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Development and acceptance of freeze-dried yogurt “powder yogurt”

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Abstract

Yogurt is the most popular fermented milk product all over the world, but its shelf life is still relatively low when compared to other dairy products. In this context, freeze dryingis a suitable toolto extend yogurt shelf life. This technology consists of drying the product through sublimation at low temperature and pressure, maintaining the nutritional, microbiological, and sensory characteristics, and resulting in a dry product that rehydrates easily.This study aimed to make a comparative study of the physicochemical, microbiological, and sensory characteristics of the traditional yogurt and rehydrated freeze-dried yogurt. The samples were characterized for moisture, a_w, ash, acidity, pH, carbohydrates, texture, lipids, protein, and metabolizable energy, they were also analyzed microbiologically to detect lactic bacteria survival and sensorial acceptance analyzes, the Tukey’s test at 5% of significance level was used for the detection of differences between means. The results showed a higher concentration of nutrients in the rehydrated product,since a lower amount of water was usedfor rehydration when compared to the water lost duringfreeze drying,aimed to obtain a more nutritious product with similar texture to the traditional yogurt.The microbiological results showed that the rehydrated yogurt kept the number of lactic acid bacteria above the values required by Brazilian law. Finally, the results of sensory evaluationevidenced that the rehydrated yoghurt had greater acceptance than the traditional yogurt, possibly due to the concentration of compounds which positively affected aroma and flavor. Therefore,it can be concluded that the rehydrated freeze-dried yogurt produced in this study met all recommendations of the Brazilian legislation for fermented milk products, and can be a promising marketing alternative for the dairy industry, since it is a differentiated product with greater acceptance, nutritional value, and shelf life when comparedwith the traditional yogurt.

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Introduction

Milk is one of the animal products most consumed in the world, due to its nutritional value; however,its shelf life is relatively low when compared to other dairy products such as cheese. As a result, fermented milk products have gained increasing importance, especially fermented milk, which is a distinct dairy product, besides being an alternative method of preservation (Tamime and Robinson, 2007; Passot *et al.*, 2012) .

Yogurt is the most popular fermented milk in the world, and consumers’ demand has growndue to current concern for healthy related issues. Therefore, functional foods are becoming increasingly popular due to the rhythm of contemporary life, leading to the production of rapid or instant foods with a longer shelf life (Tamime and Robinson, 2007; Abd El-Salam *et al.*, 2011; Saad, 2011).

Yogurt is defined by MAPA (Ministry of Agriculture Livestock and Food Supply) by normative instruction 46 (2007) as a product with or without the addition of other foodstuffs, obtained by lactic acid fermentation through the protosymbiotic action of *Lactobacillus delbrueckii* subsp *bulgaricus* and *Streptococcus thermophilus*. These bacteria should be viable during product’s shelf life in the minimum amount of 10^7CFU/g (Brazil, 2007).

Apart from being an alternative for milk conservation, yogurt is also rich in proteins, vitamins, fatty acids and minerals, especially calcium and phosphorus. Another important aspect is that the

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lactoseis partially digested by the bacteria during fermentation, thus making the final product more digestible for lactose intolerant individuals (Miller *et al*., 2006). However, the high nutritional value of yoghurt also makes it suitable for the development of some spoilage microorganisms. In this context, freeze drying appears as an alternative and effective process for conservation of the product for a longer period (Sharma and Arora, 1995; Passot *et al.*, 2012).

Freeze drying consists in reducing water from a product by sublimation, defined as the passage of water from solid to vapor, maintaining the sensory, biological, and nutritional characteristics of the dried product close to the product in natura. Freeze dried productsare easy to rehydration, quickly absorbing much of the water lost during process, which is a desirable characteristic (Marques, 2008; Velardi and Barresi, 2008; Fu and Chen, 2011).

The water reduction in yogurt contributes to greater preservation and facilitates transport and packaging due to the reduced weight of the product. Another major advantage is that low storage temperatures are not required, as those used in the conservation of traditional product, thus eliminating the need for cold chain during distribution and sale, which represents an economic advantage. Additionally, freeze drying preserves much of yoghurt microflora, since the drying process is performed at low temperatures (Kumar and Mishra, 2004; Capela *et al.,* 2006).

Besides the reduction in storage and transport costs, the yogurt powder can be used as an ingredient or supplement of juices, cookies, ice cream, sweets, milk drinks, and as powdered milk substitute in recipes, providing nutritional and functional value to the products (Kim and Bhowmik, 1990; Kumar and Mishra, 2004; Tamime and Robinson, 2007). Thus, the present study aimed to comparethe physicochemical, microbiological, and sensory characteristics of rehydrated freeze-dried yogurt and traditional yogurt.

Materials and Methods

This study was developed in the Laboratories of Food Analysis (LAA), Food Microbiology (LMA), and Animal Products (LPOA), Department of Food Technology (DTA) of the Federal University of Sergipe. UHT whole milk, skim milk powder, and sucrose were used for the formulation of traditional yogurt. The culture YF-L903 (Christian Hansen) containing *Lactobacillus delbrueckiis* sp. *bulgaricus* and *Streptococcus thermophilus* strains was used.

Manufacture of the traditional yogurt

For the production of traditional yogurt, 2L UHT whole milk (Ultra High Temperature) was mixed with 4.0% skim milk powder and 5.0% sucrose. After homogenization, the mixture was transferred to a stainless steel container and heated to 95°C for 5 minutes for pasteurization of the ingredients. Then, the mixture was cooled to 42°C for addition of the inoculum.

The inoculated milk was transferred aseptically into a sterile vessel and incubated in BOD incubator at 42°C for about 5 hours until reaching pH values between 4.5 and 4.6. Then, the yoghurt was cooled to 6°C and stored at 5°C for 24 hours to stop fermentation and to develop aroma, texture, and characteristic flavor.

Manufacture of the freeze-dried yogurt

The yogurt samples were placed in stainless steel trays and stored in a freezer (Model FE 26 Electrolux) at -25 ± 2°C for 24 hours prior to freeze drying. Then, the samples were placed in the freeze dryer (Christ Alpha 1-2 LSC) operating at -37°C in the condensation chamber under vacuum, at a minimum pressure of 0.18 mbar for 24 hours. The freeze-dried samples were ground in a micro stainless steel knife mill (MARCONI, MA048) for about 30 seconds and, sieved in 20 mesh sieves to obtain a homogeneous product, which was stored in polypropylene aluminum packages, and sealed in vacuum sealer (TEC MAQ, AP 500).

Rehydrationof the freeze-dried yogurt

Rehydration of the freeze-dried yogurt was carried out to obtain a texture similar to the traditional product, the product was rehydrated one day after freeze drying. The texture profile was measured by Texture Analyzer Brookfield CT3 25K, equipped with a TA11 / 100 AOAC standard cylinder probe (Acrylic Clear) (25.4 mm diameter and 35 mm length)and a fixed base table TA-BT-KIT. The sample consisted of 60 mL yoghurt at room temperature (± 25°C). The penetration of the probe was carried out at 50% of the initial height (4 mm) with a compression speed of 2 mm /s. Three repetitions were made for each treatment. Rehydration was carried out in samples with 3 different levels (60, 65 and 70%) of water removed from the original product in order to determine which one resulted in a more similar product the original not dehydrated one, regarding the texture profile.

The yield of freeze-dried yoghurt was calculated as follows: After freeze drying and rehydration, the yoghurt was weighed and the values were compared with the weights corresponding to the amount of traditional yoghurt used for the production of freeze-dried yogurt, as shown in Equation 1:

$$\%Yield = \frac{P_i \times 100}{P_f} \quad (1)$$

where:

P_i = weight of freeze-dried or rehydrated yogurt

P_f = weight of traditional yogurt

Physicochemical characterization

The samples were characterized for texture profile using the Brookfield Texture Analyzer as previously described. Water activity (a_w) was determined in Aqualab apparatus. Moisture content, protein, fat, carbohydrates, ash, acidity as percentage of lactic acid, pH, and metabolizable energy were also determinedaccording to the procedures of the Adolfo Lutz Institute (IAL, 2005).

Microbiological characterization

Lactic bacteria countswere carried out by pour plating method, in duplicate. The culture media M17 and MRS agar base plus acetic acid to pH 5 were used for enumeration of *Streptococcus termophillus* and *Lactobacillus bulgaricus*, respectively. The samples were previously identified, and homogenized in the packages. Then, an aliquot of 25 g was aseptically sampled and mixed with 225 mL sterile peptone water thereby obtaining a first dilution. From this dilution,successive decimal dilutions were performed to achieve a count between 25-250 CFU / g after the incubation period.

For enumeration of *Streptococcus termophillus,* the plates with the culture medium M17 were plated and incubated in BOD incubator at 37°C for 48h. For *Lactobacillus bulgaricus* counts, plates containing acidified MRS were incubated at 37°C for 72 hours in a jar Gaspack, in BOD, under anaerobic conditions (IDF, 2003).

Sensory evaluation

Affective tests were performed to evaluate the acceptability of traditional yoghurt and rehydrated freeze-dried yoghurt, regarding the attributes appearance, aroma, flavor, texture, and overall impression. For that, 60 untrained assessors regular consumers of natural yogurt evaluated both samples using a 9 point hedonic scale anchored on the left and right endswith the terms "extremely disliked" (value 1) and "like extremely" (value 9),respectively. A purchase intent scale structured with 9 points, anchored on the left and right ends with the terms "certainly would not buy" (value 1) and certainly would buy (value 9) was also used (Stone *et al*., 2008). At the end of the test, the assessors were asked to assign a market price compatible with the sensory quality perceived for each sample. The samples (25 g) were served at 8°C in 50 mL disposable cups coded with three digits, properly sanitized. The order of the samples was balanced between the assessors.

The sensory scores were subjected to analysis of variance (ANOVA) using the parameters "sample" and "judges" as sources of variation. Tukey's test at 5% significance level was used to check for differences between means, by using the software ASSISTAT 7.6 beta (Silva and Azevedo, 2006).

Statistical analysis

A completely randomized block design with three replicates for each experiment was carried out with the averages of the physicochemical determinations. The Tukey's test at 5% significance level was used for detection of differences between meansusing the software ASSISTAT 7.6 beta (Silva and Azevedo, 2006).

Results and Discussion

The texture profile of the traditional and rehydrated yoghurts and the peak force required to penetrate the samplesare shown in Figure 1, respectively. Yogurt consistency is very important for consumers' choice, and these results allow establishingthe amount of water to be added to the rehydrated yogurt to obtain a reconstituted product with texture profile similar to the traditional one. In the present study, the rehydrated yoghurt with 70% water removed by freeze drying showed texture profile close to the traditional yoghurt (Figure 1), thus this rehydration level was used to reconstitute the yogurt for sensory evaluation. However, when the product is available in the market, the amount of water used for reconstitution dependson the consumers'decision. High quantities of water lead to lower viscosity and higher yield of the reconstituted product.

Rybka and Kailasapathy (1997) and Venir *et al.* (2007) found loss of consistency in reconstituted freeze-dried yogurt, indicating an irreversible weakening of the gel network structure, probablydue to the mechanical energy required to incorporate water for rehydration. Venir *et al.* (2007) found similar results in samples with 100%, 70%,and 60% initial water contents, corresponding to 11%, 15%,and 17% total solids, respectively. Theproduct rehydration using 70% of the initial water showed the best rheological characteristics, which isan

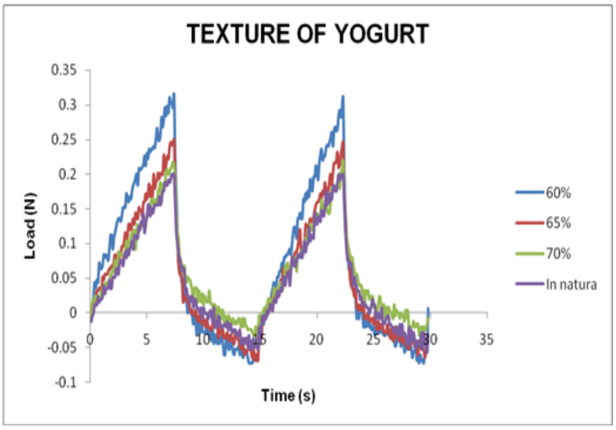

Figure 1. Probe penetration force (Newton) in the traditional and rehydrated yogurt measured by texture profile analysis.

important parameter for consumer's decision in case of commercialization.Sakin-Yilmazer *et al.*(2014) rehydrated yoghurt powderat different solids concentrations and concluded that the concentration of 30% solids provided rheological characteristics closer to those of the traditional product containing 14% solids.

Table 1 shows the yield of the dehydrated freeze driedyoghurt, when compared to the traditional yoghurt considered as 100% yield. After freeze drying, 100 g yogurt resulted in 18 g freeze dried yoghurt, containing solids that did not undergo sublimation and residual water. After rehydration using 70% of water sublimed during freeze drying, the reconstituted yoghurt reached about 75% of the weight of the traditional yoghurt. Therefore, to produce a traditional yoghurt cup of 170 g,which iscommercialized in the Brazilian market, it would be necessary 40.6 g yogurt powder (freeze dried).

The physicochemical parameters were significantly affected by the amount of water used for reconstitution of the freeze dried sample, as shown in Table 2. Thus, the rehydrated yoghurt had lower pH and was slightly acid than the traditional yoghurt. However, these values are suitable for the product, since according to Tamime and Robinson (2007), only pH values below 4.0 can lead to consumers' rejection.

Despite lower moisture content was observed for the rehydrated yogurt when compared to the traditional yogurt, probably due to the lower amount of water added, no significant differences ($p = 5\%$) were observed for the water activity values between the samples (Table 2), once the water does not easily return to the product structure during rehydration. The yogurt powder had water activity of 0.24, for a long shelf life, dehydrated foods must have water activity and moisture content of ~ 0.3 and 5%, respectively

Table 1. Yield of freeze dried and rehydrated yoghurt

YogurtYield	
Samples	(%)
Traditional	100
Freezedried	18
Rehydrated	75

(Chávez and Ledeboer, 2007). Kearney *et al.* (2009) found water activity from 0.21 to 0.32 at the end of the process of probiotic yogurt powder.

The acidity values of both traditional and rehydrated yogurts were within the recommended by the Identity and Quality Standards of Fermented Milk, since the acidity values were 0.6 to 1.5% lactic acid. Although Souza (1991) reported suitable acidity varying from 0.7% to 0.9%, values from 0.7% to 1.25% are also found in literature.

Lipids levels were also consistent with the Identity and Quality Standards, which establishes a range from 3.0 to 5.9% for whole yogurt (Brazil, 2007). Ash content, protein, carbohydrates, and metabolizable energy were higher in the rehydrated yoghurt when compared to the traditional yoghurt, since the smaller amount of water used for rehydration led to a more nutritious product (De Brito Rodas *et al.*, 2001), as also reported by Tamime and Robinson (2007).

Microbiological characterization

According tothe Identity and Quality Standards of Fermented Milk - MAPA minimum counts of 10^7CFU / g viable bacteria cells are required in the yogurt until its consumption. In line with these requirements, the bacteria counts of the traditional and rehydrated yoghurts (Table 3) were greater than those required by legislation for both samples. This has proven that freeze drying is an excellent method for maintaining the desirable microbiological characteristics of yogurt.As reported by Wang *et al.* (2005), the lactic acid in fermented milk helps maintainingbacteria viability during the freezing stage, once low pH values lead to changes in bacteria membranes that help to resist low temperatures.

A study on the survival of probiotic lactic acid bacteria in freeze dried fermented soy milk (Wang *et al.*, 2004) showed that the freeze drying maintains higher numbers of viable bacterial cells when compared tospray drying process. Immediately after freeze drying, the survival of lactic acid bacteria in the fermented milk was 74.1 to 75.1% with a population decrease of only 0.12 to 0.13 log CFU / g

Table 2. Physicochemical characteristics of the traditional and freeze dried yogurt rehydrated at 70%

Parameters	Yogurt samples[1] Traditional	Rehydrated Freezedried	MSD[2]
pH	4.53[a]	4.48[b]	0.01
Acidity (% lacticacid)	0.95[b]	1.15[a]	1.05
Wateractivity	0.987[a]	0.983[a]	0.01
Lipids (%)	3.35[b]	4.66[a]	5.37
Protein (%)	4.40[b]	6.88[a]	0.58
Moisture (%)	82.77[a]	74.09[b]	0.55
Ash (%)	0.98[b]	1.15[a]	0.17
Carbohydrates	8.49[b]	13.20[a]	1.29
Metabolizableenergy	81.78[b]	122.32[a]	3.06

[1]Means with same letters do not differ statistically by Tukey test at 5% significance level. [2]MSD = Minimum significant difference at 5% level.

The number of bacteria of the genus *Streptococcus* in both yoghurt samples (Table 3) was higher than those of the genus *Lactobacillus*, since *Streptococcus thermophlius* strains usually survive better than *Lactobacillus bulgaricus,* due to differences in cell size and structure (Fonseca *et al*., 2001). In the present study, there was no effect of sucrose concentration on the growth or maintenance of yoghurt microflora.

As can be seen in Table 3,a considerable reduction in the initial numbers of lactic acid bacteria in the traditional product was observed when compared to the rehydrated product for both *Streptococci* and *Lactobacilli*, probably due tochanges in the cell structure of the bacteria caused by freezing and drying stages. *Lactobacilli* showed a more significant reduction, which demonstrated a greater sensitivity of these bacteria to the processing steps, as also reported by Wang *et al.* (2004) and Venir *et al.* (2007).

Venir *et al.* (2007) studiedfreeze drying of three yoghurt formulations, as follows: a) skim milk yoghurt;b) whole milk yoghurt; c) yoghurt containing sucrose (10%) and blueberry (10%). The authors observed a reduction of *Lactobacilli* and *Streptococci* counts from 1.7 logCFU/g to 1.5 log CFU/g in full fat yoghurt during freezing, and from 2.5 log CFU/g to 1.9 log CFU/g during drying stage, respectively. The samples containing sucrose and blueberry maintained the viability of the bacterial cells during the process probably due to thecryoprotective effect of the sucrose. The authors also suggested that the product can be used as a nutritional source in various situations such as a space trip.

Although the number of colonies of lactic acid bacteria in the rehydrated product was lower than that found in the traditional yogurt, the rehydrated

Table 3.Lactic acid bacteria counts in the traditional and freeze dried yogurt rehydrated at 70%, containing 5% sucrose.

LacticAcidbacteria CFU/g	YogurtSamples Traditional	RehydratedFreezedried
Streptococcus	$2.76x10^9$	$4.00x10^8$
Lactobacillus	$1.26x10^7$	$7.60x10^4$
Total	$2.82x10^9$	$4.00x10^8$

product remained within the values recommended by MAPA. This demonstrates the great protection of freeze dryingagainst deterioration, although not incorporating all the amount of water lost during the process may have contributed to the maintenance of lactic acid bacteria at high numbers in the reconstituted product. Spagnol *et al.*(2005)found $2x10^7$ CFU/g viable lactic bacteria in freeze dried yogurt, whileRybka and Kailasapathy(1997) found a reduction from $1.2x10^8$CFU/g to $3x10^5$CFU/g Lactobacilli, and from $1.6x10^9$CFU/g to $7.6x10^8$ CFU/g Streptococci after freeze-drying, evidencing the high sensibility of Lactobacilli. Bozoglu *et al.* (1987) studied the survival kinetics of lactic acid bacteria during freeze drying, and found initial Lactobacilli counts of $1.61x10^8$CFU/g, which reduced to $9.21x10^4$ CFU /g after freeze drying, while Streptococci counts reduced from $1.98x10^9$CFU/g to $1.30x10^7$CFU/g.

Acceptance test

Table 4 shows the mean acceptance scores of the traditional yoghurt and the yoghurt rehydrated at 70%. The reconstituted yoghurt was significantly better accepted (p <5%) than the traditional yogurt for the attributes flavor and overall impression. No significant differences were observedfor the attributes appearance, aroma, and texture between the samples (p >5%). The mean acceptance scores of the rehydrated yoghurt were situated around 8 in the hedonic scale, which corresponds to the term “liked very much” indicating high acceptance of this sample among consumers, which wasalso observed for the traditional yogurt. Similar acceptance scores were obtained by Hekmat and Reid (2006) in probiotic and traditional yogurt; Neto *et al.*(2005)in buffalo milk yogurt, and by Singh and Muthukumarappan (2008) in yogurt fruit flavor. In contrast,Güler-Akın and Akın (2007) and Borges *et al.* (2009) found lower scores in goat yogurt and buffalo milk yogurt cajá flavor, respectively.

According to Sakin-Yilmazer *et al.* (2014), after reconstitution of yoghurt powder, it may have a

Table 4. Acceptance scores of the traditional and freeze dried yogurt rehydrated at 70%

Parameters	Yogurt samples[1]		
	Traditional	Rehydrated Freezedried	MSD[2]
Appearance	7.98[a]	8.10[a]	0.23
Aroma	7.45[a]	7.76[a]	0.32
Flavor	7.20[b]	7.80[a]	0.45
Texture	7.75[a]	7.90[a]	0.40
Overall impression	7.50[b]	7.90[a]	0.28
Purchase intente	6.98[b]	8.00[a]	0.80
Priceassigned (R$)	1.30[b]	1.64[a]	0.25

[1]Means with same letters do not differ statistically by Tukey test at 5% significance level. [2]MSD = Minimum significant difference at 5% level.

rheological behavior comparable to fresh yoghurt, since the rheological and structural properties of yogurt are closely related to the sensory quality criteria as softness and consistency, which directly affects consumer acceptance.

Rathi *et al*. (1990) studied freeze dried yogurts and found percentages of 79.44, 20.86, and 0.92, for moisture, total solids, and lactic acid, respectively, and pH 4.37 and 11.13 mg / 50 mL of diacetyl in the reconstituted product (5 g / 20 mL at 40°C). These authors obtained scores of 6.67, 6.82, 6.77, and 6.45 on a 9 point hedonic scale for the attributes appearance, flavor, taste, texture, and overall acceptance, respectively, for the rehydrated product. Pan *et al*. (1994) reported similar flavor and nutritional composition of yogurt powder rehydrated at 10-40 ºC using1:5 powder:water ratio when compared to fresh yogurt.

The purchase intention of the yogurts was significantly higher (p <5%) for the reconstituted yoghurt when compared to the traditional yogurt (Table 4). The scores suggested that consumers would buy the freeze-dried yogurt with a degree of certainty close to "definitely buy". Similarly, as shown in Table 4, consumers would be willing to pay more for the freeze-dried yoghurt, with a significant difference between the samples (p <5%) with respect to the price proposed by consumers. These results suggest that the production costs associated with freeze dryingcan be compensated not only by eliminating the need for cold chain during transportation and marketing of the product, but also by the consumers' willingness to pay a higher price for the product due to its sensory quality.

Conclusion

Freeze-drying can preserve the microbiological, nutritional, and sensory characteristics of yogurts, meeting the microbiological and physicochemical requirements established by MAPA after rehydration. The yogurt rehydrated with 70% the amount of water lost during the process was more acceptable than the traditional yogurt, since consumers'purchase intention towards the freeze dried product was greater, with willingness to pay a higher price for the product. Thus, the results of this study suggest that the freeze-dried yogurt can be a promising marketing alternative for the dairy industry as it had a good acceptability and met the parameters required by law.

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