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An exceptionally high content of kynurenic acid in chestnut honey and flowers of chestnut tree

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ABSTRACT

Kynurenic acid (KYNA) is a metabolite of tryptophan exerting a number of positive actions in rodents, including anti-inflammatory and antioxidative activity. In this study, an analysis of KYNA concentration in a number of honeys, including chestnut honey, as well as chestnut tree parts and products made from chestnuts, was performed. It was found that the content of KYNA in chestnut honey is exceptionally high, in comparison both with other types of honey investigated and with selected chestnut products, such as peeled fruit, crème, puree and flour. It was also detected that the content of KYNA in chestnut tree male flower is several times higher than in female flower. Our results indicate that chestnut honey, which is predominantly produced in Mediterranean countries, contains very high amounts of KYNA and can be an important source of dietary KYNA.

1. Introduction

Kynurenic acid (KYNA) is a substance whose presence was first demonstrated in urine (Liebig, 1853). It is formed enzymatically *via* the kynurenine pathway from tryptophan. While the main end-product of kynurenine catabolism is nicotinamide adenosine dinucleotide (NAD+), KYNA is also produced, due to the activity of kynurenine aminotransferases (Adams et al., 2012; Schwarcz et al., 2012).

It was demonstrated that KYNA is an antagonist of ionotropic glutamate receptors (Alt et al., 2004; Ganong and Cotman, 1986; Kemp et al., 1988; Kessler et al., 1989; Mok et al., 2009) and an antagonist of alpha7 nicotinic receptors (Hilmas et al., 2001). As both ionotropic glutamate receptors and alpha7 nicotinic receptors are predominantly expressed in the brain, it was stated that KYNA is present in the brain (Moroni et al., 1988; Turski et al., 1988). Studies aimed at discovering KYNA's properties and concentrations in the brain and very low blood–brain barrier penetration of KYNA (Fukui et al., 1991) prompted researchers to concentrate on analyzing peripheral KYNA. It was found that KYNA is an agonist of G-protein coupled GPR35 receptors. Furthermore, it was stated that GPR35 receptors are mainly present in the gastrointestinal tract (Wang et al., 2006). Further analyses led to results indicating that KYNA is present in the gastrointestinal tract and that its concentration increases along it (Kuc et al., 2008). The lowest concentration of KYNA was detected in human saliva (0.003 μM) and human gastric juice (0.01 μM) while the highest concentration of KYNA was found in rat's middle ileum mucus (8.08 μM) and rat's distant ileum mucus (16.10 μM) (Kuc et al., 2008). There is, however, no explanation for a gradual increase in KYNA concentration along the digestive system (Turski et al., 2013).

Abbreviations: GPR35, G-protein coupled receptor; HPLC, high performance liquid chromatography; IDO, indoleamine 2,3-dioxygenase; KYNA, kynurenic acid; SD, standard deviation; TDO, tryptophan 2,3-dioxygenase.

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It was demonstrated that KYNA protects against gastric and duodenal ulcers induced by a poisonous Atlantic shellfish (Glavin et al., 1989). Furthermore, KYNA was found to limit stress- and ethanol-dependent ulcers in rats (Glavin and Pinsky, 1989). It was also stated that KYNA decreases intestinal hypermotility and xanthine oxidase activity in experimental colon obstruction in dogs (Kaszaki et al., 2008). KYNA was also found to reduce motility and inflammatory activation in the early phase of acute experimental colitis in rats (Varga et al., 2010). There are, however, studies which show that the level of KYNA is higher in patients suffering from inflammatory bowel disease (Forrest et al., 2003) while lower in patients suffering from irritable bowel syndrome (Christmas et al., 2010; Clarke et al., 2009). Furthermore, it was shown that the level of KYNA is increased in mucus originating from either caecum or ascending colon of patients with colorectal cancer adenomas (Walczak et al., 2011). Summing up, KYNA may possess positive properties in a number of pathologies of the gastrointestinal tract, especially with regard to colitis, colon obstruction or ulceration.

Still, there is currently no clarity on the sources of KYNA in the digestive system. It was shown that KYNA might be synthesized in the human body from tryptophan along the kynurenine pathway (Hiratsuka et al., 2012; Leklem, 1971). It was, however, indicated that KYNA might be absorbed from the gastrointestinal tract into blood circulation (Turski et al., 2009). Interestingly, KYNA is also present in various foods in highly differentiated concentrations. The lowest concentrations in analysed food products were found in meat while the highest concentrations of KYNA were in vegetables, especially broccoli and potatoes (Turski et al., 2009, 2012). High content of KYNA was also detected in honey (Turski et al., 2009) but even higher concentrations of KYNA were found in some analysed herbs – St. John's wort, nettle leaf, birch leaf and peppermint leaf (Turski et al., 2011)—and in some spices characteristic of a Mediterranean diet (Turski et al., 2015).

The level of KYNA both in the diet as a whole and in separate food products should be analysed and controlled. Therefore, an analysis of KYNA concentration in a number of honeys, including chestnut honey, as well as chestnut tree parts and products made from chestnuts, was performed.

2. Materials and methods

2.1. Honeys

Honeys were shop-bought. They came from different countries and manufacturers (see Table 1 for details). Three samples were prepared from each honey jar so as to conduct analyses.

2.2. Chestnut products

Chestnut products were also shop-bought. They all came from one producer: Clement Faugier, Privas, France. Three samples were collected from each package so as to conduct analyses.

2.3. Chestnut tree parts

Leaves, root and generative organs of a chestnut tree – *Castanea sativa* – located in the Botanical Garden in Lublin (51°15′432″N; 22°30′47,7″E) were collected and dissected by an experienced botanist. First collection occurred on 14th June 2014 and 3 samples from each of 20 flowers, both uni- and bisexual, were collected. Collected flowers were then divided into female and male inflorescences in 3 phenological states: budding, beginning of flowering and full flowering. Second collection occurred on 14th July 2014 when only fruit buds were taken for further analyses. Samples were dried at 30 °C and stored at room temperature for further analysis.

Table 1
Kynurenic acid (KYNA) content in selected types of honey.

Symbol	Honey type/brand/producer	Country of origin	KYNA (μg/g) mean ± SD	$p < 0.05$ *vs*
A	Chestnut honey/Miele di Castagnio biologico [1]	Italy	601 ± 45.3	C,D,E,F,G,H,I,J,K,L,M,N,O,P,Q,R,S,T,U,V,W,X
B	Chestnut honey/Miód kasztanowy [2]	Italy	576 ± 43.1	D,E,F,G,H,I,J,K,L,M,N,O,P,Q,R,S,T,U,V,W,X
C	Chestnut honey/Chataignier – Pure Chestnut Tree Honey [3]	France	526 ± 46.3	A,D,E,F,G,H,I,J,K,L,M,N,O,P,Q,R,S,T,U,V,W,X
D	Chestnut honey/Kastanie [4]	Germany/Italy/ France	305 ± 10.4	A,B,C,E,F,G,H,I,J,K,L,M,N,O,P,Q,R,S,T,U,V,W,X
E	Chestnut honey/Organic honey from chestnut tree [5]	Greece	129 ± 7.20	A,B,C,D,F,G,H,I,J,K,L,M,N,O,P,Q,R,S,T,U,V,W, X
F	Pine honey/Miód piniowy [2]	Turkey	14.2 ± 1.26	A,B,C,D,E
G	Eucalyptus honey/Miele di Eucalipto [1]	Italy	11.3 ± 0.53	A,B,C,D,E
H	Honey made from Pueblo plants/Miód z roślin z Pueblo [2]	Mexico	3.46 ± 0.325	A,B,C,D,E
I	Sunflower honey/Miele di girasole [6]	Italy	1.73 ± 0.135	A,B,C,D,E
J	Fir honey/Miód BIO jodła [5]	Greece	1.06 ± 0.064	A,B,C,D,E
K	Clover honey/Miele di trifoglio [6]	Italy	0.746 ± 0.073	A,B,C,D,E
L	Orange honey/Orange honey [7]	Greece	0.609 ± 0.035	A,B,C,D,E
M	Linden honey/Miód lipowy [8]	Poland	0.391 ± 0.021	A,B,C,D,E
N	Clover honey/Miód koniczynowy [2]	Argentina	0.342 ± 0.017	A,B,C,D,E
O	Buckwheat honey/Miód gryczany [9]	Poland	0.329 ± 0.032	A,B,C,D,E
P	Orange honey/Miód pomarańczowy [2]	Spain	0.273 ± 0.022	A,B,C,D,E
Q	Sulla honey/Miele di sulla [6]	Italy	0.222 ± 0.011	A,B,C,D,E
R	Linden honey/Miód lipowy [9]	Poland	0.177 ± 0.008	A,B,C,D,E
S	Lavender honey/Miód lawendowy [2]	France	0.147 ± 0.007	A,B,C,D,E
T	Thyme honey [7]	Greece	0.143 ± 0.007	A,B,C,D,E
U	Multiflorous honey [7]	Greece	0.124 ± 0.005	A,B,C,D,E
V	Honeydew honey/Miód spadziowy [10]	Poland	0.122 ± 0.008	A,B,C,D,E
W	Lucerne honey/Miele di medica [6]	Italy	0.101 ± 0.004	A,B,C,D,E
X	Multiflorous honey/Miele di Millefiori [6]	Italy	0.093 ± 0.005	A,B,C,D,E

Name of producer in brackets: 1–Alce Nero, Monterenzio, Italy; 2–APIS, Apiculture Cooperative, Lublin, Poland; 3–Provence Line, Bonnieux, France; 4–Braitsamer Honig, München, Germany; 5–Smaki Peloponezu, Sosnowiec, Poland; 6–Apicoltura Il Pino, Chiusi, Italy; 7–Melissokomiki Dodecanese L.T.D., Rhodes, Greece; 8–Sądecki Bartnik, Stróże, Poland; 9–Ratos Natura, Baćkowice, Poland; 10–Pasieka pod Jaworem, Lutomiersk, Poland. Data are presented as a mean ± SD; $n = 3$. Statistical analysis was performed using one-way ANOVA with Tukey post hoc test ($p < 0.05$) *vs* respective product presented as a letter (symbol).