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Microbiological Stability of Rice Tart Stored at Ambient Temperature after Baking

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Abstract

Very popular in Belgium, rice tart is a hot pastry sold in bakeries. It is then consumed at home, for dessert or snack. This study is conducted to investigate the microbiological stability of this foodstuff, from the end of baking to end user by consumers. In this purpose, 108 rice tart samples were collected from each of seven bakeries in five Belgium provinces. Physico-chimical analysis in addition to microbiological analysis were carried out in accordance with the European Regulation EC 2073/2005 and with references methods, to enumerate the total microorganisms count, *Staphylococcus aureus*, *Bacillus cereus* as well as Enterobacteria, susceptible likely to contaminate the tarts during the production or after baking. Even when the results meet the microbiological safety standards immediately after baking in all bakeries implicated, a significant (p <0,05) increase of mesophilic aerobic bacteria (ranging from <1 to 7 log cfu/g) and *B. cereus* (> 3,7 log cfu/g) was observed during the storage at nonrefrigerated temperature (28 to 30 °C). A post-baking recontamination and other parameters like an insufficient baking time or a non uniform distribution of the oven heat could explain the observed bacteria growth. The present study shows that most rice tarts investigated are microbiologically safe. However, the possible increase of bacteria load in this foodstuff attributable to the favorable $a_{w,}$ pH and temperature conditions, highlight the importance of applying good hygienic practices and compliance with storage conditions after baking to ensure consumer safety.

Keywords: bacteria, bakery, increase, microbial load, rice tart, safety

1. Introduction

Bakery products are part of the processed food category. They include cake, pastries, biscuits, bread, breakfast cereals, and other products. They hold among foods products, a significant market share. The bakery market in Canada for example, registered total value sales of C$8.6 billion and total volume sales of 1.2 million tonnes in 2011 (Agriculture and Agri-Food Canada, 2013). Today, their production has evolved from a primitive, cottage industry into a modern manufacturing industry, promoting the increase in sale volume within the past decades (Kohn, 2000; Smith, Daifas, El-Khoury, Koukoutsis and El-Khoury 2004).

Bakery products could constitute the potential vehicles of a wide range of food poisoning microorganisms to humans because of their chemical composition that favors the proliferation of these pathogens. In fact, bakery products are an important source of carbohydrates fats, proteins, minerals and vitamins (Seiler, 1978, Saranraj and Geetha, 2012). For example, bread and biscuits contain around 7, 5 per cent to 7,8 per cent protein respectively. According to Osimani *et al*., 2016, the factors that can contribute to the production of good bakery products quality are the raw materials quality, the production technology and the plant hygiene.

The high diversity of pastries in water activity and in pH provides a variety of habitats for the bacteria growth (Novac, Sapers and Juneja 2002). Sharifzadeh, Hajsharifi-Shahreza and Ghasemi-Dehkordi *l* (2016) enumerated several microorganisms including *Coliform*, *Staphylococci* and *yeast* from cream-filled pastries made in Iran. Similarly, Voysey and Legan (2014) identified molds and yeasts as the key spoilage microorganisms in these bakery products. Microorganisms development in pastries can either speed up the organoleptic quality degradation or cause foodborne diseases. Thus, in July 2012, in the city of Lujan, Argentina, five individuals were affected after eating a handmade Viennese-style pastry at a family gathering. All of them presented with fever, joint pain, chills and non-bloody diarrhea containing mucus. Samples taken from the pastry ingredients were analyzed microbiologically. The results emphasize a contamination by *Shigella sonnei*, during the manipulation in the bakery (Della *et al*., 2015). Furthermore, Kotzekidou, (2013) emphasized a contamination and poor microbiological quality of frozen pastries. Bacteria like *Bacillus cereus*, *Salmonella* spp., presumptive *Escherichia coli* O157, *Listeria monocytogenes* and *Staphylococcus aureus* were concerned.

Rice tart is consumed at home, often sprinkled with icing sugar, for dessert or snack. According to the flow diagram, this tart specialty is made from several ingredients such as rice, eggs and cow raw milk. These ingredients could be potential sources of microbiological contamination. Several pathogens such as *Staphylococcus aureus, Bacillus cereus* and *Listeria monocytogenès* may be taken into consideration as potential hazard associated with rice tarts (AFSCA, 2015).

Rice tart is made in a variety of formats and the baking time and temperature are in each case, sufficient to destroy any vegetative microbes which are present prior to baking. However, a number of *Bacillus spp* spores are able to withstand the cooking temperature during the baking process and may grow to levels of public health concern with toxin production, if packaging and storage conditions are conductive to their growth (Bryan Guzewich and Todd *l.,* 1997, Leuschner, O'callaghan, and Arendt 1998, Rosenquist, Smidt, Anderson, Jensen and Wilcks 2005; Asadi, Maram and Kooshk 2015, André, Vallaeys, and Planchon, 2016). In respect to the organoleptic characteristics of rice tart namely the taste, appearance and smoothness traditional friendly bakers use to keep rice tarts at room temperature during 15 to 24 hours (time to sale), instead of storing them in refrigerators after production. Given that rice is part of the foodstuffs susceptible to promote the *Bacillus cereus* emetic development, the storage of tarts at room temperature could be at the origin of food safety issues for consumers.

In fact, Among the food matrices associated with *B. cereus* emetic poisoning, rice is certainly one of the most important source of contamination (Altayar and Sutherland, 2006; Ankolekar and Labbe, 2009; Shiota *et al*., 2010; Martinelli *et al*., 2013; Organji, Abulreesh, Elbanna, Osman and, Khider2015). Not only rice is easily contaminated with soil-borne spores of *B. cereus* (Sarrias Valero, Salmeron 2002; Kim *et al*., 2014), but the way rice is prepared for human consumption can promote its survival, outgrowth and toxin production during vegetative growth (Finlay Logan, Sutherland 2002). According to Jaaskelainen, (2003), rice-containing pastries accumulated high contents of cereulide, a *Bacillus* emetic toxin (0,3 to 5,5 µg/g [wet weight]),when stored at non-refrigeration temperatures (21 to 23 ℃). Cereulide is a pH and heat stable cyclic peptide toxin. (Agata, Ohta, Mori and Isobe., 1995, Rajkovic *et al*., 2008). This toxin is responsible of the emetic food poisoning syndrome, including fatal cases of food intoxications. Moreover, the increase in the number of fatal or very severe emetic outbreaks since 2000 is worthy of note (Dierick *et al*., 2005; Posfay-Barbe, Schrenzel, Frey, Studer, Korff and Belli 2008; Shiota *et al*., 2010). The cause of death or illness was attributed to liver failure, but also to acute encephalopathy and presumably acute cardiac insufficiency (Naranjo *et al*., 2011; Carlin and Nguyen-The, 2013).

The aim of this work is to study the microbiological stability of rice tart after baking. For this purpose, the development of microorganisms like total count bacteria, *Bacillus cereus, Staphylococcus aureus, as well as Enterobacteriacea* were monitored at non-refrigerated temperature (30 ℃).

2. Materials and Methods

2.1 Rice Tarts Sampling

108 medium size rice tart samples were collected from seven bakeries in five provinces of Belgium from May to July 2014. The total number of the collected samples in different bakeries is described in Table 1. Tart samples were conditioned in the sale packaging and held at 4 ℃ in a cooling box, then transported to the laboratory for analysis within approximately 40 min. Samples were stored at 4 ℃ until analysis. Cooking schedule applied at all producers are summarized in table 2. Rice tarts were then stored at nonrefrigerated temperature (30 ℃) throughout the analyses at t0, t24, t48 hours.

Table 1. Sampling protocol for Rice tarts

Provinces	Bakeries	Sample numbers
Liège	A	18
Luxembourg	B	18
Namur	C	18
Liège	D	18
Liège	E	12
Brabant Flamand	F	12
Hainaut	G	12

Table 2. Rice tart baking schedule and shelf life

Parameters/Bakeries	A	B	C	D	E	F	G
Baking temperature (°C)	260	230	240	210	270	190	260
Baking time (min)	25	30	20	40	30	45	45
Cooling temperature (°C)	20	20	20	20	20	18 °C	20
Estimated Shelf life by producers	48	12	48	48	72	48	48

2.2 Physico-chemical Analyses

Microbiological and physico-chimical analysis were carried out to enumerate the total mesophilic bacteria, *Staphylococcus aureus, Bacillus cereus* as well as Enterobacteria which could have contaminated the tarts during the production or after baking, either through the ingredients, or by product handling. The water activity and pH of rice tarts were also determined in order to measure the impact of these parameters on bacteria development.

The pH meter wtwpH340 (Mettler Toledo, Belgium) was used for the pH measurement. The tart was divided into four sections. The measurement was performed by inserting the probe of the pH meter in each part of the rice tart. The pH of the tart was calculated as the mean of the four measurements. The pH meter was calibrated before each use.

The water activity (a_w) measurement was established in a AquaLab (models series 3 and 3TE, USA) at 23±2 °C. The mirror dew point technique was used. The measurement consisted in introduction of a cup containing 1/3 of the tart volume, in the AquaLab. This tart portion was taken from inside of the tart, without the crust. The measuring device was calibrated before each measurement.

2.3 Microbiological Analysis

Microbiological analyses were carried out by an accredited environmental, toxicology and food control laboratory in accordance with the EC 2073/2005 regulation. Overall, 108 rice tarts samples were analyzed in accordance with the European Regulation EC 2073/2005. Reference methods were used for the enumeration of total bacteria count (ISO 4833-2); *Staphylococcus aureus (ISO* 6888-3*); Bacillus cereus (ISO* ISO 7932: 2004*) and Enterobacteriaceae* (ISO 21528-2) (ISO 2003; 2004; 2013). Microbiological analyses on rice tarts were carried out at three times namely immediately post baking, after 24 hours and after 48 hours. The microbiological stability of rice tarts involved in this study was assessed using criteria in the EC 2073/2005 recommendations (Table 3).

Table 3. Microbiological criteria as recommended by the Commission Regulation EC 2073 /2005 on microbiological criteria for milk based dessert (dessert, pastries)

Sectors	Microorganisms	n	c	Satisfactory (m[a])	Acceptable (M[b])	unsatisfactory	Unit	European commission 2005.
Distribution	Total bacteria count	5	2	≤ 100 000	≤ 1 000 000	> 1 000 000	ufc/g	Guide value
Distribution	*Enterobacteriaceae*	5	2	≤ 500	5000	> 5000	ufc/g	Guide value
Distribution	*B. cereus*	5	2	≤ 1000	10 000	>10 000	ufc/g	Guide value
Distribution	*Staphylococcus*	5	2	≤ 100	1000	>1000	ufc/g	Guide value

[a]In a three-class sampling plan, the "**m**" limit is used to distinguish acceptable quality units (under good manufacturing practices) from those of poor quality.

[b]The numerical value of "**M**" represents unacceptable concentrations of microorganisms. Exceeding the "**m**" level requires corrective action, *i.e*. revision of the HACCP plan. Exceeding the "**M**" value requires a recall of the product from the market.

The number of sampling units is represented by *n*

The maximum allowable number of sample(s) that yielded unsatisfactory test results is represented by "**c**".

2.4 Statistical Analysis

Experimental data were compiled in Microsoft Excel 2010 software (Manufacturer, country) for descriptive and statistical analyses and were then subjected to ANOVA analysis using Statistica 7(Manufacturer, country)with Fisher LSD. The overall least significant differences ($p < 0.05$) were calculated and used to detect significant differences among analyses. Each experiment was performed in triplicate.

3. Results and Discussion

In order to investigate the microbiological stability of rice tarts during the storage for sale, 108 tarts samples were collected through different Belgian bakeries for physico-chemical characteristic and microbiological analyses.

3.1 Physicochemical Characteristic

Averages of pH and water activity results of rice tarts analyzed are summarized in the table 4. pH values ranged from 5.9 to 6.5 while the water activity ranged from 0.970 to 0.99, 24 and 48 hours after baking. Statistically, no significant differences ($p < 0,05$) in a_w were observed during the rice tarts storage at non refrigeration temperature, in the seven bakeries. Overall, no increasing in a_w was registered. By contrast, the results showed a variability in pH values 24 and 48 hours after the baking (table 4). According to a study ranking bakery products on the basis of their $a_{w,}$ the rice tart used in this study could be considered as high moisture content product (Novak *et al*.,2002). Furthermore, a growth simulation of pathogenic microorganisms in rice tart, conducted by the FASFC (Federal Agency for the Safety of the Food Chain) in 2015, showed that with a pH of 6.5 and a_w value of 0.993, rice tarts are more likely to promote pathogenic bacteria growth at 24 ℃ and 26 ℃. In the light of the above, the rice tarts analyzed in our study should be potentially susceptible to promote spoilage by bacteria, yeast and molds under extreme ambient temperature (Smith *et al.*, 2010).

Table 4. Measurement of rice tart pH and water activity. Values are mean (± standard deviation) of triplacate pH and a_w measurements. In the same column, same bakery and same parameter; different superscript letters indicate a significant difference (p<0.05)

Bakeries	Times (h)	pH	a_w
A	t0	6.42^{f} ± 0.07	0.99^{a} ± 0.01
	t24	6.49^{f} ± 0.03	0.99^{a} ± 0.06
	t48	6.22^{de} ± 0.16	0.99^{a} ± 0,00
B	t0	5.99^{ab} ± 0.11	0.99^{a} ± 0.03
	t24	$6a^{bc}$ ± 0.09	0.98^{a} ± 0.02
	t48	6.16^{cd} ± 0.06	0.98^{a} ± 0.05
C	t0	6.12^{bcd} ±0.03	0.98^{a} ± 0.00
	t24	6.01^{abc} ± 0.04	0.98^{a} ± 0.05
	t48	6.07^{abcd} ± 0.02	0.98^{a} ± 0.02
D	t0	6.1^{abcd} ± 0.05	0.98^{a} ± 0.1
	t24	5.94^{a} ±0.05	0.98^{a} ± 0.07
	t48	6.08^{abcd} ±0.1	0.98^{a} ± 0.00
E	t0	6.39^{f} ± 0.14	0.98^{a} ± 0.01
	t24	6.09^{abcd} ± 0.05	0.98^{a} ± 0.06
	t48	6.09^{abcd} ± 0.05	0.98^{a} ± 0.08
F	t0	6.1^{abcd} ± 0.08	0.98^{a} ± 0.09
	t24	6.03^{abc} ± 0.1	0.97^{a} ± 0.01
	t48	6.03^{abc} ± 0.1	0.97^{a} ± 0.04
G	t0	6.06^{abcd} ± 0.04	0.98^{a} ± 0.00
	t24	6.21^{de} ± 0.04	0.98^{a} ± 0.01
	t48	6.36^{ef} ± 0.04	0.98^{a} ± 0.03

3.2 Microbiological Quality of Rice Tart

Tables 5 present the results of microbiological analyses performed on rice tarts, in seven different bakeries. The analyses were done at t0, t24 and t48 hours after baking. According to the results, there was lots of variability of mesophilic aerobic bacteria number between the analysis times, within the same bakery. A significant ($p <0,05$) increasing of these bacteria counts was observed at t24 and t48, in the seven bakeries concerned. No significant differences in the evolution in Enterobacteria and *S. aureus* concentration have been found at t24 and t48, for all bakeries ($p < 0,05$). Likewise, no significant variability was observed in the number of *B. cereus* and *B. cereus* spores between t0 and t24 or t24 and t48 for the bakeries D, E, F and G. But, an increasing in *B. cereus* concentration was observed at t24 and t48 for the other three bakeries (A, B, C).

Even if they are sometimes, an increasing in bacteria concentration during the rice tarts storage, these foodstuffs were found to be within microbiologically satisfactory limits, immediately (t0) after baking, for Enterobacteria, total bacteria counts, *S. aureus*, *B. cereus* and spore of *B. cereus*. The compliance regarding microbiological criteria of rice tarts, immediately after baking indicates that there is a very low likelihood of any vegetative bacteria contamination surviving the baking process. The significant increasing of bacteria count (mesophilic aerobic bacteria and *B. cereus*) during the storage (t24, t48) at non-refrigeration temperature (30 ℃) could be linked to a post-baking recontamination combined with the storage temperature, as the baking temperature is normally enough to eliminate previous contamination. Several other parameters could have influenced the quality of the rice tarts during the baking and explain bacteria growth. Among them, an insufficient baking time or a non uniform distribution of the oven heat; in these circumstances, the mean core temperature of the rice tarts could be not enough heated, allowing some heat resistant *B. cereus* spores survival and germination during the storage at ambient temperature (Lainez, Vergara, María and Bárcenas 2008; Sani, Taip, Kamal and Aziz 2014). The favorable terms created by the a_w and pH close to neutral (tables 3) of rice tarts 24 and 48 hours after baking, could probably have promoted the heat resistant *B. cereus* spore germination and the growth of bacteria commonly associated with these products (Trujillo, Yeow and Pham 2003; Lebert, Baucour, Lebert and Daudin 2005; Ureta, Olivera and Salvadori 2016; Reid *et al*., 2017). Cereulide is formed when cereulide-producing *B. cereus* strains were present at > or = 10^6 cfu/g in product with water activity values of > 0,953 and pH of > 5,6. Moreover, rice-containing pastries could accumulated high contents of cereulide (0,3 to 5,5 microg/g [wet weight]) when stored at nonrefrigerated temperature (Jääskeläinen *et al*., 2003). In the extreme temperature condition used in this study, *B. cereus* developed to a concentration > 3,5 log cfu/g. However, it is important to note that the cereulide producing *B. cereus* are rare, around 1% (Altayar and Sutherland**,** 2006). Furthermore, a study conducted by the Federal Agency for the Safety of the Food Chain (FASFC) in 2016, including pilot studies, a consumer survey and simulation tests, related to rice tarts showed that The risk related to food safety associated with the consumption of rice tarts contaminated with *Bacillus cereus* remains low at 22 °C for 12 hours of storage.

The rice tarts sampled from the bakery F remain acceptable for *S. aureus*, with a microbial load of 2,5 log cfu/g at t0 (Table 5). The significance of this microorganism in foods has been reported through different studies (Hennekinne, Marie-Laure De Buyser and Dragacci 2012; Guitiérez *et al*., 2012). In the current study, the presence of this bacterium in the rice tart could be link to a mishandling after baking or during the storage. Indeed, this pathogen can reside on the skin and mucosal surfaces of humans and animals, besides being an exceptionally well adapted opportunistic, able to survive under different conditions (Soares *et al*., 2011; Brown Cornforth, and Mideo 2012; Batista *et al*., 2016).

The incidence of foods products post-baking contamination related to the direct contact has been previously highlighted (Pérez-Rodríguez *et al*., 2007; Kotzekidou, 2013; Voysey and Legan, 2014). In the same vein, a study conducted on the evaluation of microbial contamination of pastries in confectioneries in Iran, revealed an incidences of *S. aureus* with 48,68% (Sharifzadeh *et al.*, 2016). The sources of the contamination were related to handlers and nonconformity of the hygiene standards.

Table 5. Bacteria counts of rice tarts from seven bakeries in Belgium. Values are mean(±standard deviation) of triplicate bacterial loads (in log cfu/g) measurements. In the same column, same bakery and same parameter; different superscript letters indicate a significant difference (p<0.05)

		Bacteria				
Bakeries	Times (h)	Mesophilic aerobic bacteria	*Enterobacteria*	*S. aureus*	*B. cereus*	Spore *B. cereus*
A	t0	$1.20^{a} \pm 0,35$	$1^{a} \pm 0.00$	$1.2^{a} \pm 0.35$	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$
	t24	$7.60^{hi} \pm 0,08$	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$	$3.63^{b} \pm 0.07$	$1.67^{b} \pm 0.66$
	t48	$8.13^{i} \pm 0,08$	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$	$3.72^{b} \pm 0.07$	$3.65^{c} \pm 0.06$
B	t0	$1.20^{a} \pm 0,35$	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$
	t24	$4.40^{d} \pm 1,04$	$1.2^{a} \pm 0.35$	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$
	t48	$7.75^{hi} \pm 0,06$	$3.71^{b} \pm 0.10$	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$
C	t0	$1.20^{a} \pm 0,35$	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$
	t24	$5.24^{ef} \pm 0,68$	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$	$3,54^{b} \pm 0.17$	$1^{a} \pm 0.00$
	t48	$7.25^{h} \pm 0,05$	$1.2^{a} \pm 0.35$	$1^{a} \pm 0.00$	$3,59^{b} \pm 0.09$	$1^{a} \pm 0.00$
D	t0	$1.20^{a} \pm 0,35$	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$
	t24	$3.30^{c} \pm 0,75$	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$
	t48	$6.39^{g} \pm 0,24$	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$
E	t0	$1.40^{a} \pm 0,35$	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$	$1.2^{a} \pm 0.35$	$1^{a} \pm 0.00$
	t24	$3.32^{c} \pm 0,61$	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$
	t48	$5.61^{f} \pm 0,14$	$1^{a} \pm 0.00$	1a $\pm$ 0.00	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$
F	t0	$1.40^{a} \pm 0,35$	$1^{a} \pm 0.00$	$1.69^{a} \pm 0.73$	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$
	t24	$4.85^{de} \pm 0,09$	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$
	t48	$7.77^{hi} \pm 0,25$	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$
G	t0	$1^{a} \pm 0,00$	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$
	t24	$2.52^{b} \pm 0,08$	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$	$1^{a} \pm 0.00$
	t48	$5.45^{ef} \pm 0,39$	$1^{a} \pm 0.00$	$1.8^{a} \pm 1.39$	$1.57^{a} \pm 0.98$	$1^{a} \pm 0.00$

4. Conclusion

The rice tarts were found to be within microbiologically satisfactory limits defined by the Regulation EC

2073/2005, immediately after baking for all bacteria tested, regardless the applied cooking schedule. An increase in *B. cereus* and *B. cereus* spores counts were observed 24 hours after baking, at non-refrigeration temperature (28 to 30 ℃). Although not enough to constitute a potential major hazard to consumers health in this study, *B. cereus* and its toxins can present a hazard in foods and can affect the health of the consumer. Especially, when favorable terms are created by the a_w and pH as it is the case in our study. Therefore, adequate hygienic practices are needed, mainly after baking to avoid the post-contamination of rice tarts and for the consumer protection.

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