic resources and identification of most promising ecotypes is necessary.

Considering the potential benefits of chili intake, but on the other hand the lack of thorough studies of its influence on human body and the safety of regular consumption of different amounts, we aimed to determine the effects of daily intake of 3 chili sauces with increasing pungency on a broad range of serum biochemical markers and thus evaluate the sauces as potential functional food products. The evaluated chili sauces that were produced to mimic popular commercially available sauces from chili pepper cultivars grown in the Slovenian Istria were chemically characterized for capsaicinoids content. A further aim was to assess the genetic diversity of chili peppers used as a source for sauces preparation, and other genotypes available at Slovenian market.

2. Materials and methods

2.1. Genetic analysis

2.1.1. Plant material and DNA extraction

A total of twenty-nine chili pepper cultivars grown and commercially available in Slovenian Istria were used for diversity analysis of chili pepper germplasm. The plant material was collected from local plantations, along with the chili pepper cultivars provided by Agraria Koper Ltd. (Koper, Slovenia). A total genomic DNA was extracted from young chili pepper leaves using a modified method appropriate for DNA isolation from plant tissues rich in secondary metabolites (Japelaghi, Haddad, & Garossi, 2011). DNA concentrations were measured by a Qubit TM fluorometer (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA).

2.1.2. Genotyping procedure and genetic data analysis

A set of eighteen microsatellite markers was selected for genotyping and genetic diversity analysis: CaBR23, CaBR36, CaBR53, CaBR65, CaBR67, CaBR68, CaBR77, CaBR80, CaBR82, CaBR88, CaBR116, CaBR124 (Buso, Reis, Amaral, & Ferreira, 2016), EPMS 376, EPMS 418 (Nagy, Stágel, Sasvári, Röder, & Ganal, 2007), AGi004, AGi111, AGi113 and AGi121 (Ince, Karaca, & Onus, 2010). The amplification of microsatellites was performed in a total volume of 15 µL, containing 1× supplied PCR buffer (Promega, Manheim, Germany), 2 mM $MgCl_2$, 0.2 mM of each dNTP (Sigma-ALDRICH, St. Louis, USA), 0.2 µM of each locus specific primer (IDT-DNA, Leuven, Belgium) with one of the primers in the pair elongated for the M13(−2 1) universal sequence (Schuelke, 2000), 0.25 µM of M13(−21) universal primer labelled with 6-FAM, VIC, PET or NED (Applied Biosystems, Woolston, UK), 0.375 unit of Taq DNA polymerase (Promega, Manheim, Germany), and 40 ng of chili pepper DNA. The amplification was performed in a Thermal Cycler 2720 (Applied Biosystems, Thermo Fisher Scientific, Singapore). The amplification protocol for the microsatellite loci was performed according to Schuelke (2000). Separation of amplified microsatellites was performed on automatic ABI 3130 sequencer (Applied Biosystems, Hitachi High-Technologies Corporation, Tokyo, Japan), using GeneScanTM-500 LIZ® (Applied Biosystems, Woolston, UK) for the size standards, and the data were analyzed with Gene Mapper v. 4.1 software (Applied Biosystems, Thermo Fisher Scientific, Foster City, CA, USA).

The genetic diversity and relatedness between these 29 chili pepper samples were calculated based on the proportion of shared alleles (DSA) between individuals using Populations ver. 1.2.32 (Langella, 2002). Cluster analysis was carried out by the unweighted pair-group method with arithmetic averages (UPGMA), and the UPGMA dendrogram was constructed by TreeView ver. 1.6.6 (Page, 1996).

2.2. The composition of the chili sauces

Three products were produced to mimic sauces that are among the most popular chili sauces available on the Slovenian market. All three sauces were produced by a local company Gorki Chili Ltd. which produces top-quality products from chili peppers harvested only in Slovenian Istria. Chili peppers were picked in October 2016 at the ripening stage, then washed, cleaned and stored in the freezer at −20 °C until cooking. Chili sauces were cooked and packed in glass bottles in April 2017. Two of the selected sauces on the market contain also truffle or chocolate, which were omitted for the purpose of the study, whereas the types and amounts of chili and other ingredients were preserved. Detailed composition and nutritional values are specified in the Table 1. All sauces contained the same base composed of onion, garlic, salt and lime, but differed in the amounts and cultivars of chili added to this base. Sauces 1 and 2 contained Cayenne pepper cultivars, the amount of which was double in sauce 2, and sauce 3 contained Habanero orange chili pepper cultivars; genetic analysis of the chilis was performed and is reported in Fig. 2. The three sauces for this reason differed in pungency – commercial products are labelled with arbitrary unit of pungency 3/10 for the first sauce, 6/10 for the second sauce and

Table 1
Ingredients in the formulations of tested chili products and products nutritional values.

Ingredients	Chili sauce 1	Chili sauce 2	Chili sauce 3
Chili pepper Cayenne (*C. annuum*)	50%	100%	–
Chili pepper Habanero orange (*C. chinense*)	–	–	50%
Pepper	50%	–	50%
Onion	+	+	+
Garlic	+	+	+
Salt	+	+	+
Lime	+	+	+
Nutritional value (100 g)			
Energy value kJ (kcal)	197 (47)	197 (47)	184 (44)
Total fat (g)	2.7	2.7	1.8
Saturated fat (g)	0.5	0.5	0.5
Total carbohydrates (g)	4.3	4.3	4.6
Sugars (g)	3.3	3.3	3.2
Proteins (g)	0.8	0.8	0.7
Salt (g)	0.85	0.85	0.76
Pungency	3/10	6/10	10/10