

Alternative wax recovery from *Apis mellifera*: Different combs size effect

Uma alternativa à recuperação de cera de *Apis mellifera*: Efeito dos diferentes tamanhos de favo

Recuperación alternativa de cera de *Apis mellifera*: Efecto de tamaño de peine diferente

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Abstract

The bee wax has great historical importance and has been present since the evolution of civilizations. Currently, besides several uses of the wax, its recovery is indispensable for the rational management of swarms by beekeepers. The wax can be recovered from the combs using different techniques, however the conditions of the pre-extraction combs, such as their size, can influence the wax yield. Thus, studies must still be carried out to standardize the techniques and processes for extracting the wax from the combs, in order to improve the wax yield. This study aimed to evaluate the recovery of *Apis mellifera* wax according to the size of the comb using an alternative extraction technique. First, the influence of the comb size on wax recovery was evaluated. We used brown color combs, which were broken down into four sizes, each size representing a treatment, namely: Treatment I - 25 cm²; Treatment II - 16 cm²; Treatment III - 9 cm² and Treatment IV - 4 cm². Combs were submitted to the "internal strain" technique for 30 minutes of extraction, after boiling the water. The wax recovery was superior in treatment III (P < 0.05). Wax recovery from treatment IV was similar to treatment II, which, in turn, showed similar recovery to treatment I (P < 0.05). Besides, the wax recovery using 9 cm² combs was evaluated by the commercial technique of "steam extraction" and the alternative technique of "internal strain", for a period of 30 minutes. The "internal strain" technique showed greater wax recovery than the "steam extraction" technique (P < 0.01). The use of alternative wax recovery together with 9 cm² combs size may optimize the wax extraction process, in terms that it decreases the time of exposure of the wax to heat, and thus positively affect the quality of the final product.

Keywords: Animal product; Apiculture; Innovation; Processing; Steam extraction; Technology.

Resumo

A cera apícola tem grande importância histórica e esteve presente junto à evolução das civilizações. Atualmente, dentre as diversas utilizações, é indispensável para o manejo racional dos enxames pelos apicultores. A cera pode ser recuperada dos favos por meio de diversas técnicas, porém as condições dos favos pré-extração, tais como o seu tamanho, podem influenciar no rendimento. Assim, estudos ainda devem ser realizados para padronizar as técnicas e os processos de extração da cera dos favos, a fim de melhorar o rendimento desse produto. Esta pesquisa teve como objetivo avaliar a recuperação de cera de *Apis mellifera* em função do tamanho do favo e das técnicas de extração. Primeiro, foi avaliada a influência do tamanho do favo na recuperação de cera. Os favos utilizados eram de colorações marrons, os quais foram fragmentados em quatro tamanhos, onde cada tamanho representou um tratamento, sendo eles: Tratamento I - 25 cm²; Tratamento II - 16 cm²; Tratamento III - 9 cm² e Tratamento IV - 4 cm². Eles foram submetidos à técnica da "coagem interna" por 30 minutos de extração, após a fervura da água. A recuperação de cera obtida foi superior no tratamento III (P < 0,05). A recuperação de cera do tratamento IV foi semelhante ao tratamento II, que, por sua vez, apresentou recuperação semelhante ao tratamento I (P < 0,05). No segundo experimento, avaliou-se a recuperação de cera dos favos de 9 cm² pela técnica alternativa da "coagem interna" e a comercial de "extração a vapor", pelo tempo de 30 minutos. A técnica da "coagem interna" apresentou maior recuperação de cera que a técnica de "extração a vapor" (P < 0,01).

Palavras-chave: Apicultura; Extração a vapor; Inovação; Processamento; Produto de origem animal; Tecnologia.

Resumen

La cera de abeja tiene una gran importancia histórica y ha estado presente desde la evolución de las civilizaciones. Actualmente, además de varios usos de la cera, su recuperación es indispensable para el manejo racional de enjambres por parte de los apicultores. La cera se puede recuperar de los peines utilizando diferentes técnicas, sin embargo las condiciones de los peines de pre-extracción, como su tamaño, pueden influir en el rendimiento de la cera. Por lo tanto, aún deben realizarse estudios para estandarizar las técnicas y procesos de extracción de la cera de los peines, con el fin de mejorar el rendimiento de la cera. Este estudio tuvo como objetivo evaluar la recuperación de cera de *Apis mellifera* según el tamaño del peine mediante una técnica de extracción alternativa. Primero, se evaluó la influencia del tamaño del peine sobre la recuperación de cera. Usamos peines de color marrón, los cuales se dividieron en cuatro tamaños, cada tamaño representaba un tratamiento, a saber: Tratamiento I - 25 cm²; Tratamiento II - 16 cm²; Tratamiento III - 9 cm² y Tratamiento IV - 4 cm². Los peines se sometieron a la técnica de "tensión interna" durante 30 minutos de extracción, después de hervir el agua. La recuperación de cera fue superior en el tratamiento III (P <0,05). La recuperación de la cera del tratamiento IV fue similar al tratamiento II, que, a su vez, mostró una recuperación similar al tratamiento I (P <0,05). Además, se evaluó la recuperación de cera utilizando peines de 9 cm² mediante la técnica comercial de "extracción de vapor" y la técnica alternativa de "deformación interna", por un período de 30 minutos. La técnica de "deformación interna" mostró una mayor recuperación de cera que la técnica de "extracción con vapor" (P <0.01). El uso de la recuperación de cera alternativa junto con peines de 9 cm² de tamaño puede optimizar el proceso de extracción de la cera, en términos de que disminuye el tiempo de exposición de la cera al calor, y por tanto afecta positivamente la calidad del producto final.

Palabras clave: Apicultura; Extracción de vapor; Innovación; Producto animal; Procesamiento; Tecnología.

1. Introduction

Beeswax has been used by civilizations since the beginning of their development. The Egyptians used wax and propolis to embalm pharaohs and important members of society. Even the term "mummy" is derived from the Latin word "moum" which means wax. There are descriptions of the use of wax for the production of candles by the ancient Egyptian, Greek, Roman and Chinese peoples. Wax was also an important mercantile product in the period of the great Portuguese-Spanish navigations (Benson et al., 1978; Crane, 1999; Gomes, 2019).

Due to its low allergenic potential (Kacaniova et al., 2012), beeswax is widely used in cosmetic industry for manufacture of make-up, depilatory wax, moisturizer, among other applications (Witherell, 1975; Bogdanov, 2012). In pharmaceutical industry, it is used as a coating for tablets, and component of ointments and plasters (Lucente et al., 1996; Al Waili, 2003; Al Waili et al., 2006; Moustafa & Atiba, 2015). It is also present in the electronic (Lima, 1977; Feeburg, 1986; Bradbear, 2009) and agrifood industries (Nasrin et al., 2020). In addition, it is also used by the beekeeping industry itself, in the manufacture of honeycomb blades.

The wax present in the newly constructed combs has a whitish yellow color, being "purest". As the subsequent uses by the bees, the wax gets brown tones and, later, black. This happens due to the deposition of fecal material from the larvae, deposition of food, application of propolis during cleaning by bees, among other substances that are absorbed by the wax comb (Couto & Couto, 2006; Bradbear, 2009; Bogdanov, 2012). In addition to darkening, the thickness of combs cell walls also increases, and consequently, decreases its internal volume. This happens due to the presence of the silk cocoon produced by the larva at the end of this life stage. As a result, the aging comb cell is reduced in internal space, which makes it harmful to the development of the offspring and the storage of food (Krivtsov & Lebedev, 1995), reaching the point that the queen bee rejects it for oviposition and later, the workers for the deposition of food. Thus, in rational beekeeping, these old combs must be exchanged before being rejected by the bees (Bogdanov, 2012).

To extract the wax present in these combs, physical and chemical techniques can be used, however, the most common among beekeepers are the physical ones. These physical techniques are based on the use of heat; however, little is known about an optimized protocol for extraction. Regardless of the different extraction techniques, the conditions of the pre-extraction combs, such as their size, can influence the yield of recovered wax. According to the principles of chemistry, the smaller the particle, larger is its contact surface, therefore more reactive to the factors of the environment (Peruzzo & Canto, 2003). Thus,

combs cut in smaller sizes for the extraction of wax may have an increase in their surface of contact with heat, which may result in greater obtaining of wax (Couto & Couto, 2006), even though it still needs a proper scientific evaluation of such practice. Thus, the objective of this study was to present a beeswax recovery protocol for *A. mellifera* in which the size of the comb that shows the highest yield was determined. In this way, different honeycomb sizes were first tested in wax extraction using an alternative technique, followed by a comparison of the best result obtained with a commercial technique.

2. Material and Methods

The experiments were carried out in the Wax Laboratory of Apiculture sector, in the Animal Science Department of the Universidade Federal dos Vales do Jequitinhonha e Mucuri - UFVJM, located at Campus JK, Rodovia MGT 367 - Km 583, nº 5000, Alto da Jacuba in Diamantina-MG.

Due to the lack of information in the literature about yield testing methodologies for comb processing, an analytical routine was developed, exclusively at UFVJM, to carry out these experiments.

The honeycombs of *Apis mellifera* bees used in the experiments were obtained from the apiary of UFVJM and beekeepers partners from the region of Diamantina. Honeycombs of light brown to dark brown colors were selected. We make sure that honeycombs did not present young bees, food nor moth attack, in terms that it may alter the proportion of wax in relation to the other components of the comb. Yellow and black combs were also discarded, since lighter colored combs have a higher proportion of wax in relation to the other components added by bees, such as propolis and silk, and the reverse happens in black colored combs (Krivtsov & Lebedev, 1995). The use of these types of combs could significantly influence the amount of wax recovered.

We first analyzed the influence of the comb size on the wax extraction yield. For this, the combs were broken down into four sizes, namely: Treatment I - 25 cm² (5 x 5 cm), Treatment II - 16 cm² (4 x 4 cm), Treatment III - 9 cm² (3 x 3 cm) and Treatment IV - 4 cm² (2 x 2 cm). To minimize the effect of the wax origin, after the combs being fragmented, the particles were homogenized according to their size. Each treatment consisted of seven repetitions, each repetition with 500 g of fragmented combs.

The wax extraction technique used in this step was the "internal strain". To do so, initially, comb fragments were placed at the bottom of a 20 L container with a sieve (0.2 cm mesh) fixed on the top. Then, the entire set was covered with water until it passed the sieve by approximately 7 cm and thus heated on an industrial stove. The extraction time was 30 minutes, started to count as soon as the solution started to boil. At the end, the equipment was removed from the stove to cool down the wax. After 24 hours, the wax block that formed on the surface of the water was removed with a stainless steel spatula and later cleaned with a nylon bristle brush and water. After dry outdoors, the wax block was weighed on a digital scale with a precision of 0.1 g.

The second step of this study consisted of comparing the yield of honeycomb extraction using the commercial technique of "steam extraction" and the alternative technique of "internal strain" using the size of the comb that obtained the best result from the first step of this study. The combs used in each technique were fragmented, homogenized and divided into seven repetitions, each with 500 g.

With the "steam extraction" equipment leveled and, after reaching the boiling water, two extra extractions were performed in order to impregnate the interior of the equipment with wax, and thus avoiding under estimation. During the 30 minutes of extraction, the combs were mixed twice, every 10 minutes. Subsequently, the extracted liquid wax passed through a 0.2 cm mesh sieve, placed over a container with water, where it was collected. After each repetition, the residues from the previous extraction retained in the internal sieve of the equipment were removed. When solidified, the wax was cleaned with a brush of nylon bristles in running water, dry outdoors and then weighed on a digital scale with 0.1 g precision.

Data were arranged in a completely randomized design. For the statistical analyzes, the program "R" was used (R Development Core Team, 2018). Initially, the data were tested for homogeneity of variance, by Shapiro-Wilk test, and for normality, by Barlett test, both at 5% significance. After checking the assumptions, analysis of variance (ANOVA) was carried out via ExpDes package (Ferreira et al., 2014). In the first experiment, for comparisons of treatment means, Tukey test at 5% significance was used and in the second experiment, as there were only two means, the analysis of variance and the F test at the level of 1% significance were used to distinguish between means.

3. Results and Discussion

The results obtained of wax recovered using the "internal strain" technique with different sizes of combs, are shown in Table 1. The wax recovery of Treatment III was superior to the others ($P > 0.05$). The performance of the fourth treatment did not differ from the second treatment, which in turn did not differ from the first treatment ($P > 0.05$).

Table 1. Wax recovery (average, in grams and percentage in relation to the weight of the samples) of *Apis mellifera* combs, considering different sizes, using the "internal strain" technique.

Treatment	Sizes of combs (cm ²)	Wax recovered		
		Weight (g)*	SD**	%
I	25	76.54 ^c	12.39	15.31
II	16	91.92 ^{bc}	23.17	18.38
III	9	123.60 ^a	9.51	24.72
IV	4	102.22 ^b	21.18	20.44

* Means followed by different letters on the same column differ statistically at 5% level by Tukey test

** Standard deviation

Source: Authors.

As observed, the third treatment showed a better wax recovery compared to the first two, probably due to its greater contact surface. This result corroborates with the chemical principles of specific surface, in which the more fragmented the solid is, the larger this contact surface will be (Peruzzo & Canto, 2003). Thus, the smaller the size of the comb, the greater its contact surface, therefore, the greater the area will be exposed to the action of heat in wax extraction techniques. Besides, the extraction time seemed to be insufficient for the efficient release of the wax available in the first and second treatments.

On the other hand, the fourth treatment showed a different result than expected. Even having a specific surface larger than the others, the recovery of wax was less compared to the third ($P > 0.05$). This lower rate of recovery may have occurred due to combs of this size having undergone a greater warming action. In this way, the fragments were completely undone, releasing the silk cocoons that were trapped below the sieve, blocking the passage of some wax to the surface.

Brown to black combs are composed of wax and other substances, such as propolis (used to clean the combs by the bee); silk (produced by the larvae at the end of this life cycle); and components of food and larval excretions that may have been absorbed; and thus, the amount of wax to be recovered will always be less than the weight of the comb. According to Krivtsov & Lebedev (1995), light brown combs have 60.0% of wax and dark brown combs, 49.0%, so the mixture of these combs, as used in this study, should provide about 54.5% of their weight in wax. Thus, considering the total expected amount of wax, the estimated yield of wax extraction in the fragmented combs in this study was 28.08; 33.73; 45.35 and 37.51% for combs of 25, 16, 9 and 4 cm², respectively. These results show how limited the efficiency of wax recovery using physical techniques may be, in terms that the maximum yield during the 30 minutes of extraction, in brown combs, did not exceed 46%.

This was also observed by Bogdanov (2012), who obtained a wax recovery limited to a maximum of 50% efficiency when using physical techniques applied to darkened combs.

Moreover, Table 2 shows the comparison of wax recovery using the alternative technique of "internal strain" and the commercial technique of "steam extraction", for fragments of combs with 9 cm² size. The average recovery obtained by the "internal strain" technique was superior to the "steam extraction" technique (P > 0.01). This can be explained by the fact that, in the "steam extraction" technique, part of the extracted wax remained in contact with the extraction residues on the internal sieve of the equipment, so it could not flow and, consequently, be recovered.

Table 2. Wax recovery (in grams and percentage) from *Apis mellifera* combs broken down into 9 cm² size using the commercial technique "steam extraction" and alternative "internal strain".

Technique	Wax recovery		
	Weight (g)*	SD**	%
Steam extraction	60.91 ^a	7.82	12.18
Internal strain	123.60 ^b	9.51	24.72

* Means followed by different letters on the same column differ statistically at 1% level by F test

** Standard deviation

Source: Authors.

Peranovich et al. (2009), when using the commercial "steam" wax extraction technique obtained an average recovery of 204.9 g of wax in 838.0 g of combs, which represents 24.45% in relation to the weight of the sample. This value is higher than those achieved in this study for the "steam extraction" technique. However, in the abovementioned study, in addition to the combs having been exposed to heat for much longer, about 4 hours and 30 minutes, the authors did not undergo any color and purity selection, which may have influenced the results. Although the average amount of wax recovered in the commercial "steam extraction" technique in this study be less than that obtained by Peranovich et al. (2009), the amount of wax obtained by the "internal strain" technique using fragmented combs was similar in only 30 minutes, demonstrating its efficiency.

4. Conclusion

In the "internal strain" wax extraction technique, the fragmentation of *Apis mellifera* combs in sizes of 9 cm² statistically increased the recovery of wax from brown combs compared to the other tested sizes. Moreover, using combs fragmented in 9 cm², the alternative wax extraction technique of "internal strain" obtained statistically superior wax recovery than the commercial "steam" wax extraction technique considering a time of extraction of 30 minutes.

As far as we know, research demonstrating the importance of fragmentation of combs for the recovery of wax is still very rare. If applied, it has a great impact on the recovery of wax. The technique used on this study may optimize the wax extraction process, in terms that it decreases the time of exposure of the wax to heat, which positively influences the quality of the final product.

However, aiming to improve this innovative technique presented in this work, the next steps should seek to better study the equipment's processing capacity and the duration of the recovery process.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Credit authorship contribution statement

Gleydson Luiz de Oliveira Neto: Conceptualization, Investigation, Methodology, Project administration, Resources, Writing - Original Draft, Writing - Review & Editing. Lucas Lima Verardo: Formal analysis, Writing - Original Draft, Writing - Review & Editing. Cleube Andrade Boari: Writing - Review & Editing. Rodrigo Diniz Silveira: Conceptualization, Resources, Supervision, Writing - Original Draft, Writing - Review & Editing.

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