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REGULAR ARTICLE

Development of biscuits with green banana flour irradiated by ^{60}Co: Preservation in modified atmosphere packaging

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ABSTRACT

Banana it's one of the most consumed fruits in the world but 20% of the production is wasted, mostly due to post-harvest losses, or because it doesn´t fit in consumers standards. Nonetheless, Green Banana Flour (GBF) can be an alternative to minimize these losses, further being a good source of nutrients, fiber, resistant starch and micronutrients. Considering the interesting possibilities of GBF utilization in the agrofood sector, the aims of this study, was the development of GBF cookies and preservation during three months in two different types of modified atmosphere. The experimental design considered GBF radiation with gamma radiation (1 kGy and 3 kGy), with additional packaging in modified atmosphere, using 100% CO_2 or a gas mixture containing 2% O_2; 88% N_2 e 10% CO_2. As a control, non-radiated GBF was used. In general, it was observed that samples submitted to 3 kGy radiation, had the best results. Besides, packaging with gases mixture showed higher efficiency for GBF cookies preservation, due to CO_2.

Keywords: Green banana flour; Gamma radiation; Cookies; Modified atmosphere packaging.

INTRODUCTION

Banana fruit is one of the economically most important fruit produced and consumed worldwide It has high energetic value (about 90–100 kcal per 100 g edible product), but its composition strongly depends on cultivation (Arias et al., 2003; Davey et al., 2007). GBF, or green banana, are rich in minerals, fiber, total phenolics (campesterol, stigmasterol, β-sitosterol), vitamins (B_1, B_2, C, E) and β-carotene (Lii et al., 1982; Someya et al., 2002; Hernandez-Nava et al., 2009; Haslinda et al., 2009; Tribess et al., 2009; Farage and Zandoni, 2013), while also have an high content (Lajolo, 2001) of complex carbohydrates (mainly resistant starch, 40 – 60% (m/m) on a dry basis). In this context, it must be pointed that, as resistant starch is not digested, in the small intestine becomes available as a substrate for fermentation by anaerobic bacteria in the colon. Thus, complex carbohydrates of GBF share many characteristics and benefits attributed to dietary fiber in the gastrointestinal tract (Uehara, 2011). Besides, as additional prophylactic characteristic, GBF also prevents or reduces high levels of cholesterol and colon cancer, further being an alternative for gluten (*i.e.,* free products used in ready to eat foods, namely spaghetti with up to 15% of banana starch addition).

GBF is produced through dehydration (freeze-drying or natural or under controlled atmosphere drying) of green product or purée. Nevertheless, lyophilization, although presenting a higher cost, furnishes better color, aroma and flavor to GBF (Folegatti and Matsuura, 2012). Addition of GBF to wheat, corn or rice flours also increases the nutritional value of the flour blend used in pizzas, breads,

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pastas, biscuits and other products (Fasolin et al., 2007; Uehara, 2011). Yet, the shelf life of GBF derived products might increase using different preservation methods.

Gamma irradiation is an alternative method for food preservation extending the shelf life of food products, namely because can destroy harmful bacteria. It also minimizes nutrients losses (relatively to canning, drying, heat pasteurization and sterilization), without raising the temperature of processed food (Wood and Bruhn, 2000). However, the irradiation dose of food products varies with the final objective (doses greater than 10 kGy, are intended for food sterilization; doses, ranging between 1 to 10 kGy, exert a pasteurizing effect increasing the shelf life; doses lesser than 1 kGy, control infestation by parasites and insects, and decrease the senescence process in most fresh fruits and vegetables budding) (Andrews et al., 1998). Moreover, the maximum dose is established on the basis of appearance of undesirable changes in sensory characteristics (Kilcast, 1994; Uehara, 2011). At a chemical level, doses up to 10 kGy, and even considerably beyond, do not affect the levels of energetic nutrients (*i.e.* carbohydrates, lipids, proteins) (Diehl and Josephson, 1994; Wood and Bruhn, 2000). Also, essential amino acids, essential fatty acids, minerals and most vitamins suffer no significant losses (Diehl and Josephson, 1994; Wood and Bruhn, 2000).

Preservation through packaging further keeps food properties intact and increases shelf life. Besides, modified atmosphere packaging (MAP) is crucial to reduce the physical, enzymatic and microbial deterioration food products. MAP is a food preservation method that replaces air inside the package by a mixture of gases, such as molecular oxygen, carbon dioxide and nitrogen. Accordingly, this method increases commercial validity, facilitating the commercialization of food products and reduces deterioration losses because retards microbial (namely of pathogenics) growth (Janjarasskul et al., 2016).

In the particular case of bakery, the lower moisture content (namely in cookies) are generally identified with longer shelf life due to lower a_w values. Therefore, the main causes of quality decay are related with water absorption from the surrounding environment or due to lipid peroxidation (Alamprese et al., 2017).

This study assesses the influence of gamma radiation (1 kGy and 3 kGy) during three months of preservation, in two types of modified atmospheres (100% CO_2 and a gas mixture containing 2% O_2, 88% N_2, 10% CO_2), on biscuits quality produced with GBF. It is further intended to develop biscuits based on GBF that are healthy and sensory appealing, without food additives.

MATERIALS AND METHODS

Samples preparation

GBF irradiation was performed using a ^{60}Co Gammacell 220 (AECL) source, at a dose rate of about 2.16 kGy h^{-1}, applying dose 1 kGy and 3 kGy (dose uniformity factor, 1.13). Dosimetric mapping was previously performed by Fricke dosimetry.

Biscuits were processed (75 samples x 15 types per sample) using three different types of GBF incorporation. For the production of about 450 units of GBF Cookies (> then 75 samples x 5 storage conditions), whipped eggs (6 - L class), sugar (600 g) and salt (10.8 g) were mixed for 2 minutes. Then, margarine (240 g), previously melted in a water bath, was added and GBF (600 g) was slowly mixed with corn flour (600 g). Finally, powder milk (420 g), maize starch (90 g) and baking powder (90 g) was added, being all the ingredients mixed. The dough was kneaded (by hand) for about 5 minutes and therefore rested for about 1 hour at 4 °C. Thereafter, dough was stretched in a spreading machine and biscuits were cut and cooked at 160 °C/15 minutes. After cooling to room temperature, biscuits were packaged. Samples preparation considered the following treatments (Table 1): control (T) - samples prepared with GBF but without irradiation; samples prepared with GBF irradiated with a dose of 1kGy and 3kGy (1K and 3K, respectively); samples submitted to a mixture gases containing 2% O_2, 88% N_2 and 10% CO_2 or to 100% CO_2 (M and C, respectively).

For packaging, the modified atmospheres were injected into the bags (Co-extruded PA/PE Cast film) containing the cooled biscuits, followed by sealing. Thereafter, GBF

Table 1: Experimental design for biscuits preservation

Storage conditions		Samples		
MAP	Time	Without irradiation	Irradiated	
		Control	1 kGy	3 kGy
Without MAP	0 days	0T	01k	03k
2% O_2 + 88% N_2 + 10% CO_2	1 month	1MT	1M1k	1M3k
2% O_2 + 88% N_2 + 10% CO_2	3 months	3MT	3M1k	3M3k
100% CO_2	1 month	1CT	1C1k	1C3k
100% CO_2	3 months	3CT	3C1k	3C3k

biscuits were stored sheltered from light at a temperature of *ca.* 20 °C, during 3 months. Samples were evaluated after 1 (1M) and 3 months (M).

Physical and chemical analyses

Gas measurement was performed by the PBI Dansensor gas meter (Ringsted, Denmark), using a needle attached to the machine to drill in the center of the package, allowing measuring the percentage of CO_2 and O_2 present inside the package.

Moisture, ash, fat and total nitrogen were determined followed the AOAC methods (AOAC, 1990). The protein values were determined by multiplying the total nitrogen by 6.25. The nutritional value was performed according to the Regulation (EU) Nº 1169/2011. The water activity (a_w) was determined using a GBZ FA-St/1(Scientific Instruments, Romans-sur-Isère, France) apparatus (Santos et al., 2017).

Resistant starch (RS) was measured by the official method AACC 32-40.01/AOAC 2002.02, with a Megazyme Kit (Megazyme International Ireland Ltd). Color determination and digital image analysis was performed using five replicates, according to Dias et al. (2015, 2017, 2018).

A TAHD*i*® texturometer from Stable Micro Systems (Godalming, United Kingdom) was used. In five replicates of each sample, a 2 mm diameter aluminum probe was used. The simple test was programmed under the following conditions: test speed 1.00 mm s^{-1} and sample penetration depth 10 mm. Data obtained was analysed with a Texture Expert Software for Windows.

Microbiological analysis

Aerobic colony count (ACC) were performed (0 days, 1 month and 3 months of storage) according to the ISO 4833-1 (2013), on plate count agar (PCA; Oxoid, Hampshire, United Kingdom), after incubation at 30 °C for 48 h.

Sensory analysis

For the sensory test, a hedonistic panel of fifty tasters, aged between 20 and 35 years, was randomly recruited, to taste and give a relative evaluation of the 3 (at day 0) and 6 samples (at 1 month and 3 months, which corresponded to the shelf life response). The evaluated attributes were the appearance, smell, texture, banana aroma, flavor and global appreciation. The intensity of the sensory attributes was scored on a 7 points scale (1 and 7 corresponding to very unpleasant and very pleasant, respectively).

Statistical methods

The results obtained were analyzed by the "STATISTICA" program, with analysis of variance and comparison of means in ANOVA, according to the Scheffé test, for a 95% confidence range ($p < 0.05$). Data considered the average, standard deviation and analysis of variance. A principal component analysis (PCA) was performed to evaluate differences between samples using multiple attributes simultaneously (Alvarenga et al., 2011). The factor loadings were ranked following the classification reported by Liu et al. (2003) and Palma et al. (2009).

RESULTS AND DISCUSSION

The nutritional value of all samples did not vary significantly since the amount of ingredients was the same in all formulations (Table 2), and gamma irradiation did not influence the related caloric value (Control – 301.05 Kcal 100 g^{-1}; 1 kGy 01K – 297.34 Kcal 100 g^{-1}; 3 kGy 03K – 303.44 Kcal 100 g^{-1}).

Mean values in the same row marked with different letters (a, b, c, d, e, f, g) are significantly different ($P<0.05$, n=3, Scheffé test).

The amount of RS in the biscuits (5.5 to 6.2 g/100 g) are similar to those observed by (Agama-Acevedo et al., 2012) in cookies partially substituted with unripe banana flour. Packaging type also did not affect the amount of RS in the biscuits. The irradiation dose of green banana flour (without irradiation, 1kGy and 3kGy) also did not influence the RS content. However, an increase of the RS content was observed after storage for 3 months. These results agree with previous studies by (Carreira et al., 2004), being the amount of RS in the green banana flour biscuits higher than those found in traditional wheat bread (Amaral et al., 2016).

During storage a_W increased, with samples without irradiated flour (1MT and 3MT) showing more constant and highest values. Moreover, moisture content also increased (but showing the lowest values) during storage in samples package with CO_2. Nevertheless, in general terms, during storage the rheological parameters did not show significant differences among all samples. However, the sample not irradiated and with 3 months storage revealed a decreased hardness (3CT e 3MT).

The ACC counts of GBF cookies (control, 1k and 3k), at 0 days, 1 and 3 months of storage (0, 1, 3) in samples with two different forms of preservation (M and C) (Fig. 1), were classified as satisfactory (if $< 10^4$ cfu g^{-1}), acceptable or borderline (if $\geq 10^4$- $< 10^6$ cfu g^{-1}) or unsatisfactory (if $> 10^6$ cfu g^{-1}), based on the limits proposed in different sources (Gilbert et al., 2000; HPA, 2009) for ACCs (30 ºC 48 h^{-1}) in ready-to-eat foods (type bakery and confectionery products without dairy cream).

Table 2: Mean values, standard deviation and results of analysis of variance of samples with different level of radiation and different storage conditions

Samples	L	a	b	R	G	B	Y	a_w	Moisture (g/100g)	Hardness (N)
0T	53.1 (1.87)^{a}	3.34 (0.35)^{bcd}	22.6 (1.29)^{ab}	136.4 (1.88)^{cd}	116.5 (1.26)^{bc}	59.95 (2.09)^{df}	116.0 (1.22)^{bc}	0.48 (0.05^{a}	5.35 (0.16)^{abc}	10.96 (4.15)^{a}
1CT	53.7 (1.75)^{a}	2.94 (0.15)^{bcd}	20.9 (1.19)^{b}	139.5 (0.48)^{cd}	116.4 (0.37)^{bc}	66.06 (0.55)$^{b-f}$	117.6 (0.36)^{bc}	0.37 (0.00)^{e}	4.48 (0.21)^{bc}	11.97 (4.26)^{a}
1MT	54.6 (1.77)^{a}	3.01 (0.22)^{c}	21.38 (1.12)^{a}	141.9 (1.76)^{cd}	112.7 (2.01)^{bc}	60.44 (2.65)^{df}	115.47 (1.98)^{bc}	0.40 (0.00)^{d}	5.09 (0.25)^{abc}	13.77 (2.20)^{a}
3CT	54.6 (1.40)^{a}	2.72 (0.47)^{bcd}	22.32 (0.87)^{ab}	161.1 (5.04)^{b}	135.7 (5.64)^{a}	77.38 (7.52)$^{a-d}$	136.65 (5.66)^{a}	0.48 (0.00)^{a}	5.22 (0.22) ^{abc}	9.13 (2.37)^{a}
3MT	54.5 (1.02)^{a}	2.77 (0.29)^{bcd}	21.45 (0.69)^{ab}	146.5 (3.64)^{cd}	117.9 (3.52)^{bc}	62.416 (3.69)^{adf}	120.09 (3.56)^{bc}	0.43 (0.00)^{b}	5.68 (0.19)^{a}	9.09 (2.33)^{a}
01k	54.9 (1.72)^{a}	3.637 (0.55)^{b}	23.3 (1.17)^{a}	142.2 (0.99)^{cd}	123.8 (0.92)^{b}	67.51 (2.94)$^{b-f}$	122.9 (0.89)^{b}	0.30 (0.00)^{g}	4.95 (0.11)^{abc}	12.50 (1.97)^{a}
1C1k	53.0 (2.91)^{a}	3.66 (0.33)^{b}	22.2 (1.23)^{ab}	136.7 (2.27)^{cd}	108.7 (3.09)^{c}	54.34 (3.44)^{f}	110.9 (2.75)^{c}	0.32 (0.00)^{f}	4.74 (0.37)^{abc}	12.67 (3.39)^{a}
1M1k	53.9 (2.47)^{a}	3.48 (0.44)^{bc}	21.47 (1.24)^{ab}	134.9 (2.46)^{d}	107.9 (2.94)^{c}	60.39 (2.62)^{fd}	110.54 (2.74)^{c}	0.36 (0.00)^{e}	4.31 (0.24)^{c}	11.06 (3.25)^{a}
3C1k	54.9 (0.77)^{a}	2.41 (0.15)^{d}	20.64 (0.88)^{b}	161.9 (5.25)^{ab}	136.7 (5.53)^{a}	81.58 (6.23)^{abc}	137.93 (5.52)^{a}	0.40 (0.00)^{d}	4.70 (0.09)^{abc}	11.55 (2.77)^{a}
3M1k	53.1 (8.90)^{a}	2.74 (0.61)^{bcd}	21.89 (0.98)^{ab}	160.5 (2.08)^{b}	136.4 (2.19)^{a}	83.02 (2.45)^{ab}	137.51 (2.18)^{a}	0.41 (0.00)^{cd}	5.60 (0.41)^{a}	11.55 (2.77)^{a}
03k	53.6 (1.02)^{a}	3.32 (0.16)^{bcd}	21.9 (0.54)^{ab}	134.3 (2.25)^{d}	116.9 (2.48)^{bc}	65.77 (2.96)$^{b-f}$	116.3 (2.45)^{bc}	0.31 (0.01)^{fg}	4.93 (0.18)^{abc}	11.36 (2.785)^{a}
1C3k	54.8 (0.81)^{a}	3.09 (0.31)^{bcd}	20.6 (0.23)^{b}	139.5 (1.46)^{cd}	117.1 (1.41)^{bc}	69.19 (1.21)$^{b-f}$	118.32 (1.38)^{bc}	0.35 (0.01)^{e}	4.28 (0.27)^{c}	11.39 (2.74)^{a}
1M3k	53.9 (1.35)^{a}	3.19 (0.14)^{bcd}	21.47 (0.96)^{ab}	141.5 (2.14)^{cd}	114.2 (2.52)^{bc}	64.73 (3.10)$^{b-f}$	116.76 (2.43)^{bc}	0.37 (0.00)^{e}	4.98 (0.23)^{abc}	11.68 (1.86)^{a}
3C3k	55.3 (1.61)^{a}	2.54 (0.15)^{cd}	21.002 (0.85^{b}	171.8 (7.10)^{a}	145.4 (7.96)^{a}	76.66 (19.22)$^{a-d}$	145.44 (8.14)^{a}	0.42 (0.008)^{bc}	4.97 (0.39)^{abc}	10.32 (2.09)^{a}
3M3k	54.9 (1.20)^{a}	2.6 (0.18)^{cd}	20.73 (1.02)^{b}	169.2 (2.89)^{ab}	145.1 (3.62)^{a}	90.29 (4.44^{a}	146.03 (3.48^{a}	0.43 (0.001)^{b}	5.51 (0.19)^{ab}	11.15 (2.79)^{a}

Mean values in the same row marked with different letters (a, b, c, d, e, f, g) are significantly different (P<0.05, n=3, Scheffé test).

The ACC (30 ºC 48 h^{-1}) is an indicator of quality used as part of a general quality assessment (HPA, 2009). Satisfactory result indicates a good microbiological quality, whereas an unsatisfactory result implies an investigation into the origin of such results. Typically, as the duration of storage increases the ACC also increases, as such it can be used in food shelf-life testing (HPA, 2009).

In this framework, regardless of the MAP (C or M) and irradiation dose, ACC (30 ºC 48 h^{-1}) increased from 0 to 30 days of storage. This was probably due to the increase of a_w in the cookies as well as to packaging degradation, which favors microbial development. 3CT and 3C1k samples showed acceptable microbiological quality (ACCs > 10^4 < 10^5 cfu g^{-1}), while all other samples were microbiologically satisfactory (ACCs < 10^4 cfu g^{-1}). thus, all the studied situations revealed good product stability throughout the preservation period. MAP treatment, as well as the higher irradiation dose (3k), appeared to be the most effective in maintaining microbiological stability, revealing consistent ACC values lesser than 10^4 cfu g^{-1} (Fig 1). The results obtained for the microbiological stability are consistent with those obtained in other studies for bakery products made with GBF (Borges et al., 2010; Sotiles et al., 2015).

Considering the sensory attributes, relatively to appearance, less scored samples had GBF not irradiated (the least scored was sample 3MT) and the most appreciated sample was irradiated with 3kGy. Relatively to the smell, in day zero the panel appreciated all samples, independently of being or not irradiated, but this attribute decreased along storage period. The preference of texture was on samples at day zero with 3kGy irradiation (03k). In control samples (T), during storage a notorious dislike prevailed in samples packed with the gas mixture (3MT), although this pattern remained lower with CO_2 packaging. Irradiated samples with 1kGy, packed with CO_2 (1C1k and 3C1k), were more appreciated at day

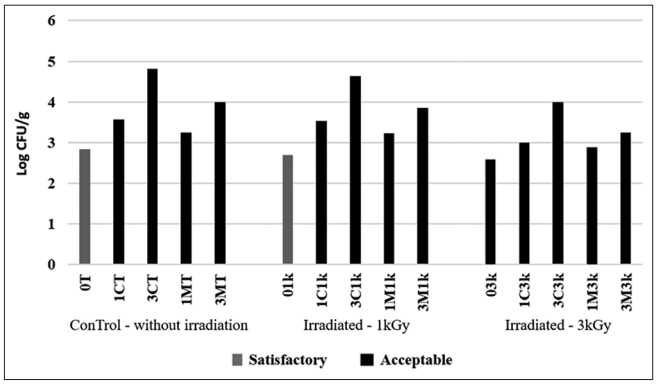

Fig 1. The ACCs means (log CFU g^{-1}) at 0 days, 1 and 3 months of storage (0, 1, 3) of different samples of GBF cookies, prepared with non-irradiated green banana flour (control), and with irradiated flour (1kGy and 3kGy), preserved under two different MAP conditions, namely 2% O_2, 88% N_2 and 10% CO_2 (M), and 100% CO_2 (C).

zero (01k). The attribute banana aroma had shown higher value in samples of 3 kGy GBF, mainly with CO_2 packaged (1C3k and 3C3k), whereas the less scored incorporated GBF without irradiation. Thus, the most appreciated samples incorporated 3 kGy irradiation GBF, mainly 03k, 1M3k and 1C3k. In the meanwhile, the less appreciated cookies had GBF not irradiated and, above all, the sample storage for 3 months in the mixture of gases (3MT). As a general remark the panelists preferred the samples storage in a modified atmosphere package with CO_2 at day zero.

A PCA was also carried out (Table 3) to evaluate the differences between samples subject to different level of radiation intensities, as well as to different storage conditions (atmosphere type and age). PCA considered color attributes (R, G, B and Y), sensory attributes (appearance, smell, texture, banana aroma and overall appreciation), a_W, hardness and counts of mesophilic aerobic bacteria. The similarity map defined by the first two principal components took into account 77.7 % of the total variance. The first component (PC1) by itself condensed 43.24 % and the second component (PC2) represented 34.53 % of the total variance.

*Marked values were considered moderately correlated with the PC; ** marked values were considered strongly correlated with the PC, following the classification used previously (Liu et al., 2003; Palma et al., 2009).

Taking into account PCA results (Fig 2), regarding the day 0, it was found that the sample 03k (3 kGy of radiation) is the most left-handed in the plane. Thus, in this sample the positive attributes predominate. In fact, as the intensity of radiation decreases (01k and 0T), samples will move to the right side, indicating the loss of positive attributes and increasing mesophilic aerobic bacteria, additionally pointing to a shelf life a longer period in samples with higher irradiation.

On the other hand, following the storage period, samples with 3kGy irradiated flour, at the end of first month of storage, moves slightly to the right of the plane (samples 1C3k and 1M3k), indicating a slight loss of positive attributes. This loss of quality becomes notable after three months of storage (3C3k and 3M3k samples were already in the right quadrant of the plane, which is explained by the negative attributes). This behaviour was found in samples with 1 kGy, although slightly prevailing in the right side of the plan (*i.e.,* loss quality). In control samples, the pattern remained similar, but since the starting point is already close to the center of the plane, it was observed that 1 month samples (1CT and 1MT) were already on the positive side PC1. Accordingly, after one month these samples had already negative attributes. At the end of 3 months storage (3CT and 3MT), these samples were at the positive end of CP1, thus being strongly affected by storage time. Concerning at the different packing atmospheres (C and M), the proximity of the samples indicated that both MAP had a similar behaviour to this product.

Table 3: Factor loading of physical, sensory and microbiological parameters on two principal components (PC1 and PC2)

Parameters	PC1	CP2
R	0.64*	0.68*
G	0.54*	0.79**
B	0.42	0.79**
Y	0.57*	0.79**
a_w	0.79**	–0.10
Hardness (N)	–0.58*	–0.05
Appearance	–0.61*	0.67*
Smell	–0.84**	0.19
Texture	–0.79**	0.53*
Banana aroma	–0.59*	0.69*
Overall appreciation	–0.71**	0.62*
Mesophilic aerobic bacteria	0.65*	0.38
Eigenvalue	5.19	4.14
% Variance explained	43.24	34.53
% Cumulative variance	43.24	77.7

*Marked values were considered moderately correlated with the PC;
** marked values were considered strongly correlated with the PC, following the classification used previously (Liu et al., 2003; Palma et al., 2009).

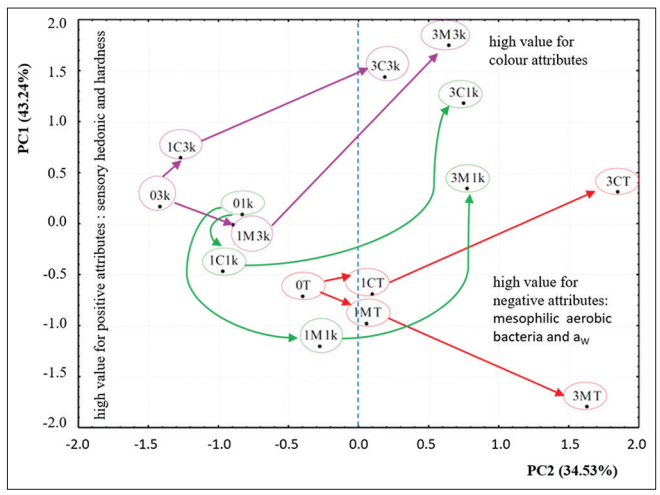

Fig 2. PC1 vs. PC2 projection of samples. The most important parameters for the definition of the two components are shown on the plot, indicating the direction in which the value of the parameter increases. Different samples of GBF cookies prepared with non-irradiated green banana flour (control) and whit irradiated flour (1 kGy and 3 kGy), preserved under two different MAP conditions, namely 2 % O_2, 88 % N_2 and 10 % CO_2 (M), and 100 % CO_2 (C), during 0 days, 1 and 3 months of storage (0, 1, 3)

CONCLUSION

Both MAP samples had an acceptable behaviour in sensory, microbiological and physical and chemical parameters. Moreover, CO_2 packaged samples showed some collapse due to CO_2 absorption by cookies, which

might be consequence of its porosity. Therefore CO_2 modified atmosphere is not the most adequate for this type of product. Hence, N_2 to a CO_2 atmosphere must be added to avoid any eventual collapse. Relatively to the irradiation level, it seems that samples with green flour incorporation have best results (sensory attributes and microbial parameters) after a 3 month storage period if irradiated with 3kGy. The less acceptable samples had GBF not irradiated (control sample – T) Indeed, after 3 months storage, the control sample (3MT) has a decreased hardness, an increase in moisture content and a_w. Finally, the technology of food irradiation may be recommended in GBF preservation.

Authors' Contributions

The contribution of all authors remained similar.

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