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Development of Shelf Life for Labaneh Using Protective Cultures of *L. paracasei* and *Propionibacterium freudenreichii* subsp. *shermanii*

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Abstract

Labaneh (strained yogurt) is a traditional dairy product. It is usually produced without chemical preservatives as Jordanian standards for this product prevent their addition. Its shelf life is limited to two weeks. This research aims to develop the shelf life of labaneh using eco-friendly methods, via the addition of protective probiotic cultures. Protective probiotic cultures were prepared by activating DVI freeze-dried *Propionibacterium freuendereichii*, subsp. *shermanii* and *Lactobacillus paracasei* in sterilized skim milk. Fifty kg of fresh milk was divided into two portions, the first containing the traditional yogurt starter culture (2%) and the second processed by adding both the starter (2%) and protective probiotic cultures (1%). After fermentation, two types of yogurts were produced, and refrigerated for several hours. They were then salted, strained separately to about 25% total solids in a special cloth to produce labaneh paste. The paste was then distributed into two groups of sterilized jars (21 for each) and placed in refrigerated storage. Both types of labaneh (traditional and developed) were evaluated at different time intervals of refrigerated storage (0, 3, 7, 10, 14, 21, 30 days) for their counts of coliform bacteria, yeast and mould, and lactic acid bacteria (LAB), and for their pH, ash, acidity, moisture, protein, fat and titratable acidity. The probiotic count was also evaluated for the developed labaneh. The shelf life of the developed labaneh was determined based on the acidity, yeast and mould counts and organoleptic characteristics. No obvious and significant undesirable changes were seen at 30 days of refrigeration or went beyond the Jordanian standard for the developed labaneh, compared to the traditional one which started to spoil after 14 days. No coliform bacteria were found after 30 days of refrigerated storage in the traditional and developed labanehs. The counts of yeast and mould were generally higher in the traditional labaneh than in the developed one, while the number of LAB was lower in the traditional labaneh than in the developed one. The pH and moisture of the developed labaneh were generally lower than that of the traditional labaneh, but the titratable acidity, ash, protein and fat were higher in the developed one. Regarding the sensory properties, most of the panelists organoleptically preferred the developed one during the storage period. Therefore, it is concluded that the protective probiotic cultures were effective in extending the shelf life of labaneh.

Keywords: Labaneh; *L.paracasei*; *Propionibacterium freudenreichii* subsp. shermanii; Protective sultures; Fermented dairy products; Jordan

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1 Introduction

Labaneh is a semi-solid dairy product, a strained yogurt used as a breakfast food in Jordan. Its characteristics are similar to both soft white cheese and yogurt. According to the Jordanian standards of 2003, it usually contains 23-25% total solids and 10% milk fat. Starter cultures are those microorganisms (bacteria, yeasts and moulds or their combinations) that initiate and carry out the desired fermentation essential in the manufacturing of cheese and fermented dairy products. Furthermore, starter cultures were defined as an "active microbial preparation, deliberately added to initiate desirable changes during the preparation of fermented products such as yogurt, sour cream and kefir (Hati et al., 2013). The production of lactic acid by fermenting lactose is the major role of dairy starters. The acid is responsible for the development of the body and texture characteristics of fermented milk products. It contributes to the overall flavour and enhances preservation. Starter cultures are being considered as burgeoning "cell factories" to produce a host of functional biomolecules such as bio thickeners, bacteriocins, vitamins, bioactive peptides, amino acids and conjugated linoleic acid (cla) (Hati et al., 2013).

The relationship between the two species of starter cultures in yogurt is symbiotic (Robinson, 2002). The main starter cultures in yogurt are *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. The function of starter cultures is to ferment lactose to produce lactic acid and flavour compounds. The increase in lactic acid decreases pH and causes milk to clot or form the soft gel characteristic of yogurt (Robinson, 2002). Lactobacillus produces acid that makes the yogurt sour and a less suitable place for other microbes. This is why there is a dramatic decrease in the number of microbes that live in yogurt when compared to milk (Aslim et al., 2005). Other bacterial cultures, such as *Lactobacillus acidophilus, Lactobacillus paracasei* and Bifidobacteria may be added to yogurt as probiotic cultures. Probiotic cultures may benefit human health. It may improve lactose digestion and gastrointestinal function, and it may stimulate the immune system (Robinson, 2002). There is a recent and growing interest in the probiotic potential of these bacteria. In addition to the production of vitamin B12 and the inhibition of undesirable microflora in fermented food by the release of organic acids and bacteriocins, they may beneficially modulate colon flora in animals (Pérez Chaia et al., 1999) and in humans, mainly by improving the indigenous bifidobacterial population (Bouglé et al., 1999).

Probiotics that are specific to the Lactobacillus genus are found in foods and food supplements (Yildiz, 2009). Although it may inhabit the intestine and the vagina, the purpose of this friendly microbe is to prevent other "bad" microbes that cause disease. This could be done by its proliferation in the intestine. Thus, the *Lactobacillus* microbe acts as a defense system. This is achieved through a variety of mechanisms. Other potential probiotic responses include improving lactose absorption and digestion in people who are lactose intolerant, improving the immune response, and alleviating symptoms of bowel syndrome (Yildiz, 2009). It may help with folic acid and B-vitamin synthesis and enhance mineral bioavailability (Yildiz, 2009).

In addition to their desired health benefits and clinical properties, probiotics must meet several basic requirements for the development of marketable probiotic products. The most important requirements are that probiotic bacteria survive in sufficient numbers in the product so that their physical and genetic stability during storage of the product can be guaranteed and that all their essential properties, to express their health benefits after consumption, can be maintained during the manufacture and storage of the product. Additionally, probiotics should not have adverse effects on the taste or aroma of the product and should not enhance acidification during the shelf life of the product. Finally, methods should be available to identify probiotic strains.

The objective of our study was to develop a novel labaneh from a traditional Jordanian labaneh by adding protective cultures of *Propionibacterium freuendereichii* subsp. *shermanii* and *Lactobacillus paracasei*, with its usual starter cultures (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*). The study also investigated the chemical, microbiological and sensorial characteristics of this developed labaneh.

2 Materials and methods

2.1 Milk

In this study, two types of cow's milk were used, a commercial UHT skimmed milk for activation of the starter and probiotic protective cultures (skimmed UHT, Almarai Co.) and a raw whole milk, purchased from Abu-Hammour Dairy Co., which was brought from a known farm in Al-Salt City. The two types were analysed by milk analyser (milk ana^{TM}, Turkey). Table 1 shows their analysis.

2.2 Activation of Cultures

The DVI starter cultures and the protective probiotic cultures were purchased as freeze-dried sachets from Saco (Japan) and Danisco (Denmark), respectively. The starter culture sachet is for yogurt and contains both *S. thermophilus* and *L. bulgaricus* (50:50), while the probiotic protective cultures sachet contains both *L. paracasei* and *Propionibacterium freudenreichii* subsp. *shermanii*. Both starter and protective cultures were activated using a commercial UHT-skimmed milk in a presterilised screw-capped round bottle. Two bottles of skimmed UHT milk (250 ml) were inoculated with either 2% of the starter culture or 1% of the protective cultures. The starter and protective probiotic cultures were then incubated at 43^{o}C for 4 hours and 37^{o}C for 72 hours respectively, and then refrigerated at 4^{o}C until used.

2.3 Counting of bacterial starter cultures

After the curd formed in the above two bottles the number of starter and protective cultures were counted on MRS agar, using a pour plate method, to determine the number per gram in the curd after they were refrigerated. The counts for starter cultures were performed aerobically, whereas those for probiotic counts were carried out anaerobically. The counts were then recorded to set a limit for the cultures to be used in the production of labaneh.

2.4 Labaneh production

Fifty kilograms of raw milk were pasteurised at 95 ^{o}C for 5 minutes, and then divided into two equal portions before separate inoculation with suitable cultures. Two culture treatments were used: a traditional activated yogurt culture (*S. thermophilus+ L. bulgaricus*) as the control, and a mix of traditional activated yogurt culture and protective culture (*Propionibacterium fruendrichii* subsp. *shermanii + L. paracasei*). The inoculations were 2% from each previously activated culture. The inoculated cultures were mixed in milk, before the milk was incubated at 43 ^{o}C for 4 hours until the two portions coagulated and became yogurt, and then they were refrigerated at 4 ^{o}C. Salt was added to the curd at 0.9%, and then mixed and evenly distributed in the yogurt. The two separate portions of yogurt were placed in two bags of special white cloth, and then hung separately overnight in a refrigerator, to allow the drainage of whey, so to reach the thick viscous texture of labaneh at nearly 25% of total solids. The two types of labaneh were removed from the cloth and placed separately in 42 jars (250 g), 21 jars for each type, and then stored at 4 ^{o}C for different time intervals (0, 4, 7, 9, 14, 21 and 30 days). Three of the 21 jars were removed at each time interval of the above- mentioned storage days, and were then subject to 3 examinations: microbiological, chemical and sensory tests.

2.5 Chemical tests

pH, titratable acidity, protein (Kjeldahl) and fat (Gerber) were measured for fresh product in both traditional and developed labanehs. Samples of the two types were dried by oven (105 ^{o}C) and then the mineral content was measured. A muffle furnace at 550^{o}C was used to determine the ash in labaneh.

2.6 Microbiological tests

Microbiological tests were performed using the pour plate method, which included the testing of yeast and moulds (PDA), coliform bacteria (VRB agar) and LAB (MRS agar). The LAB

Table 1: Approximate composition (%) for the two types of milk used in this study.

Milk type	Water	T.S.	Fat	protein	Lactose	Ash
UHT skimmed milk	90.7	9.3	0.5	3.3	4.7	0.8
Whole raw milk	87.1	12.9	3.9	3.4	4.9	0.7

count was carried out under reduced aerobic conditions. Samples for the three microbiological tests were incubated at 25^{o}C, 37^{o}C and 37^{o}C, for 5 days, 1 day and 3 days, respectively.

2.7 Sensory tests

Organoleptic assessment of the developed and traditional labanehs was performed by 12 trained panelists from Al-Balqa Applied University (six women and six men). Samples with predefined codes were served at room temperature. Bottled water and bread as a neutralizing agent were used by panelists between tasted samples. The 9-point hedonic scale was used to measure the samples of labaneh by choosing 9 for 'extremely like' and 1 for 'dislike extremely'.

2.8 Statistical analysis

Data from a completely randomized design experiment was used to analyse the difference between the traditional and developed labanehs. The t-test and ANOVA with probability were used to calculate the significant difference between variables.

3 Results and Discussion

3.1 pH and Titratable Acidity

The pH and titratable acidity of labaneh containing starter cultures differ from labaneh containing both starter and protective cultures (Table 2). pH for both products gradually decreased. The pH started at 4.1 for both products. The final pH, at 30 days refrigerated storage, was 3.9 and 3.7 for the traditional and developed labanehs, respectively. The titratable acidities on average were 1.55 and 1.37 (% lactic acid) for the developed and traditional labanehs, respectively. Our results met with the 2003 Jordanian standards, which state that titratable acidity for labaneh should not exceed 2.5% lactic acid.

Generally, fermented dairy products that contain probiotic additions tend to be slightly more acidic compared to those without probiotic additions. Probiotics are live bacteria or yeasts that are favorable for our digestive system. They help convert lactose, the milk sugar, into lactic acid through the fermentation process. During fermentation, LAB digest lactose in milk and produces lactic acid as a byproduct. This lactic acid accumulation is responsible for the characteristic tangy taste and acidic nature of fermented dairy products such as yogurt, kefir and some types of cheese. When probiotics are added to dairy products, they contribute further bacteria to the fermentation process. This results in increased bacterial activity and a higher production of lactic acid, leading to a little more acidic product compared to the one without probiotic additions.

3.2 Total solids

The total solids in the developed and traditional labanehs were 26% (74% water) and 23% (77% water), respectively. It was noticed that the addition of protective probiotic cultures to labaneh may slightly improve its total solids as well as its mineral content. The percentage of moisture in certain dairy products influences their shelf life. Excess moisture in the product promotes microbial growth, making the product more perishable. The total solids content in fermented dairy products can be influenced by various factors, including the presence of probiotic additions. While there is no universal rule that applies to all fermented dairy products, it is possible for products with probiotic additions to have slightly higher total solids compared to those

Table 2: pH of traditional and developed labanehs during cold storage (4^{o}C) for different time intervals within and after their shelf life.

Day	Traditional labaneh		Developed labaneh	
	pH	Titratable Acidity	pH	Titratable Acidity
0	4.17±0.03^a	1.19±0.02^a	4.17±0.05^a	1.19±0.03^a
3	4.12±0.05^a	1.21±0.4^a	3.93±0.04^a	1.25±0.03^a
7	4.06±0.03^a	1.31±0.04^a	3.89±0.06^a	1.50±0.06^b
9	3.99±0.07^a	1.35±0.03^a	3.85±0.05^a	1.75±0.06^b
14 *	3.97±0.06^a	1.40±0.03^a	3.82±0.04^a	1.77±0.02^b
21	3.94±0.07^a	1.49±0.05^a	3.80±0.06^a	1.80±0.02^b
30	3.89±0.04^a	1.52±0.05^a	3.77±0.06^a	1.85±0.03^b
Average	4.02	1.31	3.89	1.64

*: standard shelf life

without probiotic additions. However, it's important to note that the exact composition can vary depending on the specific product and manufacturing process.

One potential reason for the higher total solids content in fermented dairy products with probiotic additions is the metabolic activity of the added probiotic bacteria during fermentation. Probiotics, such as certain strains of LAB, digest lactose and other components present in milk, producing various byproducts, including lactic acid and carbon dioxide, and sometimes additional bacterial biomass. These byproducts contribute to the total solids content of the final product. Furthermore, probiotic bacteria can enhance the fermentation process, resulting in a more efficient conversion of lactose into lactic acid and other compounds. This can lead to a higher concentration of solids in the fermented dairy product.

3.3 Protein, fat and ash content

The fat content in the developed labaneh was 12% whereas it was 11% in the traditional labaneh. The 2003 Jordanian standards for labaneh state that it must contain not less than approximately 10 % fat, depending on the milk properties associated with the breed, nutrition and stage of lactation of the dairy cows (Bauman & Lock, 2006). The protein content in the developed labaneh was 9% (36.25% on dry basis) compared to 8% (33.25% on dry basis) in the traditional labaneh. The ash contents on dry basis were between 5.9% and 6.7% in both the developed and traditional labanehs.

3.4 Mineral content

Table 3 shows the chemical analysis of traditional and developed labanehs. There were no significant differences found in nitrogen, sodium, chloride and electrical conductivity between the traditional and developed labanehs but there were significant differences found in phosphorus, potassium, calcium and magnesium between both types. The increase in potassium, calcium and magnesium and decrease in phosphorus in the developed labaneh may be due to the probiotic action, i.e., its metabolic effect on the labaneh.

3.5 Coliform

No coliform bacteria weres observed in traditional and developed labanehs during the time intervals of refrigerated storage.

3.6 Yeast and mould count

The yeast and mould count in labaneh should be as low as possible as it has a direct effect

Table 3: Chemical analysis of traditional and developed labanehs on dry basis.

Test Name	Traditional labaneh	Developed labaneh	Test Method No.
N (wt/wt) %	5.4±0.3[a]	5.8±0.4[a]	AOAC 978.02
Na (wt/wt) %	1.6±0.1[a]	1.5±0.1[a]	AOAC 983.02
Cl (wt/wt) %	1.9±0.1[a]	1.7±0.1[a]	AOAC 928.02
P (ppm)	570.4±10[a]	417.7±25[b]	AOAC 977.01
K (ppm)	2968.2±22[a]	3382.8±31[b]	AOAC 983.02
Ca (ppm)	2962.5±51[a]	3231.6±55[b]	AOAC 965.09
Mg (ppm)	317.9±3.2[a]	341.8±5.3[a]	AOAC 965.09
EC (dS/m)*	5.6±0.1[a]	5.5±0.2[a]	AOAC 973.04, Wet basis

* (dS/m): decisiemens per meter = mmhos/cm
* different letters on the measurements for a named test mean a significant difference is found.

Table 4: Yeast and mould count in developed and traditional labanehs.

Refrigerated Storage (day)	**Traditional (cfu/g)**	**Developed (cfu/g)**
0	11±1[a]	3±1[b]
3	29±3[a]	10±2[b]
7	40±5[a]	23±2[b]
14	115±11[a]	57±5[b]
21	1052±101[a]	101±12[b]
30	11852±213[a]	254±23[b]

on the shelf life of labaneh. As seen in Table 4, the yeast and mould count in the developed labaneh is generally lower than the traditional labaneh. Counts in the developed labaneh were 3, 10, 23, 57, 101 and 254 cfu/g whereas in the traditional labaneh they were 11, 29, 40, 115, 1025 and 11852 cfu/g after 0, 3, 7, 14, 21 and 30 days of refrigerated storage, respectively. This difference could be due to the probiotic action on labaneh composition. The addition of protective cultures could contribute to the production of organic acids, such as lactic acid, which help lower the pH of the product and inhibit the growth of spoilage microorganisms. This suggests further investigations on the chemical composition of labaneh in the near future.

Different countries and international organisations have varying standards and guidelines for yeast and mould in fermented dairy products. The European Union (EU) regulations for food safety and quality, specifically EC No 2073/2005 on microbiological criteria for foodstuffs, provide guidelines for microbial limits in fermented dairy products such as yogurt and sour cream. These regulations set the limit for yeast and mould at 10^3 CFU/g (Swanson, 2011). The United States Food and Drug Administration (US-FDA) has established general guidelines for dairy products. The FDA's Grade "A" Pasteurized Milk Ordinance (PMO) suggests a limit of 10^4 CFU/g for yeast and mould in dairy products (Boor et al., 2017). Based on the above-mentioned microbiological regulations and guidelines, our developed probiotic product remains within acceptable limits for yeast and mould contamination.

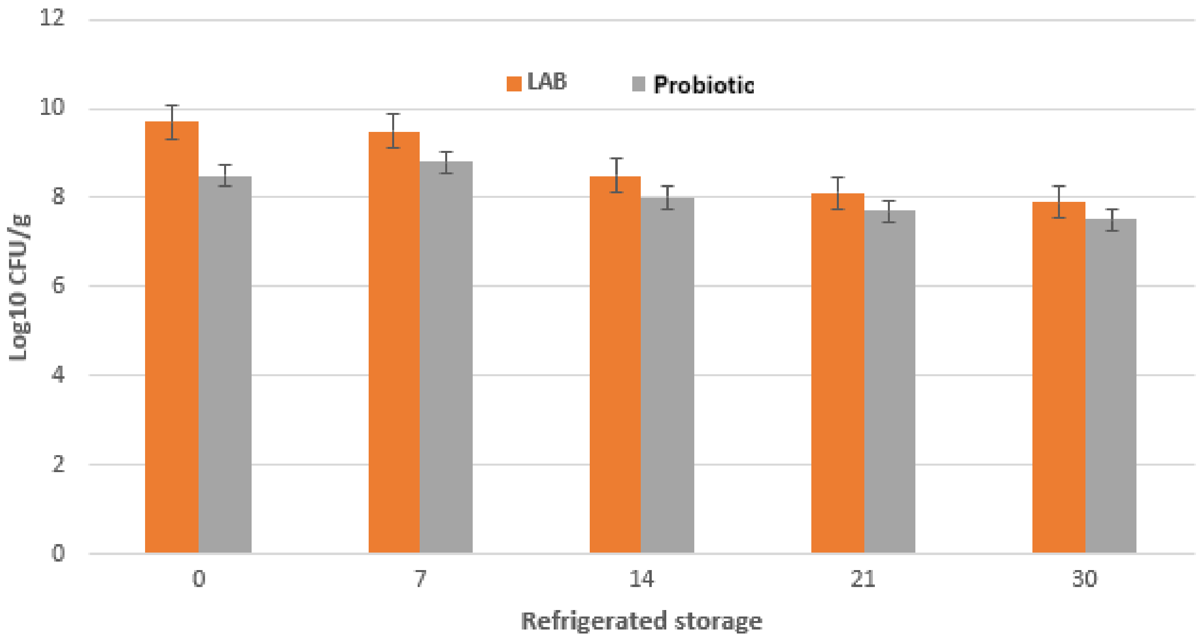

Figure 1: Comparative analysis of probiotic bacteria and lactic acid bacteria in the developed labaneh during refrigerated storage

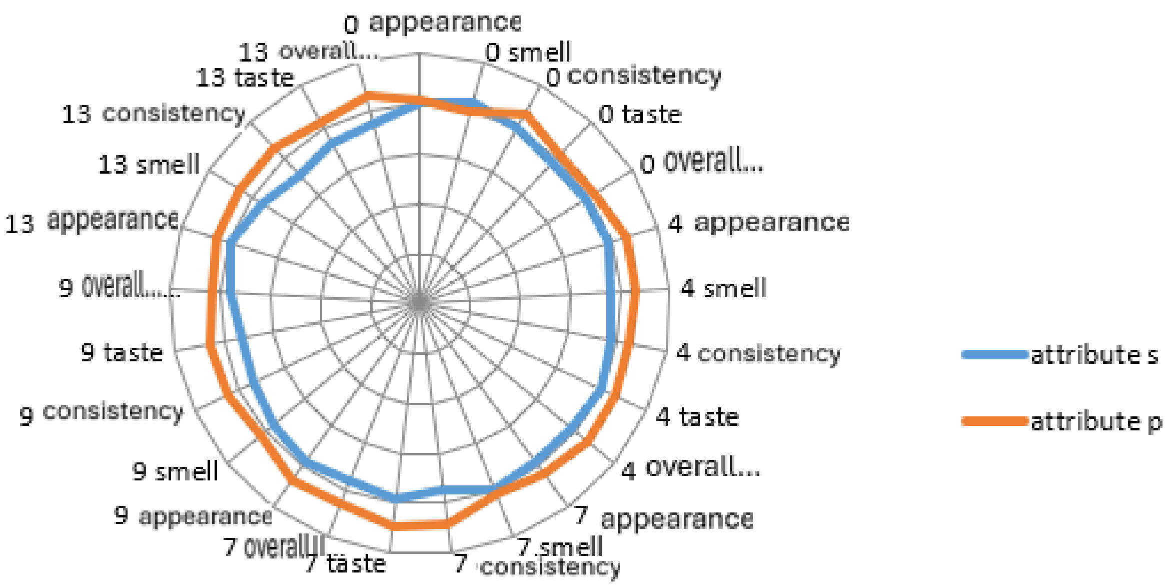

Figure 2: The average sensory evaluation of the traditional and developed labanehs after one month of refrigerated storage

3.7 Counts of LAB and probiotic protective bacteria in the developed labaneh

Generally, probiotic counts were slightly lower than LAB counts in the developed labaneh during 30 days of refrigerated storage, but both showed the same trend. The LAB counts were between 9.7 to 7.9 $\log_{10}$ cfu/g, whereas the probiotic counts were between 8.5 to 7.5 $\log_{10}$ cfu/g during 30 days of refrigerated storage. As can be seen in Figure 1, LAB and probiotic counts in the developed labaneh decreased gradually during refrigerated storage.

3.8 Sensory evaluation

Figure 2 shows the sensory evaluation of developed and traditional labanehs. All sensory characteristics were better for the developed one after 30 days of refrigerated storage. Protective cultures usually produce exopolysaccharides (EPS) during fermentation, contributing to the texture and viscosity of the dairy product. This can result in a creamier and smoother mouthfeel, enhancing the overall sensory properties. In addition, it improves the dairy flavour. For instance, *Propionibacterium freudenreichii* subsp. *shermanii* is known for its role in producing flavour compounds, like propionic acid, which contribute to the nutty and sweet taste. Based on the sensory evaluation results, the panelists prefer the developed labaneh which contains both protective cultures and starter cultures to the traditional labaneh which only contains starter cultures.

4 Conclusions

The developed labaneh has lower counts of yeast and mould than the traditional labaneh. Panelists prefer the developed labaneh to the traditional one. Protective cultures can promote the starter cultures and reduce the number of lactic acid bacteria. This could be seen through the extended shelf life and delayed spoilage of the developed labaneh. Therefore, the use of protective cultures, *Lactobacillus paracasei* and *Propionibacterium freudenreichii* subsp. *shermanii*, has several positive effects on the properties of labaneh, including enhanced shelf life, improved texture and mouthfeel, flavour development and control of undesirable microorganisms such as pathogens. Protective cultures can contribute to a more efficient fermentation process, reducing the need for additional preservatives or stabilisers. This can lead to cost savings in production whilst maintaining product quality.

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