Performance of 2% and 20% glucose-containing potato agar for quantitative and qualitatively detecting molds and yeasts from non-inspected honey samples

Performance do ágar batata contendo 2% e 20% de glicose para a detecção quantitativa e qualitativa de bolores e leveduras de amostras de mel não inspecionadas

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ABSTRACT

The Brazilian Official Methodology had established the employment of two culture media for determining molds and yeasts in honey samples, being one containing 2% glucose and other 20%. From 2003 onward, it has been established the use of 2% glucose-containing medium only for this purpose. Variation in glucose concentration into culture media may induce interference on osmotic pressure, which may cause difference on fungi identification and counting results. In this study**,** 30 samples of honey informally traded in the city of São Paulo were analyzed for determining the occurrence of molds and yeasts through spread plate on potato agar media containing 2% and 20% of glucose. No significant difference in fungi counting (CFU.g $^{-1}$) was found when the cultures in both media were compared, although higher numbers of molds (CFU.g $^{-1}$), mainly *Penicillium* spp, were observed in 2% medium. The 20% glucose-containing medium showed the best performance in detecting yeasts (CFU.g $^{-1}$). In view of the honey be considered as an adverse environment for mycotoxigenesis, although susceptible to yeast fermentation, the 20% glucose medium should be chosen for performing the honey microbiological quality analyses.
Key words. honey, osmotic pressure, culture medium, yeasts, molds, fungi.

RESUMO

Até o ano de 2003, a Metodologia Oficial Brasileira determinava o uso de dois meios de cultura para executar a análise de bolores e leveduras, um contendo 2% e outro com 20% de glicose; atualmente, recomenda-se apenas o de 2%. Concentrações distintas de glicose implicam em pressões osmóticas diferentes, o que pode influenciar no resultado da quantificação e da identificação dos fungos. No presente trabalho, 30 amostras de mel comercializadas informalmente na cidade de São Paulo foram analisadas visando o isolamento de bolores e leveduras por meio de semeadura em superfície em ágar batata contendo 2% e 20% de glicose. Não houve diferença estatística significante entre as contagens de fungos (CFU.g^{-1}) nos dois tipos de meios, embora tenham sido observados números maiores de bolores (CFU.g $^{-1}$), especialmente *Penicillium* spp nas amostras cultivados em meio contendo 2% de glicose. O meio com 20% de glicose apresentou melhor desempenho para detectar leveduras (CFU.g $^{-1}$). Considerando que o mel é um ambiente muito adverso para a micotoxigênese, mas passível de fermentação pelas leveduras, pode-se inferir que o meio com 20% de glicose seja a melhor alternativa para avaliar a qualidade microbiológica desse produto.
Palavras-chave. mel, pressão osmótica, meio de cultura, leveduras, bolores, fungos.

INTRODUCTION

Honey is a very hostile environment to microbial metabolism due to its high acidity, low water activity and low humidity. Brazilian Official Standards for honey allows a maximum of 20% humidity, demands the absence of fermentation signs and the pH between 3.3 and 4.6[1,2]. The water activity in the honey varies from 0.54 and 0.75[3,4]. In such conditions the fungi are the most viable microorganisms, especially the xerophilic yeasts and the osmophilic molds.

Both yeasts and molds can spoil the honey. Yeasts can cause fermentation and molds grow on the surface causing a repugnant aspect[3]. From the public health point of view, neither yeasts nor molds represent hazards. Yeasts are not implicated in food poisoning and the micotoxin production by molds in honey can be considered unlikely due to the fact that products with low water activity and poor in starch do not favour the micotoxigenesis[5].

In the past, two media (with 2% and 20% of glucose) were required by Brazilian official methodology[6] for honey analysis but currently just the medium with 2% of glucose is demanded[7]. Despite this, a recent study suggested that the performance of these media varies in detecting yeasts or molds from honey[4].

Based on these facts, the purpose of this study was to evaluate the influence of the two glucose concentrations in potato agar (2 and 20%) in detecting yeasts and molds from non inspected honey traded in the city of São Paulo.

MATERIAL AND METHODS

Thirty honey samples from the open-air market trade were analyzed between December 2005 and February 2006. Dilution was carried out in 0.1% pepton water until 10^{-2} and 0.1mL of each dilution was spread over the culture medium, in duplicate; 1mL of 10^{-1} dilution was plated in 5 dishes, in duplicate, to reduce the detection limit. Potato agar with 2% and 20% of glucose were used. Plates were incubated at 25ºC for 5 days. After registering the Colony Forming Units per milliliter (CFU), the various colonies which were formed were identified and evaluated. One of each colony was streaked along 2% glucose potato agar slant. Yeasts were identified by their macroscopic and microscopic characteristics. All colonies were also subjected physiological tests, according to the literature[8,9,10]. Filamentous fungi were identified by macroscpic and microscopic characteristics based on literature[9,11,12]. The Wilcoxon Signed Rank Test was used to compare the CFU counts from 2% and 20% glucose agar, for the groups: fungi, molds and yeasts.

RESULTS AND DISCUSSION

There was no statistical difference (p=0.399) in CFU.g $^{-1}$ of fungi between the 2% and 20% glucose potato agar. Results can be observed in Table 1 (median, minimum and maximum values). These results agree with those obtained by Denardi et al.[4]. The better performance of the 20% medium to detect low contamination levels, characterized by fewer occurrences of "<5 CFU.g^{-1}" (Table 2) also corroborates with Denardi's et al.[4] findings. Molds were the predominant group in both media. *Penicillium* represented 97.7% and 81.3% of isolates from 2% and 20% glucose potato agar, respectively (Table 3). Matuella and Torres[13] also found more molds than yeasts in honey, identifying *Penicillium, Aspergillus* and *Mucor*.

In addition, there was a difference (p<0.001) when molds from each agar were compared. The same difference applied to yeasts. A larger number of CFU.g $^{-1}$ of molds was observed in 2% of glucose agar while the counts of yeasts were higher in 20% agar. This result indicates that the media choice will be determinant in the growth of molds or yeasts.

Except for *Penicillium*, all the other molds or yeasts were more isolated in the 20% glucose medium, as shown in Table 4.

Considering that fermentation caused by yeast deteriorates the product and that micotoxigenesis in honey is unlikely, due to its limiting conditions as pointed out by Dragoni et al.[5], the 20% of glucose agar should be chosen for a more accurate microbiological analysis of honey.

Some interesting results presented in Tables 3 and 4 are the isolation of *Epidermophyton* (Harz) Langeron; Milochevitch, *Trichophyton schoenleinii* (Lebert) Langeron; Milochevitch and *Streptomyces*. There is no evidence in the literature of the presence of the two former microorganisms on food. They are associated to dermatophytosis and are not common in Brazil[14,15,16]. The latter is actually a bacteria which resembles fungi in their branching filamentous structure and can be isolated from media usually employed for fungi detection[17]. *Streptomyces* strains that tolerate high sugar levels

Table 1. Minimum, median, and maximum values of fungi counts in 2% and 20% glucose agar. São Paulo, December 2005 – February 2006.

Values / Culture Medium	2% glucose potato agar	20% glucose potato agar
Minimum value (CFU.g $^{-1}$)	<5*	<5
Median (CFU.g $^{-1}$)	$2.5x10^1$	$3.5x10^1$
Maximum value (CFU.g $^{-1}$)	$1.1x10^4$	$6.1x10^3$

* means the threshold of the technique; no colony was present.
CFU: colony forming units

Table 2. Distribution of samples in fungi count interval, according to the medium employed. São Paulo, December 2005 - February 2006.

Medium / Fungi Count	<5 CFU.g $^{-1}$ *	5|—| 100 CFU.g $^{-1}$	>100 CFU.g $^{-1}$
2% glucose potato agar	7/30 (23.33%)	12/30 (40%)	11/30 (36.66%)
20% glucose potato agar	5/30 (16.66%)	20/30 (66.66%)	5/30 (16.66%)

* means the threshold of the technique; no colony was present.
CFU: colony forming units

Table 3. Identification of microorganisms isolated from 2% and 20% glucose potato agar, as well as the number and frequency of colonies from each medium. São Paulo, December 2005 - February 2006.

Microorganisms	Agents	Culture Medium	
		2% glucose	20% glucose
Molds	*Penicillium*	848 (97.7%)	308 (81.3%)
	Geotrichum	8 (0.9%)	33 (8.7%)
	*Streptomyces**	0	6 (1.6%)
	Alternaria	0	2 (0.5%)
	Scopulariopsis	5 (0.5%)	14 (3.7%)
	Aspergillus	3 (0.3%)	8 (2.1%)
	Fusarium	1 (0.1%)	3 (0.8%)
	Gliocladium	0	5 (1.3%)
	Stemphylium	1 (0.1%)	0
	Epidermophyton	1 (0.1%)	0
	Trichophyton schoenleini	1 (0.1%)	0
Total of molds		868	379
Yeasts	*Trichosporon pullulans***	3 (75%)	18 (21.9%)
	Rhodotorula	1 (25%)	1 (1.2%)
	Candida krusei	0	63 (76.8%)
Total of yeasts		4	82
Total of fungi		872	461

* bacteria that forms mycelium similar in appearance to the mycelium of some fungi.
** *Trichosporon pullulans* (Lidner) Diddens & Lodder[8,10].

Table 4. Microorganisms that showed different CFU.g $^{-1}$ in 2% and 20% glucose potato agar, and the medium that better recovered the agent and the p value of the difference. São Paulo, December 2005 - February 2006.

Microorganisms	Medium	p value
Penicillium	*2%*	*p<0.001*
Geotrichum	20%	p<0.001
*Streptomyces**	20%	p=0.014
Aspergillus	20%	p=0.020
Gliocladium	20%	p=0.003
Scopulariopsis	20%	p=0.003
*Trichosporon pullulans***	20%	p<0.001
Candida	20%	p=0.004

* bacteria that forms mycelium similar in appearance to the mycelium of some fungi.
** *Trichosporon pullulans* (Lidner) Diddens & Lodder[8,10].

have also been isolated from honey in Zambia. Zambian honey has also showed low levels of streptomycin. PCR analysis of honey and environmental samples has shown the presence of the str gene cluster which indicates that Streptomycetes are producing streptomycin in the environment from which the Zambian honey is harvested[18].

CONCLUSION

The osmotic pressure of the agar, given by the glucose concentration (2 or 20%), had no influence on fungi quantification in honey, however had a significant effect on the kind of fungi recovered. Potato agar with 2% of glucose favours *Penicillium* spp. while the one with 20% of glucose gives better support to yeasts and other molds than *Peniclillium* such as *Geotrichum* spp., *Scopulariopsis* spp. and *Aspergillus* spp. among others.

Finally, the 20% glucose agar should be used to enumerate fungi in honey in order to evaluate its microbiological quality instead of the 2% glucose agar.

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