Easter Eggs and Other Cocoa (*Theobroma cacao* L) Products Quality: Insects, Mites, Fungi and Packaging versus Critical Control Points

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Abstract

The samples collected were sold in Florianopolis, Southern Brazil, and were tested for light filths (insects/larvae/rodent hair/mites-live/dead/fragments),as well as for fungi(total load/genera/species) and packaging (material type/sealing). From the total samples studied, different biological contaminants were detected in 53% of them. Fungi were isolated in 41% of the total samples, mainly Penicillium, Aspergillus and Rhyzopus. Regarding packagings, despite of their number of different material layers, 9% did not comply to the current packaging regulation regarding perforations stains and insect/larvae presence. In addition, a total of 15% of the inner contents howed darker spots, oxidation/rancid odor and perforations too. Indeed, the chocolate products of those insects damaged packages, showed also live larvae in the chocolate mass, quite disgusting to consumers.The contamination CPs of the factory processing steps identified were: raw materials reception; chocolate manufacturing; packaging; storage; transport and commercialization (sold too close to shelf life expiring date).

Keywords: cocoa, *Theobroma cacao*, chocolate, Easter egg, packaging, insects, fungi

1. *Introduction*

According to the Cocoa, Peanuts and Candies Brazilian Industries Association (ABICAB), the country chocolate production has shown an increase of 12 % in the last years, from 562,000 tons in the year 2010 to 790,000 tons in 2013, setting the country as the third largest market in the world, just behind the United States and Germany (ABICAB 2014). Only in 2013 the country produced 18,000 tons of chocolate (80 million Easter eggs) just for the country´s Easter season. One of the main reasons for that high consumption is the current Class C Brazilian population increasing income (CEPLAC 2013; ABICAB 2014).

Regarding the quality of chocolate products, it depends upon several factors involving different stages of processing. Although chocolate is prepared from a mixture of cocoa paste, sugar and milk, its production starts far behind, from the cocoa bean (*Theobroma cacao* L.) cultivation / harvesting / fermentation / drying / roasting & grinding for cocoa paste formation which is sold to the chocolate manufacturers for further processing (Beckett 1994; Nachtigall 1999; Oetterer *et al.* 2006).

For the production of a good and safe quality chocolate, it is important to pay attention on the two main processing steps: the (a) *cocoa bean to paste preparation* and (b) *chocolate mixture to final product release*, in which the temperature levels and their variation are crucial. In (a), for the processing steps of bean fermentation, drying, roasting and paste formation it is necessary to keep the temperatures at 50, 55, 120 and 42°C, respectively. On the other hand, for (b) the chocolate processing of mashing (ingredients mixing), refining, maturation, conching, tempering and molding the temperatures need to be around 45/65, 50, 50, 46/70, 40/50 and 18°C, respectively (i.e, temperatures of melting and formatting) (Beckett 1994; Bastos 2003; Oetterer *et al*. 2006). However, none of them are enough to kill some biological contaminants such as insects and their larvae and so fungi spores (Beckett 1994).

All those steps and mild temperatures (up to the ready-made product), followed by storages, transportation and commercialization present conditions to allow the entry and/or proliferation of several biological contaminants, such as insects, (their larvae / pupa stages) mites and contact to rodents (indicated by hair presence) (Lorini *et al*. 2002; Lorini *et al*. 2008; Mello *et al*. 2004; Gredilha *et al*. 2007; Kreibich 2013). These contaminants (called light filth) are important microorganisms carriers (fungi, bacteria, viruses) including their residues and metabolites (faeces / toxins) to food and temperature resistant (Koerich de Souza 2013; Kreibich 2013). The mites, which develop in stored products, can trigger consumers' allergic reactions. In addition, improper packaging can allow both, contaminants as well as aroma transfer to chocolate components, leading to mycotoxin formation and/or lipid oxidation (Mello *et al*. 2004; Gredilha *et al*. 2007).

For those reasons, chocolate products should be manufactured with selected raw materials (cocoa paste), made with healthy and clean (free of insects / parasites / plant waste / animal and earthy matter) cocoa beans, as well as with utilization of packaging that protect the final product adequately and well / tightly sealed to avoid contaminants entry. However, there are little information and lack of proper inspection on those stages of cocoa paste preparation (in the factories) or chocolate products (in the industries facilities). In addition, Easter eggs, which are highly produced to be consumed, in a short period of time, need to be checked for quality / safety conditions and to learn what is done with seasons left overs; not sold products.

Therefore, this study aimed to evaluate the quality and safety of Easter eggs and other chocolate products sold in the Southern of Brazil for either macro and stereo / micro (light filth - microganism carriers) biological agents contamination, as well as to assess and recommend implementation of systems (critical points control) to improve the quality of chocolate through manufacturing procedures.

2. Material and Methods

2.1 Material

2.1.1 Samples: chocolate products (34), milk and semi-sweet Type, of different brands, in the formats of ***Easter eggs*** [plain and stuffed], ***bars*** [with and without fruit, nuts, and/or mixtures of both], ***bonbons*** [(a) plain and (b) stuffed with (b.1) fruit/nuts/grains or (b.2) artificial fruit flavors cream],***truffles***[creamy or filled with fruit/nuts] and ***smarties*** [milk Type tablets with colored cover]. Table 1 shows the Easter eggs and cocoa product samples different characteristics (format/chocolate Type/filling/brands/weight/number).

2.1.2 Chemicals and culture media:(*a) chemicals* - potassium hydrogen phosphate, sodium nitrate, potassium chloride, magnesium sulfate, ferrous sulfate and sodium lauryl sulfate, all from Vetec (Duque de Caxias, RJ, Brazil); liquid petrolatum, sucrose, glycerol, chloramphenicol, all from Kyma (Americana, Brazil); lactophenol cotton blue, Laborclin (Pinhais, PR, Brazil) and *(b) culture media* - potato dextrose agar (PDA), malt extract (MEA) and bacteriological peptone, all from Himedia (Curitiba, Brazil), Czapek-dox, 25 % glycerol nitrate (G25N), Czapek yeast extract (CYA), all Vetec (Duque de Caxias, Brazil).

2.1.3 Equipment*:* stereo microscope, model MZ-16 (Wetzlar, Germany); light microscope, model CH-Bl45-2, Olympus (Tokyo, Japan); microscope video printer, model Image, Pro Express (Baltimore, USA); semi-analytical scale, Kern (Balingen, Germany); heater platform, Dist (Florianopolis, Brazil); stirring hot plate, model AV-50, Dist(Florianopolis, Brazil); autoclave, Phoenix (Araraquara, Brazil); microwave, Sharp (Sao Paulo, Brazil); sample homogenizer, model MA440/CF, Marconi (Piracicaba, Brazil); microbiological oven, Quimis (Diadema, Brazil); colony counter, model CP608, Phoenix (Araraquara, Brazil); heating block, model EQ-18MT, Tecnal (Piracicaba, Brazil); ultra-violet cabin model model 118, (short & long wavelength: 254 & 360 nm, respectively), Dist (Florianópolis, Brazil) and vacuum pump, model TE058, Millipore (São Paulo, Brazil).

2.1.4. Others: Wildman filth bottle trap (Erlenmeyer-2 L with metal rod and rubber cap – 250 and 60/30/50 mm for length and lower/upper/height, respectively), Dist (Florianopolis, Brazil); Buchner funnel (13 cm diameter); glass bottles with polyethylene cap (200 mL); granulometric sieves (200 mesh), Bertel (Sao Paulo, Brazil); filter paper No. 4, Whatman (Maidston, England); magnetic bar (250/80 mm, length/width) and Software Pro-Express 4.0, Windows 95/NT/98, (Silver Spring, MS, USA).

2.2 Methods

2.2.1 Sample collection and Preparation:*(a) collection* - chocolate products (eggs*/bars/bonbons truffles and smarties) were randomly collected from supermarkets in the city of Florianópolis, state of Santa Catarina, Southern Brazil, from March/2013 to March/2014 (*Easter eggs were only collected during both years Easter time – around 2 months before and up to 2 days after Easter –as those product are only commercialized during Easter seasons). Samples were registered, inspected for sealing integrity and stored in a light free area for further analyses. *(b) preparation* - each chocolate sample was aseptically packaging opened followed by *(b.1) packaging* and *(b.2) internal content* (chocolate product), separation in two different containers for the following analysis: *packaging/label* (MACRO: characteristics/integrity) and *internal content* (MACRO: characteristics / integrity / composition / expiring date *versus purchasing date*&STEREO/MICRO: light filth - insects/mites/rodent hair and mycology - total load/genera/species/toxigenicity).

2.2.2 Packaging and Internal Product Characteristics & Integrity Evaluation: both, packaging and the inner chocolate content were evaluated macro/stereoscospicaly, by checking visual (naked eye/stereoscope) alterations. *(a) packaging* - samples were evaluated for their type (material used / number of protective layers), integrity (presence of perforations / color changes / stains / defective sealing/ format deformation) according to the method of de Souza Koerich, et al (2013). On the other hand, the packaging functions such as product containment; type of protective materials, ease of handling / storage / transportation, regarding the Brazilian and international regulations, were also evaluated by applying the Moura and Banzato (1997) method. *(b) internal content* - the following characteristics of each product were macro / stereo evaluated as well as obtained from the labels information: format / chocolate type / plain or stuffed / weight / batch number / expiring date / apart from products sensorial characteristics (color/odor/texture), integrity (damages on/in/the products) and composition (cocoa/lipids/sucrose).

2.2.3 Light Filth Analysis: was carried out according to the AOAC (2005) method, art. 965.38 by stereoscopy for cocoa, chocolate and candies. Briefly, *(a) fat extraction* - a portion of the grind sample (100 g), had sodium lauryl sulfate 2% (500 mL) added, followed by sieve transfer, with hot water added (until the fat sample did not pass over the sieve); *(b) transfer* - the content was transferred to a Wildman filth bottle trap maintained under heating (10 min). Next, the flask was cooled (room temperature) and liquid petrolatum added, followed by water (until completion-1L). The flask trap stirring rod was held with tweezers above the liquid surface (2 immiscible phases) and added a magnetic stirring bar to accelerate separation, with further heating (5 min). At the end of this step, the flask was allowed to stand (30 min); *(c) light filth separation and microscopic identification* - petroleum phase (with a light filths) was removed by vacuum filtration and dried at 105°C. The filter paper was transferred to the stereo microscope, where light filths reading (counting) and image identification (filths characterizations) through an Image-Pro Express software, was performed.

2.2.4 Mycology Tests: *(a) total fungi count* - samples (25g) were transferred into sterile polyethylene bags and added 0.1% peptone water (225 ml), followed by homogenization in a stomacher. Each diluted sample, had a volume of 100 µL (0.1 ml) inoculated to PDA medium surface (n = 2) containing chloramphenicol (100 mg/L) in a laminar flow hood and incubated at 25°C±1°C for 7 days (Silva *et al*. 2010). The colonies developed were counted and expressed as colony forming unit per gram (CFU / g). *(b) colonies isolation* - the colonies were then transferred to MEA, G25N & CYA, incubated (25°C±1°C for 7 days) and observed macroscopically (diameters and individual characteristics) followed by microscopy identification of fungi genera/species (Raper and Fennel 1965; Pitt 1979; Barnett and Hunter 1986) and *(c) genera/species identification* - a support was added on a glass slide in a Petri dish, then cubes (5 cm) of G25N grown colony were placed in the middle and lid covered. Inside the plate, was added a piece of moist cotton. After incubation, the stained slides were viewed under light microscope and identified according to Raper and Fennell (1965); Pitt (1979); Barnett and Hunter (1986).

2.5 Contamination Critical Control Points: the following stages of chocolate production were evaluated to identify the possible critical points (CP) of contaminants (rodents/insects/mites/fungi) entry / proliferation, including temperatures conditions - *raw material reception* (quality, damage, moisture content), *processing* (temperature, time), *storage* (type of warehouse, with / without ventilation, temperature, time) and *transport* / *selling* (careful handling, temperature, expiring date) points. From the CP identification, control measures for reduction / prevention of those contaminant were recommended.

Figure 1 shows the flowchart of all steps involved in the study on biological contaminants of the Easter eggs and chocolate products.

3. Results and Discussion

From the data obtained, it was possible to register some macro & micro alterations caused by the presence of biological contaminants and so their detection in the Easter eggs and chocolate products surveyed. They were detected, either in the *packaging* (material/sealing damages) and *inner contents* (chocolate mass perforations / live & dead larvae presence). Also light filth (which can only be detected by means of a prior solvent extraction from chocolate sample and through stereo microscopy) were registered in some samples and so storage fungi. Figure 2 and Tables 1-4 show the packaging & chocolate internal products macro alterations, light filth (insects, larvae and mites -whole/fragments) and fungi data, respectively.

3.1 Packaging and Internal Products Macro Characteristics and Integrity

(a) Packaging: as far as chocolate products packaging and quality are concerned, most of them (91.2 %) were in good conditions (not presenting damage or stains), made of resistant material (polyethylene, aluminum and cardboard in several layers) and sealed tight (nor faulty, unbroken or loose sealing), indicating the use of adequate materials and calibrated sealing equipment (Table 1). On the other hand, the other 8.8 % samples packaging (Easter egg, chocolate bar and truffles - one sample each) showed visible alterations as follows. The Easter egg, although was double packaged into two separated material types (a transparent cellophane wrapping and a hard cardboard box), which should protect it, had visible insect damages (perforations and stains), apart from the presence of live larvae, contributing to the inner product contamination (Figures 2a.1, 2a.2). Apart from that, the thin cellophane sheet was only wrapped around the chocolate egg tight with a loose silky lace or top (a insects way in). On the other hand, the box was not sealed at the bottom on top (only closed together by folded flaps) (Figure 2a.1). All, indicating the packaging protection fragility. Those lack of sealing, might have allowed biological contaminants entry, thus causing those detected damages. Indeed, live larvae were detected inside the egg (Section 3.2). Regarding the chocolate bar sample (containing raisins and cashew nuts), it also presented packaging perforations and stains (Figure 2.a.3) and so larvae proliferation (Section 3.2). Regardless of the damages and contamination detected, the alterated packaging samples (Easter egg and bar) were within their shelf life validity period. However, somewhat short ie., only at 3 and 4 months to their expiring date, respectively (Table 1). The type of packaging/sealing utilized together with the storage time (closer to the expiring date) and temperature would certainly allow insects proliferation (conditions for eggs to hatch). Regarding the information that ensures the time for chocolate commercialization / consumption – i.e. date of batch/lot production, none of the packaging evaluated had referred the *date they were manufactured on the label* (so why shelf life setting if there is no starting point?). That leaves the consumer with the uncertainty on when the product was actually produced or whether it was sent to be re-processed (end of season's stores left overs returned to industry for new chocolate processing). There is no enforcement by regulation to refrain that.

(b) Internal Product: CHOCOLATE MASS - as expected, apart from the 85.3% in good conditions internal products samples, those packaging integrity alterations detected above (Section 3.1.a), led to similar alterations (chocolate mass perforations and visible contaminants detection) of those chocolate samples (Figure 2b, Table 1). In addition, apart from the two samples (Easter egg and bar), other chocolate products (bars and truffles) also had alterations corresponding to 14.7% (5) of them. It was observed whitish stains (discoloration in some chocolate parts of the products), high fat flavor, chocolate mass perforations and larvae (live) presence in the surveyed samples. They were mainly in bars, followed by the Easter egg and truffle in 8.85, 2.95 and 2.95 % of the samples, respectively (Figure 2b, Table 1). In the positive total chocolate bar samples, their contamination probably occurred due to the mild temperatures applied in the processing steps including tempering. Important to emphasize that, the temperatures of 40-50°C, do not kill or interfere on the insects proliferation (larvae to pupa or eggs hatching), either during the chocolate mass melting or solidification stages.

Especially after packaging (micro environment) and storage (temperature & time), if eggs are also present. As far as chocolate products ingredients (mono/di- carbohydrates), such as *sugar* (sucrose) and *dried fruits* (fructose) are concerned, they can attract/bring ants (*Linnepithema* genera) into the product (Lorini 2002). Apart from *cocoa beans*, also the *cocoa paste* may carry insects such as moths (*Ephestia* genera) allowing further product infestations (Lorini and Schneider 1994; Lorini 1998) getting into the paste, either during its preparation or storage (Kreibich 2013). Although the chocolate mass *fat* content (esterified soya oil and cocoa butter) does not add to the biological contaminants, it may harm consumers' health as its percentage was rather high (2.9-5.2 g *in a total 16 g chocolate portion*), and so the *sugar* (7.0-12.0 g *per 16 g*), the possible source of insect contamination (ants) detected in the current work (Table 1). CHOCOLATE FILLING & STUFFING - regarding the other ingredients included in the chocolate products, either as a *filling* (mixed in the chocolate mass – Figure 2b.6) or *stuffing* (added into a chocolate cavity - Figures 2b.2, 2b.3), they were of different groups: *(a) dry fruits* (raisins / sherries / strawberries*), (b) nuts* (cashew / peanuts / hazel / coconuts), *(c) cereal* (barley malt / corn & rice flakes), *(d) soy bean creams* (different artificial flavors added) and *(e) liquor*. Those ingredients could also contribute to the inclusion of some biological contaminants into the chocolate samples (Figures 2b.3, b.6). The only detected were those of cocoa (larvae: *Ephestia elutella*) and sugar/fruits characteristics, **(sugar ants of the genus *Camponitus* and *Linepithema* and dry fruit beetle of the genus *Carpophilus*). See details in Section 3.2. Important to emphasize that, dry fruits have been reported being fungi infected which may lead to toxin contamination (Scussel *et al*. 2002; Souza Koerich *et al*. 2010), thus care on selecting the raw materials and ingredients are quite crucial. However not highly detected in the current work (Section 3.3 - Tables 2,4) (Drusch and Ragab 2003; Souza Koerich *et al*. 2010).As far as the type of the chocolate mass, are concerned, some chocolate products had in there compositions: milk whey /soya oil / different percentages of sugar, which also could make a difference on contaminants presence (less in esterefied soya oil creams or milk whey).

3.2. Light Filth Identification, Quantification versus Regulation

A total of 53% of the samples surveyed, present some type of biological impurities that usually are not visible in the samples (Section 3.1). They need to be submitted to solvents extraction and concentration (for possible stereoscopy visualization) prior identification and quantification. They are the light filth, presented whole or as fragments (ground during processing), and considered indicators of bad quality and handling conditions of raw material utilized. In the current study, insects, either whole or fragments and so their larvae and pupa (growing stages) were identified. Tables 2 and 3 show the positive samples percentage, each light filth type detected, their characteristics and effects on food/consumers. From the positive samples (eggs, bars, bonbons or truffles), 29.4(10) and 23.5(8) % of them had either only one or more than one type (2 to 5) of light filth per sample, respectively.

The detected ones were (a) insects (a.1) whole & fragments of ants (*Camponitus consobrinus* L.; *Linepithema humile*) and (a.2) *larvae & pupa stages*, inactive immature insect form between larva an adult of chocolate moth (*Ephestiaelutella*) (Figure 3),followed by *(b) whole mites (Aceria anthocoptes*). They were found in 20 / 20 / 10 / 50 % of eggs / bars / bonbons / smarties samples, respectively. Only the truffles did not have light filth detected. Regarding the source of insects infestation, i.e., the entrance of *ants*, they probably came from the ingredients (sugar / dried fruits) added at the chocolate mixture and / or the filling / stuffing inclusion step (lack of fruits/nuts quality / factory environment cleaning) or got into the sugar/dry fruits during their storage - prior mixture. On the other hand, the *chocolate moth larvae*, which are commonly found (accepted by regulation though - FAO, 2014), might came from either through cocoa paste infestation (at its factory), ingredients mix (during industry chocolate production) and/or the final products moth eggs hatching inside packaging (Figure 2.a) As they are resistant and persistent to the mild processing temperatures and commercialization storage at selling stores. Important to emphasize that the total moth life cycle is 35 to 50 days. Adult phase lasts 10 to 20 days; the eggs delivery take 4 to 7 days to hatching; has 4 to 8 weeks and pupa has 5 to 10 days, respectively, as well as the harm to food & consumers they can cause (Lorini and Schneider 1994; Lorini 2008).

On the other hand, *mites* (allergy promoters) were detected in 17.64 (5) % of the samples, mainly in the chocolate bars, followed by the Easter eggs and only one bonbon and smartie sample (Table 2). Fortunately, rodents' hair was not detected in any of the samples, which indicates that both processing plants (cocoa paste factories and chocolate industries) applied rodent control programs, thus preventing several rats transmitted diseases related to products (Table 3) (Giordano *et al*. 2008; Lorini 2008; Kreibich 2013).

QUALITY/SAFETY INDICATORS - the main purpose of controlling the light filths in food, is that they are indicators of raw material, ingredients and processes sanitary quality and safety. They are considered vectors of several diseases (bacteria/fungi/virus) that may affect consumers and also can cause alteration to the final products (reduction of dry matter / nutritional value/ sensory characteristics) leading to fermentation and/or deterioration. Therefore, entry of light filth in food should be minimized and/or controlled to protect consumers and keep food/chocolate products sensory and nutritional quality. Under the Brazilian Resolution14/2014, it is considered harmful to human health different matters detected either macroor microscopically apart from insects also (at any development stage,living or dead, whole or in fragments) recognized as vectors; parasites; insects excrement; hard or sharp objects that may cause injury to the consumer. Although, larvae do not harm health directly, they will bevery difficult to be accepted byconsumertofindgrubsandinsectsinsidethechocolate (BRASIL 2014).

3.3 Fungi Isolated in the Chocolate Products and their Identification

As expected, the chocolate products samples total fungi load was not high, as they have low humidity (moisture content and water activity of <3% and <0.2, respectively), thus not optimum environment for them to grow (BRASIL 1978). Tables 2 and 4 show the fungi quantitative data and the genera/species isolated from the different chocolate sample types. A high percentage of the samples (59 %) did not present fungi growth (NG) ie., no spores contamination that could allow them to grow on the mycological media utilized (Table 2). Only 41 % of the samples had them grown, whoever at rather low count (1-3x10 UFC/g) and below the maximum allowed by the Brazilian regulation ($<1x10^3$) (BRASIL 1978). The Easter eggs were more contaminated and surprisingly no truffles samples had fungi isolated (even with their varied types of stuffing utilized). Despite of the low fungi load, from the 41% of the samples fungi strains isolated, 19, 18 and 4% were of *Aspergillus*, *Penicillium* and *Rhysopus* spps, respectively (Table 4). Important to emphasize that one isolated strain was aflatoxigenic, indicating the importance to control the samples fungi growth conditions as well as to select safe/high quality raw materials (aflatoxin free) to avoid final product contamination. That sample did not contain either nuts or cereal stuffing (which could be the fungi & toxin vectors). Studies conducted have reported high fungi growth, mainly on cocoa beans (raw material) due to faulty/inefficient (heterogeneous/high humidity content) drying processes (Samson *et al*. 2002;Pitt and Hocking 2009; Copetti *et al*.2011; Genovese 2009). In addition to beans fungi deterioration and consequent influence on the quality of cocoa paste and chocolate, their metabolites (mycotoxins) can remain stable in the final products due to whole processing mild temperatures / conditions applied (Scussel 2002; Neto 2009).

3.4 Whole Processing Stages of Contamination Critical Control Points

From the different samples macro packaging / inner product damages / insects visualization and the light filth biological contaminants detected, it was possible to identify the most prone critical CPs of their entry & proliferation, thus to recommend some control measures to be applied. ***(a) Contaminants critical control points****:* the main CPs that contamination can entry the processing stages were at the *(a.1) factory reception* due to the use of low quality *raw materials* (cocoa paste infested - use of moldy/insect infested cocoa beans and so sugar with ants), as well as during the *(a.2) chocolate manufacturing* (and / or reprocessing) with proliferation or access of those contaminants to food. Also from *the packaging* (rupture or its poor quality), *transport*, *storage* and / or *commercialization* (lack of environment cleaning). ***(b) Recommended control measures:*** among the entry points of contamination and its possible control measures to be adopted, it was observed that these contaminants must have gotten into the products during the steps: *reception* of raw materials (quality cocoa and sugar paste), *processing* (cleaning machine / stops during processing), *packaging* (packaging / sealing of low quality); *handling* (during transport and marketing) and *shelf life* (about the time allowed). Actions should be applied from crop management (to get good quality cocoa beans followed by their proper fermentation and drying. The same showed occurs with sugar and thefat (to be added) cocoa butter and sterified soil to raw materials (Good Management Practices), in the manufacture of food during the production stages (HPCC, ISO), and work to clarify the responsibility of producers, transporters, distributors, and marketers of foods that contribute to the reduction of population exposure to consumption of contaminated food and consequently the reduction of health risk .

Important to emphasize that the light filths are transmitter of deterioration, pathogenic and/or toxigenic microorganisms to food, which may allow bacterial and fungal spreading, as well as promote the development of diseases and intoxications (mycotoxins).

4. Conclusion

Most of the Easter eggs and cocoa products packaging characteristics and integrity were adequate, however only some of them had visible alterations. Despite that, no consumer would like to see/consume a cocoa product within a damaged pack, which is an indication of finding further problems inside (confirmed in the current study: live larvae inside pack and product).

Regarding the shelf life date, the Easter eggs samples had it too close from purchasing (expiring date) and none of the cocoa products surveyed had the processing date registered on the label. Chocolate reprocessing i.e., left over's returned from selling stores to the industries to be re-processed into new chocolate products should not be allowed, however there is no regulation to enforce that yet.

On the other hand, the samples inner content showed more damages than those wrapped in the damaged packaging. They had higher percentage of stains, rancid fat flavor, and larvae perforations. Over 50 % of chocolate products – Easter eggs and bars - contained some light filth (whole/fragmented insects, live/dead larvae/pupa and mites). Reduced number of mites is allowed by law as long as it doesn't mean the final product hazards health consumption). Based on current regulation, it allows sample evaluated for consumption (except the sample with whole insects - ants).

The fungi assessment, allowed us to register Aspergillus, Penicillium and Rhyzopus samples, in low count though, below current regulation. However, an aflatoxigenic strain was isolated indicating need for care on processing and selection of raw material (mainly cocoa bean) used, since that are aflatoxins producers.

Despite that Regulations do not consider light filth presence as harm; no consumer accepts seeing live (moving) or dead larvae in the chocolate mass/stuffing.

There is a need of standard implementation of the established processes applied to the chocolate industry in order to solve these PCs and ensure products integrity, safety to assure consumers acceptability and so the left over re-processing.

The current study can serve as subsidy for chocolate industries to reduce insect infestation; regulatory agencies to future biological contaminants adjustments and applications on preventive and control measures to improve product quality.

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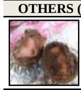
Table 1: Characteristics and Integrity Evaluation of Packaging and Internal Content of Easter Eggs and Other Chocolate Products Sold in Florianopolis, Southern Brazil (March 2013 to March 2014)

CHOCOLATE PRODUCTS						PACKAGING					INNER CONTENT												
						Characteristics			Integrity		Characteristics					Integrity			Composition* (16 g)				To expiring date gap (months)
												Odor		Texture		Damage / alterations			Lipid				
Format	Type	Filling	Brand	Weight/N°	Produced in	Type	Layers	Sealing	Damage	Stains	Color	Chocolate	Stuffing	Base	Filling	Surface	Base	Thickness	Sat/unsat(g)	Trans	Total g(%)	Sucrose g(%)	
EGGS (n=10)																							
	Milk	Ns	D	200/1	SP	Cb/Al	2	Gl/Fo	P	I	Bw2**	Chr	NA	F	NS	La**	La***	P*	1.7/1.2	No	2.90(18.1)	8.6(53.8)	2
		Ns	J	250/1	PR	Cb/Al/	3	Gl/Fo	ND	ND	Bw2	Chr	NA	F	NS	N	N	N	4.35/0.17	No	4.52(28.3)	NI(NA)	3
		Ns	K	300/1	SP	Al	1	Fo	ND	ND	Bw2	Fa	NA	F	NS	N	N	N	NI	No	NI(NA)	NI(NA)	3
		Ns	A	230//1	SP	Cb/Al	2	Gl/Fo	ND	ND	Bw2	Chr	NA	F	NS	N	N	N	2.62/2.31	No	4.93(30.8)	NI(NA)	5
		Ns	A	230/1	PR	Cb/Al	2	Gl/Fo	ND	ND	Bw1	Chr	NA	F	NS	N	N	N	2.8/3.0	No	4.80(30.0)	NI(NA)	5
		Ns	E	230/1	SP	Cb/Al	2	Gl/Fo	ND	ND	Bw1	Chr	NA	F	NS	N	N	N	2.0/1.5	No	3.50(21.9)	NI(NA)	3
		Ns	L	230/1	SP	Cb/Al	2	Gl/Fo	ND	ND	Bw1	Chr	NA	F	NS	N	N	N	2.9/1.9	No	4.80(30.0)	NI(NA)	3
		Ns	M	200/1	SP	Cb/Al	2	Gl/Fo	ND	ND	Bw1	Chr	NA	F	NS	N	N	N	4.5/0.7	No	5.20(32.5)	NI(NA)	4
		Ns	N	200/1	RS	Cb/Al	2	Gl/Fo	ND	ND	Bw2	Chr	NA	F	NS	N	N	N	2.4/2.1	No	4.50(28.1)	NI(NA)	4
	Semiswee	Ns	L	230/1	SP	Cb/Al	2	Gl/Fo	ND	ND	Bw3	Chr	NA	F	NS	N	N	N	2.6/4.3	No	4.30(26.9)	NI(NA)	3
BARS (n=10)																							
	Milk	Cashew / raisins	A	150/1	ES	Pe/Al	1	Ht	P	I	Br2**	Chr	NA	F	NS	La*	La*	P*	2.4/2.7	No	5.06(31.6)	8.3(51.9)	4
		Cashew / raisins	A	150/1	ES	Pe/Al	1	Ht	ND	ND	Bw2	Chr	NA	F	NS	N	N	N	2.4/2.7	No	5.06(31.6)	8.3(51.9)	10
		Cashew / raisins	A	150/1	ES	Pe/Al	1	Ht	ND	ND	Bw2	Chr	NA	F	NS	N	N	N	2.4/2.7	No	5.06(31.6)	8.3(51.9)	10
		Cashew / raisins	A	150/1	ES	Pe/Al	1	Ht	ND	ND	Bw2	Chr	NA	F	NS	N	N	N	2.4/2.7	No	5.06(31.6)	8.3(51.9)	10
		Cashew / raisins	A	150/1	ES	Pe/Al	1	Ht	ND	ND	Bw2	Chr	NA	F	NS	N	N	N	2.4/2.7	No	5.06(31.6)	8.3(51.9)	10
		Cashew / raisins	A	150/1	ES	Pe/Al	1	Ht	ND	ND	Bw2	Chr	NA	F	NS	N	N	N	2.4/2.7	No	5.06(31.6)	8.3(51.9)	10
		Cashew / raisins																					
		Cashew / raisins																					
		Cashew / raisins																					
		NI	A	150/1	SP	Pe/Al	1	Ht	ND	ND	Bw2	Chr	NA	F	NS	N	N	N	2.9/2.2	No	5.06(31.6)	9.0(56.3)	7
		NI	C	130/1	PR	Pe/Al	1	Ht	ND	ND	Bw1	Chr	NA	F	NS	G	N	Ae	2.9/2.0	No	4.90(30.6)	9.0(56.3)	8
		NI	B	170/1	ES	Pe/Al	1	Ht	ND	ND	Bw1	Chr	NA	F	NS	Op	N	Ae	2.8/1.8	No	4.60(28.8)	9.6(60.0)	9
	Semisweet	NI	A	150/1	SP	Pe/Al	1	Ht	ND	ND	Bw3	Chr	NA	F	NS	N	N	N	2.8/2.4	No	5.20(32.5)	9.0(56.3)	10
BONBONS (n=10)																							
	Milk	Af (grape)	H	9/50	SP	Pe	2	Ht	ND	ND	Bw1	Pt	Pt	S	S	N	N	N	1.6/0.6	No	2.20(13.8)	12.0(75.0)	10
		Af (strawberry)	H	9/50	SP	Pe	1	Ht	ND	ND	Bw1	Fa	Fa	S	S	N	N	N	1.6/0.6	No	2.20(13.8)	12.0(75.0)	10
		Cashew / Peanut	B	22/5	SP	Pe	2	Ht	ND	ND	Bw2	Chr	Pt	F	S	N	N	N	2.3/2.4	No	4.70(29.4)	9.7(60.6)	8
		Cherry/liquor	E	16/12	SP	Cb/Al	2	Gl/Fo	ND	ND	Bw2**	Chr	Ch/Li	S	S	N*	N	N	2.0/1.5	No	3.50(21.9)	9.7(60.6)	8
		Cream / Peanut	F	15/13	SP	Pe	1	Ht	ND	ND	Bw1	Fa	Pt/Fa	S	S	N	N	N	2.4/2.6	No	5.00(31.3)	7.0(43.8)	5
		Coconut	G	18/22	ES	Cb/Pe	2	Ht	ND	ND	Bw1	Chr	Cn	S	S	N	N	N	2.3/1.0	No	3.30(20.6)	NI(NA)	5
		Malted	G	28/14	ES	Cb/Pe	2	Ht/Fo	ND	ND	Bw2	Fa	Chr	F	S	N	N	N	0.35/2.55	No	2.90(18.1)	NI(NA)	5
		Peanut	G	19/21	SP	Cb/Pe	2	Ht	ND	ND	Bw2	Chr	Pt	F	S	N	N	N	2.2/2.0	No	4.20(26.3)	NI(NA)	7
		Rice flakes	G	16/25	ES	Cb/Pe	1	Ht/Fo	ND	ND	Bw2	Chr	Chr	S	S	N	N	N	1.1/1.0	No	2.10(13.1)	NI(NA)	7
	Milk whey	Af (peanut)	I	15/40	SP	Pe	1	Ht/Fo	ND	ND	Bw2	Chr	Pt/Fa	F	S	N	N	N	1.0/2.0	No	3.00(18.8)	NI(NA)	9
OTHERS (n=4)																							
 Truffles	Milk	Strawberry	D	30/4	SP	Pe	1	Fs	P	I	Bw2	Chr	Chr	F	S	La**	P**	P	3.1/2.0	No	5.10(31.9)	7.7(48.1)	6
	Soy	Hazelnut	J	13/6	RS	Cb/Al	2	Fs	ND	ND	Bw2	Chr	Fa	S	Cr	N	N	N	2.5/1.3	No	3.80(23.8)	7.7(48.1)	7
Smarties	Milk	Ns	B	80/1	PR	Pe	1	Ht	ND	ND	Bw2	Chr	NA	F	S	N	N	N	2.1/3.1	No	5.20(32.5)	NI(NA)	10
		Ns	K	52/1	SP	Pe	1	Ht	ND	ND	Bw2	Chr	NA	F	S	N	N	N	1.2/0.8	No	2.00(12.5)	8.8(55.0)	10

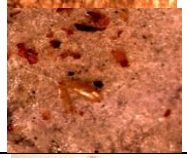
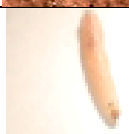
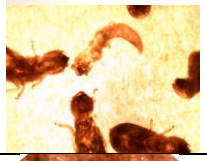
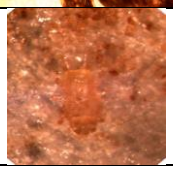
Ae: aerated
Af: artificial flavor
Al: aluminum
Bw1: light brown
Bw2: medium brown
Bw3: dark brown
Cb: cardboard
Chr: characteristic
Ch: cherry
Cn: coconut
Cr: cream
ES: Espirito Santo State
F: firm
Fa: fat
Fo: folding
Ld: larvae (dead)
G: glossy
Gl: glue
Ht: heat sealed
I: identified
La: larvae (alive)
Li: liquor
N: normal
NA :not applicable
No: does not contain
ND: not detected
NS: not stuffed
NI: not informed
Op: opaque
P: presence
Pe: polyethylene
PR: Parana State
Pt: peanut
RS: Rio Grande do Sul state
S: soft
SP: Sao Paulo State
* obtained from packaging label
** small white spots
*** several white spots
✱no information on production date

Table 2: Different Light Filth and Fungi Detected in Easter Eggs and Other Chocolate Products Commercialized in Florianopolis, Santa Catarina State, Southern Brazil (March 2013 to March 2014)

Chocolate Product		Light Filth							Fungi	
		INSECTS		STAGE		Rodent Hair	Mites	Total / Sample	Total Count (Cfu/G)	Genera
Type	Number	Whole	Fragment	Larvae	Pupa					
EASTER EGGS (n=10)										
	01	ND	02	12	02	ND	ND	16	$3x10^1$	Aspergillus
	02	ND	ND	ND	ND	ND	01	01	$1x10^1$	Aspergillus
	03	ND	01	ND	ND	ND	ND	01	NG	NA
	04	ND	ND	ND	ND	ND	ND	ND	NG	NA
	05	ND	ND	ND	ND	ND	ND	ND	NG	NA
	06	ND	ND	ND	ND	ND	ND	ND	NG	NA
	07	ND	ND	ND	ND	ND	ND	ND	NG	NA
	08	ND	01	ND	ND	ND	ND	01	$2x10^1$	Aspergillus
	09	ND	ND	ND	ND	ND	ND	ND	$2x10^1$	Aspergillus
	10	ND	01	ND	ND	ND	01	02	$1x10^1$	Rhysopus
BARS (n=10)										
	01	ND	ND	ND	ND	ND	ND	ND	NG	NA
	02	ND	ND	02	ND	ND	01	03	$1x10^1$	Aspergillus
	03	ND	01	ND	ND	ND	02	03	$1x10^1$	Penicillium
	04	ND	ND	ND	ND	ND	ND	ND	NG	NA
	05	ND	ND	ND	ND	ND	ND	ND	NG	NA
	06	ND	01	ND	ND	ND	ND	01	NG	NA
	07	ND	01	ND	ND	ND	ND	01	NG	NA
	08	ND	01	ND	ND	ND	ND	01	NG	NA
	09	ND	ND	ND	ND	ND	ND	ND	NG	NA
	10	ND	ND	ND	ND	ND	ND	ND	NG	NA
BONBONS (n=10)										
	01	ND	ND	ND	ND	ND	ND	ND	NG	NA
	02	ND	03	ND	ND	ND	ND	03	$1x10^1$	Penicillium
	03	ND	ND	ND	ND	ND	ND	ND	NG	NA
	04	ND	01	ND	ND	ND	ND	01	NG	NA
	05	ND	ND	ND	ND	ND	01	01	$1x10^1$	Penicillium
	06	ND	03	ND	02	ND	ND	05	$1x10^1$	Penicillium
	07	ND	ND	ND	ND	ND	ND	ND	NG	NA
	08	ND	ND	ND	ND	ND	ND	ND	NG	NA
	09	ND	ND	ND	01	ND	ND	ND	NG	NA
	10	13	02	ND	03	ND	ND	18	$1x10^1$	Penicillium
OTHERS (n=4)										
	01	ND	01	03	01	ND	ND	04	NG	NA
	02	ND	ND	ND	ND	ND	ND	ND	NG	NA
	03	ND	02	ND	02	ND	ND	04	NG	NA
	04	ND	ND	ND	ND	ND	01	01	NG	NA
STATISTICS										
Positive samples(%)		01(2.9)	11(32.4)	03(8.8)	05(14.7)	00(NA)	06(17.7)	NA(NA)	11(32.4)	11(32.4)
Light filth total		13	21	17	11	00	07	67	NA	NA
	Min	ND	01	02	01	00	01	01	1x10	NA
	Max	13	03	12	03	00	02	18	3x10	NA
	ND/NG	33	13	17	23	34	27	NA	23	23

ND: not detected CFU: colony forming unities: total fungi count NG: no growth NA: not applicable LMT: maximum tolerable level (for light filth and/fungi)

Table 3: Light Filth Characteristics Detected in Easter Eggs and Other Cocoa (*Theobroma Cacao* L.) Products Commercialized in Florianopolis, Their Possible Contamination Risk Points and the Effects on Food / Consumers

LIGHT FILTH			POSSIBLE CONTAMINATION RISK CRITICAL POINTS								VECTOR OF	LIGHT FILTH EFFECTS ON:	
TYPE	MAGE	SAMPLES	COCOA BEAN	PASTE		CHOCOLATE			ackaging	COMMERCIALIZATION		FOOD	CONSUMER✱
		Positive/total	Harvesting	rocessing	orage	Processing*	Re-processing			Storage (ºC)	Shelf life	(reduces)	(causes)
sects hole)		1/34	NA	NA	NA	NA	NA		A	A	A	Bacteria fungi / virus (pathogenic /toxigenic)	Dry matter Nutritional value Sensory characteristic	Microorganisms proliferation (bacteria &fungi) Allergies
ragment)		11/34	A	A	A	A	A		NA	NA	NA	Bacteria ** fungi / virus (pathogenic /toxigenic)	Nutritional value Sensory characteristic	Microorganisms proliferation Contact with fungi/toxins Allergies
rvae ve)**		3/34	NA	NA	NA	NA	NA		A	A	A	Bacteria fungi / virus (pathogenic /toxigenic)	Dry matter Sensory characteristic Quality(deterioration &Brew)	Bacteria / fungi proliferation
pa*** hole)		5/34	NA	NA	NA	NA	A		A	A	A	Bacteria fungi / virus (pathogenic /toxigenic)	Dry matter Sensory characteristic Quality(deterioration& fermentation)	Bacteria / fungi proliferation
tes		6/34	NA	A	A	A	A		A	A	A	Bacteria fungi / virus (deteriorating / pathogenic)	Dry matter Sensory characteristic Quality (deterioration & fermentation)	Allergies

NA: not applicable A: applicable *raw material (cocoa/sugar/fat storage) and their mixing / melting mild temperatures (45-55oC) applied **observed prior Wildman trap bottle light filth test ***moth (Ephestia elutella L.) metamorphosis stage □indicator of possible disease

□Chocolate INSECT: moth bio-cycle timing

(a) Whole cycle: 35-50 days

(b) Adult phase: 10-20 days

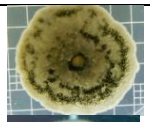
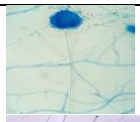
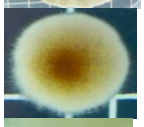
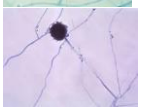
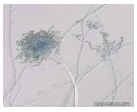
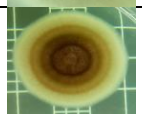
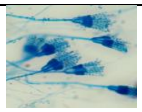
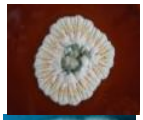
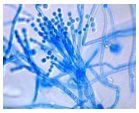
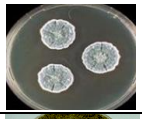
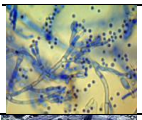
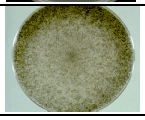
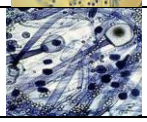
(c) Eggs delivery: 04-07 days (150-300 eggs)

(d) Larvae: 4-8 weeks

(e) Pupa: 5-10 days (to adult phase reach)

Total time allowed for chocolate products commercialization (shelf life date): the expiration date may be considered for a contractual requirement and a warranty issue stipulated by manufacturer. However, it is not something scientific bromatological and is only indicative that if the product is consumed before that" due date" should ensure their quality and pose no risk to consumer health.

Table 4: Fungi Genera and Species Characteristics Isolated from Easter Eggs and Other Cocoa (*Theobroma Cacao* L.) Products Sold in Florianopolis, Southern Brazil (March 2013 to March 2014)

FUNGI STRAINS			POSITIVE SAMPLES		TOTAL COUNT		CHRACTERISTICS	
GENERA	SPECIES	TOXIGENICITY	NUMBER	(%)	(CFU)	(%)	COLONY	MICROSCOPY
Aspergillus	A. flavus	AFL	02	6	1 x 10^1 3 x 10^1	07		
	A. flavipes	NT	01	3	1x10^1	04		
	A. niveus	NT	02	6	2x10^1 2x101	07		
Penicillium	P. glabrum	NT	01	3	1 x 10^1	04		
	P. camemberti	NT	02	6	1 x 10^1	07		
	P. chysogenum	NT	01	3	1 x 10^1	04		
	P. griseofulvum	NT	01	3	1 x 10^1	04		
Rhysopus	R. stolonifer	NT	01	3	1x10^1	04		
			Total: 11/34		18/19/4=41%			

AFL: aflatoxins NT: not toxigenic

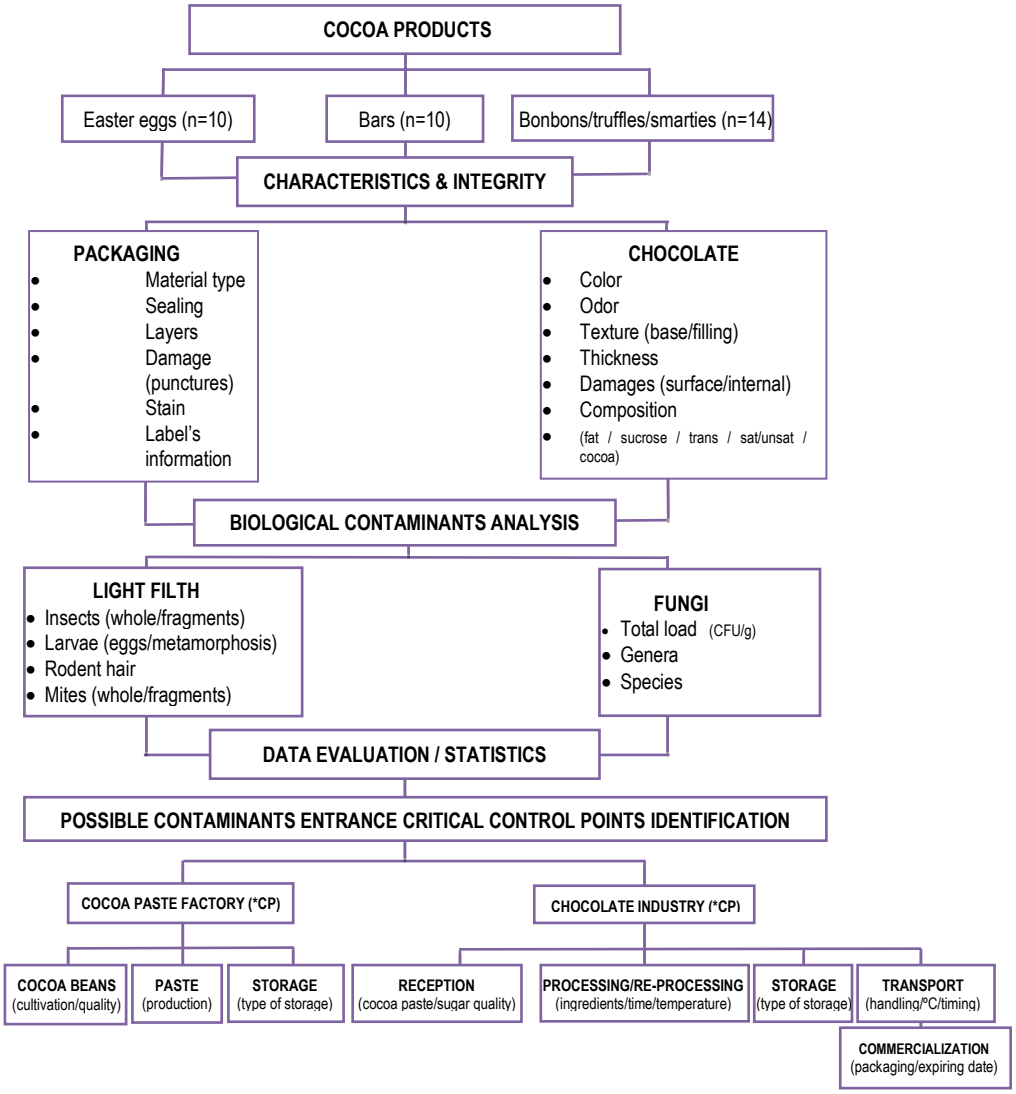


Figure 1: Flowchart of Biological Contaminants Evaluation of Easter Eggs and Other Cocoa (*Theobroma Cacao* L.) Products Sold in Florianopolis, Southern Brazil, in the Period from March 2013 to March 2014 (Cp: Control Point)

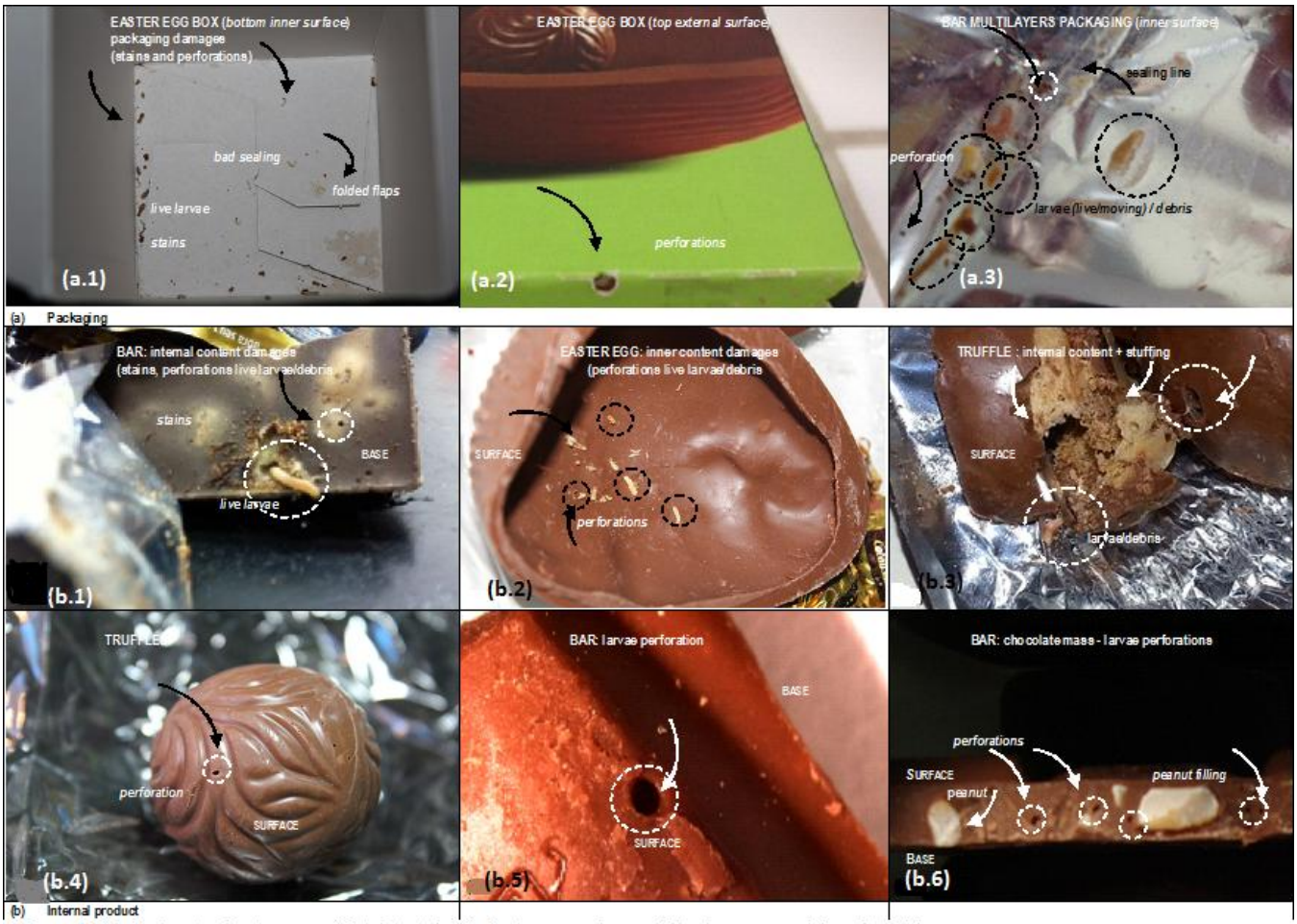


Figure 2 Biological contaminants macro effect detected on/in Easter egg and cocoa (*Theobroma cacao* L.) products (a) packaging and (b) internal content.

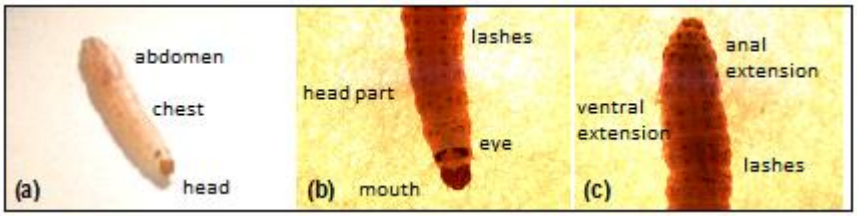


Figure 3: Chocolate Moth (*Ephestia Elutella*) Characteristics at Larvae Stage Isolated from Chocolate Bar with Raisins in the Current Survey: (A) Whole Body, (B) Head and (C) Bottom View (Stereoscopy)