Scholars LITERATURE

Journal of Food and Nutritional Health

Short Communication

DOI: https://doi.org/10.47275/2692-5222-115
Volume 3 Issue 1

Effect of Honey on the Maintenance of Taste Components in the Chub Mackerel *Scomber Japonicus* (Houttuyn, 1782)

Hiroko Seki[1]* and Misaki Kikuchi[2]

[1]School of Bioscience and Biotechnology, Tokyo University of Technology, Tokyo, Japan
[2]Panasonic System Solutions Japan Co., Ltd., Tokyo, Japan

Abstract

The primary taste component of chub mackerel is inosinic acid (IMP). Inosinic acid is generated by ATP degradation after fish death. However, it is degraded to non-taste components by IMP-degrading enzymes (IMPases) over time. Therefore, to maintain the taste of chub mackerel, IMPase activity should be inhibited. Chub mackerel is often processed using liquid seasoning including sugar. Honey is one of the sugar-rich products. As sugar has been reported to inhibit the activity of some types of enzymes, honey is also expected to inhibit IMPase activity. Therefore, in this study, we investigated the effects of honey, its main components (glucose and fructose), and its characteristic components (hydroxymethylfurfural (HMF) and melanoidin) on IMPase activity in chub mackerel. The results indicated that IMPase activity decreased with increasing honey concentration. Furthermore, a relatively high inhibitory effect of fructose was confirmed. Although HMF slightly inhibited IMPase activity, it did not decrease with increasing HMF concentration. In contrast, the IMPase activity decreased with increasing melanoidin concentration. These results indicate that honey has an inhibitory effect on IMPase activity in chub mackerel, and fructose and melanoidin are the main components inhibiting IMPase activity. Thus, honey could be an effective ingredient for processing chub mackerels.

Keywords: Inosinic Acid; IMP Degrading Enzyme Activity; Fructose; Melanoidin; Maillard Reaction

Correspondence to: Hiroko Seki, School of Bioscience and Biotechnology, Tokyo University of Technology, Tokyo, Japan; Tel: +81-42-637-2193; E-mail: sekihrk@stfteu.ac.jp

Citation: Seki H, Kikuchi M (2022) Effect of Honey on the Maintenance of Taste Components in the Chub Mackerel *Scomber Japonicus* (Houttuyn, 1782). J Food Nutr Health, Volume 3:1. 115. DOI: https://doi.org/10.47275/2692-5222-115

Received: May 06, 2022; **Accepted:** May 18, 2022; **Published:** May 23, 2021

Introduction

Chub mackerel are small fish that migrate over a wide area and contribute to a good catch [1]. Chub mackerel are consumed throughout the world because of their good taste. Similar to that, in other fish, the major taste component of chub mackerel is inosinic acid (IMP), which is generated by the degradation of ATP-related components as follows: ATP→ADP→AMP→IMP→HxR→Hx.

ATP is degraded to IMP relatively quickly, and then, IMP accumulates in the fish muscle [2]. However, IMP is degraded to non-taste components inosine (HxR) and hypoxanthine (Hx) by IMP-degrading enzymes (IMPases) over time. Therefore, IMPase activity should be inhibited to maintain the taste of fish. IMPases consists some types of enzymes, and their composition and structure depend on fish species [3]; thus, IMPase in each fish species needs to be investigated. In addition, the conditions under which inhibit IMPase activity is inhibited in chub mackerel should also be investigated to preserve the taste.

Chub mackerel is often stored in liquid seasoning, brine, vinegar, miso, or sweet sake. Salt has been reported to inhibit IMPase activity in chub mackerel [2]; thus, processing methods using salt or liquid seasoning including salt can preserve the taste of chub mackerel. Sugar is often added to vinegar, miso, or sweet sake liquid seasoning. However, the effect of sugar on IMPase activity in chub mackerel has not been reported in detail. The main component of sugar is sucrose, which inhibits IMPase activity in the chicken grunt *Parapristipoma trilineatum* (Thunberg, 1793), Japanese amberjack *Seriola quinqueradiata* (Temminck & Schlegel, 1845), and Pacific cod *Gadus macrocephalus* (Tilesius, 1810) [4]. Fructose has been reported to inhibit sucrase activity in rats [5]; thus, it is expected that these saccharides will also have an inhibitory effect on IMPase activity in chub mackerel. Honey contains glucose and fructose and is one of the sugar-rich products. Honey has been reported to inhibit acetylcholinesterase, which is associated with Alzheimer's disease [6]. It also inhibits the activity of angiotensin I-converting enzyme [7]. Therefore, honey is expected to inhibit IMPase activity in chub mackerel. Hydroxymethylfurfural (HMF), which is generated by heating honey, has been reported to exert enhanced immunostimulatory effects [8]. Melanoidin, the color component in honey, is reported to possess antioxidant activity [9]. Furthermore, HMF inhibits an increase in the growth of ethanol-generating yeast [10]. Similarly, melanoidin inhibits carboxypeptidase [11], angiotensin I-converting enzyme, and lipase activities [12]. Therefore, it is expected that these components could also inhibit

IMPase in chub mackerel. To the best of our knowledge, there have been no studies on the inhibitory effect of honey on the activity of IMPase. Therefore, in this study, we aimed to examine the effect of honey and sugar on IMPase activity in chub mackerel. Furthermore, we investigated the effects of individual components of honey, HMF, and melanoidin on IMPase activity in chub mackerel.

Materials and Methods

Materials

Chub mackerels were purchased in retail from a local market near the Tamagawa University, and Japanese Pure Hundred Flower Honey (Ou Youhouen Co., Ltd., Akita, Japan) was used for the subsequent experiments. IMPase was then extracted as an enzyme solution from the fish samples. White meat chub mackerel samples were collected and homogenized in three volume equivalents of water. The homogenate was dialyzed against ultra-pure water for 2 days at 10°C; then, the dialysate was filtered (No. 1; Advantec Co., Ltd., Tokyo, Japan) and diluted twice to adjust the concentration of the enzyme solution. This enzyme solution was then passed through a 0.20-μm filter to remove all bacteria.

Methods

Effect of honey on IMPase activity in chub mackerel: IMPase activity was determined using standard reaction mixtures. A 3.5-mL reaction mixture comprising 0.5 mL of IMP (25 mM), 2 mL of buffer (50 mM maleic acid/Tris/NaOH, pH 6.0), 0.5 mL of honey prepared at 2-10-fold dilutions, and 0.5 mL of enzyme solution was incubated at 30°C for 24 h, and the reaction was stopped by adding 1.5 mL of 10% perchloric acid. The resulting precipitate was separated by centrifugation (13,040 × *g*, 5 min), and the level of free phosphoric acid was determined using the molybdenum blue method. The amount of phosphoric acid was considered to reflect the enzyme activity [13].

Effect of honey, glucose, fructose, and sugar on IMPase activity in chub mackerel: A 3.5-mL reaction mixture comprising 0.5 mL of IMP (25 mM), 2 mL of buffer (50 mM maleic acid/Tris/NaOH, pH 6.0), 0.5 mL of honey (prepared at 2-10-fold dilution) or 8%-41% of glucose, fructose, glucose + fructose (1:1), or sugar (sucrose) [14], and 0.5 mL of enzyme solution was incubated at 30°C for 24 h. The reaction was stopped by adding 1.5 mL of 10% perchloric acid. IMPase activity was determined as described above.

Effect of HMF on IMPase activity in chub mackerel: A 3.5-mL reaction mixture comprising 0.5 mL of IMP (25 mM), 2 mL of buffer (50 mM maleic acid/Tris/NaOH, pH 6.0), 0.5 mL of 1%-10 % HMF (0.5 mL), and 0.5 mL of enzyme solution was incubated at 30°C for 24 h, and the reaction was stopped by adding 1.5 mL of 10% perchloric acid. IMPase activity was determined as described above.

Effect of melanoidin on IMPase activity in chub mackerel: First, a model melanoidin solution was prepared. Briefly, 0.5 g each of the L-isotopes of cysteine, glutamine, glutamic acid, isoleucine, leucine, proline, serine, tryptophan, tyrosine, and valine, along with 50 g of glucose were dissolved in 50 mL of distilled water, and this solution was autoclaved (121°C, 15 min). A 3.5-mL reaction mixture comprising 0.5 mL of IMP (25 mM), 2 mL of buffer (50 mM maleic acid/Tris/NaOH, pH 6.0), 0.5 mL of model melanoidin solution, and 0.5 mL of enzyme solution was incubated at 30°C for 24 h, and the reaction was stopped by adding 1.5 mL of 10% perchloric acid. IMPase activity was determined as described above.

Statistical Analysis

Data were subjected to one-way analysis of variance using the least significant difference method. T-tests were used for pairwise comparisons; statistical significance was set at $p < 0.05$ by Microsoft Excel.

Results

Effect of honey on IMPase activity in chub mackerel

Figure 1 shows the effect of honey on IMPase activity in chub mackerel. The IMPase activity without the addition of honey was 28 PO_4 ng·g^{-1}min^{-1}. However, the addition of honey inhibited the IMPase activity. Interestingly, the extent of inhibition reduced with increasing dilution of honey (2-10-fold dilution of honey [$p < 0.05$]), as demonstrated by the increased IMPase activity (4.7 to 18 PO_4 ng·g^{-1}min^{-1}).

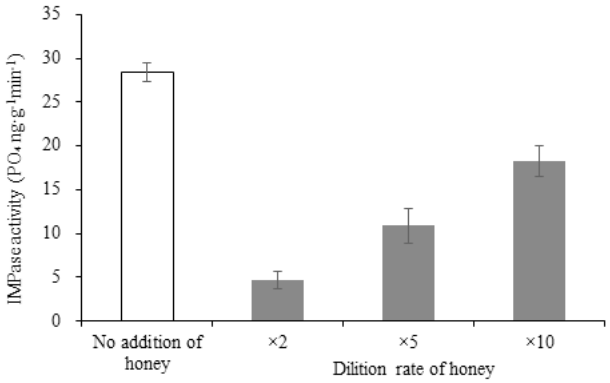

Figure 1: Effect of honey on inosinic acid-degrading enzyme (IMPase) activity in chub mackerel.

The white column shows IMPase activity with no addition of honey and the grey columns show IMPase activity in the presence of honey diluted 2–10-fold (n = 3).

Effect of honey, glucose, fructose, and sugar on IMPase activity in chub mackerel

Figure 2 shows the effect of glucose, fructose, glucose + fructose (1:1), and sugar on IMPase activity in chub mackerel. The IMPase activity without any addition was 65 PO_4 ng·g^{-1}min^{-1}. However, the addition of honey inhibited the IMPase activity. Interestingly, the

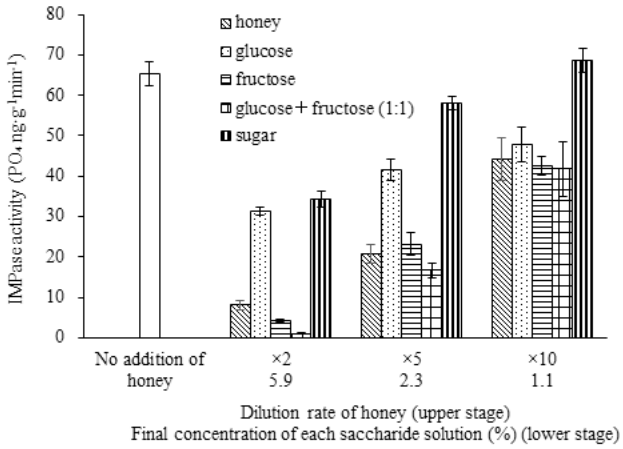

Figure 2: Effect of honey and individual saccharides on inosinic acid-degrading enzyme (IMPase) activity in chub mackerel (n = 3).

extent of inhibition reduced with increasing dilution of honey (2-10-fold dilution of honey [$p < 0.05$]), as demonstrated by the increased IMPase activity (8.2 to 44 PO_4 ng·g^{-1}min^{-1}). Furthermore, the IMPase activity increased from 31 to 48 ng·g^{-1}min^{-1} with glucose, from 4.3 to 43 ng·g^{-1}min^{-1} with fructose, from 1.1 to 42 ng·g^{-1}min^{-1} with glucose + fructose (1:1), and from 34 to 69 ng·g^{-1}min^{-1} with sugar, as the final concentration of these saccharides decreased from 5.9% to 1.1% ($p < 0.05$). There was no difference in IMPase activity between the addition of sugar at a final concentration of 1.1% and no addition of sugar ($p > 0.05$); however, all other test concentrations showed low enzyme activity compared with the activity without any additions ($p < 0.05$). Glucose and sugar addition at a final concentration of 5.9% showed higher activity than that with honey addition ($p < 0.05$), whereas the addition of fructose and glucose + fructose (1:1), at the same final concentration, showed lower activity than that with the addition of honey ($p < 0.05$). At a final concentration of 2.3%, a higher activity was observed with glucose and sugar addition than that with honey addition ($p < 0.05$). However, there was no significant difference in activities between the addition of fructose or glucose + fructose (1:1) and the addition of honey ($p > 0.05$). Similarly, at a final concentration of 1.1%, a higher activity was observed with sugar addition than with honey addition ($p < 0.05$). However, there was no significant difference in enzyme activity with the addition of glucose, fructose, or glucose + fructose (1:1) compared to that with the addition of honey ($p > 0.05$).

Effect of HMF on IMPase activity in chub mackerel

Figure 3 shows the effect of HMF on IMPase activity in chub mackerel. The IMPase activity without any addition was 45 PO_4 ng·g^{-1}min^{-1}. However, with the addition of HMF, the IMPase activity varied from 32 to 38 PO_4 ng·g^{-1}min^{-1} with final concentrations of HMF ranging from 0.14-1.4%, and no differences were observed among final HMF concentrations ranging from 0.14%-1.4% ($p > 0.05$). The addition of HMF resulted in lower IMPase activity that that without any addition ($p < 0.05$).

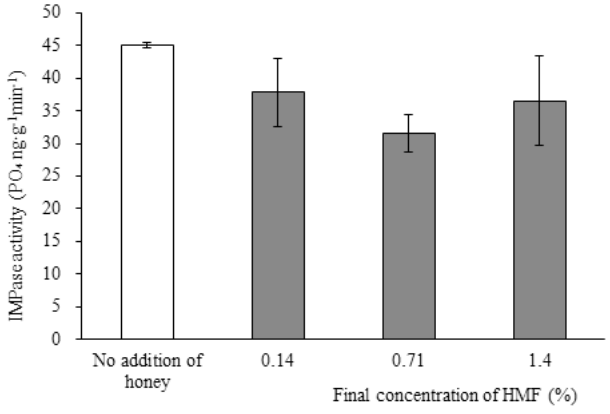

Figure 3: Effect of hydroxymethylfurfural (HMF) on inosinic acid-degrading enzyme (IMPase) activity in chub mackerel.

The white column shows IMPase activity with no addition of honey and the grey columns show IMPase activity with HMF addition.

Effect of melanoidin on IMPase activity in chub mackerel

Figure 4 shows the effect of model melanoidin on IMPase activity in chub mackerel. The IMPase activity without any addition was 55 PO_4 ng·g^{-1}min^{-1}, which decreased to 26 PO_4 ng·g^{-1}min^{-1} when the final concentration of melanoidin was 0.057% ($p < 0.05$). The IMPase activity

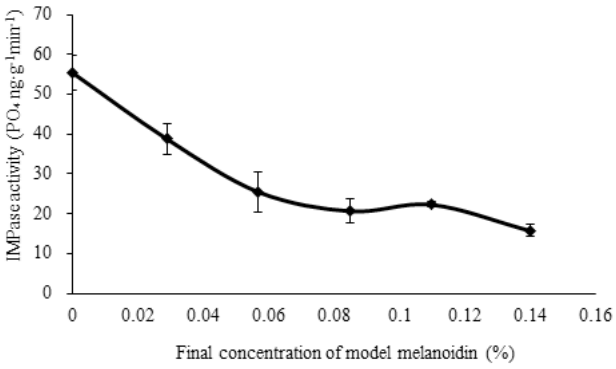

Figure 4: Effect of model melanoidin on inosinic acid-degrading enzyme (IMPase) activity in chub mackerel.

varied between 21 and 26 PO_4 ng·g^{-1}min^{-1} with final concentrations of melanoidin between 0.057% and 0.11%, and there were no differences in IMPase activity at final melanoidin concentrations of 0.057% to 0.11% ($p > 0.05$); however, the IMPase activity decreased to 16 PO_4 ng·g^{-1}min^{-1} at a final melanoidin concentration of 0.14% ($p > 0.05$). The IMPase activity upon melanoidin addition at all tested concentrations was lower than that with no addition ($p < 0.05$).

Model melanoidin was prepared using 0.5 g each of L-cysteine, L-glutamine, L-glutamic acid, L-isoleucine, L-leucine, L-proline, L-serine, L-tryptophan, L-tyrosine, and L-valine and 50 g of glucose. They were dissolved in 50 mL of distilled water (n = 3).

Discussion

Effect of honey on IMPase activity in chub mackerel

Honey affects the activity of several enzymes. The inhibition of angiotensin I-converting enzyme by honey [7]; inhibition of α-glucosidase by the polyphenols in honey [15]; and increased serum cardiac marker enzymes (creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH), and aspartate transaminase (AST)) in rats by honey [16] have been reported. Similarly, in this study, it was demonstrated that honey inhibited IMPase activity.

Effect of honey, glucose, fructose, and sugar on IMPase activity in chub mackerel

Sucrose has been reported to inhibit IMPase activity in the chicken grunt, Japanese amberjack, and Pacific cod. The IMP decomposition rate significantly decreased from 60% to 40% when sucrose increased from 2% to 4% in the chicken grunt and Pacific cod [4]. Similarly, when sucrose was added to Japanese amberjack fish meat, the 5'-NTase activity was inhibited with increasing sucrose concentration [17]. In this study, although IMPase activity was inhibited with increasing sugar concentration, showing a similar trend to that in previous reports, sugar had the lowest inhibitory effect among all examined saccharides. Fructose showed a stronger tendency to inhibit IMPase activity than glucose. Consistent with this result, higher inhibition of enzyme activity has been reported with fructose than with glucose for peroxidase in apples and carrots [18] and for glucokinase-like enzyme activity in the liver of Atlantic salmon (*Salmo salar*) [19]. However, fructose did not show strong inhibition of strawberry polyphenol oxidase and peroxidase activities [20]. This suggests that the effects of glucose and fructose may differ depending on the enzymes. Fructose is a typical competitive inhibitor and glucose is a classical non-competitive inhibitor of invertase in *Tropaeolum majus* leaves [21]. Similarly, in

Saccharomyces cerevisiae, partial non-competitive inhibition, and competitive inhibition of invertase by glucose and fructose, respectively have been reported [22]. Differences in the inhibition mechanisms of sugars in Pacific cod result in differences in the inhibitory effect of sugars on IMPase activity [23]. Similar differences in the inhibitory effect on IMPase activity was observed in this study, which could also be due to variations in the inhibition mechanisms.

Effect of HMF on IMPase activity in chub mackerel

HMF is a heterocyclic aldehyde formed during the Maillard and caramelization reactions. It is the most consistent marker for honey freshness or quality deterioration and is used in national, regional, and international standards as a quality control parameter because its quantity increases upon heat treatment and storage. Furthermore, HMF causes cytotoxicity in humans through reduced granulocyte metabolism [24]. Therefore, the maximum limit for this parameter established by international standards is 40 $mg \cdot kg^{-1}$, and in honeys of tropical origin, the European directives, and the Codex Alimentarius allow a maximum HMF concentration of 80 $mg \cdot kg^{-1}$ [25]. In this study, although we examined the effect of HMF on IMPase activity at extremely high concentrations, we did not observe a substantial inhibitory effect. HMF promotes the activities of alcohol dehydrogenase and acetaldehyde dehydrogenase, and inhibits the pyruvate dehydrogenase complex in the white-rot fungus *Trametes versicolor* [26]. HMF from instant coffee selectively inhibits the activities of mammalian DNA polymerase λ and terminal deoxynucleotidyl transferase; whereas, it does not influence the activities of replicative DNA polymerases such as α, δ, and ε, and DNA polymerase β [27]. According to these reports, HMF shows different effects on different enzymes. Here, the IMPase activity of chub mackerel was found to be slightly inhibited by HMF.

Effect of melanoidin on IMPase activity in chub mackerel

Melanoidin has been reported to inhibit carboxypeptidase A and B enzymes, with their maximum reaction rate decreasing with increasing melanoidin concentration [11], similar to that in the present study. Melanoidin has been found to exert an adverse effect on the activity of α-amylase, and its inhibitory effect increases with increasing molecular weight of melanoidin [28]. Furthermore, the enzyme inhibitory effect may depend on the constitution of melanoidin, as Maillard reaction products formed from the reaction of glucose or fructose with glutathione reportedly show greater inhibition of purified apple polyphenol oxidase activity than those formed from the reaction of hexoses with cysteine [29]. The composition of melanoidin in honey is not uniform, as it depends on the type of source flower. Melanoidin from buckwheat honey has been reported to contain multi-component polymers including the protein-polyphenol-oligosaccharide complex [9]. However, as buckwheat honey has a higher polyphenol content than honey from other nectar sources [30], polyphenols are thought to be one of the components of melanoidin. As the content of polyphenols in the Japanese Pure Hundred Flower Honey used in this study was considerably lower than that in buckwheat, melanoidin in the honey used in this study was considered general melanoidin consisting of sugar and amino acids. In this study, we investigated the effect of a model melanoidin on IMPase activity and observed that the IMPase activity decreased with increasing melanin concentration. As honey contains high-molecular weight melanoidin (65–235 kDa) [9,31], it could be one of the main components that inhibit IMPase activity. This study had some limitations. It is difficult to handle actual samples of both honey and mackerel because of their large individual differences.

Conclusion

In this study, we investigated the effects of honey and the major saccharides in honey, HMF, and melanoidin on IMPase activity for maintaining the taste components in chub mackerel. Fish and honey have differences in their taste. With the addition of honey, the IMPase activity decreased with increasing honey concentration, indicating that honey has an inhibitory effect on IMPase activity. Similarly, high IMPase activity inhibition was observed with fructose, a component of honey. With HMF addition, inhibition of IMPase activity was observed. However, there was no correlation between the increase in HMF concentration and inhibition of IMPase activity, and this inhibition was relatively low. The enzyme activity decreased with increasing melanoidin concentration. These results indicate that honey has an inhibitory effect on IMPase activity in chub mackerel, mainly due to fructose and melanoidin. Thus, honey is an effective ingredient for processing chub mackerels.

It is difficult to identify the specific component of honey responsible for inhibiting the activity of IMPase in chub mackerel, as it is a complex mixture of various components. However, saccharides are the main components of honey; thus, we studied the effect of major polysaccharides in honey on the activity of IMPase. The results indicated that they exerted the greatest inhibitory effect on IMPase activity. However, the components of honey depend on the type of pollen collected. Therefore, in the future, we will investigate the effect of various types of honey on IMPase activity and clarify the effect of honey on the taste components of chub mackerel in greater detail.

Abbreviations

HMF: hydroxymethylfurfural

Hx: hypoxanthine

HxR: inosine

IMP: inosinic acid

IMPase: inosinic acid degrading enzyme

Acknowledgments

We thank Dr. Jun Nakamura. This work was supported by the Mishima Kaiun Memorial Foundation.

Conflict of Interest

The authors declare that they have no conflicts of interest.

References

1. Saito H (2011) Understanding the mechanism and forecasting of fish species alternation by means of system science approach: Results of the research project "SUPRFISH" and the perspective. Aquabiology 33: 69-75.

2. Seki H, Inoue N, Hamada-Sato N (2018) Preservation of fish taste component using bittern. J Japan Soc Taste Technol 16: 30-36. https://doi.org/10.11274/bimi.16.2_30

3. Ooizumi T (2010) Effect of sodium chloride on inosine monophosphatase activity in fish meats and degradation of inosinic acid during drying process of salted fish meats. Salt Sci Res Foundation Annual Res Rep 2010: 133-140.

4. Tomioka K, Endo K, Yang S. 1993. Decomposition of inosinic acid in flesh during cooking. J Home Econ Japan 44: 11-16. https://doi.org/10.11428/jhej1987.44.11

5. Han XY, Hu JN, Wang Z, Wei SN, Zheng SW, et al. (2017) 5-HMF attenuates liver fibrosis in CCl_4 plus alcohol-induced mice by suppression of oxidative stress. J Nutr Sci 63: 35-43. https://doi.org/10.3177/jnsv.63.35

6. Baranowska-Wojcik E, Szwajgier D, Winiarska-Mieczan A (2020) Honey as the

potential natural source of cholinesterase inhibitors in Alzheimer's disease. Plant Foods Hum Nutr 75: 30-32.v https://doi.org/10.1007/s11130-019-00791-1

7. Leon-Ruiz V, Gonzalez-Porto AV, Al-Habsi N, Vera S, Andres MPS, et al. (2013) Antioxidant, antibacterial and ACE-inhibitory activity of four monofloral honeys in relation to their chemical composition. Food Funct 4: 1617-1624. https://doi.org/10.1039/c3fo60221d

8. Ota M, Ishiuchi K, Xu X, Minami M, Nagachi Y, et al. (2019) The immunostimulatory effects and chemical characteristics of heated honey. J Ethnopharmacol 228: 11-17. https://doi.org/10.1016/j.jep.2018.09.019

9. Brudzynski K, Miotto D (2011) Honey melanoidins: Analysis of the compositions of the high molecular weight melanoidins exhibiting radical-scavenging activity. Food Chem 127: 1023-1030. https://doi.org/10.1016/j.foodchem.2011.01.075

10. Kanna M, Kimura N, Yamashita K, Yanagida T, Matsumura Y (2013) Effect of inhibition substances on Monod equation of yeast growth. J Japan Pet Inst 56: 326-330. https://doi.org/10.1627/jpi.56.326

11. Ibarz A, Garza S, Pagan J (2008) Inhibitory effect of melanoidins from glucose-asparagine on carboxypeptidases activity. Eur Food Res Technol 226: 1277-1282. https://doi.org/10.1007/s00217-007-0655-3

12. Miura M (2002) Physiological function of melanoidine (brown pigments). J of Brew Soc Japan 97: 253-256. https://doi.org/10.6013/jbrewsocjapan1988.97.253

13. Seki H, Hamada-Sato N (2015) Effect of various salts on inosinic acid-degrading enzyme activity in the white and dark muscle of saury. Fish Sci 81: 365-371. https://doi.org/10.1007/s12562-014-0840-9

14. Ministry of Education, Culture, Sports, Science and Technology. Standard tables of food composition in Japan. Japan.

15. Ding Y, Xu M, Lu Q, Wei P, Tan J, et al. (2019) Combination of honey with metformin enhances glucose metabolism and ameliorates hepatic and nephritic dysfunction in STZ-induced diabetic mice. Food Funct 10: 7576-7587. https://doi.org/10.1039/C9FO01575B

16. Khalil MI, Tanvir EM, Afroz R, Sulaiman SA, Gan SH (2015) Cardioprotective effects of Tualang honey: Amelioration of cholesterol and cardiac enzymes levels. BioMed Res Int 2015: 1-8. https://doi.org/10.1155/2015/286051

17. Endo K, Yang S (1992) Processing effects on the decomposition of inosinic acid in yellowtail muscle. Res J Life Sci 38: 51-57.

18. Nicoli MC, Elizalde BE, Pitotti A, Lerici C (1991) Effect of sugars and Maillard reaction products on polyphenol oxidase and peroxidase activity in food. J Food Biochem 15: 169-184. https://doi.org/10.1111/j.1745-4514.1991.tb00153.x

19. Tranulis MA, Dregni O, Christophersen B, Krogdahl A, Borrebaek B (1996) A glucokinase-like enzyme in the liver of Atlantic salmon (*Salmo Salar*). Comp Biochem Physiol B: Biochem Mol Biol 114: 35-39. https://doi.org/10.1016/0305-0491(95)02119-1

20. Chisari M, Barbagallo RN, Spagna G (2007) Characterization of polyphenol oxidase and peroxidase and influence on browning of cold stored strawberry fruit. J Agric Food Chem 55: 3469-3476. https://doi.org/10.1021/jf063402k

21. Isla MI, Vattuone MA, Gutierrez MI, Sampiero AR (1988) Acid invertase from tropaeolum leaves. Phytochemistry 27: 1993-1998. https://doi.org/10.1016/0031-9422(88)80084-0

22. Nagamune T, Nakamura T, Endo I, Inoue I (1991) Simulation of sucrose hydrolysis by yeast. Kagaku Kogaku Ronbunshu 17: 462-469. https://doi.org/10.1252/kakoronbunshu.17.462

23. Hamada-Sato N, Seki H (2014) Effects of salt and bittern on inosinic acid- and inosine-degrading enzyme activity in Pacific cod muscle. J Aquac Marine Biol 1: 00001. https://doi.org/10.15406/jamb.2015.01.00001

24. Belay A, Haki GD, Birringer M, Borck H, Lee YC, et al. (2017) Enzyme activity, amino acid profiles and hydroxymethylfurfural content in Ethiopian monofloral honey. J Food Sci Technol 54: 2769-2778. https://doi.org/10.1007/s13197-017-2713-6

25. Makhloufi C, Taiebi K, Ait AL (2020) Characterization of invertase and diastase activities, 5-hydroxymethylfurfural content and hydrogen peroxide production of some Algerian honeys. Iranian J Sci Technol Trans A Sci 44: 1295-1302. https://doi.org/10.1007/s40995-020-00936-x

26. Kudahettige NRL, Holmgren M, Madavi B, Nilsson RT, Sellstedt A (2016) Adaptability of *Trametes versicolor* to the lignocellulosic inhibitors furfural, HMF, phenol and levulinic acid during ethanol fermentation. Biomass Bioenerg 90: 95-100. https://doi.org/10.1016/j.biombioe.2016.03.030

27. Mizushina Y, Yagita E, Kuramochi K, Kuriyama I, Shimazaki N, et al. (2006) 5-(Hydroxymethyl)-2-furfural: A selective inhibitor of DNA polymerase λ and terminal deoxynucleotidyltransferase. Arch Biochem Biophys 446: 69-76. https://doi.org/10.1016/j.abb.2005.11.015

28. Miura M, Gomyo T (1993) Effect of the brown pigment melanoidin on digestive enzymes in human saliva. J Japan Soc Nutr Food Sci 46: 309-316. https://doi.org/10.4327/jsnfs.46.309

29. Billaud C, Brun-Merimee S, Louarme L, Nicolas J (2004) Effect of glutathione and Maillard reaction products prepared from glucose or fructose with glutathione on polyphenoloxidase from apple—I: Enzymatic browning and enzyme activity inhibition. Food Chem 84: 223-233. https://doi.org/10.1016/S0308-8146(03)00206-1

30. Schramm DD, Karim M, Schrader HR, Holt RR, Cardetti M, et al. (2003) Honey with high levels of antioxidants can provide protection to healthy human subjects. J Agric Food Chem 51: 1732-1735. https://doi.org/10.1021/jf025928k

31. Brudzynski K, Miotto D (2011) The recognition of high molecular weight melanoidins as the main components responsible for radical-scavenging capacity of unheated and heat-treated Canadian honeys. Food Chem 125: 570-575. https://doi.org/10.1016/j.foodchem.2010.09.049