HONEY DETECTION, THROUGH PROLINE ANALYSIS IN HARD HONEY CARAMELS

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Summary

This work is suggesting a new technique to detect honey in hard honey caramels, through proline analysis by spectrophotometry, that allows detecting adulterations. The caramels genuineness was taken as an index, as well as the presence of the proline amino-acid in the final product, as this amino-acid is brought by bees into honey, where its presence is chiefly to be found in the same. Although there is a certain quantity of amino-acid that is denaturalized, decomposed, and thus destroyed, the residual proline converts itself into a good marker of the honey presence in the product.

From the legal point of view, there are no tools able to discover an adulteration, as the Argentine Alimentary Codex, does not specify any honey contents to be added to caramels, as to become possible for those to be labelled as made "of honey".

For proline determining the spectrophotometric method was used, recommended by the IRAM standard No.15940/1 (1995) for honey[19] .

The average content of caramels was 0.99 mg/100 g of caramels, with a minimum of 0.34 mg/100 g, and a maximum of 2.21 mg/100 g, that is the amino-acid is to be found in caramels in quantities that can be easily measured. Besides, there was checked out the caramels that have no honey in their composition, do not reveal the presence of proline.

Key words: caramels / proline / adulteration / analytical methods / honey

Introduction

It is worth stressing the importance of adding honey to other foods, with a view of transferring the benefic properties that are allotted to it. Presently, there are on the market a large variety of alimentary products that contain honey, as an essential or minority component. It occurs the today's alimentary trends, inclined towards organic, ecologic, healthy, fresh, natural products etc., led the industrial producers to incorporate into traditional foods, matters that are considered as actual symbols of purity, health, and energy, as it is the case of honey. Nevertheless, that is only a trade strategy, meant to stand out the properties of a product, replaced by other, that are not so noble.

Given the good price honey reaches on the market, its adulteration is frequent, as well as of other foods that contain it, therefore being replaced by sugar syrups, nutrients, and additives (mainly essences).

Checking up if a certain food contains honey or not, is many a time a difficult and complex operation, above all if there are taken into account the physical, chemical, biologic, and organoleptic changes that occur in the raw materials that form the product, during its different stages of its working up. Moreover, if the final product was submitted to high temperatures, as for instance in a cooking process, as it is the case of caramels. Thus, for determining the genuineness of a product that contains honey in its composition, there is necessary to find and identify some matter that would be added to that food only by honey, and that is able to remain in the final product after its elaboration process.

This work suggests a technique for detecting honey in hard honey caramels, through proline analysis by spectrophotometry. As index of the caramels genuineness there was taken the presence of the amino-acid proline in the final product, as it is an amino-acid bees add to honey[1], and its presence is to be chiefly found in honey. As it is about caramels, a sweet morsel of a very simple composition, formed by a balanced mix of sugars, water, and additives, the search is much simplified, since there are no interferent matters. Besides, proline can only reach the honey caramels if those contain honey, that is, a matter that is absent in the rest of the caramel components. That is, if we are talking about an amino-acid, thus a matter with biological activity, that could be affected by various factors[16] , as it is, for instance, the heat. The industrial manufacturers incorporate honey at the end of the process, when the syrup was already prepared, so the losses of proline, produced during elaboration, are never total, owing to the little time honey stays at higher temperatures, and its residual content is transformed into a good index of the honey presence in the product.

The Argentine food law[5] is lacking clarity in respect to those products, and, moreover, as they are generally sold without a label, the consumer does not know the composition of the same, and therefore their adulteration is facilitated. On the other hand, as the consumption of those products is linked to a medicinal application, mainly in the winter months, owing precisely to the therapeutic properties allotted to honey, there is necessary to know well the quality of the same, in order to keep the consumers' health.

Material and methods

21 hard honey caramel samples were analyzed, 4 elaborated according to an artisan way, and 17 trade samples. From the artisan samples there was also obtained the honey, that was used in the elaboration process, and served as reference mark to calculate the losses of proline during the caramel confectioning process. There were also analyzed 3 caramel samples that contained no honey, mint, orange, or cherry flavor, in order to confirm the absolute absence of proline, and thus the effectiveness of the suggested method. The samples were grinded in a mortar to render easier their dissolution in water. The obtained solution formed the sample-solution to be analyzed.

Method

For the analysis, the procedure was adopted, recommended by the IRAM Standard No. 15940+1 (1995) for honey[19].

Procedure

5.0 g of sample (honey caramels) are weighed, after being previously grinded in a mortar, and are placed in a precipitation vessel of 50 ml. They are then dissolved in destilated water, and are transferred to a gauged glass vessel of 15 ml, the volume is then completed with water, the vessel is corked and agitated. A 33.3% solution of mass in volume is obtained. 0.5 ml of the previous solution are pipetted into a trial tube, 1,0 ml of formic acid and 1,0 ml of ninhydrin are added. The tube is then well plugged, and agitated well.

Two such tubes are placed in a double boiler and boiled for 15 minutes. They are then cooled at 22º C for 5 minutes. The plug is then removed, and 5 ml of isopropanol (1+1) are added to each tube. Tubes are then agitated well, and the absorbancy at 520 nm is determined, against a target prepared by substituting the sample by 0.5 ml of water, and proceeding as it was described above for the honey caramel samples. Tubes are then read within the 35 minutes of cooling.

The caramel colour is corrected, determining the absorbancy of a solution which contains 0.5 ml of caramel solution, obtained as above, 2.0 ml of water, and 5.0 ml of isopropanol (1+1). The previously calculated reagent value is then deducted from the sample.

Standard Curve

The calibration curve is prepared, as there is indicated in the procedure, using a diluted solution of the proline-type, instead of the caramel solution, with the following dilutions: Tube 1: 1.94 µg/ml of proline; Tube 2: 6.94 µg/ml of proline; Tube 3: 12.25 µg/ml of proline; Tube 4: 15.89 µg/ml of proline; Tube 5: 22.33 µg/ml of proline; Tube 6: 29.91 µg/ml of proline; Tube 7: 52.22 µg/ml of proline; and Tube 8: 102.08 µg/ml of proline. It was graphically represented the proline concentration against the obtained absorbancy, and, through simple linear regression by the method of square minima, the equation of the straight line of adjustment between both variables, defined by the equation:

$$y = 93.248 \,.\, x - 0.4678$$

The proline concentration was calculated, using the formula that results from the equation of the straight line. The determination coefficient R^2 (0.9977) is highly significative, that allowed us to work within the linearity range of the curve.

The proline content was expressed in mg/100 g of caramel.

Results discussion

From the obtained results for industrially manufactured caramels, there was possible verify that more or less all of them contained proline, which confirms the presence of honey in the product (Table I).

Table I

Proline contents in the industrially manufactured caramels

Samples	Proline (mg/100 g)
1	0.79
2	0.34
3	0.45
4	2.21
5	1.22
6	1.08
7	0.59
8	0.99
9	1.90
10	0.62
11	0.94
12	1.11
13	2.14
14	1.56
15	1.04
16	0.73
17	0.86
Minimum	**0.34**
Maximum	**2.21**
Average	**1.09**
Standard deviation	**0.56**
Variance	**0.31**

Besides, there was effected a microscopic observation of the residue obtained by centrifugating a caramel solution, in order to visually verify the presence of pollens proceeding from honey. In all cases, the observed residua contained pollens (Datas non-shown).

The average proline content of those caramels reached 0.99 mg/100 g of caramel; with a minimum of 0.34 mg/100 g, and a maximum of 2.21 mg/100 g (see Table I), and showing a standard deviation of 0.56. Even if there is unknown in this case the quality of the honey used in the caramel confection, and therefore the initial value of proline, there was verified the same is to be encountered in the caramels, in easily measurable quantities.

According to the data brought in by the literature, the proline contents in honeys vary depending on various factors such as, the bee itself, the flora, the pollen richness of honey etc[25, 17, 14], by which the proline residual content in caramels would be different, according to the honey sort used in the confection.

Besides, there were analysed artisan-type caramels, considering the proline is a compound endowed with biological activity, and as such the same will be denaturalized and/or destroyed by the effect of heat[16], reason for which the proline content was determined, in the honeys used as raw material (see Table II). In this way, losses of this aminoacid in the caramels could have been valuated, according to their initial content in honey.

Table II

Proline content in honey

Samples	Proline mg/100 g
1*	86.80
2**	106.49
3***	66.76

** Corresponds to the sample # 19 of the artisan-type caramel; ** Corresponds to the sample # 20 of the artisan-type caramel; *** Corresponds to the sample # 18 and 21 of the artisan-type caramel*

Other factor to be taken into account is the moment when honey is added in the process of the caramel confection, depending if it occurs at the beginning, with the other sugars, or if it is left at the end, before the syrup reaches the “caramel point”.

As concerns the honeys we analyzed for achieving this work, the encountered proline values vary from 66.76 to 106.49 mg/100 g of sample (see Table II). Therefore, it is possible to see that, even if the proline contents are variable, they are nevertheless sufficient, for a total loss not to occur during the caramels confection. This can be noticed in Table III, where the proline residual content in the artisan-type caramels varies from 1.28 to 9.68 mg/100 g. There was verified, in that caramel class the proline residual content is higher than those encountered in the industrially manufactured caramels, what we suppose is due rather to the use, in the latter, of better quality honey or lesser proportion of honey, than to the differences in the confection process; this is because in the industrial processes there are generally used boilers that work under vacuum, what regulates the decomposition of the caramel compounds (as such, the proline content should have been higher), while artisanly this is done under normal conditions of pressure and temperature, and the decomposition of sugars cannot be controlled any more.

Table III

Proline content in the artisan-type caramels

Samples	Proline mg/100 g
18	2.83
19	4.07
20	9.68
21	1.28

As concerns the quantity of proline lost in the confection, that varies from 70%, as a minimum, to 90%, as a maximum (see Table IV), and it only could be calculated in the artisan-type caramels, where the initial quantity of proline in the added honey is known, as well as the moment when the same was incorporated into the syrup.

Table IV

Percentage of proline, that is lost during confection

Samples	Percentage of loss
18	85.90%
19	84.40%
20	69.70%
21	90.40%

Therefore, it can be noticed, when honey is added at the end of the confection process, the residual proline quantity in the final product would be higher (see sample # 20 of the artisan-type caramels). That sample suffered a loss of about 70% of the initial proline content (see Table IV). But if honey is added before the syrup formation, increases the time of honey being maintained at the cooking temperature, by what a larger quantity of proline is destroyed (see samples # 18, 19, and 21 of the artisan-type caramels). In the latter case, the percentage of loss was estimated at about 90% (see Table IV). In these home-made preparations there were used higher proportions of honey (30% of honey in the samples 18, 19, and 20; and 20% of honey in the sample 21), what justifies the high content of proline encountered in the artisan-type caramels.

There is also to be taken into account that a caramel, processed in the latter conditions, would have lost a large part of its nutritive value, ceded by the honey, and there will be attacked not only the proline, but also other aminoacids, enzymes, antibiotioc factors, hormones, and volatile compounds responsible for the aroma, and flavor of honeys. Therefore, it is necessary to signal that, in the artisan-type confections the work was carried out voluntarily, under conditions little recommendable (the temperature cannot be well regulated, and the pressure is not controlled any more), as concerns the integrity of the matters endowed with biological activity, that confirms that, the most severe be the confection treatment, a small quantity of proline keeps being preserved up to the end.

That is to say, the analysis of the artisan-type caramels, as well as their confection, were effected with the purpose of proving with certitude the above, given that in the industrially manufactured caramels there is not known the quality of the honey used as raw material. In this way, we could check up proline could be considered a parameter proving the presence of honey in caramels.

Table V

Determining proline in hard aromatized caramels

Samples	Proline (mg/100 g)
Mint	Non-detected
Orange	Non-detected
Cherry	Non-detected

The absence of proline in caramels manufactured without honey (see Table V), has confirmed the hypothesis that, if there is no honey among the raw material, there will be no proline in the caramel manufacturing, save a few exceptions (caramels manufactured with proteic raw materials). With that aim in view, three caramel samples were analyzed, tasting of mint, orange, and cherry, that have shown no absorbancy in the ninhydrin reaction, what would indicate they contain no proline. Besides, there has been checked up the absence of honey in these products, through microscopic observation, without finding any pollen grains (Data yet not published).

Conclusions

Determining the proline content in the honey caramels, is a feasible parameter for detecting adulterations, both by the easy application of the method, as well as by the simple composition of the sample. Therefore, the method could be used as an index of genuiness. Thus, the caramels that were manufactured with honey, contain proline.

Proline is not completely destroyed during the manufacturing process, and the residual contents are easily detectable.

Of the analyzed honey caramels, all show the presence of proline, and the low values encountered suggest little honey was added during the manufacturing process. Given the fact it is about a food that is largely used, and to which therapeutic properties are allotted, there should be recommended the following:

- Be manufactured with good quality honeys, that have not suffered thermic treatments.
- A larger quantity of honey should be added to their manufacturing.
- Be manufactured taking into account honey is a natural product, that has to reach the consumer with the lesser possible changes, without essential modifications in its components and sensory features; only in this way, a good quality food product can be achieved.

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