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Review article

The influence of selected process factors on the physicochemical and biological properties of honeys: A review

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Abstract: *Honey is one of the world's most valued natural food products. Characteristic, pleasant aroma, golden colour, sweet taste and health-promoting properties cause a continuous increase of honey consumption in the European Union. The most wanted is regional honey. However, imported honey available on the market has often lower prices. The honey obtained can be heat treated, which reduces water content and can slow down the growth of the microorganisms, which are responsible for the undesirable fermentation of honey. Unprocessed honey appears to be the healthiest, but in practice, it's often heat treated to slow down or back up the crystallization process and to make its dosage during technical processes easier. It is widely believed that heating of honey may have a harmful impact on its properties. There are countless articles of popular science, that warn of heating and cooking with honey. Should honey never be heated?*

The aim of this work is to give an overview of the influence of technical processes on the physicochemical and health-promoting properties of honey. The results demonstrated that the physicochemical and bioactive properties of honey are significantly affected by thermal treatment. As a result of the temperature, it comes to the Maillard reaction, during which HMF is synthesized. Furthermore, heating affects the activity of the enzymes contained in honey, among others, diastase and inverses. However, few studies made in this field show that honey heat treatment may work in favour of antioxidant properties. Depending on the type of honey, melanoidins may increase or decrease the antioxidant activity.

Keywords: *honey, maillard reaction, hydroxymethylfurfural, antioxidant activity, heating.*

Introduction

Honey is considered as one of the most popular food products. Consumers enjoy it for the characteristic pleasant aroma, golden colour, naturalness and health-promoting attributes which are assigned to this product. It is believed that honey has anti-oxidative, antibiotic, antiphlogistic and antidiabetic properties. It also exhibits a positive influence on red blood cells and the respiratory system. What's more, honey is a natural product, made by honeybees *Apis mellifera* and other insects such as hornets.

Due to the properties of honey, this product has been used in medicine and cosmetics since ancient times. Honey has such a wide application – it's used not only as a nutritional product, but also in folk medicine as an alternative to conventional medicine. It is believed that this product will make wounds heal quicker and even that it has some anticarcinogenic properties. As a product high in sugar and low in fat, characterized by low water activity, honey which is kept in a closed container, can be stored at room temperature for a long time. A characteristic acid reaction (pH) is the result of the contents of organic acids such as citric or amber. The low pH level of honey contributes to its antibiotic properties.

Honey contains an average of 0.35% protein. The most abundant amino acid is proline. The quantity of this informs us about the quality of honey [1]. Besides proline there are 23 other amino acids, among them, for example: glutamic acid and aspartic acid, glutamine, histidine and glycine [2]. Furthermore, it's worth noting that honey, as a product of vegetable origin, contains only a small amount of vitamins such as vitamin C, some B complex vitamins (riboflavin, pantothenic acid, pyridoxine, biotin, nicotinic acid). The pleasant aroma of honey is caused by the presence of volatile alcohols, aldehydes, esters and ketones. The contents of the phenolic compounds are the reason for the high antioxidant activity and characteristic colour. However the colour of honey may indicate its mineral content. The mineral content ranges from 0.04% in lighter honey types to 0.2% in dark types. To the main macro and microelements belong minerals such as potassium, magnesium, calcium, iron, sodium, manganese, zinc, cobalt, nickel, cooper, selenium and arsenic. The sweet taste of honey is caused by the large number of sugars about 75-80%, especially monosaccharides, glucose and fructose, followed by disaccharides, sucrose, maltose, isomaltose, trehalose and some trisaccharides matotriose and melezitose [3]. Color, smell and taste are determined by the botanic and geographic origins [4].

Furthermore, important aspects which affect sensory properties, are weather conditions, technological factors and the time and temperature of the storage of honey [5]. The physicochemical properties of honey, like, for example, water content, may be information which tells us about the degree of maturity or characteristic storage conditions. Mature honey is separated from honeycomb in honey extractors by spinning. Next it is filtered through sieves to separate the beeswax, bee pollens and other impurities. The honey obtained can be heat treated, which reduces water content and can slow down the growth of the microorganisms, which are responsible for the undesirable fermentation of honey [6]. Unprocessed honey appears to be the healthiest, but in practice it's often heat treated to slow down or back up the crystallization process and to make its dosage during technical processes easier. The limited availability of honey is the reason for their high price. That is why we can see a lot of attempts to manipulate and falsify honey production and so there was a need to identify the quality parameters, which, besides the falsifications in the honey harvesting review, would be helpful in estimating the hygienic conditions during production [7].

Legal norms

According to the data of Honey Market Presentation of European Commission the European Union is the second largest (after China) producer of honey in the world, the third largest producer is Turkey. Each year, the EU produces around 250.000 tons of honey. However, EU production does not cover the demand: import of honey into the EU is about 200.000 tons, of which 40% come from China and almost 20% from Ukraine [8]. The most popular among consumers, as always, is honey in liquid form, so-called strained honey. One of the reasons why non-crystallized honey is considered a product more attractive for consumers is the belief, that its liquidity determines its freshness.

However it's important to emphasise that honey crystallization is a natural process. The crystallization rate depends on a lot of factors, such as: geographic and botanic origin, the temperature of storage, and the water and sugar content. As a result of the huge popularity of liquid honey in industry, it is necessary to recrystallize them. This process requires the product to be heated to 65°C, and then filtered in order to get rid of crystal fibres. The next step is to cool the liquid honey to 49°C and distribute it by pouring it into individual containers [9]. However, the heat treating of honey arouses a great deal of controversy. Crystallized honey, so-called granular honey, is characterized by higher water activity than strained honey (liquid form). It may have some influence on microbiological safety and be a reason for its fermentation, which is a serious problem in tropical countries. Native honey, when kept at an ambient temperature, ferments in a couple of days, because of the high content of water and yeast. In order to prevent fermentation, honey is filtered and heat treated. The heating of honey reduces the number of microorganisms which are responsible for undesirable fermentation and reduce the water content. Furthermore, the liquid form of honey is the most suitable for technical processes.

There are a couple of substations which may be used as indicators of honey heat treatment. One of them is the 5-hydroxymethylfurfural (HMF) – compound which is manufactured during the Maillard reaction by the dehydration of sugar moiety (caramelization for example) under the influence of temperature [10]. The requirements of HMF content in honey have been identified in national law and international standards. The most important of these is the World Standard approved in 2001 by the Codex Alimentarius Commission [11]. In accordance with its provisions, the HMF content in honey must not exceed 40 mg/kg. The member States of the European Union are required to repeatedly apply the amended Council Directive 2001/110/EC, which regulates all the requirements connected with honey quality [12]. Similar to the World Standard, it restricts HMF content to the level of 40 mg/kg of honey. The above directive has applied in Poland since the time of Poland's accession to the European Union in 2004. What's more, in order to harmonize Polish current quality of honey parameters with the requirements of European Union law, the Minister of Agriculture and Rural Development issued a regulation from 2004 [13]. In this legal act it is also stated, that the current limit of HMF content in honey is 40 mg/kg.

Controlling the amount of permitted HMF in honey is important because of food safety. A lot of research tagged with a radioactive carbon isotope HMF confirms that HMF from received food is metabolized and then excreted in urine. However in the experiment with rats, it was found that shortly after administration, HMF is noticeable in both the liver and kidneys [14]. It is suspected that this compound may have a mutagenic, carcinogenic and cytotoxic effect [15]. In an acidic medium, HMF may occur at lower temperatures, although its concentration increases with temperature or with the length of storage [16]. The influence of temperature and heating time on the formation of HMF in the honeydew honey and polyfloral honey was analyzed in the research of Turhan and co-author [17]. Samples were heated to 75, 90 and 100°C for 15-90 minutes. Next, the amount of HMF was examined using HPLC. It was found that the amount of HMF for honeydew honey heated at 90°C for 75 minutes, as well as for polyfloral honey heated to the same temperature for 90 minutes, does not exceed the allowed limit of 40 mg/kg. However, these values were exceeded for honeydew honey heated for 90 minutes at 75°C and for polyfloral honey heated at 100°C for 15 minutes. It was noticed, that the heat treatment of honey in mild conditions for both types of honey does not significantly influence the increase in the amount of HMF and may be safely used to decrystallize and reduce the viscosity of honey. Turhan and co-authors also discovered the dependence between HMF formation and the botanic origin of the honey. On the other hand, Kroh observed that, besides temperature, the pH level and higher water content are both very important for the formation of the compound [18].

Honey also displays a lot of health benefits, which are conditional on the bioactive substance content derived from the nectar and the bee's bodies. Most of these compounds are sensitive to high temperature. Using heat treatment, for example, in order to decristallize the honey, has an impact on reducing the biologically active ingredients in honey. The amount of the losses is proportional to the temperature applied and the time of heating. A negative influence of temperature may be marked by a measurement of the honey's quality indicators. Besides HMF, described above, the diastase number is also considered as a quality parameter [19]. Diastase is the main enzyme found in honey. It's part of glycoside hydrolases and it comes from the bee's glands in the digestive track. It has been proven that an increase of temperature has an influence on the diastase's activity [20]. It is presumed that HMF content, as well as the diastase number is indicators of the honey's quality, however, these substances should not be treated as direct indicators of heating honey. So some of the native honey have a higher HMF content or low diastase activity and as a result they could be incorrectly identified as heat treated. As an example we can take lemon honey, which is characterized by a low diastase number. According to the Codex Alimentarius, the diastase number for honey shouldn't be lower than 8, except honeys with a naturally low diastase number. For these, it should be a minimum of 3.

The effect of heat treatment on the honey properties

There is a lot of research about the impact of heat treatment on the properties of the honey and its components. An interesting aspect of these considerations is the impact of heat on the honey's antioxidant activity. Namely, it has been observed that heat treatment affects the change of the honey's antioxidant properties. As demonstrated, conventional heating may reduce or increase the antioxidant activity of honey [21]. Due to the action of microwave radiation of 1, 26 W/g power, an increase of antioxidant activity for lemon honey and buckwheat honey has been seen. An increase of antioxidant properties after heating a sample of honey in bath-water at 90°C has been found for lemon honey, which correlated with an increase in the polyphenol content.

Polymers of high molecular weight are formed by the Maillard reaction – melanoidins, which are characterized by a high antioxidant activity [22]. The formation of melanoidins may contribute to the changes of the olfactory characteristics of food products such as: colour, aroma or texture. The impact of the type of heat treatment on the composition of melanoidins and their influence on the nourishing and functional characteristics of honey is unknown. However the antioxidant properties of honey, due to the formation of melanoidins, change depending on the type of honey [23]. Moreover, a change of antioxidant activity is conditioned on a change of the polyphenol profile, a change of the colour [24], time and temperature to which honey had been subjected. Honey rich in the polyphenols, such as buckwheat honey, have a greater antioxidant potential than honey with low levels of these substations, for example acacia honey [25]. It has been proven that polyphenols are part of the melanoidin molecules. The Melanoidin fraction from dark buckwheat honey contains the most polyphenols compared with light clover honey and manuka honey [26]. However, polyphenol content in the melanoidin fraction changes after thermal treatment, which has a positive influence on the antioxidant properties of melanoidins. It has been noted that there is a strong relationship between polyphenol content and their antioxidant activity. Chemical analysis of melanoidins demonstrated the presence of proteins, polyphenols and oligosaccharides. Size-exclusion chromatography (SEC) and activity-guided fractionation of honeys allowed Brudzynski and Miotto the isolation of high molecular weight brown compounds, ranging in size from 66 to 235 kDa. They confirmed that melanoidins exhibited peroxyl radical-scavenging activity of heated honeys[27]. However, it is worth mentioning that the concentration, antioxidant activity and the degree of browning of melanoidins in honey increases with temperature. Also, the honey darken during storage [28].

The speed of the browning depends on the physical properties of honey, such as: pH, water activity and the temperature at which the honey is stored. It is presumed that products of Maillard's reaction are mainly responsible for browning.

The high molecular weight of melanoidins indicates a protein content. Analysis confirms the presence of protein complexes with polyphenolic compounds. Based on research by SDS-PAGE, it has been observed that the heat treatment of honey has a bad influence on the amount of proteins contained in melanoidins fraction.

Chaikham and Prangthip [29] have analyzed the effect of the hydrostatic pressure, ultrasounds and temperature on the antioxidant properties of honey from longan flowers. In the wake of studies, it has been found that, as a result of the increased pressure, the amount of phenolic compounds and antioxidant activity may decrease. This effect is noticeable especially in samples used to treat at 500 MPa for 20 minutes. Using ultrasounds turns out to have a similar effect. It also increases the amount of bioactive compounds, which are responsible for antioxidant properties. Authors suppose that the reason may be the disintegration of pollen compounds from honeys. In contrast to the high pressure and ultrasounds, heat treatment at temperatures of 50 and 70°C doesn't change the antioxidant activity, while the sample heated at a temperature of 100°C showed reduced amounts of bioactive compounds and a decrease of antioxidant activity. Fauzi and co-authors [30] were led to the same conclusion, when they were analyzing the impact of high blood pressure on the health promoting properties of manuka honey. Exposing the sample for 10 days at a pressure of 600MPa at room temperature resulted in an increase in the antioxidant activity of 30% and didn't result in a change of colour. However, samples treated at both a higher pressure and a higher temperature didn't show any changes in antioxidant properties, although a change in the honey's colour has been observed.

Honeys display antibacterial activity, which is the result of the presence of polyphenols, flavonoids and melanoidins. Furthermore, other substances which are formed by the Maillard reaction, such as glyoxal or methylglyoxal exhibit cytotoxic properties. They are seen as the main compounds responsible for the antibacterial activity of manuka honey [31]. There is a noticeable correlation between the colour of the honey and, its antioxidant status and antibacterial status against *E. coli* [32]. It was also found that there is a correlation between the antibacterial activity and content of the Maillard reaction's products in native honeys [27]. The impact of the microwave heating to the antibiotic properties of Slovakian honeys was also examined. Samples were treated with microwaves, as well as conventional heating. Next, the antibacterial activity of honeys was evaluated. As a result of studies, it was found that microwave heating has a negative impact on the enzymes defensin-1 and glucose oxidase activity. Defensin-1 is an abiotic peptide derived from the organism of bees. It is believed that this enzyme suppresses the growth of the gram-negative bacteria, although some studies also show antibiotic activity in regard to gram-positive bacteria [33].

Glucose oxidase is a substance contained in the secretions of bees, which are mixed with nectar during transport from the flower to the beehive. This enzyme catalyzes electron transport on the molecular background. That's why bactericidal hydrogen peroxide is synthesized, which plays an important role in honey preservation. Microwaves have a negative impact on the antibacterial potential of honey, because they inhibit the activity of two of the most important enzymes with abiotic properties [34]. However, a reduction of antibacterial potential isn't observed in the case of conventionally heated honey.

Furthermore, the microwave radiation of honey results in a faster HMF growth than conventional heating. While fresh honey doesn't contain any or contains only trace amounts of HMF, honeys which are heated or stored for a long time are characterized by a higher HMF concentration. Scientists did a lot of research on the effect of the temperature on the formation of HMF in honey. 20% water content in honey relates to the efficient operation of microwave pulses, because water is a main absorber of microwave energy. As a result, the higher the humidity the more effective the result of thermal treatment. As opposed to conventional heating, microwave energy enters into the relation with a heated product and leads to the formation of heat influx and consequently to its rapid heating. Loss of water causes higher viscosity, which promotes the flow of heat in a sample. In a study conducted in 2002 researchers compared the effect of microwave heating with conventional heating by the formation of HMF. A sharp increase in the HMF amount together with the time of the sample's microwave irradiation with power between 11, 9 and 16W/g has been observed. However, these amounts don't exceed the maximum HMF quantity [12]. What's more, it turned out that among the different combinations of time and power applied during the irradiation of honey samples, the least invasive in terms of the resulting quantity of HMF, is the usage of higher power and a shorter time of heating rather than less power and a longer time [35]. Research done by Kowalski confirms this conclusion [21]. The author noted that honeydew, because of the higher water content and acidity, is much more sensitive to microwave pulses than other honeys, such as: buckwheat honey, lemon honey or acacia honey. Both in the case of conventional heating and microwave heating, the most intensive HMF growth was observed in honeydew honey. However, in the case of conventional heat treatment, the pace of this process is lowest for acacia honey and highest for lemon honey.

High viscosity makes honey cumbersome to use and in contrast, crystallized honeys are not popular with consumers. Honeys in liquid form pose an economic problem, both in transportation and during storage. In recent years, attempts at drying honey have been made to obtain so-called honey powder. This form of honey may be an additive to many food products, such as: yoghurts, beverages, sauces or snacks. Furthermore, dried honey may be used as a dietary supplement or could be eaten directly. Usage of dried honey as an addition to pastries could positively affect the value and sensory qualities of them. It can also enhance aroma, taste and texture. One of the opportunities for pulverizing honey is microwave drying [36]. It is believed that heating by microwave provides a more uniform and quicker flow of heat than conventional heat treatment. Also, conventional heat treatment using a high temperature and longer time of product exposure may lead to negative changes in its quality, such as: a change of colour, aroma or nutritional values [37]. During the microwave drying process, honey in an autoclave is heated and evaporated in 10 minutes to less than 10% water content. During microwave honey drying, it is very important to control time as well as the temperature of this process. Too high a temperature and too long a time of drying lead to a loss of nutritional properties, colour, aroma and taste of the honey powder. In research by Zheng-Wei, efforts to optimize this process were made [36]. It was found that microwave honey drying using 330 Watt power and 30 mbar pressure is the most beneficial for obtaining honey powder. The colour of the powder obtained hasn't changed compared to the colour of unprocessed honey. Also, the concentration of sugars such as fructose, glucose, maltose or sucrose changed significantly. It was noted that from the most important components of honey responsible for characteristic nice smell, ester and alcohol content, most of them were virtually unchanged, while volatile acids were reduced in contrast to aldehydes and ketones. Their amount in the honey powder was increased.

Shi and co-authors [38] analysed the impact of the carrier type on the properties of the obtained honey powders. Maltodextrin, whey protein or a mix of both substances were used as a carrier. It was not possible to dry honey without a carrier by the spray method. Both in the case of using maltodextrin as well as whey protein, honey powder was obtained. Powders obtained from maltodextrin were characterized by a larger molecular size than those with whey protein. What's more, the size of the molecules increased in proportion to the amount of maltodextrin contained in the carrier mixture. However, both water content in the obtained product as well as the sample's hygroscopic decreased with the increasing amount of maltodextrin. It has been noted that adding a whey protein to maltodextrin reduces the quantity of the required carrier and has a beneficial effect on the drying performance. The amount of protein required to efficiently carry out the drying process depends on the concentration of protein on the surface of the drop. It doesn't depend on the total concentration in the solution of honey and a carrier.

There are many processes used to obtain a powder honey. Besides microwave drying it's worth mentioning the spray drying method. A mixture consisting of honey, a carrier, for example maltodextrin, and water in the drying bucket, through which hot air at a temperature about 160-200°C flows, which leads to rapid evaporation of the mixture and the powder collecting on the bottom of the bucket, is sprayed during this process. In literature, publications which describe the properties of honey powders obtained by above method are available. Dried honeys are characterised by their low water content and low water activity. In the research of Samborska and Czelejewska [39], the effect of thermal treatment and spray drying using Arabic gum with the flower honey and rape honey were compared. Samples of honey were heat treated at temperatures of 50-90ºC for 15-120 minutes. For every sample of the flower honey, a decrease of the diastase number was seen. Heating honey at a 90ºC temperature for more than 30 minutes resulted in a decrease of the diastase number to a value lower than required by the legislature. In the case of rape honey, the diastase number decreased in all samples except these treated at a temperature of 50ºC for 15 and 30 minutes. For samples heated at a temperature of 70ºC t for 120 minutes and all samples heated at a temperature of 90ºC the diastase number didn't reach the required minimum. The Diastase number turned out to be a factor more sensitive to the temperature changes than the quantity of the rising HMF. In both honeys a slight HMF growth was observed after heating at temperatures of 50 and 70ºC. However, with the increase in temperature a rapid increase in the amount of the HMF was discovered, which for samples heated for 120 minutes, led to exceeding the maximum number of 40 mg/kg. It was determined that the honey most associated with the formation of HMF as a result of an increase in temperature is rape honey. As a result of spray drying, the diastase numbers of both samples hadn't changed and the HMF concentration increased in both samples, However, in the rape honey the allowed value was exceeded. It's worth pointing out that, although the temperature of the samples during spray drying was 70ºC and the contact between the hot air and the sample was really short, the increase in HMF was higher than in the honeys heat treated at the same temperature.

In research carried out by Samborska and Bieńkowska [40] Polish flower honey was dried to 1 to 2.1% water content and maltodextrin and dextrin were used as a carrier. On the other hand, according to Nurhadi's research honey dried with maltodextrin was evaporated to 2.2% water content [41]. However, when Arabic gum was used as a carrier, this value was 4.4%. The powder obtained was characterized by good solubility – it was noted that this property depends on a good carrier and drying parameters. Powder prepared with the use of maltodextrin dissolves faster than that using dextrin. The authors noticed a decrease in the HMF concentration compared to the starting product. The Diastase number also has been reduced.

The interesting objectives of Ram’s study [42] were to produce a honey powder containing retrograded starch as a drying agent and use it as an alternative to sucrose in bread formulations. A solution comprising 20% honey, 30% retrograded corn starch an 50% water was spray dried at 200°C. All powder samples indicated the moisture content between 3.83% and 5.53%. The spray dried powder had good morphology and were dispersed rather than being aggregated. He demonstrated, that breads baked with honey powder indicated a lower rate of retrogradation as compared to the bread baked with sucrose. Moreover the loaf volume was significantly higher for the formulation with honey powder than with sucrose.

In the research of Kruszewski and co-authors [5], it was proposed that drying honey should be preceded by alcoholic fermentation in order to reduce the sugar content. Polish buckwheat honey which had fermented with a yeast strain *Saccharomyces cerevisiae* was subjected to this process. As a carrier, Arabic instant gum and tapioca starch were used. A 20% fermentation solution was mixed and homogenized with the carrier, then it was dried in inlet air temperature 180ºC. The obtained powder was characterized by a lower level of acidity in comparison to the raw material. Furthermore, it was also observed that the diastase number fell which could be the result of the partial inactivity of enzymes caused by the action of the temperature. Buckwheat honey powders contained a large number of polyphenols and this was characterized by a high antioxidant activity, which could be the result of a synthesis reaction of antioxidant compounds in the Maillard reaction. A negative aspect of this reaction is increasing the concentration of HMF, which exceeded the standard as defined in European law.

Samborska and Bieńkowska studied the effects of powder honey storage obtained from the Arabic gum and sodium caseinate on their physical properties [40]. After 12 weeks, it was found that the colour had darkened and the redness and yellowing was reduced. However, samples with the addition of 2% caseinate were found to be stable in terms of colour. The storage of powders also affected the particle size. Particle sizes from all samples, except those with Arabic gum, decreased. What's more, the water content and water activity increased, in contrast to the hygroscopicity.

Conclusion

The thermal treatment of honey significantly affected their health-promoting properties. As a result of the temperature, it comes to a non-enzymatic browning of honey, the so-called Maillard reaction, during which HMF is synthesized – a substance suspected of having carcinogenic and mutagenic properties. The requirements for the HMF content in honey have been defined in national law and international standards. Furthermore, heating affects the activity of the enzymes contained in honey, among others, diastase and inverses. Both the amount of the HMF and the enzyme activity provide information about the quality of honey.

However, few studies made in this field show that honey heat treatment may work in favour of antioxidant properties. As a result of the reaction of polymerization of sugars and amino acids arise polymers having a high molecular weight and brown colour – melanoidins. Depending on the type of honey, melanoidins may increase or decrease the antioxidant activity.

Nowadays, in scientific literature, there are only a few publications about the influence of temperature on the formation of the above-mentioned polymers in honey and their influence on the health properties of honey. Therefore, there is a need for a thorough analysis of the above phenomenon.

The process of honey decrystallization using microwaves inhibit the enzymatic activity responsible for the abiotic properties of honey, which isn't observed in the case of heating by the conventional method. Besides the antimicrobial properties, honeys also show strong antioxidant activities. The antioxidant activity may be increased by subjecting honey to increased hydrostatic pressure or ultrasounds.

The physical and chemical properties of honey mean that its use in the food industry is very small – the high density and viscosity make it difficult to dose. A solution to this problem could be the production of honey powder. Such a product could be a dietary supplement, be included in desserts and be used as a sweetener. The properties of honey powder depend on the type of carrier, pressure and drying temperature and the raw material used.

Autors contribution

All authors have contributed equally to the work.

Conflict of interests

The authors declare that they have no potential conflict of interest in relation to the study in this paper.

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