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IMPACT OF CORN AND RICE SYRUP ADULTERATION ON PHYSICO-CHEMICAL PROPERTIES OF *TILIA* HONEY

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Abstract: *The aim of this study was to highlight the impact of corn and rice syrups adulteration on physico-chemical parameters of tilia honey. For this purpose, tilia honey was adulterated by addition of concentrated corn and rice syrups in different percentages: 5%, 10%, 20% and 50%. The parameters chosen to study the influence of adulteration agents in honey were the following: pH, free acidity, electrical conductivity, color, moisture content, hydroxymethyl furfural content and the sugar content (fructose, glucose, sucrose, maltose, melesitose, turanose, trehalose, raffinose and F/G ratio). The result indicated that the adulteration with corn and rice syrup caused significant chances on most of physico-chemical parameters values. The moisture content of adulterated honey with both types of syrup increased depending on the degree adulteration from 16.75% in authentic honey to 19.39% for 50% adulterated honey with corn syrup and to 17.03% for adulterated honey with 50% rice syrup. The hydroxymethyl furfural content of honey samples did not exceed the maximum allowed limit (40 mg/kg). The free acidity content decreased from 14.56 meq/kg in authentic honey to 8.96 meq/kg in adulterated honey with 50% corn syrup and increased from 14.56 meq/kg to 18.17 meq/kg in adulterated honey with 50% rice syrup. Electrical conductivity decreased 1.91 times in adulterated honey with 50% corn syrup and increased 1.41 times in adulterated honey with 50% rice syrup.*

Keywords: *tilia honey, adulteration, syrups, physico-chemical parameters*

1. Introduction

Honey is a natural produce with a characteristic taste and known for its therapeutic properties (antioxidant, antimicrobial, anti-inflammatory, anti-tumor) [1]. The composition of honey include about 200 substances, mainly sugars and water but also other substances such as proteins, organic acids, enzymes, vitamins, minerals, phenolic compounds, pigments and solid particles (results from honey harvesting) [2]. Honey composition depends on various factors (floral source, soil, and climate) [3]. Due to a high worldwide demand for bee products, the adulteration of honey has become an increasingly common problem. Illegal honey producers add various types of syrups at a lower cost price, seeking to obtain greater economic benefits. Thus, consumer confidence in the honey quality and safety is undermined, having also a negative effect on the competitiveness and profitability of honest producers [4]. In recent years, honeybee has been adulterated with different concentrated carbohydrate syrups such as: sugar cane, inverted sugar, rice syrup, agave syrup, date syrup, corn syrup, HFCS (high-fructose corn syrup), fructose, glucose, zaharose and maltose syrups[5]. At present, guaranteeing the quality and authenticity of honey represents a very

important issue for both the national and international market. Therefore, it is necessary to establish an analytical procedure for the detection of adulterated honey [4].

Rice syrup is obtained from rice in three stages: hydrolysis (acid or enzymatic), refining and concentration. The main chemical components of rice syrup are glucose, maltose, trisaccharides, tetrasaccharides, etc. [6]. Corn syrup is obtained from corn starch by enzymatic reaction using the enzymes α-amylase and glucoamylase.If the reaction is continued with the enzyme glucose isomerase, high fructose corn syrup (HFCS) of different degrees of isomerization (HFCS-90, HFCS-55, HFCS-42) can be obtained[7]. The chemical composition of these syrups is similar to that of authentichoney, but thenutritionalvalueismuchlower. Thus, the detection of adulterated honey with corn and rice syrups has become an important topic for the production and quality of honey, but also for the nutrition and health of consumers. Adulterated honey by addition of sugars may present changes in some physico-chemical parameters (color, free acidity, electrical conductivity etc.) and/or enzymatic activity, the content of various compounds such as fructose, glucose, sucrose, fructose/glucose ratio, maltose, proline and hydroxymethyl furural[8].

In this study, the influence of corn and rice syrups adulteration on the physico-chemical parameters of Romanian tilia honey is presented.To highlight the differences between authentic and adulterated honey samples, the analysis of variance (ANOVA) was used.

2. Materials and methods

Tilia sp. honey was purchased from a local beekeeper located in Suceava County, Romania. The syrups produced in South Korea, were purchased from importing companies: DAESANG EUROPE B.V. for corn syrup and from PANAISIA DE HANDELS GMBH for rice syrup.

Authentic honeywas adulterated with these types of syrups by addition in different percentages (5%, 10%, 20% and 50%) (w/w).

The physico-chemical characteristics analyzed were: color, electrical conductivity, moisture content, pH, free acidity, hydroxymethyl furfural content and sugar content. Before performing the analysis, all the samples were liquefied at 50° and homogenized.

To measure the color of honey samples two instruments were used: a portable chromameter CR-400 (Konica, Minolta, Japan) and a photometer Pfund HI 96785 (Hanna Instruments, USA).

The electrical conductivity was measured with a portable conductometer HQ14d (HACH, USA). For this purpose, 20 g of honey were dissolved in 100 ml of distilled water. The results were expressed in micro Siemens per centimeter ($\mu S \cdot cm^{-1}$).

The moisture content was analyzed using an Abbé refractometer (Leica Mark II Plus), based on refractometry. This is a method that determines the refractive index of honey and the determination of the water content (%) is done using the Chataway table [9].

A 10% honey aqueous solution was prepared to measure the pH with a METTLER TOLEDO FiveGo pH-meter (Mettler Toledo, USA).

The free acidity was determined by titrimetric method with a TITROLINE easy device (Schott Instruments, Germany). The results were expressed in milliequivalents/kg of honey.

The hydroxymethyl furfural (HMF) presence in honey was determined using the method proposed by White [10].The absorbance of the reference samples (containing 0.2% sodium bisulphite solution) and samples (containing distilled water) was read at 284 nm and 336 nm using a UV-VIS-NIR 3600 spectrophotometer (Schimadzu

Corporation, Japan). The results were expressed in mg/kg honey.

High performance liquid chromatography (HPLC) was used to determine the sugar content according to the method published by Bogdanov& Baumann [11]. The mobile phase was the mixture acetonitrile: water (80:20) and standard substances used were: fructose, glucose, sucrose, maltose, melesitose, turanose, trehalose and raffinose.

One-factor analysis of variance (ANOVA) was the statistical analysis used to present the differences between samples. The ANOVA is based on the law of total variance.

3. Results and discussion

Color

Honey color is an important factor that depends largely on the polen content and the source of nectar. The various pigments that derive from these such as phenolic acids, flavonoids, anthocyanins along with the mineral content constitute the basic color of honey[12].

Depending on the degree of adulteration, L*, a*, b*, ΔE* color parameters showed significant (p<0.001) or partially significant (p<0.01) changes for both types of syrups. The values of L* ranged between 37.76 for authentic honey and 40.81 for adulterated honey with 50% corn syrup and between 37.76 for authentic honey and 27.10 for adulterated honey with 50% rice syrup.The value of L* for corn syrup was 38.22 and for rice syrup was 34.13. Ribeiro et al. [13] observed an increase of L* parameter for adulterated honey with high fructose corn syrup.

a* parameter decrease for adulterated honey with corn syrup from 3.01 in authentic honey to 0.48 in adulterated honey with 50% syrup and increase for adulterated honey with rice syrup from 3.01 to 9.02 in adulterated honey with 50% syrup. The variation of b* parameter was between 28.88 and 31.17 for adulterated honey with corn syrup and between 28.88 and 13.41 for adulterated honey with rice syrup. ΔE* parameter ranged from 0.66 to 4.47 for adulterated honey with corn syrup and from 2.82 to 19.73 for adulterated honey with rice syrup.

Positive values for both coordinates (a* and b*) indicate that all the samples had nuance of color between yellow and red (first quadrant of CIE L*a*b* color space).

On the Pfund scale adulterated with corn syrup decreased significantly from 35.64 in autentic honey to 27.72 in adulterated honey with 50% syrup (acording to the Pfund scale, the color changed from extra light amber to white), and for adulterated honey with rice syrup increased significantly from 36.64 to 52.47 in adulterated honey with 50% syrup (the color changed from extra light amber to light amber acording to the Pfund scale). The value for corn syrup (100%) was 33.46 and for rice syrup (100%) was 41.98.

Depending on the adulteration agent all the parameters from CIE L*a*b* space showed significant changes (p<0.001). All these results are shown in Table 1 and Table 2.a. and Table 2.b.

pH

A low pH prevents the development of microorganisms. The acidic content of honey bee is relatively low, influencing its texture, stability and term of validity [14].

Depending on the degree of adulteration, the pH results for adulterated honey with corn syrup were not significant (p>0.05), the values being between 4.05 for authentic honey and 4.27 for adulterated honey with 50% syrup (Table 2.a.). For adulterated honey with rice syrup the results were less significant (p<0.05), existing as in the case of adulterated honey with corn syrup an increase, with values between 4.05 for authentic honey and 4.36 for adulterated honey with 50% syrup (Table 2.b.).

Ribeiro et al. [13] observed an increase in the case of adulterated honey with high fructose corn syrup.

Depending on the adulteration agent, the changes were not significant, the pH values being 4.13 for corn syrup and 4.15 for rice syrup (Table 1).

Free acidity

Free acidity is given by the presence of organic acids and organic ions. A high degree of acidity may indicate the fermentation of sugars into organic acids [15].

Adulterated honey with corn syrup presented a significant decrease of free acidity, against to adulterated honey with rice syrup that presented a significant increase (p<0.001). The values ranged from 14.46 meq/kg for authentic honey to 8.96 meq/kg and 18.17 meq/kg, for adulterated honey with 50% corn syrup and 50% rice syrup, respectively, as shown in Table 2.a. and Table 2.b. Depending on the adulteration agent, all the changes were significant, the values being 12.65 meq/kg for corn syrup and15.78 meq/kg for rice syrup (Table 1).

None of the analyzed samples showed free acidity values superior to 50 meq/kg [16].

Electrical conductivity

Electrical conductivity depends on various factors such as the content of ash, proteins, organic acids, some sugars and polyols[14].

The electrical conductivity values decreases significantly (p<0.001) for adulterated honey with corn syrup from 333.63µS·cm^{-1} in authentic honey to 270 µS·cm^{-1}in adulterated honey with 20% syrup and to 175.02 µS·cm^{-1}for adulterated honey with 50% syrup (Table 2.a.).The values for adulterated honey with rice syrup has shown a significant increase from 333.63 µS·cm^{-1}in authentic honey to 397.12 µS·cm^{-1} (for adulterated honey with 20% syrup) and to 492.36 µS·cm^{-1} (for adulterated honey with 50% syrup) (Table 2.b.). Regardless of the adulteration agent, the changes were significant, too. The value for corn syrup was 279.70 µS·cm^{-1} and for rice syrup was 387.60 µS·cm^{-1} (Table 1).

All the analyzed honey samples showed values of electrical conductivity lower than the maximum allowed limit of 800µS·cm^{-1}[16].

Moisture

The moisture content of honey varies from year to year depending on the environmental conditions and the manipulation of beekeepers during the harvest period. High moisture content could increase the activity of water with negative effects on the development of yeasts and accelerate the crystallization of certain types of honey [15]. Depending on the degree of adulteration, the moisture content presented a significant increase for adulterated honey with corn syrup, the values ranged from 16.75% (authentic honey) to 19.39% (adulterated with 50% syrup) (Table 2.a.).For adulterated honey with rice syrup the increase was not significant (p>0.05), values ranged from 16.75% to 17.03% (adulterated honey with 50% syrup), as it can be observed in Table 2.b.

Ribeiro et al. [13] observed also an increase for the adulterated honey by high fructose corn syrup (HFCS).

Depending on the adulteration agent, the changes were significant. The values for corn and rice syrups were 17.65% and 16.85% (Table 1). The moisture content did not exceed the maximum allowed limit (20%) established by Codex Alimentarius and EU Honey Directive for any sample.

Hydroxymethyl furfural content (HMF)

The HMF content is a parameter that indicates the degree of freshness of honey. This is a product of the Maillard reaction, the maximum allowed value being 40 mg/kg of honey [12].

In this study, the HMF content did not exceed the established limit. Depending on the degree of adulteration and on the adulteration agent the changes for HMF

content were significant (p<0.001). For adulterated honey with corn syrup the HMF content decreased from 13.34 mg/kg in authentic honey to 8.07 mg/kg in adulterated honey with 50% syrup and for adulterated honey with rice syrup the HMF content presented a significant increase from 13.34 mg/kg to 20.03 mg/kg adulterated honey with 50% syrup (Table 2.a. and Table 2.b). The values for corn and rice syrups (100%) were 11.55 mg/kg and 15.70% mg/kg (Table 1).

Table 1.

Physico-chemical parameters of adulteration agent

Parameter	Adulteration agent		F – ratio	Interaction
	Corn syrup	Rice syrup		Agent – Degree of adulteration
L*	38.22(3.93)a	34.13(1.50)b	311.07***	118.24***
a*	2.78(1.27)b	6.01(2.12)a	10248.90***	2186.62***
b*	30.12(1.04)a	24.65(6.19)b	947.05***	327.26***
ΔE*	1.91(1.63)b	6.70(7.27)a	11377.98***	3755.05***
Color (mm Pfund)	33.46(3.14)b	41.98(6.16)a	1214.70***	317.96***
pH	4.13(0.09)a	4.15(0.12)a	1.16ns	0.32ns
Free acidity (meq/kg)	12.65(2.1)b	15.78(1.36)a	1157.91***	316.39***
Electrical conductivity (µS/cm)	279.70(59.52)b	387.60(59.63)a	2429.31***	664***
Moisture (%)	17.65(1.01)a	16.85(0.21)b	52.21***	14.36***
HMF (mg/kg)	11.55(1.98)b	15.70(2.62)a	2176.06***	595.20***
Fructose (%)	27.93(6.30)a	27.97(6.26)a	0.04ns	0.01ns
Glucose (%)	25.67(5.45)a	27.88(3.02)a	164.13***	44.83***
Sucrose (%)	0b	0.02(0.03)a	1874.70***	506.89***
Turanose (%)	0.29(0.07)a	0.29(0.07)a	0.03ns	0.03ns
Maltose (%)	0.95(0.21)a	0.95(0.21)a	0ns	0ns
Trehalose (%)	1.21(0.27)a	1.20(0.27)a	0ns	0ns
Melesitose (%)	6.58(6.43)b	6.77(6.64)a	11.56**	3.17ns
Raffinose (%)	0.17(0.01)a	0.15(0.03)a	96.94***	26.25***
F/G ratio	1.07(0.02)a	0.98(0.13)b	173.69***	56.41***

ns – not significant (p>0.05), * - p<0.05, ** - p<0.01, *** - p<0.001, a-e –significant differences between sample

Sugar content

For adulterated honey with corn syrup the significant changes (p<0.001) were in the case of fructose, glucose, turanose, maltose, trehalose and melesitose, the partially significant were in the case of raffinose (p<0.01) and for sucrose and F/G ratio the changes were not significant (p>0.05).

In the case of fructose, glucose, turanose, maltose, trehalose, raffinose and F/G ratio for adulterated honeywith corn syrup was a decrease from 33.65% to 16.85% (fructose), 30.61% to 16.08% (glucose), 0.36% to 0.18% (turanose), 1.15% to 0.58% (maltose), 1.46% to 0.73% (trehalose), 0.19% to 0.15% (raffinose) and 1.09% to 1.04% (F/G ratio). An increase was in the case of melesitose from 0.75% to 17.89%. Sucrose was not present in either authentic honey or corn syrup. For adulterated honey with rice syrup the changes were significant (p<0.001) for all sugar content analyzed in this study.

A decrease was in the case of fructose (from 33.65% to 16.95%), glucose (from 30.61% to 22.57%), turanose (from 0.36% to 0.18%), maltose (from 1.15% to 0.57%), trehalose (from 1.46% 0.72%), raffinose (from 0.19% to 0.09%), F/G ratio (from

1.09 to 0.74). The content of sucrose and melesitose increased from 0% in authentic honey to 0.07% in adulterated honey with 50% syrup (sucrose) and from 0.75% to 18.47% (melesitose).

Regardless of the adulteration agent, the changes were not significant for fructose, turanose, maltose, trehalose (p>0.05).

The values of fructose, glucose, trehalose, melesitose, raffinose and F/G ratio were 27.93% for corn syrup and 27.97% for rice syrup (fructose), 25.67% for corn syrup and 27.88% for rice syrup (glucose), 1.21% for corn syrup and 1.20% for rice syrup (trehalose), 6.58% for corn syrup and 6.77% for rice syrup (melesitose), 0.17% for corn syrup and 0.15% for rice syrup (raffinose) and 1.07 for corn syrup and 0.98 (F/G ratio) (Table 1).

All these results are shown in Table 1 and Table 2.a. and Table 2.b.

Table 2.a.

Physico-chemical parameters of tilia honey adulterated with corn syrup

Tilia honey analysis results						
Parameter	**Degree of adulteration for corn syrup**					**F - ratio**
	0.00%	**5.00%**	**10.00%**	**20.00%**	**50.00%**	
L*	37.76(0.54)bc	36.8(0.52)c	37.48(0.54)bc	38.24(0.54)b	40.81(0.58)a	15.98**
a*	3.01(0.04)c	3.93(0.06)a	3.55(0.05)b	2.95(0.04)c	0.48(0.007)d	1963.05***
b*	28.88(0.41)b	30.48(0.44)a	29.14(0.42)b	31.17(0.45)a	30.93(0.44)a	11.85**
ΔE*	0e	2.08(0.03)c	0.66(0.008)d	2.33(0.03)b	4.47(0.06)a	4756.80***
Color (mm Pfund)	35.64(0.51)a	35.64(0.51)a	34.65(0.49)ab	33.66(0.48)b	27.72(0.40)c	95.64***
pH	4.05(0.06)b	4.07(0.06)b	4.09(0.06)b	4.14(0.06)ab	4.27(0.06)a	4.63ns
Free acidity (meq/kg)	14.56(0.21)a	13.99(0.20)b	13.44(0.19)c	12.32(0.18)d	8.96(0.13)e	296.82***
Electrical conductivity (µS/cm)	333.63(4.77)a	317.77(4.54)bc	301.91(4.31)c	270.19(3.86)d	175.02(2.50)e	478.56***
Moisture (%)	16.75(0.24)c	17.01(0.24)c	17.28(0.25)bc	17.80(0.26)b	19.39(0.28)a	34.08***
HMF (mg/kg)	13.34(0.19)a	12.81(0.18)b	12.28(0.17)c	11.23(0.16)d	8.07(0.12)e	314.67***
Fructose (%)	33.65(0.48)a	31.96(0.46)b	30.29(0.43)c	26.92(0.39)d	16.85(0.24)e	533.57***
Glucose (%)	30.61(0.43)a	29.15(0.42)b	27.70(0.40)c	24.80(0.36)d	16.08(0.23)e	480.28***
Sucrose (%)	0	0	0	0	0	0ns
Turanose (%)	0.36(0.007)a	0.34(0.008)b	0.32(0.002)c	0.28(0.005)d	0.18(0.0005)e	354.91***
Maltose (%)	1.15(0.02)a	1.09(0.01)b	1.03(0.01)c	0.92(0.01)d	0.58(0.007)e	467.11***
Trehalose (%)	1.46(0.02)a	1.38(0.01)b	1.31(0.02)c	1.16(0.01)d	0.73(0.007)e	621.39***
Melesitose (%)	0.75(0.007)e	2.46(0.03)d	4.17(0.06)c	7.60(0.11)b	17.89(0.25)a	5749.03***
Raffinose (%)	0.19(0.007)a	0.18(0.002)ab	0.17(0.006)ab	0.17(0.0007)b	0.15(0.001)c	21.53**
F/G ratio	1.09(0.01)a	1.08(0.02)a	1.08(0.02)a	1.07(0.02)ab	1.04(0.01)b	3.09ns

ns – not significant (p>0.05), * - p<0.05, ** - p<0.01, *** - p<0.001, a-e –significant differences between sample

Table 2.b.

Physico-chemical parameters of tilia honey adulterated with rice syrup

Tilia honey analysis results						
Parameter	**Degree of adulteration for rice syrup**					**F - ratio**
	0.00%	**5.00%**	**10.00%**	**20.00%**	**50.00%**	
L*	37.76(0.54)a	36.39(0.52)b	35.36(0.50)b	34.08(0.49)c	27.10(0.39)d	144.83***
a*	3.01(0.042)d	5.43(0.078)c	5.41(0.078)c	7.23(0.11)b	9.02(0.13)a	1211.89***
b*	28.88(0.41)a	29.38(0.42)a	27.05(0.39)b	24.52(0.35)c	13.41(0.19)d	657.06***
ΔE*	0e	2.82(0.04)d	3.86(0.05)c	7.09(0.10)b	19.73(0.28)a	6285.09***
Color (mm Pfund)	35.64(0.51)d	39.6(0.57)c	38.61(0.55)c	43.56(0.62)b	52.47(0.75)a	231.60***
pH	4.05(0.06)b	4.08(0.06)b	4.11(0.06)b	4.17(0.06)b	4.36(0.06)a	8.89*
Free acidity (meq/kg)	14.56(0.21)d	14.92(0.22)cd	15.28(0.22)c	16.00(0.23)b	18.17(0.26)a	80.33***
Electrical conductivity (µS/cm)	333.63(4.77)e	349.50(4.99)d	365.38(5.22)c	397.12(5.67)b	492.36(7.04)a	254.31***
Moisture (%)	16.75(0.24)a	16.78(0.24)a	16.81(0.24)a	16.86(0.24)a	17.03(0.24)a	0.41ns
HMF (mg/kg)	13.34(0.19)e	14.03(0.20)d	14.73(0.21)c	16.12(0.23)b	20.30(0.29)a	297.30***
Fructose (%)	33.65(0.48)a	31.98(0.46)b	30.31(0.43)c	26.97(0.38)d	16.95(0.24)e	526.97***
Glucose (%)	30.61(0.43)a	29.80(0.43)ab	29.00(0.41)b	27.39(0.39)c	22.57(0.32)d	128.54***
Sucrose (%)	0d	0.009(0.002)c	0.01(0.003)c	0.03(0.001)b	0.07(0.001)a	506.89***
Turanose (%)	0.36(0.007)a	0.35(0.008)b	0.32(0.002)c	0.28(0.005)d	0.18(0.003)e	348.06***
Maltose (%)	1.15(0.02)a	1.09(0.01)b	1.03(0.01)c	0.92(0.01)d	0.57(0.009)e	454.88***
Trehalose (%)	1.46(0.02)a	1.38(0.01)b	1.31(0.02)c	1.16(0.01)d	0.72(0.008)e	615.74***
Melesitose (%)	0.75(0.007)e	2.52(0.04)d	4.29(0.06)	7.84(0.11)bc	18.47(0.26)a	5746.84***
Raffinose (%)	0.19(0.007)a	0.17(0.005)b	0.16(0.005)b	0.15(0.0008)c	0.09(0.001)d	129.01***
F/G ratio	1.09(0.01)a	1.06(0.02)ab	1.03(0.02)b	0.97(0.02)c	0.74(0.01)d	162.51***

ns – not significant (p>0.05), * - p<0.05, ** - p<0.01, *** - p<0.001, a-e – significant differences between sample

4. Conclusion

In this study the impact of honey adulteration with corn and rice syrups on the physico-chemical properties was analyzed.

Statistical analysis indicated that most of the physico-chemical parameters presented significant differences (p<0.001) between authentic and adulterated honeys.

Free acidity, electrical conductivity, moisture and HMF contents did not exceed the maximum allowed limit for any analyzed sample.

pH values had a low increase from 4.05 to 4.27 for adulterated honey with corn syrup and from 4.05 to 4.36 for adulterated honey with rice syrup. The color parameters on both color CIE L*a*b* and Pfund scales showed significant changes (p<0.001 in the case of adulterated honey with rice syrup and significant and partially significant (p<0.01) for adulterated honey with corn syrup, due to the intensity degree of color of the adulteration agent.

The content of fructose, glucose, turanose, maltose, trehalose and melesitose decreased significantly for adulterated honey with corn and rice syrups. For turanose, maltose and trehalose the changes were similar in the adulterated honey with both types of syrups.

Depending on the adulteration agent the changes for the following parameters were not significant (p>0.05): pH and sugar content (fructose, turanose, maltose and trehalose).

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