

VALORIZATION ALTERNATIVES OF COLOMBIAN BEE-POLLEN FOR ITS USE AS FOOD RESOURCE – A STRUCTURED REVIEW

ALTERNATIVAS DE VALORIZACIÓN DE POLEN APÍCOLA COLOMBIANO PARA SU USO COMO RECURSO ALIMENTICIO – UNA REVISIÓN ESTRUCTURADA

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ABSTRACT

Background: Pollen of honey-bee *Apis mellifera* L. is a product gathered for human consumption and marketed as a nutritional and functional resource. However, some studies indicate that it is necessary to develop transformation processes to modify the external structure of pollen, which is extremely difficult and prevents the nutrients and bioactive compounds to be completely digested in the gastrointestinal tract. The bee-pollen productivity in Colombia is up to five times higher than in countries traditionally recognized for its marketing, making this a valuable business opportunity. **Objectives:** To describe the chemical structure and the most important nutritional and functional components in bee pollen, as well as to understand the availability of compounds and some backgrounds reported for the opening of the bee-pollen grain, both in a natural and artificial manner. **Methods:** The literature search involved the use of different terms, alone or combined, by using logical operators; some terms were: pollen, bee-bread, bioactive and nutritional compounds, bioavailability, pollen structure, exine, fertilization. Selected search fields were the title or abstract of the publication in the following databases: *Directory of Open Access Journals, Emerald, Pubmed, Redalyc, Scientific Electronic Library Online, Science Direct, Springer Journal, Taylor & Francis, Wiley Online Library*. The selected literature was that found mainly between 2008 and 2014 in Spanish and English. **Results:** The nutritional and functional composition of bee-pollen is widely reported; nevertheless, few studies on transformation processes to improve the availability of the compounds present in this product were found. The natural fermentation process occurred within the hive to obtain the product known as “bee-bread” is highlighted, since an emulation of this transformation could be developed in a controlled manner for a scaled production; despite this, further research in the involved biotechnological aspects is still required. **Conclusions:** Efforts have been made to characterize the bee-pollen from a physical-chemical point of view, but no significant progress in the development of processes of transformation has been reached. The future trends should aim at developing engineering processes emulating the phenomena occurred in nature where structural changes in the outer layer of bee-pollen can be achieved.

Keywords: *Apis mellifera*, food quality, biochemical processes, nutrition, food composition.

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RESUMEN

Antecedentes: El polen de la abeja *Apis mellifera* L. es un producto recolectado para consumo humano y comercializado como recurso nutricional y funcional. Sin embargo, algunos estudios indican que es necesario desarrollar procesos de transformación para modificar la estructura externa del polen, la cual es muy resistente e impide que los nutrientes y compuestos bioactivos sean completamente digeribles en el tracto gastrointestinal. La productividad del polen en Colombia es hasta cinco veces superior a la de países tradicionalmente reconocidos por su comercialización, convirtiéndose en una oportunidad de negocio valiosa. **Objetivos:** Describir la estructura química y los componentes nutricionales y funcionales más importantes del polen. Además, comprender la disponibilidad de los compuestos y algunos antecedentes reportados para la apertura del polen, tanto natural como artificialmente. **Métodos:** La búsqueda bibliográfica comprendió el uso de diferentes términos, solos o combinados mediante el empleo de operadores lógicos; algunos fueron: polen, pan de abejas, compuestos bioactivos y nutricionales, biodisponibilidad, estructura del polen, exina, fertilización. Los campos de búsqueda fueron seleccionados en las siguientes bases de datos: *Directory of Open Access Journals*, *Emerald*, *Pubmed*, *Redalyc*, *Scientific Electronic Library Online*, *Science Direct*, *Springer Journal*, *Taylor & Francis*, *Wiley Online Library*. La bibliografía seleccionada principalmente, fue aquella encontrada entre 2008 y 2014 en español e inglés. **Resultados:** La composición nutricional y funcional del polen es ampliamente reportada; sin embargo, son escasos los trabajos sobre procesos de transformación para mejorar la disponibilidad de los compuestos presentes. Se destaca el proceso natural de fermentación ocurrido al interior de la colmena para la obtención del producto conocido como “pan de abejas”, ya que se podría desarrollar una emulación de esta transformación de manera controlada para una producción a escala; a pesar de esto, todavía se requiere de una mayor investigación en los aspectos biotecnológicos involucrados. **Conclusiones:** Desde el punto de vista fisicoquímico, se han hecho esfuerzos para caracterizar el polen; sin embargo, no hay avances significativos en el desarrollo de procesos de transformación. Las futuras tendencias deben estar encaminadas a desarrollar procesos ingenieriles, emulando fenómenos ocurridos en la naturaleza en donde se logren modificaciones estructurales de la capa externa del polen.

Palabras clave: *Apis mellifera*, calidad de alimentos, procesos bioquímicos, nutrición, composición de alimentos.

INTRODUCTION

Pollen is the male gametophyte of flowers used as a mean for the reproduction of plants. Different insects, including bees, take advantage of pollen as a source of protein, fat, vitamins and minerals (1). When bees visit flowers, they cover their bodies with pollen dust and form pellets with their saliva; they then attach the pellets to the corbiculae of their hinder legs to carry them to the hive and thus provide nutrients to their offspring (2). Although there are over 500 species of bees (3), the term “bee-pollen” is usually used for the product collected by species *Apis mellifera* L. Beekeepers have designed traps set in the hives to collect the excess of bee-pollen, and then, to undergo it to basic processes of suitability, especially those of drying and removal of impurities, before being offered on the market (4).

In recent years, the recognition of bioactive and nutritional value of bee-pollen has promoted its incursion as food for humans (5-14). However, regardless of the characteristics of this food, there are reports

that show a reduced availability of nutrients and bioactive compounds once the bee-pollen is ingested. This is mainly due to the strong chemical structure that covers the outer layer of the grain (4,5), which suggests that the bee-pollen for human consumption must undergo transformation processes; one, as a strategy to increase its shelf life, and two, to improve the nutritional and functional quality indicators.

In Colombia, reports about physical-chemical and palynological analyses as well as traceable systems for bee-pollen collection, have been described in specialized literature (15-18). In this country, beekeepers have recognized the geographical advantages of the region known as the Cundiboyacense Highland, where about 90% of the bee-pollen domestic production is concentrated (19). This area presents yields per hive of near 40 kg of product / year, compared to other countries that dominate most of the market such as Spain, Portugal, China, Brazil or Argentina, which do not reach 15 kg / hive / year (4, 19-20). The geographical localization of the Cundiboyacense Highland is shown in Figure 1.

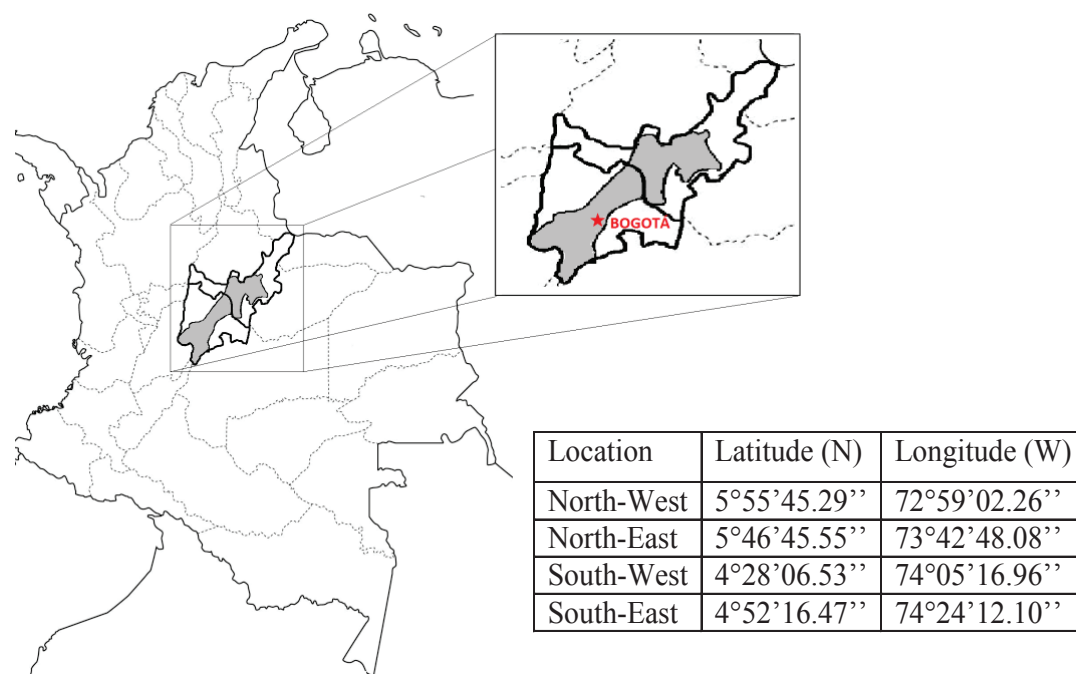


Figure 1. Localization of the colombian Cundiboyacense Highland (area shaded in gray). Source of coordinates: (21)

Even though there are plenty of studies that focus on the research of the characterization of physical-chemical properties of bee-pollen, there are actually few reports on potential treatments to suit it as a food with enhanced nutritional and bioactive value for humans. Consequently, the need to strengthen the beekeeping productive chain, particularly in aspects related to innovation and new product development, is clear (19). The objective of this review is both to contribute to the systematization of information related to both the bee-pollen structure and worldwide reports of chemical and physical composition. The availability of nutrients and the backgrounds for bee-pollen grain rupture is also discussed. Finally, some further stages which require the pollen for alimentary purposes in humans are suggested.

METHODS

Bibliographic information was collected by revising investigations related to bee-pollen composition, structure and transformation processes. Available bibliographic tools at Universidad Nacional de Colombia were employed; these were: *Directory of Open Access Journals*, *Emerald*, *Pubmed*, *Redalyc*, *Scientific Electronic Library Online*, *Science Direct*, *Springer Journal*, *Taylor & Francis*, *Wiley Online Library*. Research was made in both English and Spanish. Different terms, alone or combined by

using logical operators, were used such as: pollen, bee bread, bioactive and nutritional compounds, bioavailability, pollen structure, exine, fertilization, pollen transformation.

Selected search fields were the title or abstract of the publication. The specialized literature selected was that found mainly between 2008 and 2014. In addition, the bibliographic administrator employed was *Mendeley Desktop* ver. 1.11. The criteria for the inclusion of articles were based on the contribution of investigations in the topics for which this article has been structured: pollen structure, nutritional and bioactive potential of bee-pollen, availability of nutrients and bioactive compounds in bee pollen and backgrounds for exine rupture. On the other hand, exclusion criteria were based on those articles which had topics beyond those mentioned in the objective of this work, such as: antimicrobial or medicinal activity of bee-pollen, and phenotypic or genotypic characterization of bacteria isolated from bee-pollen.

RESULTS

More than 3500 articles related to the search criteria were identified; however, only 60 records were selected and referenced in this review. In figure 2, the search process and the results obtained in the investigation are illustrated.

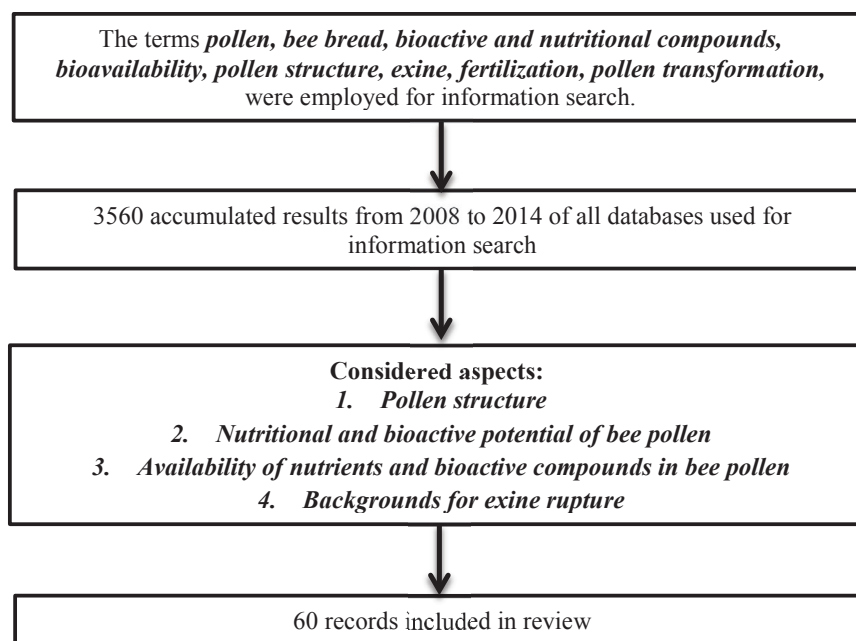


Figure 2. Search flowchart

Pollen structure

Pollen is a powder of variable appearance, from fine to coarse, composed by a multitude of microscopic particles (6–200 μm in diameter) with variable shape, usually spherical or oval. The pollen cell walls consist of a series of stratified concentric layers. The outer wall is known as exine and is very flexible, elastic, strong and firm; it is made of sporopollenin, a compound that provides chemical resistance to pollen and preserves the compounds which are within it (Figure 3) (22). The exine is mainly composed of sporopollenin and other components unknown up to date (23).

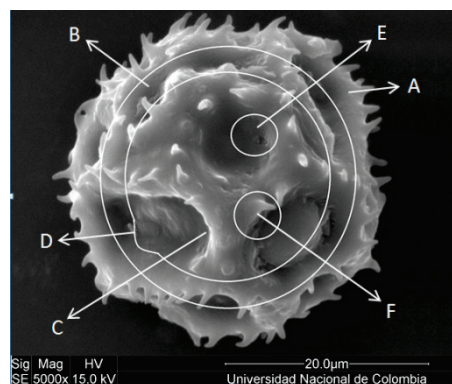


Figure 3. Structure of the pollen cell. Outer part: (A) exine. Inner part (depicted in imaginary white lines): (B) intine; (C) endoplasmic reticulum; (D) aperture; (E) vegetative nucleus; (F) nucleus of the generative cell

In addition, the inner layer is called intine, which consists of cellulose, and is structurally similar to the cell wall of a plant (24). From reports based on specialized literature, it is known that the exine is rich in antioxidant compounds, while the intine has the rest of the nutritional and bioactive compounds (25).

The chemical analysis of sporopollenin has been limited due to the difficulty of obtaining large quantities of exine without affecting or modifying the structure of this molecule (24). Atkin *et al.* recently found that this molecule has an empirical formula $\text{C}_{90}\text{H}_{144}\text{O}_{27}$ (22).

Nowadays, it is suggested that the sporopollenin consists of a series of biopolymers, homologous to the compounds cutin, suberin and lignin. Cutin is the structural component of the plant cuticles, a layer that covers the outer surface of cells, synthesized from oxygenated fatty acids (C_{16} and C_{18}), and derived mainly from the oleic acid, together with a small amount of phenolic acids such as p-coumaric and ferulic acid (26).

The suberin protects the inner parts of the plant such as roots and tubers, and it is also found in the coating of seeds of certain plants (27). It is a mixed polymer of long-chain fatty acids (C_{18} – C_{30}) and a moderate content of phenylpropanoids such as p-coumaric and ferulic acid, and compounds such as tyramine, and some monolignols. Lignin, on

the other hand, is composed entirely of phenolic compounds known as monolignols (27).

Nutritional and bioactive potential of bee pollen

Bee-pollen has been used for centuries in the human diet and traditional medicine due to its nutritional and physiological properties (5, 8-9). Different studies have shown that, besides protein richness, the bee-pollen is a good source of carbohydrates, dietary fiber, fatty acids and antioxidant components, making it a product with functional properties based on the health effects caused by its periodic consumption (2).

With respect to Colombian bee-pollen from the Cundiboyacense Highland, several reports have described its physicochemical composition (15, 28-30). As for the botanical species, there is a tendency to a greater presence of dandelion (*Hypochaeris radicata*) and red clover (*Trifolium pratense*) pollen (31).

Table 1 shows the average composition of Colombian bee pollen of the Cundiboyacense Highland (15), the reported composition ranges in specialized literature and the values suggested by the standard regulations described by Campos *et al.* which set the ranges of the nutritional conditions to be met by bee-pollen (2). This literature also reports other components of interest to the bee-pollen, although they are not part of the normativity, such as minerals and vitamins. Potassium (5,600 to 5,800 mg/kg), magnesium (1,000 - 1,200 mg/kg) and calcium (1,400 to 1,700 mg/kg) are the predominant elements (12, 32). Moreover, the vitamins make an important contribution to the bee pollen, being the most significant the β -carotene (10-200 mg/kg) and vitamin C (70 - 560 mg/kg), followed by niacin (40-110 mg/kg), thiamine (6-13 mg/kg), folic acid (3-10 mg/kg) and biotin (0.5 to 0.7 mg/kg) (2, 14, 32).

Among the biologically active substances that have been reported in bee-pollen, phenolic compounds, flavonoids and anthocyanins are found (10, 12-13). Flavonoids are secondary components important in bee-pollen. They are found as glycosides, called aglycones, responsible for the color of the grain (yellow, brown, red, purple, etc.) and its characteristic bitter taste (4). This aspect receives special attention in the field of functional foods, since some studies have shown some therapeutic benefits of the anti-radical activity, the inhibition of lipid peroxidation and the cellular and humoral response suppression (6-7, 38).

On the other hand, different regulations have defined minimum limits according to the Recommended Daily Intake (RDI) for each nutrient and bioactive compound that should be present in the portion of a food matrix in order to be labeled as a potentially significant source of such substance (39-42). Based on national and international standards, Table 2 lists the nutritional and bioactive components of bee-pollen, according to its contribution to the RDI. Calculations were performed on a portion of bee-pollen of 15 g/day as suggested by some authors (2,32); nevertheless, other researchers advise the possibility of increasing the portion up to 40 g/day (4, 43), which would increase the contribution of bee-pollen to the RDI of the compounds described.

Table 1. Interval of composition of bee pollen from diverse origins and suggestion for standard regulations

Component	Content Min-Max g/100 g dry weight	Average content Colombian bee-pollen g/100 g dry weight	Suggestion for standard regulation according to (2)	References
Proteins	10-40	23.8	No less than 15g/100g	(2,4, 15, 29, 33-36)
Lipids	1-13	6.86	No less than 1.5g/100g	(2,4, 15, 29, 33-36)
Total carbohydrates	13-55	39.8	No less than 40g/100g	(2,4, 15, 29, 33-36)
Total dietary fiber	-	14.5	-	(34, 37)
Ash	2-6	2.47	No more than 6g/100g	(2,4, 15, 29, 33-36)

Table 2. Contribution of nutrients and bioactive compounds of bee-pollen

	MV	Recommended Daily Intake (RDI)	Quantity contained in 15 g of bee pollen	Contribution of bee-pollen to the RDI	Reference
Protein	At least 12% of the energetic value	50 g/day	Between 1,5 and 6 g	More than 28% (13 kcal protein/ 45 kcal bee-pollen, approx.)	(42)
	At least 20% of the nutritional composition			Between 20% and 27%	(40)
β -carotene	At least 15% of the RDI	0.9 mg/day	Between 0.15 and 3 mg	Between 17% and 333%	(5, 39, 41, 43-44)
Vitamin E		13 mg/day	Between 0.65 and 4.80 mg	Between 5% and 37%	
Vitamin B1		1.1 mg/day	Between 0.09 and 0.20 mg	Between 8% and 18%	
Vitamin B2		1.3 mg/day	Between 0.09 and 0.30 mg	Between 7% and 23%	
Folic acid		0.4 mg/day	Between 0.04 and 0.15 mg	Between 11% and 38%	
Biotin		0.045 mg/day	Between 0.008 and 0.010 mg	Between 17% and 23%	
Zinc		8.5 mg/day	Between 0.43 and 3.74 mg	Between 5% and 44%	
Fiber	6 g Fiber / 100 g Food	20 - 35 g/day	2,18 g	14.5 g Fiber / 100g bee-pollen	(42)

MV = minimum value for a food to be considered a rich source of this nutrient or bioactive compound

Availability of nutrients and bioactive compounds in bee-pollen

Despite the large content of nutrients and bioactive compounds that are found in bee-pollen, the quality, in terms of nutrition, depends mainly on the digestibility (parameter used to measure the nutritional value of a food) and bioavailability (part of the nutrient that the body digests, absorbs and uses in physiological functions), which appears to be closely related to some morphological characteristics of grains, such as porosity and thickness of the outer wall (4, 45).

Previous investigations suggest that the availability of usable components of bee-pollen by humans, including nutritional and bioactive compounds is limited and, therefore, so is its use as food (45). In the past years, there have been some doubts on the ability of the human digestive system to break the outer layer overlying the pollen and to absorb nutrients and bioactive substances that are inside it. For instance, different simulations *in vitro* of human digestion suggest that the bee-pollen is partially digested -between 48% and 59%- (5), and anthropological studies found coprolites (dried human excretions) with intact pollens of the periods between 1400 - 200 BC, which has been used by some researchers as evidence of the strength of the outer wall of pollen, even to gastric acid (46).

This reflects the need to find alternatives for transforming bee-pollen for an integral use of this product. Despite the absence of conclusive data, the common view of different researchers is that bee-pollen is insufficiently digested and a breaking of this outer wall would improve the digestibility and bioavailability (5, 46).

Backgrounds for exine rupture

The backgrounds to describe the different processes known, in which the exine aperture is achieved, can be divided into two categories. The first one corresponds to those transformations in the structure of pollen occurring in nature; the second one refers to the developments made by humans in order to achieve the same goal, mainly through biotechnological processes.

Aperture processes of exine occurring in nature

There are two phenomena recognized for achieving the aperture of the exine. The first one is the natural process of plant fertilization, in which the pollen-flower interaction favors the passage of genetic information for the reproduction of plants. The other one is related to the process occurred in hives where the bees, with the help of digestive enzymes and microorganisms, put the pollen in cells where a

fermentation process that structurally modifies the exine and facilitates the aperture, occurs.

Plant fertilization

It is important to note that the main purpose of pollen is to ensure that genetic information arrives in the best condition to another plant of the same species, in order to continue with its reproductive and evolutive cycle. Possibly, the rigidity of the exine is useful to protect DNA in the pollen grain regardless of environmental conditions.

Both the pollen and the recipient flower, have a mechanism that facilitates the exine aperture favoring fertilization (47). This process starts in the stigma, which traps and discriminates pollen grains related and unrelated to the plant (48). Little is known about the pollen-stigma interaction (49), but some reports agree that in the pollen aperture process, a complex mixture of saturated and unsaturated long chain triacylglycerols and carbohydrate-rich aqueous solutions is involved (47-48). During the process, some enzymes such as peroxidases, polygalacturonases, glucanases and esterases show maximum peaks of activity. Although the enzyme function is unknown, it is suggested that they are useful for pollen recognition and to facilitate the aperture process (50).

Unfortunately, the research done so far only clarifies some aspects of this phenomenon, but the understanding of what happens at a biological level may be useful to be applied in the development of a technological transformation process. The reports exposed show the importance of enzymes in the weakening and opening of the pollen exine layer, but it is still necessary to prove which of the reported enzymes exerts an appreciable effect on the grain.

Biological transformation process of pollen in the hive

It is known that in the hives bees rarely consume pollen directly, but they do it after it has been stored in cells in order to put it through to a biochemical transformation process and convert it into a product known as “bee-bread”; this increases the availability of nutrients and bioactive components (51). Despite the improved nutritional and functional value of bee bread with respect to pollen, this product is still very rare and expensive –up to 208 USD / kg (Source: Apiarios Sao Braz, Lages SC, Brazil)– due to the inherent difficulty of extraction that obligates the beekeeper to partially destroy the hive.

Del Risco, based on his research, mentions that the process of obtaining bee-bread occurs in a progression of appearance/disappearance of colonizing microorganisms according to the conditions of both the cell of the hive and the substrate. This fermentation process would occur in three different stages: initially, the bee deposits the pollen grain in one of the cells used for this purpose inside the hive, where salivary secretions and some honey are also added. Honey works as a source of carbohydrates for the initial growth of microorganisms, as well as a seal for generating anaerobic conditions within the cell (52).

At this point, it is clear that the microbiome is abundant and diverse, not only because of the microorganisms naturally present in the bee-pollen, but because of those who live in the digestive tract of the bee. Several studies show that different species of the bacteria *Pseudomonas* sp. and yeast *Torulopsis* sp. are the colonizers in the first stage (51). These microorganisms induce fermentation under aerobic conditions, and it is estimated that the main results include a decrease in the pH of the medium, with a consequent increase in the acidity, the production of vitamins of the B complex by yeasts, and the depletion of oxygen remaining in the cell (52). Similarly, various investigations have attributed to bacteria of the genus *Pseudomonas* sp. a high enzyme activity and, particularly, a production of the enzyme cutinase, which degrades cutin, one of the structural components of the exine (53).

The oxygen depletion and the decline in pH cause a decrease in the population of *Pseudomonas* sp., a situation that is exploited by different species of *Bacillus* and *Streptococcus*, which are the colonizers of the substrate in the second stage of fermentation; at this point, the process is carried out under anaerobic conditions (52). These microorganisms apparently have the ability to pre-digest the pollen as well as to inhibit the growth of other microorganisms that can affect the health of the hive (52). Some authors also believe that the enzymes produced (pectinases, cellulases, amylases, proteases, β -glucanases and isomerases) have some effect on the breaking of exine under anaerobic fermentation (4, 54), but it is still unknown if these enzymes are an influential factor for obtaining bee bread inside the hive.

The third stage begins with a decrease in the population of *Bacillus* and *Streptococcus*, and the colonization of the substrate from various types of Lactic Acid Bacteria (LAB). These bacteria produce lactic

acid quickly, up to a point in which it is sufficiently toxic for microorganisms (approximately 3% of lactic acid present in the product), and the result is the

disappearance of the LAB and yeasts that remained in the bee bread (52). Table 3 presents some of the microorganisms isolated from bee bread.

Table 3. Main microorganisms found during the process of transformation of bee pollen into bee bread (55).

Bacilli	Lactobacilli	Yeasts	
<i>B. subtilis</i>	<i>L. kunkeii</i>	<i>Candida parapsilosis</i>	<i>Torulopsis globosa</i>
<i>B. megaterium</i>	<i>L. jensenii</i>	<i>Candida reukauffii</i>	<i>Torulopsis inoscipua</i>
<i>B. licheniformis</i>	<i>L. fructosus</i>	<i>Candida tenuis</i>	<i>Torulopsis magnoliae</i>
<i>B. circulans</i>	<i>L. lantarum</i>	<i>Torulopsis candida</i>	<i>Torulopsis sake</i>
<i>B. pumilus</i>		<i>Torulopsis etchellsii</i>	<i>Torulopsis stellata</i>
		<i>Torulopsis famata</i>	

Biotechnological processes for the aperture of exine of pollen based on natural phenomena

Regarding pollen transformation processes, specialized literature is still very scarce. Most studies related to the breaking or weakening of the exine of pollen have been made mainly as a botanical research approach (56-57), rather than as a technological tool to make more available the nutrients and bioactive compounds for its use as a dietary supplement.

Some studies have been conducted to use the exine in food and pharmaceutical industries as a carrier of different substances; however, the employed techniques promote the destruction of all nutrients (26). Xu *et al.* managed to break the exine using supercritical CO₂ at 45 MPa for 10 minutes, but only with the aim of obtaining an extract rich in unsaturated fatty acids (58 g/100 g of fatty acids), mainly linoleic acid (C18:2) (12.9 g/100 g of fatty acids) and linolenic acid (C18:3) (38.6 g/100 g of fatty acids) (58).

The most notable advances have been achieved by simulating the process that occurs in the hive for bee bread making at laboratory scale. Del Risco and Fuenmayor represented the anaerobic fermentation of bee-pollen carried out by acid lactic microor-

ganisms to produce bee bread (33,52). Del Risco ensures that in the presence of heat and humidity, the bee pollen loses the exine layer and starts lactic acid fermentation (52).

Fuenmayor evaluated different bioprocesses aiming at improving nutritional indicators of bee-pollen as a contribution to the development of a protein nutritional supplement. This investigation showed that it is possible to induce lactic acid fermentation in solid phase in a matrix composed primarily of bee pollen, using an inoculum of *Lactobacillus acidophilus*, but it is also highlighted that further studies are needed to describe the phenomenon of rupture of the outer layer of pollen, which would allow a better understanding of the phenomenon (33). Del Risco *et al.* mentioned that from pollen silage with lactic acid bacteria native from bee bread (*L. plantarum*, *L. coryniformis* and *L. delbrueckii*), it is feasible to obtain an acid product with an acceptable microbiological quality (51). In comparison with pollen, bee-bread has an average of 3% of lactic acid, a greater content of vitamin K (6500 mg/kg and 7400 mg/kg in bee-pollen and bee-bread, respectively) and higher values of leucine, threonine and valine aminoacids as shown in table 4 (52, 59-60).

Table 4. Aminoacid composition in bee-pollen and bee-bread (60).

Aminoacid	Average content in bee-pollen µg/g	Average content in bee-bread µg/g
Leucine	70	110
Threonine	35	50
Valine	40	50

It can be observed that bee pollen transformation techniques established are still not enough, and therefore, it is of great importance to assess different processes that generate a solution to the issues mentioned, factors that have not been evaluated yet despite the potential of this product. It would be interesting to emulate the fermentation process for the scaled production of bee-bread, through the development of a transformation process, with the goal of improving the nutritional and functional indicators. However, this emulation is only possible if certain conditions of the process are figured out and the roles of enzymes and microorganisms involved in the transformation of the pollen are completely known.

In this review, a general panorama on composition and transformation alternatives of bee-pollen is given, with the objective of focusing future research in these areas. Nevertheless, the wide varieties in pollen species, the recognized link of pollen to geographical origin and the lack of knowledge in some aspects commented in this article, restrict the generalization of the results. Additionally, due to space limitations, to provide a broader picture in topics, such advances in the extraction of bioactive compounds or the inclusion of bee-pollen directly in several food matrixes is a future task.

CONCLUSION

While it is true that there are several investigations for the physicochemical characterization of bee pollen, the availability of the compounds present in such product, once it is consumed by humans, is still unknown. Additionally, the current information is not enough to generate guidelines that serve to define a transformation process of bee-pollen that promotes the availability of nutrients and bioactive compounds. Future research for transformation should focus on the application of treatments of various kinds for breaking the exine and, in turn, assessing whether this process improves their nutritional and functional indicators, measured *in vitro* and *in vivo*. It would be interesting to emulate the fermentation of bee-pollen that occurs in the hive for the scaled production of bee bread, through the development of a process of transformation. The colombian beekeeping chain currently sells products directly, with no kind of transformation processes; thus, there is neither innovation in the market nor guidelines for the

creation of value. The recognition of beekeeping as a productive chain, by the Ministry of Agriculture and Rural Development of Colombia in 2012, commits this sector to improve its technological development indicators and create new products useful for market diversification.

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Conflict of interest

Each author declares there is no conflict of interest with any result of this review.

REFERENCES

1. Seeley T. Ecología da abelha: Um estudo de adaptação na vida social. Porto Alegre, Brazil: Paixao; 2006. 256 p.
2. Campos M, Bogdanov S, Almeida-Muradian L, Szczesna T, Mancebo Y, Frigerio C, Ferreira, F. Pollen composition and standardisation of analytical methods. J Apicult Res. 2008 Aug 5; 47(2):156–163.
3. Vit P, Pedro S, Roubik D. Pot Honey. A legacy of Stingless Bees. New York, USA: Springer; 2013. 654 p.
4. Bogdanov S. The Bee Pollen Book. Bern, Switzerland: Bee Product Science; 2011. 42 p.
5. Campos M, Frigerio C, Lopes J, Bogdanov S. What is the future of Bee-Pollen?. J ApiProduct ApiMedical Sci. 2010 Oct 1; 2(4):131–144.
6. Almaraz-Abarca N, Campos M, Ávila-Reyes J, Naranjo-Jiménez N, Corral J. Antioxidant activity of polyphenolic extract of monofloral honeybee-collected pollen from mesquite (*Prosopis juliflora*, Leguminosae). J Food Compos Anal. 2007 Nov 1; 20(2):119–124.
7. Leja M, Mareczek A, Wyżgolik G, Klepacz-Baniak J, Czekońska K. Antioxidative properties of bee pollen in selected plant species. Food Chem. 2007 Nov 28; 100(1):237–240.
8. Ulusoy E, Kolayli S. Phenolic Composition and Antioxidant Properties of Anzer Bee Pollen. J Food Biochem. 2013 Apr 17; 38(1):73–82.
9. Basuny A, Arafat S, Soliman H. Chemical analysis of olive and palm pollen: Antioxidant and antimicrobial activation properties. J Food Technol. 2013 Apr 2; 1(2):14–21.
10. Morais M, Moreira L, Feás X, Estevinho L. Honeybee-collected pollen from five Portuguese Natural Parks: Palynological origin, phenolic content, antioxidant properties and antimicrobial activity. Food Chem Toxicol. 2011 Feb 1; 49(5):1096–1101.
11. Graikou K, Kapeta S, Aligiannis N, Sotiroidis G, Chondrogianni N, Gonos E, Chinou I. Chemical analysis of Greek pollen - Antioxidant, antimicrobial and proteasome activation properties. Chem Cent J. 2011 Jun 23. 5(1): 1–9
12. Rebiai A, Lanez T. Chemical composition and antioxidant activity of *Apis mellifera* bee pollen from northwest Algeria. J Fund App Sci. 2012 Dec 1; 4(2):26–35.

13. Saric A, Balog T, Sobocanec S, Kusic B, Sverko V, Rusak G, et al. Antioxidant effects of flavonoid from Croatian *Cystus incanus* L. rich bee pollen. *Food Chem Toxicol*. 2008 Dec 16; 47(3):547–554.
14. Yang K, Wu D, Ye X, Liu D, Chen J, Sun P. Characterization of Chemical Composition of Bee Pollen in China. *J Agr Food Chem*. 2013 Jan 23; 61(3):708–718.
15. Fuenmayor C, Zuluaga C, Diaz C, Quicazán M, Cosio M, Mannino S. Evaluation of the physicochemical and functional properties of Colombian bee pollen. *Rev MVZ Córdoba*. 2014 Jan 1. 19(1):4003–4014.
16. Vivas N, Maca J, Pardo M. Caracterización cualitativa del polen recolectado por *Apis mellifera* L en tres apiarios del municipio de Popayán. *Rev Bio Agro*. 2008 Jul 10. 6(2):94–98.
17. Chamorro-García F, León-Bonilla D, Nates-Parra G. El polen apícola como producto forestal no maderable en la cordillera oriental de Colombia. *Colomb Forest*. 2013 May 15. 16(1):53–66.
18. Salamanca G, Osorio M, Gutierrez A. Sistema trazable en el proceso de extracción y beneficio del polen corbicular colectado por *Apis mellifera* L. (Hymenoptera: Apidae) en la zona Altoandina de Boyacá, Colombia. *Zootec Trop*. 2011 Mar 18. 29(1):127–138.
19. Ministerio de Agricultura y Desarrollo Rural. Plan Estratégico de Acción 2011–2025. Bogotá: Cadena de las Abejas y la Apicultura; 2006. 40 p.
20. Laverde J, Egea L, Rodríguez D, Peña J. Agenda prospectiva de investigación y desarrollo tecnológico para la cadena productiva de las abejas y apicultura en Colombia con énfasis en miel de abejas. Bogotá, Colombia: Ministerio de Agricultura y Desarrollo Rural; 2010. 224 p.
21. IGAC-ORSTOM. Estudio regional integrado del Altiplano Cundiboyacense: estudio general de suelos. Bogotá, Colombia: IGAC; 1984. 137 p.
22. Atkin S, Barrier S, Cui Z, Fletcher P, Mackenzie G, Panel V, et al. UV and visible light screening by individual sporopollenin exines derived from *Lycopodium clavatum* (club moss) and *Ambrosia trifida* (giant ragweed). *J Photoch Photobio B*. 2010 Dec 21; 102:209–217.
23. Suzuki T, Masaoka K, Nishi M, Nakamura K, Ishiguro S. Identification of *kaonashi* mutants Showing Abnormal Pollen Exine Structure in *Arabidopsis thaliana*. *Plant Cell Physiol*. 2008 Oct 10; 49(10):1465–1477.
24. Blackmore S, Wortley A, Skvarla J, Rowley J. Pollen wall development in flowering plants. *New Phytol*. 2008 Jan 10; 174(3):483–498.
25. Wakil A, Mackenzie G, Diego-Taboada A, Gordon J, Atkin S. Enhanced Bioavailability of Eicosapentaenoic Acid from Fish Oil After Encapsulation Within Plant Spore Exines as Microcapsules. *Lipids*. 2010 May 22; 45(7):645–649.
26. Thomasson M, Baldwin D, Diego-Taboada A, Atkin S, Mackenzie G, Wadhawan J. Electrochemistry and charge transport in sporopollenin particle arrays. *Electrochem Commun*. 2010 Aug 7; 12:1428–1431.
27. Carrington E, Hernes P, Dyda R, Plante A, Six J. Biochemical changes across a carbon saturation gradient: Lignin, cutin, and suberin decomposition and stabilization in fractionated carbon pools. *Soil Biol Biochem*. 2012 Jan 5; 47:179–190.
28. Diaz C, Zuluaga C, Fuenmayor C, Martinez T. Special features of pollen production in Colombia. 41st World Congress of Apiculture-Apimondia; Montpellier, France; 2009.
29. Zuluaga C. Análisis quimiométrico para diferenciar la huella digital de los productos de las abejas en Colombia [master's thesis]. [Bogotá, Colombia]: Universidad Nacional de Colombia; 2010. 246 p.
30. Fuenmayor C, Díaz C, Quicazán M, Mannino S. Exploring nutritional and functional features of colombian corbicular pollen. 42nd Apimondia: Buenos Aires, Argentina; 2011.
31. Montoya P. Uso de recursos florales poliníferos por *Apis mellifera* (Hymenoptera: Apidae) en apiarios de la Sabana de Bogotá y alrededores [master's thesis]. [Bogotá, Colombia]: Universidad Nacional de Colombia; 2011. 66 p.
32. Almeida-Muradian L, Pamplona L, Coimbra S, Barth O. Chemical composition and botanical evaluation of dried bee pollen pellets. *J Food Compos Anal*. 2005 Apr 12; 18(1):105–111.
33. Fuenmayor C. Aplicación de bioprocesos en polen de abejas para el desarrollo de un suplemento nutricional protéico [master's thesis]. [Bogotá, Colombia]: Universidad Nacional de Colombia; 2009. 225 p.
34. Díaz C, Zuluaga C, Morales C, Quicazán M. Determinación de fibra dietaria en polen apícola colombiano. *Vitae*. 2012 Jun 13; 19(S1):454–456.
35. Zuluaga C, Diaz C, Quicazán M. Partial least squares modelling for predicting mineral content of Colombian bee-pollen. 42nd Apimondia Buenos Aires, Argentina; 2011.
36. Carpes S, Mourao G, Alencar S, Masson M. Chemical composition and free radical scavenging activity of *Apis mellifera* bee pollen from Southern Brazil. *Braz J Food Technol*. 2009 Jul 1; 12(3):220–229.
37. de Arruda VAS, Pereira AAS, de Freitas AS, Barth OM, de Almeida-Muradian LB. Dried bee pollen: B complex vitamins, physicochemical and botanical composition. *J Food Compos Anal*. 2012 Dec 27; 29(2):100–105.
38. Gulcin I, Elias R, Gepdiremen A, Boyer L, Koksall E. A comparative study on the antioxidant activity of fringe tree (*Chionanthus virginicus* L.) extracts. *Afr J Biotechnol*. 2007 Feb 19; 6(4):410–418.
39. Fernández M. Etiquetado de propiedades nutritivas. Madrid, España: Agencia Española de Seguridad Alimentaria y Nutrición; 2009. p. 956–959.
40. Salamanca M. Requisitos de rotulado o etiquetado nutricional que deben cumplir los alimentos envasados para consumo humano. Bogotá, Colombia: Ministerio de Protección Social de Colombia; 2011. 56 p.
41. Gesellschaften für Ernährung in Deutschland, Österreich und der Schweiz. Referenzwerte für die Nährstoffzufuhr [Internet]. Rostock (Germany): DGE-MedienService; 2000 Jan 1 [cited 2012 Feb 28]. Available from: <http://www.dge.de/modules.php?name=Content&pa=showpage&pid=3&page=1>
42. Fontelles J, Korkeaioja J. Regulation (EC) No 1924/2006 of the European Parliament and of the Council on nutrition and health claims made on foods. Brussels, Belgium: European Parliament and Council; 2006. p. L12/3 - L12/18.
43. Oliveira KCLS, Moriya M, Azedo RAB, Almeida-Muradian LB de, Teixeira EW, Alves MLTMF, et al. Relationship between botanical origin and antioxidants vitamins of bee-collected pollen. *Quim Nova*. 2009 May 28; 32:1099–1102.
44. Zapatero V. Norma de etiquetado sobre propiedades nutritivas de los productos alimenticios Madrid, España: Agencia Española de Seguridad Alimentaria y Nutrición; 1992. p. 382
45. Cook S, Awmack C, Murray D, Williams I. Are honey bees' foraging preferences affected by pollen amino acid composition? *Ecol Entomol*. 2003 Sep 18; 28(5):622–627.
46. Rimpler M. Von Bienen gesammelte Blütenpollen: Eigenschaften und Verwendung. *Ärztezeitschrift für Naturheilverfahren*. 2003 May 1; 44(3):158–165.
47. Hiscock S, Allen A. Diverse cell signalling pathways regulate pollen–stigma interactions: the search for consensus. *New Phytol*. 2008 Jul 1; 179:286–317.
48. Gao X, Zhu D, Zhang X. Stigma factors regulating self-compatible pollination. *Front Biol*. 2010 Apr 1; 5(2):156–163.
49. Lenartowska M, Lenartowski R, Smoliński D, Wróbel B, Niedojadło J, Jaworski K, et al. Calreticulin expression and localization in plant cells during pollen–pistil interactions. *Planta*. 2009 Oct 7; 231(1):67–77.
50. McInnis S, Emery D, Porter R, Desikan R, Hancock J, Hiscock S. The role of stigma peroxidases in flowering plants: insights from further characterization of a stigma-specific peroxidase

- (SSP) from *Senecio squalidus* (Asteraceae). *J Exp Bot.* 2006 May 12; 57:1835–1846.
51. Del Risco C, Pérez A, Álvarez V, Rodríguez G, Leiva V, Puig Y, *et al.* Bacterias ácido-lácticas para ensilar polen apícola. CENIC. 2012 May 10; 43(1):17–21.
52. Del Risco C. Polen-Pan de Abejas: Composición, Nutrición, Acción en la Salud Humana y Microbiología [dissertation]. [La Habana, Cuba]: Centro de Investigaciones Apícolas; 2011. 13 p.
53. Dutta K, Sen S, Veeranki V. Production, characterization and applications of microbial cutinases. *Process Biochem.* 2009 Sep 23; 44(2):127–134.
54. Čeksterytė V, Račys J, Kaškonienė V, Venskutonis P. Fatty acid composition in beebread. *Biologija.* 2008 Oct 6; 54(4):253–257.
55. Gilliam M. Microbiology of pollen and bee bread: The genus *Bacillus*. *Apidologie.* 1979 Mar 4; 10(3):269–274.
56. Chichiricò G, Pacini E. *Cupressus arizonica* pollen wall zonation and in vitro hydration. *Plant Syst Evol.* 2008 Feb 1; 270(3):231–242.
57. Jiang J, Zhang Z, Cao J. Pollen wall development: the associated enzymes and metabolic pathways. *Plant Biol.* 2012 Dec 17; 15(2):249–263.
58. Xu L, Sun I, Dong L, Zhang H. Breaking the cells of rape bee pollen and consecutive extraction of functional oil with supercritical carbon dioxide. *Innov Food Sci Emerg Technol.* 2009 Aug 28; 10:42–46.
59. Andelkovic B, Jevtic G, Mladenovic M, Markovic J, Petrovic M, Nedic N. Quality of pollen and honey bee bread collected in spring. *J Hyg Eng Des.* 2012 Mar 23; 1: 275–277.
60. DeGrandi-Hoffman G, Eckholm B, Huang M. A comparison of bee bread made by Africanized and European honey bees (*Apis mellifera*) and its effects on hemolymph protein titers. *Apidologie.* 2013 Jun 19; 44:52–63.