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Research Article

Pork Liver Freshness Evaluated through Spoilage Microbiota and a Consumer Test in Shelf Life Extension Experiment

Rita O. Silva,[1] Margarida I. F. C. Rouxinol,[2] and Luís A. S. C. Patarata[1,3]

[1]*Universidade de Trás-os-Montes e Alto Douro, Vila Real 5000-081, Portugal*
[2]*Irmãos Monteiro S.A., Aveiro 3830-527, Portugal*
[3]*Centro de Ciência Animal e Veterinária, UTAD, Vila Real 5000-081, Portugal*

Correspondence should be addressed to Luís A. S. C. Patarata; lpatarat@utad.pt

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Pork liver is an affordable product, very much appreciated by some consumers. Packaged pork liver has a short shelf life that can represent an additional cost to the producers. This work aimed to assess the relationship between consumer acceptability, evaluated through both a test made live with photography and samples to be smelled and an internet survey platform based only on photography, and the counts of the main spoilage microorganisms, on pork liver samples stored from the actual end of shelf life (ESL) and two extra days of storage times, ESL + 2 and ESL + 4. The results indicate limited usefulness of microbial counts, once they were generally very similar between accepted and nonaccepted samples, with total viable count below 7 Log CFU/g. Both methodological approaches revealed that there is no margin to extend the shelf life from the three days previously established by the manufacturer. It was observed that pork liver packaged with modified atmosphere had similar evaluations of freshness using internet-based or live test with consumers, with a drop in purchase intention from 87.5% at ESL to 13.2% at ESL + 2, when the assessment was made through the internet-based test. When the test was made live, the purchase intention had the same trend, but the drop was smaller, from 61.5 at ESL to 21.2% at ESL + 2. The purchasing intention was lower when the consumers had the opportunity to smell the samples but considering the decision of defining the end of shelf life based on 50% of consumers accepting, it was similar in almost all the cases.

1. Introduction

Pork liver is a budget product, rich in protein, minerals like iron, zinc, and manganese, and many essential nutrients such as amino acids and fatty acids with an interesting ratio PUFA/SFA [1, 2]. It is a product very appreciated by a niche of consumers accustomed to traditional cuisine. As with meat and other offals, the liver has a limited shelf life that might have difficulty in its commercialization [3]. Shelf life can be defined as the length of time the product remains suitable for consumption, and it may be limited by microbial, chemical, and physical modifications that result in detectable sensory characteristics recognized as spoilage or might represent an increased risk for consumers' health [4]. In products that undergo a cooking procedure, the biological hazards will be controlled, and it is the sensorial features that determine the end of shelf life [5].

The responsibility of defining the adequate shelf life lies in the meat packaging industry that has to ponder the benefits of extending shelf life with the drawback of having unsatisfied consumer due to not-so-fresh products sold inside the shelf life period [6]. One common strategy used to study the shelf life of perishable products is to evaluate the spoilage microbiota. There are recommendations in the literature suggesting that red meats and poultry shelf life should be limited to the period until the total viable count does not exceed 6 to 8 Log CFU/g [7, 8]. Concerning the liver and other offals, it was believed that they have a shorter shelf life due to better support of microbial growth. However, considering offal composition, pH, and moisture content, there is no obvious reason to have a higher spoilage rate in offal than in meat, for similar storage conditions [3, 9].

To our knowledge, besides few works made on the eighties and nineties on the microflora of offal in different storage conditions [10–13], there is only one publication [14] on the shelf life estimation for beef liver that considered the product sensorially unacceptable after 7 days of storage at 5°C, due to the total microbial count reach values between 7 and 8 Log CFU/g. If we consider that microbial criteria of total viable count, once this is an indirect measure of what might be occurring in terms of sensorial detectable spoilage, the results might be biased, and the shelf life is incorrectly established, because products might have low microbial counts but are sensorially unacceptable, mainly due to autolysis and oxidation modifications, or have a high microbial count but are still accepted by consumers.

Aging tests are commonly used to establish shelf life, which reports the sensory changes that the food experiences during its storage. The use of sensory analysis to describe the characteristics of the product, namely, quantitative descriptive analysis or sensory profiling performed with a trained panel [15, 16], can be used to evaluate the modifications the food experiences during the shelf life. However, with these techniques, a problem arises, which is the establishment of the cut-off point distinguishing the fresh products from those already considered spoiled [17]. Once the aim of the industry is having products perceived by the consumers as fresh when they are bought before the end of the shelf life, it is possible to use directly that perception of the consumers. Sensory analyses are performed periodically using a group of consumers who are questioned about their willingness to consume or to purchase a product with certain storage time, taking into account its freshness [4]. Shelf life is then limited by the storage time and the proportion of consumers who accept the product. It is usually accepted to have at least 50% of the consumers accepting the product to consider the product still in the shelf life. That proportion results from the principle that at the end of shelf life there are only a limited number of packages to be sold, and, for that reduced number of packages, the proportion of 50% of consumer acceptance is still acceptable for products of general consumption. For high priced products, the industry usually defines a higher proportion of acceptance, 75% or more [6].

One of the biggest problems to define the shelf life of pork liver, or other perishable products, is the need to assess its willingness to consume at very short intervals, with very complex logistics to make consumers tests with a large number of consumers. In a purchasing situation, the aspect of pork liver is the only sensory characteristics that consumers can evaluate, to decide if they consider it fresh enough [18]. The use of photographs instead of real products can be an alternative to simplify the execution of consumer tests. That strategy was successfully used to evaluate the freshness of a fruit salad showing a robust relationship with an analysis performed by a trained sensory panel [19]. In a previous study with chicken breast, it was found that the use of images in a consumer test was an adequate and useful strategy to evaluate the freshness and establish the shelf life [17]. The aim of this work was (1) to evaluate the relationship between pork liver freshness evaluated through a consumer test and the spoilage microbiota aiming at the shelf life extension and to (2) assess the possibility of using a photography based consumer test to study the freshness perception and willingness to buy.

2. Materials and Methods

2.1. Pork Liver Samples. The self-life of pork liver defined by manufacturers was three days and in the present study the shelf life was studied up to four days after the expiration of the current shelf life. Thus, there were three moments of analysis: the end of self-life (ESL), two days after the end of shelf life (ESL + 2), and four days after the end of shelf life (ESL + 4). The pork liver samples, packed in modified atmosphere (MAP: 70% O_2, 20% CO_2, and 10% N) and vacuum, from three different production batches, were collected in the industrial unit in the day after packing. Each package contained one whole liver. Samples were transported to the laboratory under refrigeration and stored at 2 ± 1°C until the time of analyzing. From each packaging method, nine packages were stored (3 batches × 3 sampling times).

At each sampling, time samples were photographed and still packaged and were then opened in an aseptic environment and two portions of ca. 25 g were cut from each sample for microbial analysis. After collecting samples for microbial analysis, unpackaged samples were photographed on a no-reflective polystyrene tray. Photography conditions included the use of a white light flash and were oriented at nearly 120°C to the axis of the image capture. Only images of unpacked pork liver were used in the consumer test because the brightness and reflection on the packaging material made the images not clear enough, and we did not want to manipulate the image to reduce the brightness, once it could alter the general aspect. Immediately after being photographed, the samples were cut into sections about 2 × 2 × 8 cm, always including sections of the surface of the liver. These portions were introduced into 50 ml Falcon tubes (previously deodorized by washing in hot water and drying at 60°C) and frozen for subsequent odor evaluation.

2.2. Microbiological Test. In the three analysis times, 10 g of the liver was weighted from the ca. 25 g sample cut before the photography. An initial dilution was made with 90 ml of peptone water and serial ten-fold dilutions were prepared in the same solution. Mesophilic total count (MTC), psychotropic total count (PTC), *Enterobacteriaceae*, *Pseudomonas* spp., and lactic acid bacteria (LAB) were counted as described elsewhere [20].

2.3. Consumer Test. The consumer test was conducted in two different ways. (1) A test was performed with the 52 consumers. It presented the photographs of the different samples (printed on photographic paper, 10 × 15 cm) and the Falcon tube with the respective sample thawed for 24 h at 2°C, with the temperature stabilized at ca. 20°C. The

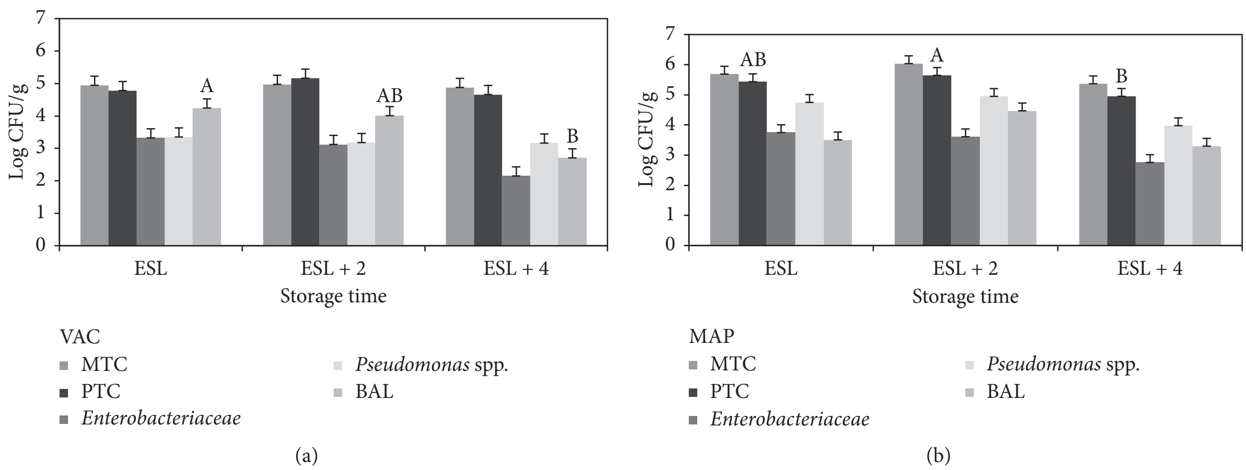

Figure 1: Counting of total mesophilic (MTM) and psychotropic (MTP) microorganisms, *Enterobacteriaceae*, *Pseudomonas* spp., and BAL in vacuum and map packed pork liver. Results expressed as mean values and CI (95%). Bars followed by different letters are different ($p < 0.05$); bars without letters are similar ($p > 0.05$). (a) VAC. (b) MAP.

consumers were asked to rate the freshness of the samples, having into consideration its aspect and the smell, on a 5-point scale, where 1 corresponded to "not fresh" and 5 to corresponded to "very fresh." Purchase intention was also asked with a yes/no question. (2) An online test using the Google Forms platform used only the photographs of the samples. Consumers were invited to participate by email or via social networks; 183 responses were recorded. Two photographs of each stipulated vacuum-packed and MAP analysis time were presented and, for each set of photographs, consumers were asked to indicate their evaluation of freshness and the purchase, as in the test with the consumers live. The questionnaire had an average duration of 8 minutes.

In both approaches, each sample was assigned a random three-digit number, and the order of presentation was obtained by the ascendant ordering of the random numbers. The gender and age group in ten-year classes of consumers were recorded, as well as their pork liver consumption habits, "are you a regular consumer" with a binary answer "yes/no." Any personal identification was recorded.

2.4. Data Analysis. The comparison of the mean values of continuous variables, microbial counts, and sensory evaluation of freshness was performed by one-way ANOVA. The difference between means was located with the Tukey-Kramer test. Purchase intention was compared through the chi-square test. All procedures were performed at XLSTAT 2018 software.

3. Results and Discussion

The composition of the liver is highly favorable to microbial growth [21]. The results of microbial counts are presented in Figure 1 for samples package in MAP and vacuum.

The counts of total mesophilic microorganisms were below 5 Log CFU/g in vacuum samples and between 5 and 6 Log CFU/g in MAP samples. In both packages, there were no differences ($p > 0.05$) between the three sampling times. Similar results were observed by Hanna and colleagues [11] on pork liver at days zero and 1, 3, and 5 days of storage at 2°C. Psychotropic microorganisms presented a trend similar to mesophilic ones. No differences were observed in vacuum samples, and a punctual difference ($p < 0.05$) was observed in MAP samples, curiously in the sense of having fewer microorganisms at ESL + 4 than at ESL + 2. The counts of *Enterobacteriaceae* and *Pseudomonas* spp. were not affected ($p > 0.05$) by the storage after the ESL in both packages. They were slightly higher in MAP than in vacuum, ca. 0.5 Log CFU/g for *Enterobacteriaceae* and 1.5 Log CFU/g for *Pseudomonas* spp. The more favorable gaseous environment was provided in the MAP, due to the presence of high amounts of oxygen [22] and due to the competition with LAB in vacuum package, where these microorganisms presented slightly higher counts, about 0.7 Log CFU/g at ESL + 2 and ESL + 4 which gave a competitive advantage to *Pseudomonas* spp. in MAP. The counts of LAB were moderate, between 3.5 and 4.5 Log CFU/g. Similar LAB counts were observed by Woolthuis et al. [3] on day 1 and day 5 after the slaughter in vacuum packaged pork liver. In the present work, no significative differences were observed on the LAB counts in MAP samples. In a vacuum, it was observed that at ESL + 4 the LAB counts were lower ($p < 0.05$) than at ESL in less than one logarithmic unit (0.84). This result is contrary to the theoretically expected, once the LAB found very propitious conditions to growth in vacuum packages [23]. We believe that the trend of low growth of LAB is due to the proximity between sampling times that did not allow us to observe higher differences. Also, despite the psychotropic character of some LAB [23], the temperature of 2°C could have contributed to the slower growth of these spoilage microorganisms.

Having in consideration these results and the suggestions found in the literature to end the shelf life when the total mesophilic count exceeds 6 to 8 Log CFU/g, all the samples of the present study should be considered still inside the shelf life window. However, when we observe the sensory evaluation of the freshness and the correspondent willing to

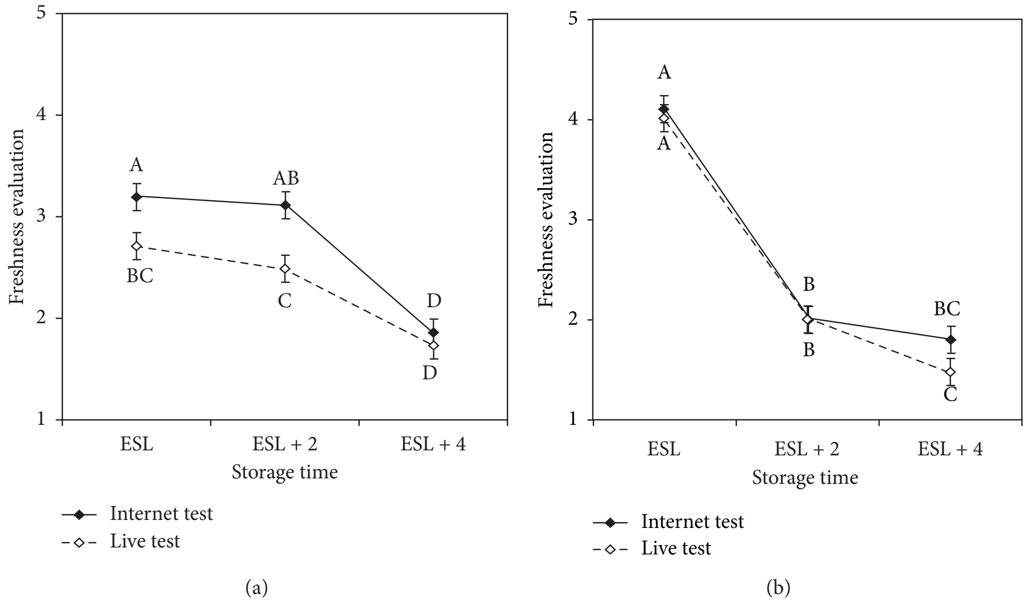

Figure 2: Freshness evaluation of vacuum-packed pig liver and modified atmosphere (MAP) from the end of the current shelf life (ESL). Results expressed as mean values and CI (95%). Means marked with different letters show significant differences ($p < 0.05$). (a) VAC. (b) MAP.

Table 1: Purchase intention of vacuum-packed and MAP pork liver.

Package	Time (days) ESL	ESL + 2	ESL + 4	p (time)
Vacuum				
Internet test	57.7 (0.1)[1]	51.1 (0.9)	9.9 (0.2)	<0.001
Live test	55.8 (−0.1)	30.8 (−1.7)	7.7 (−0.4)	<0.001
p (internet vs. live)	0.874	0.011	0.791	
MAP				
Internet test	87.4 (0.9)	13.2 (−0.6)	9.3 (0.6)	<0.001
Live test	61.5 (−1.6)	21.2 (1.2)	3.8 (−1.1)	<0.001
p (internet vs. live)	<0.001	0.185	0.259	

Results expressed as the percentage of consumers who expressed the intention calculated in the test group of the type (live or internet). [1]Standardized residuals.

purchase (Figure 2 and Table 1, resp.), it is possible to find that after the actual shelf life defined by the manufacturer (ESL), the freshness evaluation decreases abruptly, as well as the purchasing intention. According to the results presented in Figure 1, both MAP and vacuum-packed pork liver experience a significant decrease in overall freshness evaluation, with this decrease being the most noticeable in MAP, particularly because at the start-point ESL, these samples had a higher freshness score, ca. 4 points in the 5-point scale. Vacuum package samples had a lower initial evaluation at ESL, near 3 values, but it maintained that evaluation until the ESL + 2 and only decreased at ESL + 4.

When comparing the evaluation method, live or online, it was observed that for the MAP liver both approaches are nearly coincident, with no statistical differences. In vacuum packaged samples, it was observed that the freshness evaluation was considerably lower when the evaluation was made live, with the consumers having the possibility of smelling the samples, indicating that the odor contributed to the perceived lack of freshness.

The proportion of consumers who expressed their intention to purchase the products (Table 1) had a similar trend of the freshness assessment. In the vacuum-packed samples at ESL, no differences were observed in the proportion of consumers who would purchase it. Since more than 50% of consumers give a positive response, we can infer that the actual shelf life (ESL) defined by the manufacturer is correct and it is not recommended to extend it. If two extra days are added to the actual shelf life (ESL + 2), the purchase intention drops significantly to 30.8% in the live test and 51.1% in the online test. It is in the last analysis time (ESL + 4) that the percentage of consumers willing to buy the liver drops considerably. These results are in line with the results of the freshness assessment, which demonstrates consumer consistency. With liver packed in MAP, consumers of the live test, with the opportunity to smell the samples, were more exigent ($p < 0.001$) than those evaluating the liver samples only through the image assessed online. Only 61.5% of the live test consumers indicated purchase intention, but when the test was done online, this proportion was 87.4%. The sharp drop from ESL to ESL + 2 is noticeable, both in the overall freshness assessment and in the intention to purchase. A reasonable coherence was observed between both assessments, live and online tests. In the major part of the situations, the operational decision would not be affected by the strategy used to perform the consumer test, considering the level of 50% of purchasing intention as the cutting point. The only situation where there could be any ambiguity with the use of internet testing was in vacuum packaged samples at ESL + 2, where consumers who were tested via internet considered, although in the limit, samples within the shelf life, while those making the test live rejected the product, with only 30.8% of acceptance. The exclusive use of the online approach based on images would result in a longer shelf life than that obtained by the use of consumers' test live. In the same sense of the present results, Manzocco and colleagues [19] found that the shelf life estimated using fruit salad images was longer than that obtained using the product itself. They attributed these differences to the two-dimensional nature of the images, which could cause a bias on the visual comprehension of panelists. In the present work, we believe that more than the nature of the visual object to be evaluated, it was the odor of the samples that contributed to the lower perception of freshness.

In the liver vacuum and MAP packaged, the counts of spoilage microorganisms were moderate at the end of shelf life presently used by the industries (ESL). Extending the potential shelf life did not affect the microbial load, once the count of total mesophilic and psychotropic bacteria remains similar with two or four extra storage days. A similar trend was observed for specific spoilage microorganisms putatively associated with liver spoilage. Having into consideration the limit currently used of 7 Log CFU/g of the total viable count, it would be possible to extend the shelf life of the liver for extra 4 days. However, using a consumer test to define the proportion of purchasing intentions of the liver aged after the ESL, it was observed that it is not possible, once two extra storage days results in a drop in the freshness evaluation and correspondent purchasing intention to values below the generally accepted of 50%. The perception of freshness of raw liver depends on its aspect, namely, the bright red colour, absence of discoloration spots, and absence of visible slime and the odour that can be modified due to the activity of spoilage microorganisms, autolytic mechanisms, or the interaction between both [3]. The mechanisms underlying the spoiled odor of the liver are complex. It might involve degradation of the nitrogen fraction due to endogenous enzymes and due to the activity of certain microorganisms, namely, *Pseudomonas* spp. and *Enterobacteriaceae*, which have a recognized aminopeptidase and aminogenic activity and are present in the product. From these modifications on the nitrogen fraction, there is an accumulation of amines, aldehydes, and the respective acids or alcohols that have a low odor threshold, resulting in a clear sensory perception [24, 25].

Lipolytic activity and fatty acid oxidation reactions might also be involved in the liver's loss of freshness. Due to its high proportion of unsaturated fatty acids [26] that are more prone to oxidation than saturated ones, it might be expected that oxidation products contribute also for the general spoilage perception of the liver. The high amount of iron present in the liver that is an important catalyst for fatty acids oxidation would favor fatty acid oxidation [27]. On the other hand, the intermediate compounds formed during fatty acid oxidation might modify the oxidation state of the iron, contributing to less interesting red-brownish colors and the occurrence of discolorations spots [27].

The formation of sour odors might be due to the fermentation of carbohydrates that are in higher amounts in the liver than in muscle meat. Shelef [14] pointed out that the level of acidification might become intolerable when the microbial counts reach ca. 8 Log CFU/g, particularly if that count is composed mainly by LAB. Also, the levels of *Pseudomonas* spp. above 7 to 8 Log CFU/cm^2 are claimed to be associated with the detection of off-odors and the counts of *Enterobacteriaceae* above 8 Log CFU/g are responsible for the occurrence of slime in fresh meat [28]. The counts observed in the present work tend to be lower than those referred to in the literature, suggesting that autolysis, oxidation, and microbial spoilage phenomena compete for the final perception of (lack of) freshness of the liver during storage.

The correct establishment of the shelf life of fresh meat, including offal, is a concern for that industry. If it is wrongly established by excess, the product will be slightly spoiled when the consumers buy it that will have negative consequences on the brand fidelity. If it is too short, it will have costs of returning the expired product. The use of sensory analysis, due to its direct nature to the perception of the consumers, is recommended to establish shelf life in not ready-to-eat products. Among the methodological recommendations, consumers' tests to evaluate the willingness to buy, or to consume, are currently used to establish shelf life [4, 6]. Innovative methods to evaluate the consumer perception, as qualitative projective tests, conceptual profiling, polarized projective mapping, or preferred attribute elicitation [29–32], might be useful both in establishing shelf life and in understanding the determinants of that consumers' perception.

4. Conclusion

In pig liver vacuum and MAP packaged, the counts of spoilage microorganisms were moderate at the end of shelf life presently used by the industrial (ESL). Extending the potential shelf life did not affect the microbial load, once the count of total mesophilic and psychotropic bacteria remains similar with two or four extra storage days. A similar trend was observed for specific spoilage microorganisms putatively associated with liver spoilage. Having into consideration the limit currently used of 6 to 8 Log CFU/g of total viable count, it would be possible to extend the shelf life of the liver for extra 4 days. However, using a consumer test to define the proportion of purchasing intentions of the liver aged after the ESL, it was observed that it is not possible, once two extra storage days results in a drop in the freshness evaluation and correspondent purchasing intention to values below the generally accepted of 50%.

Considering that the consumers' freshness evaluation of the not ready-to-eat foods is one of the most important criteria to define the shelf life, the use of microbial counts to define shelf life of pork liver appeared to be worthless, once products rejected by the consumer had acceptable microbial counts. The use of microbial criteria would result in potential damages in the brand due to the presence of supermarket' shelves of products that sensorially would be considered not fresh.

Using sample images works reasonably well to perform consumer testing with a high number of consumers, accessed through the internet, at least as a preliminary approach; once it was observed for vacuum packaged liver, smelling samples reduced considerably the freshness evaluation and the respective purchasing intention, but with marginal implications on the decision on considering the product shelf life.

Data Availability

The data used to support the findings of this study are included within the supplementary information file.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Supplementary Materials

The data used to support the findings of this study are included in the Excel sheet provided with the article. (*Supplementary Materials*)

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