

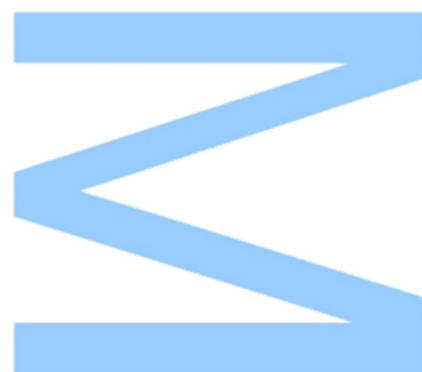
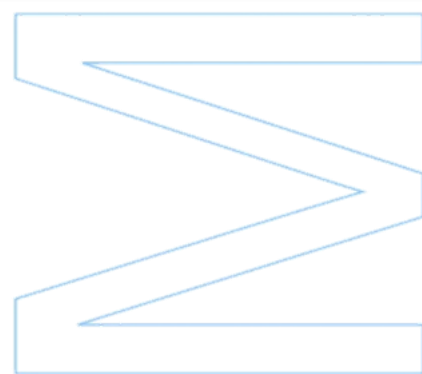
Effect of house cricket (*Acheta domestica*) content and extrusion temperature on extrudate snack properties

Liliana Patrícia da Rosa Ribeiro

Dissertação de Mestrado apresentada à
Faculdade de Ciências e à Faculdade de Ciências da Nutrição e
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Ciências do Consumo e Nutrição

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UNIVERSITAT
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DE VALÈNCIA



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Departamento de Geociências, Ambiente e Ordenamento do Território

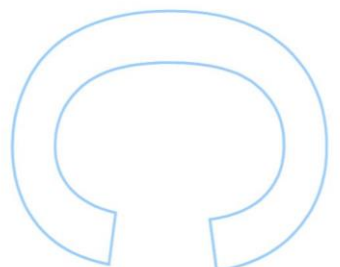
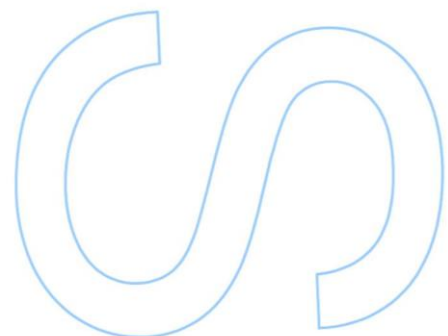
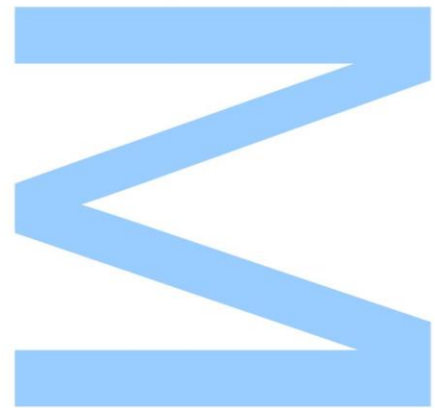
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Orientador

Doutor Javier Martínez Monzó, Professor Titular, Univeristat Politècnica de València (UPV)

Coorientador

Doutor Luís Miguel Cunha, Professor Associado, Faculdade de Ciências da Universidade do Porto (FCUP)

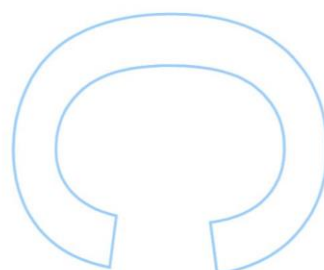
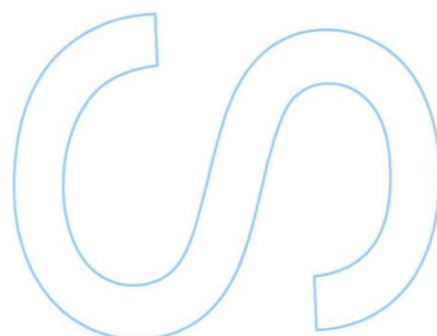
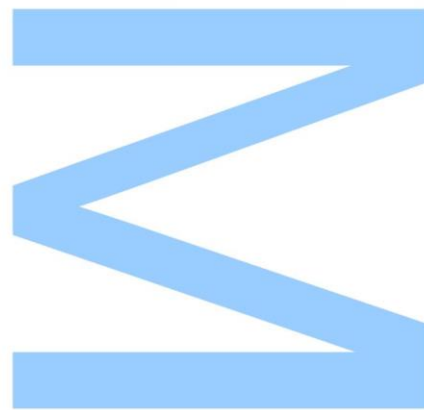




Todas as correções determinadas
pelo júri, e só essas, foram efetuadas.

O Presidente do Júri,

Porto, ____/____/____



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Resumo

O consumo de snacks tem vindo a crescer substancialmente em todo o mundo, por serem considerados produtos saborosos, económicos e convenientes, dado o atual estilo de vida agitado. No entanto, como as escolhas dos snacks por parte do consumidor nem sempre são as mais adequadas, a adição de ingredientes benéficos para a saúde a estes produtos, como as fontes de proteína alternativas, têm sido uma grande aposta. Dentro destas, os insetos edíveis têm sobressaído, apesar de o seu consumo nas sociedades Ocidentais ser algo ainda bastante rejeitado. O grilo doméstico (*Acheta domesticus*) tem sido das espécies de maior destaque, devido principalmente aos seus altos teores de proteína, mas também lípidos e até micronutrientes, além das suas vantagens a nível ambiental. Deste modo, para aumentar a sua aceitação uma estratégia que tem vindo a ser implementada é a sua incorporação em snacks extrudados, por serem produtos familiares aos consumidores e que têm vindo a ser os preferidos. Estes são produzidos a partir da tecnologia de extrusão, a altas temperaturas e num curto espaço de tempo, onde múltiplas operações ocorrem em simultâneo, o que permite a preservação das cores e sabores naturais dos alimentos, levando inclusive à melhoria de determinados atributos.

Assim, o presente trabalho teve como objetivo investigar o efeito da incorporação de diferentes quantidades de grilo doméstico e do uso de diferentes temperaturas de extrusão nas propriedades de snacks extrudados. Para tal, foram formuladas quatro misturas com farinha de milho e farinha de grilo em diferentes proporções e cada uma delas foi extrudada a 165 e a 175°C. De seguida foram analisadas as respostas do processamento, pela análise da pressão e torque ao longo da extrusão, bem como as respostas do produto pela avaliação das propriedades físico-químicas, composicionais, texturais e óticas, e ainda digestibilidade *in vitro* e bioacessibilidade proteica.

A melhor estrutura dos extrudados, caracterizada por uma elevada expansão e porosidade e, conseqüentemente, baixa densidade aparente, foi observada nos snacks com uma incorporação de 5% de *A. domesticus*, especialmente quando extrudados a 165°C. Estes apresentaram os valores mais elevados de bioacessibilidade proteica e exibiram uma coloração amarela com poucos tons acastanhados, similar aos tons que parecem agradar mais o consumidor. Os produtos extrudados enriquecidos com 15% mostraram as melhores propriedades associadas à água do produto, com baixos valores de humidade, atividade da água, índices de absorção e solubilidade, independentemente da temperatura, mas os processados a 175°C obtiveram ainda o maior teor de proteína, o suficiente para serem considerados como “fonte de proteína”.

Os extrudados com 10 e 15% de grilo doméstico incorporado foram considerados os que apresentaram melhores atributos texturais, por não serem demasiado fraturáveis, serem menos duros e mais crocantes.

Os resultados sugerem que a percentagem de incorporação de *A. domesticus* foi o fator em estudo que mais afetou as várias propriedades dos produtos obtidos. Deste modo, para o desenvolvimento de snacks extrudados com grilo doméstico incorporado é recomendado o uso de percentagens de 5 e 10% e que a extrusão ocorra a 165°C, com o intuito de manter as características únicas dos snacks apreciadas pelos consumidores, mas também de forma a que estes apresentem melhorias no teor de proteína e noutras propriedades, comparativamente aos snacks atualmente disponíveis no mercado.

Palavras-chave: Snacks, entomofagia, insetos edíveis, *Acheta domesticus*, extrusão, expansão, textura, digestibilidade *in vitro*

Abstract

The consumption of snacks has been growing substantially all over the world, as they are considered to be tasty, economical, and convenient products, given the current hectic lifestyle. However, as the consumer's choices of snacks are not always the most appropriate, the addition of health beneficial ingredients to these products, such as alternative protein sources, has been a major focus. Within these, edible insects have stood out, even though their consumption in Western societies is still rather rejected. The house cricket (*Acheta domesticus*) has been one of the most prominent species, mainly due to its high levels of protein, but also lipids and even micronutrients, in addition to its environmental advantages. Consequently, to increase its acceptance, a strategy that has been implemented is its incorporation in extruded snacks because they are products familiar to consumers and that have been the preferred ones. These are produced from the extrusion technology, at high temperatures and in a short time, where multiple operations take place simultaneously, which allows the preservation of the natural colours and flavours of the food, even leading to the improvement of certain attributes.

Therefore, the aim of the present work was to investigate the effect of incorporating different amounts of house cricket and the use of different extrusion temperatures on the properties of extruded snacks. For this purpose, four mixtures were formulated with maize flour and cricket flour in different mass ratios and each one was extruded at 165 and 175°C. The processing responses were then analysed by pressure and torque analysis throughout the extrusion, as well as the product responses by assessing of physicochemical, compositional, texture and optical properties, plus *in vitro* digestibility, and protein bioaccessibility.

The best extruded structure, characterised by high expansion and porosity and, consequently, low bulk density, has been observed in snacks with an incorporation of 5% of *A. domesticus*, especially when extruded at 165°C. In addition, these presented the highest values of protein bioaccessibility and exhibited a yellowish colour with few brownish tones, similar to the tones that seem to please the consumer the most. Extruded products enriched with 15% of *A. domesticus* showed the best properties associated with the water of the extrudates, with low values of water content, water activity, and indexes of water absorption and solubility, regardless of temperature, but those processed at 175°C still obtained the highest protein content, enough to be considered as a "source of protein". Those extruded with 10 and 15% of incorporated house cricket were considered to have the best texture attributes, since they did not fracture too easily, were less hard and were the crunchier.

The results suggest that the percentage of incorporation of *A. domesticus* was the factor under study that most affected the various properties of the products obtained. In this way, for the development of extruded snacks with incorporated house cricket it is recommended to use percentages of 5 and 10% and that extrusion occurs at 165°C, in order to maintain the unique characteristics of the snacks appreciated by consumers, but also for them present improvements in protein content and in other properties, compared to the snacks currently available on the market.

Keywords: Snacks, entomophagy, edible insects, *Acheta domesticus*, extrusion, expansion, texture, *in vitro* digestibility

Table of contents

Acknowledgements.....	i
Resumo.....	ii
Abstract.....	iv
Table of contents.....	vi
List of tables.....	ix
List of figures	xii
List of abbreviations	xiv
Nomenclatures	xv
1. Introduction.....	1
1.1. Snacks	1
1.1.1. Trends.....	1
1.1.2. Concept of snack	3
1.1.3. Snacks and health	4
1.1.4. Influential factors in the choice of snacks	6
1.1.5. Demand for healthy snacks	6
1.2. New food sources	8
1.3. Consumption of edible insects.....	9
1.3.1. Entomophagy in the West.....	11
1.3.2. House cricket (<i>Acheta domesticus</i>).....	12
1.3.2.1. Nutritional aspects	12
1.3.2.2. Economic and environmental sustainability aspects	15
1.3.3. Food security	17
1.3.3.1. Biological hazards.....	18
Bacteria.....	18
Virus	19
Parasites.....	19
Fungi	19
Prions.....	20
1.3.3.2. Chemical hazards	20
1.3.3.3 Allergens.....	20

1.3.4. Legislation.....	21
1.3.5. Consumer acceptance.....	22
1.4. Extrusion.....	23
1.4.1. Extrusion parameters	26
1.4.2. Raw materials and modifications in main components.....	27
1.4.2.1. Starch.....	28
1.4.2.2. Proteins.....	29
1.4.2.3. Lipids.....	29
1.4.2.4. Fibre	30
1.4.2.5. Minerals and vitamins.....	30
1.4.3. Characterization of extruded products	30
1.4.4. Development of promising healthy extruded products	32
2. Objectives	34
3. Material and methods	35
3.1. Raw materials and formulations	35
3.2. Extrusion processing	35
3.3. Analysis.....	37
3.3.1. Water content and water activity	37
3.3.2. Water absorption index, water solubility index and swelling index.....	38
3.3.3. Hygroscopicity.....	39
3.3.4. Expansion index.....	39
3.3.5. Bulk density and porosity	39
3.3.6. Crude protein.....	40
3.3.7. Texture.....	40
3.3.8. Optical properties.....	42
3.3.9. <i>In vitro</i> digestibility and protein bioaccessibility	43
3.3.10. Statistical analysis	45
4. Results and discussion.....	46
4.1. Extrusion process responses.....	46
4.2. Physicochemical and compositional properties of extrudates	49
4.2.1. Water content and water activity	49
4.2.2. Water absorption index, water solubility index, swelling index and hygroscopicity	52
4.2.3. Expansion index, diameter, bulk density, and porosity.....	56
4.2.4. Crude protein.....	64

4.2.5. Relationship among physicochemical and compositional properties	66
4.3. Textural properties of extrudates.....	70
4.3.1. Relationship among texture and physicochemical and compositional properties	75
4.4. Optical properties of extrudates.....	77
4.5. <i>In vitro</i> digestibility and protein bioaccessibility of extrudates.....	83
5. Conclusion.....	86
6. References	88
7. Appendix.....	109
Appendix I. Paper submitted in Journal of Insects as Food and Feed	109

List of tables

Table 1. Nutritional composition of some of the most consumed snacks. Adapted from: Brennan <i>et al.</i> (2013).	4
Table 2. Essential amino acid (EAA) content of <i>A. domestica</i> in different studies and recommendation of EAA for children and adolescents (of ages between 3-10; 11-15; 15-18) and adults (> 18) according to WHO (2007).	14
Table 3. Nutritional composition of corn grits (Maicerías Españolas S.L.) and <i>A. domestica</i> powder (Entoma Foods).....	35
Table 4. Treatment code corresponding to each extrusion temperature (T) to which each formulation with different percentage of <i>A. domestica</i> (AD) has been submitted.	37
Table 5. Mean values of torque obtained for each treatment.	49
Table 6. Mean values and standard deviations of water content of the mixture (x_w^M) formulated with different levels of incorporation of <i>A. domestica</i> (AD). The <i>p-value</i> obtained from ANOVA for AD is indicated.	49
Table 7. Mean values and standard deviations of extrudate water content (x_w^E), extrusion water loss (W_L) and extrudate water activity (a_w) of extruded products obtained for each treatment. The <i>p-value</i> achieved from ANOVA for the interaction between T and AD on each parameter is indicated.	50
Table 8. Mean values and standard deviations of extrudate water content (x_w^E), extrusion water loss (W_L) and extrudate water activity (a_w) of extruded products obtained at different temperature (T). The <i>p-value</i> achieved from ANOVA for the effect of T on each parameter is indicated and significant parameters are highlighted in bold ($p < 0.05$)...	51
Table 9. Mean values and standard deviations of extrudate water content (x_w^E), extrusion water loss (W_L) and extrudate water activity (a_w) of extruded products with different levels of <i>A. domestica</i> (AD). The <i>p-value</i> achieved from ANOVA for the effect of AD on each parameter is indicated and significant parameters are highlighted in bold ($p < 0.05$)...	51
Table 10. Mean values and standard deviations of water absorption index (WAI), water solubility index (WSI), swelling index (SWE) and hygroscopicity (Hg) of extruded products obtained for each treatment. The <i>p-value</i> achieved from ANOVA for the interaction between T and AD is indicated and significant parameters are highlighted in bold ($p < 0.05$).	53
Table 11. Mean values and standard deviations of water absorption index (WAI), water solubility index (WSI), swelling index (SWE) and hygroscopicity (Hg) of extruded products obtained at different temperature (T). The <i>p-value</i> achieved from ANOVA for	

the effect of T on each parameter is indicated and significant parameters are highlighted in bold ($p < 0.05$).....	54
Table 12. Mean values and standard deviations of water absorption index (WAI), water solubility index (WSI), swelling index (SWE) and hygroscopicity (Hg) of extruded products with different levels of <i>A. domestica</i> (AD). The <i>p-value</i> achieved from ANOVA for the effect of AD on each parameter is indicated and significant parameters are highlighted in bold ($p < 0.05$).....	55
Table 13. Mean values and standard deviations of expansion index (SEI), diameter (D_e), bulk density (ρ_b) and porosity (ϵ) of extruded products obtained at different temperature (T). The <i>p-value</i> achieved from ANOVA for the effect of T on each parameter is indicated and significant parameters are highlighted in bold ($p < 0.05$).	57
Table 14. Mean values and standard deviations of expansion index (SEI), diameter (D_e), bulk density (ρ_b) and porosity (ϵ) of extruded products with different levels of <i>A. domestica</i> (AD). The <i>p-value</i> achieved from ANOVA for the effect of AD on each parameter is indicated and significant parameters are highlighted in bold ($p < 0.05$)...	58
Table 15. Mean values and standard deviations of crude protein (CP) of extruded products obtained for each treatment. The <i>p-value</i> achieved from ANOVA for the interaction between T and AD is indicated in bold, being treatments significant for a $p < 0.05$	65
Table 16. Mean values and standard deviations of crude protein (CP) of extruded products obtained at different temperature (T). The <i>p-value</i> achieved from ANOVA for the effect of T is indicated.	65
Table 17. Mean values and standard deviations of crude protein (CP) of extruded products with different levels of <i>A. domestica</i> (AD). The <i>p-value</i> achieved from ANOVA for the effect of AD is indicated in bold, being samples significant for a $p < 0.05$	65
Table 18. Pearson correlation coefficients among studied physicochemical and compositional parameters of extruded products.	69
Table 19. Mean values and standard deviations of texture parameters of extruded products obtained for each treatment. The <i>p-value</i> achieved from ANOVA for the interaction between T and AD on each parameter is indicated and significant parameters are highlighted in bold ($p < 0.05$).....	72
Table 20. Mean values and standard deviations of texture parameters of extruded products obtained at different temperature (T). The <i>p-value</i> achieved from ANOVA for the effect of T on each parameter is indicated and significant parameters are highlighted in bold ($p < 0.05$).....	72

Table 21. Mean values and standard deviations of texture parameters of extruded products with different levels of <i>A. domesticus</i> (AD). The <i>p-value</i> achieved from ANOVA for the effect of AD on each parameter is indicated and significant parameters are highlighted in bold ($p < 0.05$).....	72
Table 22. Pearson correlation coefficients between texture parameters of extruded products and physicochemical and composition parameters.....	76
Table 23 . Mean values and standard deviations of colour coordinates (L^* , a^* , b^* , C^*_{ab} and h^*_{ab}) and total colour difference (ΔE) of extruded products obtained for each treatment. The <i>p-value</i> achieved from ANOVA for the interaction between T and AD on each parameter is indicated and significant parameters are highlighted in bold ($p < 0.05$).	79
Table 24. Mean values and standard deviations of colour coordinates (L^* , a^* , b^* , C^*_{ab} and h^*_{ab}) and total colour difference (ΔE) of extruded products obtained at different temperature (T). The <i>p-value</i> achieved from ANOVA for the effect of T on each parameter is indicated.....	80
Table 25. Mean values and standard deviations of colour coordinates (L^* , a^* , b^* , C^*_{ab} and h^*_{ab}) and total colour difference (ΔE) of extruded products with different levels of <i>A. domesticus</i> (AD). The <i>p-value</i> achieved from ANOVA for the effect of AD on each parameter is indicated and significant parameters are highlighted in bold ($p < 0.05$)...	81
Table 26. Mean values and standard deviations of <i>in vitro</i> digestibility (IVD) and protein bioaccessibility of extruded control products obtained at different temperature (T). The <i>p-value</i> achieved from ANOVA for the effect of T on each parameter is indicated and significant parameters are highlighted in bold ($p < 0.05$).	83
Table 27. Mean values and standard deviations of <i>in vitro</i> digestibility (IVP) and protein bioaccessibility of extruded products with different levels of <i>A. domesticus</i> (AD) at 175°C. The <i>p-value</i> achieved from ANOVA for the effect of AD on each parameter is indicated and significant parameters are highlighted in bold ($p < 0.05$).	84

List of figures

Figure 1. Consumption of meals and snacks reported by adults in the United States by gender and race/ethnicity. Source: Kant (2018).	1
Figure 2. Percentage over the years of men (A) and women (B) from the United States who are skipping meals and snacking. Source: McCrory <i>et al.</i> (2016).....	2
Figure 3. Entomophagy worldwide, based on data compiled by Jongema (2015). Source: Costa-Neto and Dunkel (2016).....	10
Figure 4. Edible insects sold in various local markets in Thailand. Source: a) photography of Patrick Durst (2009) taken from Costa-Neto and Dunkel (2016); b) Hanboonsong <i>et al.</i> (2013).	11
Figure 5. House cricket / <i>Acheta domestica</i> (Linnaeus, 1758). Source: Morales-Ramos <i>et al.</i> (2017).....	12
Figure 6. Quantity of GHG (left) and ammonia (NH ₃) (right) produced per kg of mass gained, for cricket, pigs, and beef cattle. Adapted from: Van Huis <i>et al.</i> (2013).....	16
Figure 7. Amount of land, feed and water required to produce 1 kg of animal mass and percentage of animal that is edible. The following are represented: house cricket, chicken, pig, and beef cattle (from bottom to top). Adapted from: Dobermann <i>et al.</i> (2017).	16
Figure 8. Some extruded products such as milk stick biscuits (a), wheat flower chips (b) and pasta (c), made with different raw materials. Source: Offiah <i>et al.</i> (2018).....	24
Figure 9. Scheme of the main parts of a screw extruder. Adapted from: Navale <i>et al.</i> (2015).	25
Figure 10. Scheme of how expansion occurs. Adapted from: Moraru and Kokini (2003).	32
Figure 11. Single screw laboratory extruder turned off (left) and during the extrusion of corn and house cricket snacks (right).....	36
Figure 12. Scheme of conditions used in the extruder to reach final extrusion temperatures of 165°C (a) and 175°C (b).....	36
Figure 13. Helium pycnometer (AccPyc 1330, Micromeritics, Norcross, USA).	39
Figure 14. Texture analyser TA–TXT2 (Stable Micro Systems Ltd, Godalming, UK). ..	40
Figure 15. A typical force-time curve of extruded products for texture measurements. S represents the area under the curve and N ₀ the number of peaks.....	41
Figure 16. Gastric phase of <i>in vitro</i> digestibility.	44
Figure 17. Variation of temperature and pressure over the processing time recorded by the temperature (T1 and T2) and pressure (P) probes of the extruder for each treatment:	

165-0 and 175-0 (a); 165-5 and 175-5 (b); 165-10 and 175-10 (c); 165-15 and 175-15 (d).	48
Figure 18. Mean values and standard deviations of expansion index (SEI) of extruded products obtained at different temperature and levels of <i>A. domestica</i> . Bars with different letters are significantly different ($p < 0.05$).	57
Figure 19. Mean values and standard deviations of diameter (D_e) of extruded products obtained at different temperature and levels of <i>A. domestica</i> . Bars with different letters are significantly different ($p < 0.05$).	59
Figure 20. Mean values and standard deviations of bulk density (ρ_b) of extruded products obtained at different temperature and levels of <i>A. domestica</i> . Bars with different letters are significantly different ($p < 0.05$).	60
Figure 21. Mean values and standard deviations of porosity (ϵ) of extruded products obtained at different temperature and levels of <i>A. domestica</i> . Bars with different letters are significantly different ($p < 0.05$).	61
Figure 22. Radial (left) and longitudinal (right) cross sections of samples extruded at 165°C with 0 (a), 5 (b), 10 (c) and 15% (d) of <i>A. domestica</i> and samples extruded at 175°C with 0 (e), 5 (f), 10 (g) and 15% (h) of <i>A. domestica</i>	63
Figure 23. Total pore area for samples with 0, 5, 10 and 15% of <i>A. domestica</i> , extruded at 165 and 175°C. Data were obtained directly from ImageJ software (Abramoff <i>et al.</i> , 2004).	64
Figure 24. Force-time curves of each treatment: all formulations, 0, 5, 10 and 15% of <i>A. domestica</i> extruded at 165°C (a) and at 175°C (b).	71
Figure 25. Reflection spectrum for each treatment: all formulations, 0, 5, 10 and 15% of <i>A. domestica</i> extruded at 165°C (a) and at 175°C (b).	78
Figure 26. Spectra distribution of the Kubelka-Munk coefficients (K/S) of control extrudates at 165 and 175°C.	78
Figure 27. Variation of colour coordinate a^* in relation to the coordinate L^* for each treatment (a) and variation of colour coordinate a^* in relation to the coordinate b^* for each treatment (b).	80
Figure 28. Extrudates obtained at 165°C for each of the formulations with 0, 5, 10 and 15% of <i>A. domestica</i> (from left to right respectively).	81

List of abbreviations

AD – *Acheta domesticus*

ANOVA – Analysis of variance

Ca – Calcium

EFSA – European Food Safety Authority

EU – European Union

FAO – Food and Agricultural Organization of the United Nations

Fe – Iron

GHG – Greenhouse gas

K – Potassium

Mg – Magnesium

P – Phosphorus

PUFA – Polyunsaturated fatty acids

WHO – World Health Organization

Zn – Zinc

Nomenclatures

x_w^M – Mixture water content ($\text{g}_{\text{water}}/\text{100g}$)

x_w^E – Extrudate water content ($\text{g}_{\text{water}}/\text{100g}$)

W_L – Water loss ($\text{g}_{\text{water}}/\text{100g}$)

a_w – Water activity

WAI – Water absorption index ($\text{g}_{\text{sediment}}/\text{g}_{\text{dry solid}}$)

WSI – Water solubility index (%)

SWE – Swelling index ($\text{mL}_{\text{swollen}}/\text{g}_{\text{dry solid}}$)

Hg – Hygroscopicity ($\text{g}_{\text{water}}/\text{100g}_{\text{dry solid}}$)

D_e – Diameter (mm)

SEI – Expansion index

ρ_b – Bulk density (g/cm^3)

ε – Porosity (%)

CP – Crude protein ($\text{mg}/\text{100g}$)

W_c – Crispness work (N.mm)

F_p – Average puncturing force (N)

F_s – Average specific force of structural ruptures (N)

N_{sr} – Spatial frequency of ruptures (mm^{-1})

N_0 – Number of peaks

IVD – *In vitro* digestibility (%)

$CIE^*L^*a^*b$ – Colour space: lightness, greenness/redness, and blueness/yellowness

h^*_{ab} – Hue

C^*_{ab} – Chroma

ΔE – Total colour difference

T – Temperature ($^{\circ}\text{C}$)

1. Introduction

1.1. Snacks

1.1.1. Trends

Lack of free time and excessive hours of work describe the current hectic lifestyle of populations, characteristic of modern life, where there is no time to prepare adequate meals (Bellisle, 2014). As a result, over the past 30 years, the habit of eating snacks has grown substantially throughout the world, since these are products considered tasty, economical and convenient, that can be consumed quickly at the same time as other activities are being carried out (Cuj-Laines *et al.*, 2018; Mattes, 2018). Therefore, among the various food sectors, the market for this type of ready-to-eat products stands out as has been becoming one of the most promising and dynamic, with growth potential (Adem *et al.*, in press; Moraru & Kokini, 2003).

Given the circumstances of lifestyles, more than ever consumers are snacking, with 94% of consumers in the United States consuming at least 1 snack during the day, whereas 50% consume 2-3 snacks per day (Singha *et al.*, 2018) (figure 1); in Europe, which is one of the largest markets for this type of products, it has also been reported that consumers snack at least once and several times a day, with consumption frequencies ranging from 4.9 to 7.0 times a day (Mattes, 2018). Considering these frequencies, snacks can thus contribute to consumer nutrition (Adem *et al.*, in press).

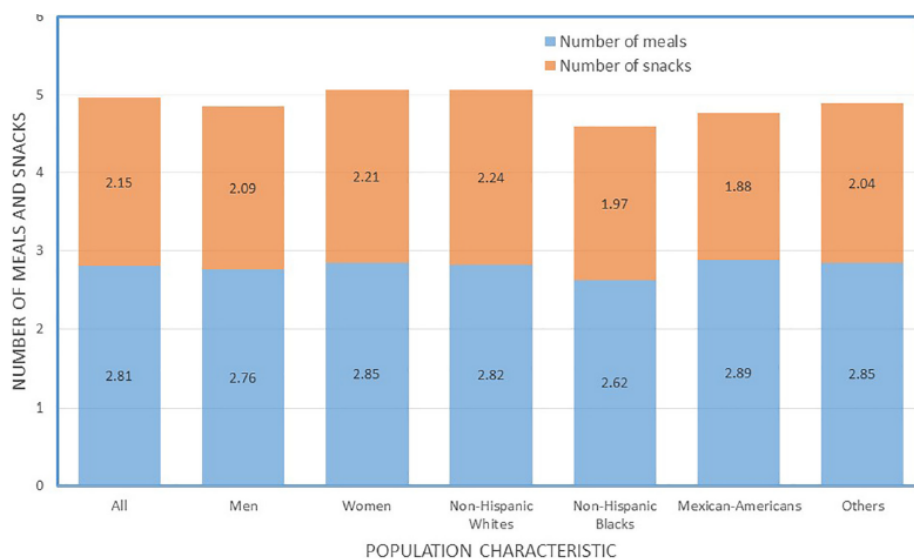


Figure 1. Consumption of meals and snacks reported by adults in the United States by gender and race/ethnicity. Source: Kant (2018).

This practice of eating snacks has become an integral part of the diet of many people, independently of culture, social status, gender and, in all age groups (Cortes *et al.*, 2014; Navarro-Cortez *et al.*, 2018). Among the most consumed snacks are sugary beverages, sweets such as chocolates, cakes, bars, jellies, cookies, popcorn, and salty snacks as chips (Ciurzynska *et al.*, 2019; Hess & Slavin, 2018). However, since snacks are increasingly replacing conventional meals, and there are a lot of people skipping these meals (figure 2), there has also been a growing demand for alternative snacks, especially the most nutritious ones, being already 33% of consumers consuming healthier snacks (Rathod & Annapure, 2016; Singha *et al.*, 2018).

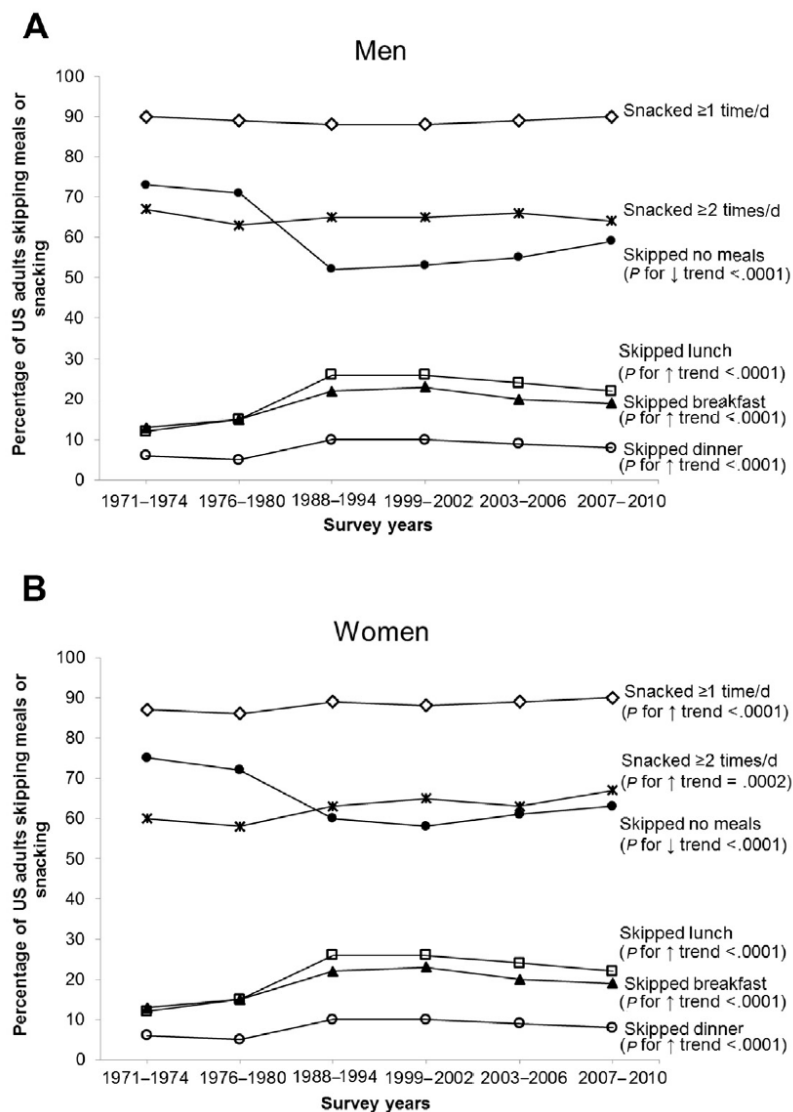


Figure 2. Percentage over the years of men (A) and women (B) from the United States who are skipping meals and snacking. Source: McCrory *et al.* (2016).

1.1.2. Concept of snack

Currently there is no consensual definition for snack and various approaches have been used to define them according to the type and amount of food consumed, the place of consumption, the energy content ingested, the time of day when the occasion for eating occurs, or these definitions may be based on combinations of many of these factors or on characteristics of the individual himself (Potter *et al.*, 2018).

A large part of the research seems to be based mainly on the time of an eating occasion, followed by the energy content, as regards the definition of snack, although there is no unanimity. Even the few countries that provided a specific definition were based on one of these two assumptions (Potter *et al.*, 2018). Thus, considering these criteria, in order to define snack, one will inevitably have to define meal, because although both contribute differently to the intake of nutrients and to the energy balance of the body, the concepts themselves end up being indirectly related, since the definition of one allows to know automatically the definition of the other (Bellisle, 2014).

In relation to energy content, an eating episode would be considered a meal if it contributes with more than 15% to the total daily energy intake and all other eating occasions would be classified as snacks (Murakami & Livingstone, 2016).

Regarding to the time of the occasion to eat, snacks can be defined as food episodes located outside the main meals, being considered all types of foods, regardless of whether they are rich or poor in nutrients. Consequently, meals would be defined as occasions to eat between 6 am and 10 am, 12 am and 3 pm, 6 pm and 9 pm, and all other occasions outside these times would be considered snacking occasions (Murakami & Livingstone, 2016). This information can be summarized based on the definition given by Ciurzynska *et al.* (2019) which states that snacks are products consumed between the main meals and can be both "healthy" and "unhealthy".

Although there is not exactly one definition, the daily diet of Western societies seems indirectly to adopt the last criterion mentioned as well, since it is typically divided into three main meals, breakfast, lunch and dinner and everything that is consumed in the middle of the morning, mid-afternoon and late evening is assumed to be a snack, in addition to the called accidental snacks consumed outside any of these hours (Bellisle, 2014; Shah *et al.*, 2019).

1.1.3. Snacks and health

Most of the snacks available on the market are made from starches and are usually characterized as products with few or no health benefits (Cuj-Laines *et al.*, 2018; Do Carmo *et al.*, 2019). In addition, it is well known that snacks dense in energy, which have high amounts of sugar, salt, fat, and consequently low levels of nutrients, are the most popular in many countries (table 1) and therefore their consumption is associated with something negative for health (Ramos *et al.*, 2017).

Table 1. Nutritional composition of some of the most consumed snacks. Adapted from: Brennan *et al.* (2013).

	Energy values (Kcal /100 g)	Fat (g/ 100 g)	Starch (g/ 100 g)	Sugars (g/ 100 g)	Protein (g/ 100 g)	Dietary fibre (g/ 100 g)
Popcorn	480	20.0	15.5	62.1	2.1	4.5
Potato chips	530	34.2	52.6	0.7	6.2	5.3
Tortilla chips	459	22.6	58.9	1.2	7.6	6
Breadstick	392	8.4	67.5	5	11.2	3.8
Cereal bar	468	22.2	28.3	27.6	10.4	4.8
Kit kat	500	26	12.9	50.1	7.5	1.4
Crispbread	308	0.6	67.4	3.2	9.4	11.7
Rice krispies	382	1	82.5	10.4	6.1	0.7
Corn flakes	376	0.9	81.4	8.2	7.9	0.6

Children are one of the main groups of snack consumers, since most of these products are linked to food for this age group (Shah *et al.*, 2019). Adolescents between the ages of 12 and 19 also eat snacks regularly, with 70% consuming two or more snacks during the day (Hess & Slavin, 2018). These, along with individuals aged 19-30 are the ones who make the least healthy choices, selecting preferably nutrient-poor snacks that are not suitable for health (De Vlieger *et al.*, 2017).

Frequent consumption and inadequate choices have led a number of studies to make associations between snack consumption and obesity, considering that snacks provide extra calories to the diet (Kachurak *et al.*, 2019). This is of particular concern to families living in low-income social contexts, whose access to healthy food is limited, which makes convenience stores that mostly offer several unhealthy snacks their only options

(Njike *et al.*, 2016). However, even outside these settings, there is excessive consumption of unhealthy snacks, especially in children, which is alarming since eating preferences and habits are formed at these ages and can be maintained during adolescence and adulthood, so their choices will influence their eating behaviour and the potential risk of overweight (Jacquier *et al.*, 2017). Parents are often primarily responsible for these selections because they mostly provide children with calorie-filled snacks that can contribute to weight gain (Younginer *et al.*, 2016).

On the other hand, other researchers shown that there are no association between snack consumption and obesity and others have showed that their frequent consumption can even be beneficial for health (Hess & Slavin, 2017).

There are few specific recommendations for adults and adolescents, so most of them are for the overall population or are more focused on children, as they have high energy needs in order to endure their growth and development (Potter *et al.*, 2018). In some European countries, for example, to satisfy possible nutritional needs, the practice of giving children a morning snack, such as school milk distribution, still persists (Jacquier *et al.*, 2017). Numerous studies mention that snacks are essential and can met the requirements of all segments of the population (Bhat *et al.*, 2019), being even recommended to consume 2 snacks per day in several countries (Potter *et al.*, 2018).

Snacking several times a day between meals has also been associated with something positive, which improves the quality of the diet, not affecting the quantity of calories ingested at the next meal (Hess *et al.*, 2016). Based on an intervention study in adults with a normal weight, consuming snacks between main meals “can act as a facilitating mechanism for energy compensation and adjustment to energy requirements”, so frequent snack consumers have a higher level of dietary compensation than those who consume fewer and bigger meals, which may be related to maintaining body weight (Bellisle, 2014). Thus, excessive consumption in the next meal can be suppressed with the practice of snacking between the main meals, so leading to reduced hunger and this habit also affects satiety by increasing fullness, with products rich in protein being the ones that most prolong this feeling (Ciurzynska *et al.*, 2019; Njike *et al.*, 2016).

Even though numerous studies have been conducted using different types of snacks, different populations and different methodologies, the absence of a consistent definition of snacks often still makes it difficult to determine whether its consumption brings benefits or harm to health (Njike *et al.*, 2016).

1.1.4. Influential factors in the choice of snacks

The impact of snacks on health, rather than depending on a concrete definition of snack, is related to the choices of the snack itself by consumers (Hess & Slavin, 2017). Choices are influenced by several personal level factors, both demographic and biological. Among these are variations in genes that are quite important, since they are in charge of the taste receptors, which affect the perception of taste and then the preferences for certain snacks. Emotions are also very relevant, and emotional consumers under stress or pressure tend to consume large quantities of snacks considered poor in nutrients. Consequently, having knowledge about snacks and having an idea of their constitution is also an important factor, since this is a great determinant for the choice of snacks suitable for health (Njike *et al.*, 2016).

In addition, other equally important factors are to be highlighted: the consumption of snacks in the absence of hunger that leads to increased consumption of products rich in sugar, salt and fat, inducing increased energy intake, which can cause eventual weight gain; location, which affects not only the choice of snack type but also the portion size, as products usually consumed outside the home have less micronutrients and are presented in larger portions; the social environment and food culture; the distracted eating, that is related to the consumption of snacks while doing other activities; and hedonic hunger, which consists of the consumption of snacks motivated by the comforting properties of the products (Hess *et al.*, 2016; Hess & Slavin, 2018).

Generally, the factors mentioned lead to the consumption of unhealthy snacks in most cases (Hess *et al.*, 2016). This consumption is due, in part, to food marketing that often associates unhealthy snacks with tastier and more satisfying foods. Several studies even show that when people intend to eat more healthily, they tend to feel less pleasure in the act of consumption. There is an urgent need to develop options that are more beneficial to health but, just as unhealthy snacks, appeal to taste (Schlinkert *et al.*, 2020).

1.1.5. Demand for healthy snacks

Although individuals' choices regarding the consumption of snacks are not always the most appropriate, awareness of a healthy lifestyle is increasing in the various age groups (Kumar *et al.*, 2018), with a greater perception of the consequences of consuming unhealthy snacks (Ramos *et al.*, 2017) and a greater interest in knowing the nutritional advantages of any product (Dalbhagat *et al.*, 2019).

There has been a significant change in consumer demands for food products, increasing the request for snacks whose nutritious and functional properties have been improved (Uribe-Wandurraga *et al.*, 2019). Consumers then look for snacks that are not only low in sugar, salt and fat, and fortified with the necessary nutrients, but also meet their hunger needs, enhance their physical and mental status and have a fair price compared to conventional snacks (Poliszko *et al.*, 2019; Rathod & Annapure, 2017).

For the development of healthy snacks, it is important to keep in mind the concept of healthy snack and the way consumers define it, considering their knowledge and perception (Bucher *et al.*, 2017). There are five features that allow a snack to be characterized as healthy: the suitability of nutrients, with essential nutrients to be provided in the right amounts by snacks (Shah *et al.*, 2019), according to the recommendations, which promote diets composed of more vegetables, fruits, and cereals (Njike *et al.*, 2016); in addition to quantities, it is also important that no group of nutrients is overlooked; the control of calories, which allows one to know whether the energy provided by the snack is sufficient to keep consumers within their daily energy needs; the variety of ingredients of the different groups; and the satiety, that should leave the body in a metabolic condition where the consumption of more food is no longer necessary as the desire is satisfied (Shah *et al.*, 2019).

Given the demands, the development of promising snacks for health is a growing area resulting from cooperation between food producers and nutritionists (Ciurzynska *et al.*, 2019). Among the various products, extruded snacks that are rich in protein and fibre are those that have gained preference among consumers, but also for the producers themselves (Beck *et al.*, 2018; Rathod & Annapure, 2016). These snacks have the characteristics desired, for being produced from the extrusion technology (Azzollini *et al.*, 2018). A strategy that has been implemented to produce healthier snacks is the addition to these snacks of ingredients with health benefits, such as the alternative food sources (Yadav *et al.*, 2018), addressed in the following section.

These requirements are especially challenging for the food industry, since the incorporation of new ingredients can make the final snack something unacceptable to the consumer (Shah *et al.*, 2019). This way, it is crucial to be able to create tasty products with the desired appearance and, at the same time with nutritional benefits, in order to promote the inclusion of healthy snack options on the market (Cuj-Laines *et al.*, 2018; Yovchev *et al.*, 2017), because according to the study by Schlinkert *et al.* (2020), the snack considered ideal for people is one that is tasty and healthy at the same time.

Several snacks are already being reformulated from the addition of macro and micronutrients to improve their nutritional value (Pensamiento-Nino *et al.*, 2018). Therefore, the possible negative impacts of snack consumption could be mitigated with the increased availability and choice of healthy snacks, which may contribute to the promotion of nutrient-dense diets (Hess *et al.*, 2016).

1.2. New food sources

Nowadays, the world population is about 7.7 billion, and this number is increasing at a rate of 1.07% per year (Fasolin *et al.*, 2019), leading the Food and Agricultural Organization of the United Nations (FAO) to predict a growth in world population to 9 billion by 2050 (Tao *et al.*, 2017). On the one hand, this situation is alarming in developing countries, where FAO expects that food availability will not be able to meet demands, which may lead to inadequate protein consumption and malnutrition, that already affects around 1 billion people worldwide (Churchward-Venne *et al.*, 2017; Ferri *et al.*, 2019).

On the other hand, as a result of growth population, there will be an increase in the production and consumption of meat, with production tending to grow to 460 million tonnes, 76% up on current levels, especially in countries where meat represents a relevant part of the diet (Alexandratos & Bruinsma, 2012; Ferri *et al.*, 2019). However, these unsustainable levels are due not only to population growth, but also to the propagation of eating dietary habits from Western to developing countries (Ribeiro *et al.*, 2018), so that only in these countries the consumption of meat has been growing at a percentage of 5% per year (Ferri *et al.*, 2019).

These indications should give cause for concern, since there are several problems associated with exacerbated meat production and consumption, such as environmental degradation (Govorushko, 2019). The agricultural sector has a massive impact on greenhouse gas (GHG) emissions, with 2/3 being due to the cattle sector (Beusen *et al.*, 2008; Steinfeld *et al.*, 2006), which leads to other problems as soil acidification, loss of biodiversity, water pollution, deforestation, among others (Fasolin *et al.*, 2019; van Huis & Oonincx, 2017). Besides that, the rise in high density of animal production can lead to an increased incidence of infectious diseases in animals and the excessive consumption of red meat is associated with an increase in the incidence of cardiovascular disease, colorectal cancer and mortality in humans (Aykan, 2015; Pan *et al.*, 2012).

It is also important to note that meat production is quite inefficient because the consumption of edible protein by humans is higher than the amount that is produced, that is, for each kg of animal protein produced, 6 kg of vegetable protein are spent as food (Pimentel & Pimentel, 2003). In addition, economic sustainability will also be questioned, as by 2050 world prices for agricultural crops will rise more than 30% in beef, pork, and poultry prices (Nelson *et al.*, 2009). Thus, although improvements have been made to production systems, alternatives to meat production and, above all, alternative protein sources are required to ensure a safe future (Raheem *et al.*, 2019).

In view of the concerns related to the present and future sustainability of meat production and consumption, there is a crucial need to change global eating habits worldwide so that they become not only more environmentally and economically sustainable, but also guarantee a healthier lifestyle for present and future generations (Burlingame & Dernini, 2012). One of the strategies that has generated the most interest is the possibility of using alternative food sources (Van der Spiegel *et al.*, 2013), such as micro and macroalgae, pulses, seeds, insects, to safeguard the aforementioned requirements (Fasolin *et al.*, 2019). Among these, insects have stood out because they are becoming a very valuable source for meat replacement in Western countries (Smetana *et al.*, 2019).

1.3. Consumption of edible insects

Entomophagy, the consumption of insects, is a practice that dates to the beginning of the human species when insects were consumed abundantly because they were easily harvested (Ferri *et al.*, 2019). Today, it is estimated that these are consumed by around 2 billion people worldwide, as part of their daily diet, but mostly in Africa, Asia, Central and South America, and Australia (Van Huis *et al.*, 2013) (figure 3).

There are over than 2000 species of edible insects, according to what Jongema (2015) documented. Among the most consumed are the insects belonging to the Coleoptera order (beetles), since they are the group that comprises about 40% of all recognised edible species, Lepidoptera (caterpillars), Hymenoptera (bees, wasps, ants), Orthoptera (crickets, locusts), Hemiptera (cicadas, leafhoppers), Isoptera (termites), Odonata (dragonflies) and Diptera (flies) (Raheem *et al.*, 2019). Besides the diversity in the quantity of species, insects can also be consumed in different states of their life cycle, being for example, insects of the order Coleoptera consumed in the adult and larval

states, whereas insects of the orders Orthoptera, Hemiptera and Isoptera are mostly eaten in the adult stages (Govorushko, 2019).

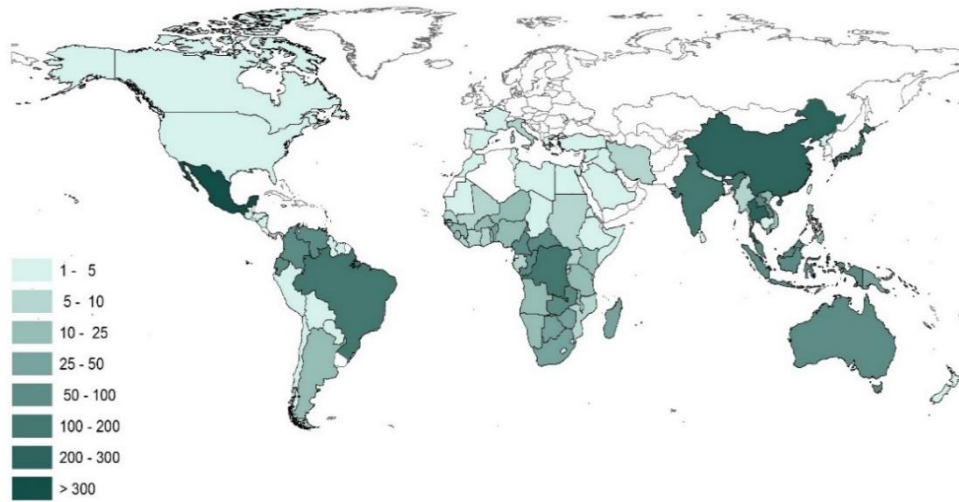


Figure 3. Entomophagy worldwide, based on data compiled by Jongema (2015). Source: Costa-Neto and Dunkel (2016).

The consumption of insects is often assumed as a regular practice in places where farming conditions are not favourable, as is effectively the case in tropical, however there are several reasons to explain the fact of their consumption is so high in these places (Lesnik, 2017). The main justification is their enormous diversity and abundance in these areas, where they can aggregate in significant numbers, which facilitates their harvest and the prediction of that same harvest, and can still be found during all year, whereas in temperate zones they hibernate to endure cold winters (Govorushko, 2019). Additionally, insects are still widely consumed in the countries of these regions, not only because they are nutritionally beneficial and their taste is appreciated (Nonaka, 2009), but also because they are a source of income for many people, thus being an integral part of the economy of the country (van Huis & Oonincx, 2017) (figure 4) .

Initially, insects were eaten alive, but over the years they began to be cooked (Yen, 2010). Insects can be consumed in different styles: in some countries they are eaten in their recognizable form (the whole insect), being submitted to some kind of processing such as drying, roasting, cooking, frying or bleaching before their integration in different meals (Melgar-Lalanne *et al.*, 2019); or else they are transformed into an unrecognizable form and later added to different products (Ferri *et al.*, 2019).



Figure 4. Edible insects sold in various local markets in Thailand. Source: a) photography of Patrick Durst (2009) taken from Costa-Neto and Dunkel (2016); b) Hanboonsong *et al.* (2013).

1.3.1. Entomophagy in the West

Insect consumption in Western societies is the contrary of what happens in developing countries, where insects remain a relevant part of the diet (Ferri *et al.*, 2019). In a place where protein is still principally derived from domesticated animals and protein seeds, the consumption of edible insects is seen as unpleasant and considered unacceptable for many cultures (Raheem *et al.*, 2019).

There are several reasons for this rejection of entomophagy by Western societies, in particular disgust to insects (Ferri *et al.*, 2019), which is a feeling triggered by psychological reasons and not by logic (Belluco *et al.*, 2013). Other factors should also be considered, such as the notion that the consumption of insects is a primitive practice, caused by starvation, being deemed just a mechanism of survival (Garino *et al.*, 2019).

However, in recent years the attitude of the Western countries is changing and given that the consumption of whole insects or part of them is something not accepted by consumers, it is important to focus on techniques for incorporating insects into products (Montowska *et al.*, 2019). In fact, it has been demonstrated that there are several alternatives, such as the incorporation of insects in a ground, convenient and familiar form, i.e. not visible, which leads to greater acceptability and predisposition of people to try them (Dobermann *et al.*, 2017); the main alternative that has been used is the incorporation in the form of protein isolates or powder (Klunder *et al.*, 2012).

Europeans and North Americans do not mind consuming insects if they are in a processed and unrecognizable form, according to some works (Melgar-Lalanne *et al.*,

2019), what indicates that hiding them in products will make their consumption easier (Raheem *et al.*, 2019). There are even already insects and products with embedded insects being marketed and projects and companies that continue to study the best ways to incorporate it into daily diets. Most of the products sold then involve the use of insect flour, added to a wide variety of products such as hamburgers, sweets, canned products, snacks, among others (Churchward-Venne *et al.*, 2017).

1.3.2. House cricket (*Acheta domesticus*)

The house cricket (*Acheta domesticus*) (figure 5), belongs to the Orthoptera order and is one of the most cultivated species worldwide (Udomsil *et al.*, 2019) and, consequently, one of the most consumed, with the demand for products based on this increasingly high, due to its unique characteristics. This species shares several nutritional benefits with other edible insects, as well as environmental and economic advantages from the production point of view, standing out in some of these benefits (Raheem *et al.*, 2019), which will be addressed below.



Figure 5. House cricket / *Acheta domesticus* (Linnaeus, 1758). Source: Morales-Ramos *et al.* (2017).

1.3.2.1. Nutritional aspects

The nutritional value of insects varies according to the species, sex, state of the life cycle in which they are found, diet, habitat and even the preparation and processing done before consumption (Govorushko, 2019). However, most edible insects are valuable sources of protein, lipids and even micronutrients (Ferri *et al.*, 2019).

Proteins are the main component of insects and their levels can differ between 7 and 91% (on a dry basis) for the various species (Fasolin *et al.*, 2019), with an average crude

protein content per species of 51.17%. The protein content of the house cricket shows values above this average, ranging from 64.38 to 70.75%, according to the state of the life cycle in which it is found (Rumpold & Schlüter, 2013). These levels are comparable to those recorded for other high-quality animal protein sources (Churchward-Venne *et al.*, 2017; Govorushko, 2019), such as fish (71%) and poultry (69.1%) (Bosch *et al.*, 2014). *A. domesticus* and the other edible insects have a high nutritional value, which is dependent on two factors that must also be evaluated, since they define the quality of the protein fraction and subsequently this nutritional value, which are: the amino acid composition and digestibility (Belluco *et al.*, 2013).

Overall, insects have proteins with appropriate amino acid profiles, with nutritionally high levels of all essential amino acids (Nongonierma & FitzGerald, 2017). The sum of these essential amino acids varies between 320 and 480 mg/g protein, identical values to those recorded for soy (439 mg/g protein) (Yi *et al.*, 2013), fish (417 mg/g protein) (Makkar *et al.*, 2014), beef (475 mg/g protein) and pigs (453 mg/g protein), but lower than the amino acid levels for chicken (508 mg/g protein) (Tessari *et al.*, 2016).

Regarding house cricket, their amino acid composition covers the requirement established for adults by the World Health Organization (WHO) (2007) and according to the studies of Rumpold and Schlüter (2013) and Yi *et al.* (2013), it also covers the requirement established for children and adolescents (3-18 years); however, considering the works of Finke (2002) and Makkar *et al.* (2014), there is a small deficit of the essential amino acids methionine + cysteine and tryptophan for these age groups (table 2). In any case, a supplementation of cricket-based products with other proteins easily covers this requirement (Makkar *et al.*, 2014).

Protein digestibility can be defined as the "balance of amino acids across the small intestine" and has been determined by numerous authors stem from multienzyme *in vitro* systems to evaluate the digestibility of the protein fraction of insects (Churchward-Venne *et al.*, 2017). It also presents satisfactory values (between 77.9 – 98.9%) for insects belonging to the order Orthoptera (Apolo-Arévalo & Lannacone, 2015), and in some species may be comparable to the values verified for eggs (95%) or beef (98%) (Kouřimská & Adámková, 2016).

Table 2. Essential amino acid (EAA) content of *A. domestica* in different studies and recommendation of EAA for children and adolescents (of ages between 3-10; 11-15; 15-18) and adults (> 18) according to WHO (2007).

EAA	<i>A. domestica</i> (mg/g protein)				Recommendation of WHO (2007) (age in years)			
	Finke (2002)	Rumpold and Schlüter (2013)	Yi <i>et al.</i> (2013)	Makkar <i>et al.</i> (2014)	3-10	11-14	15-18	> 18
His	23	22.7	21	23	16	16	16	15
Ile	46	36.4	36	44	31	30	30	30
Leu	100	66.7	66	98	61	60	60	59
Lys	54	51.1	53	54	48	48	47	45
Met+Cys	23	29.3	25	22	24	23	23	22
Phe+Tyr	81	74.2	92	82	41	41	40	38
Thr	36	31.1	35	36	25	25	24	23
Trp	6	7.6	9	6	6.6	6.5	6.3	6
Val	52	48.4	55	51	40	40	40	39

Abbreviations: His – Histidine; Ile – Isoleucine; Leu – Leucine; Lys – Lysine; Met + Cys – Methionine + Cysteine; Phe + Tyr – Phenylalanine + Tyrosine; Thr – Threonine; Trp – Tryptophan; Val – Valine.

Lipids represent the second largest component of the nutritional composition, varying between 2 – 50% on a dry matter basis (Mezes, 2018). Insects have high levels of polyunsaturated fatty acids (PUFAs), which are quite beneficial from a nutritional point of view, with these percentages varying in the house cricket from 29 to 31% (Fernandez-Cassi *et al.*, 2019). These values are higher than those found for beef and pigs (Rumpold & Schlüter, 2013) and comparable to fish (Garino *et al.*, 2019). However, they are also harmful to the process capability and shelf life of the products, since these type of fatty acids are susceptible to oxidation by free radicals, leading to lipid degradation, which consequently may lead to the development of unpleasant flavours (Arab-Tehrany *et al.*, 2012; Udomsil *et al.*, 2019).

Within PUFAs stand out the known healthy lipids, which include omega-3 and omega-6 fatty acids, whose ratio is quite large in most species (Ghosh *et al.*, 2017). According to Santurino *et al.* (2016), the lipid extract from the cricket has a higher omega-6 contribution than other edible insects and, according to Paoletti and Dreon (2005), when

this is processed into flour, it contains at least twice the omega-3 contribution compared to other processed foods of animal origin.

The carbohydrate content in insects varies between 6.71 and 15.98% (Govorushko, 2019), and these are mostly represented by chitin, which is a glucosamine polymer (N-acetyl-D-glucosamine) (Kim *et al.*, 2019). This is the main constituent of the exoskeleton of insects and is considered indigestible, being one of the elements that can lead to decreased protein digestibility, since some amino acids in the cuticular protein are bound to this component difficult to digest (Mezes, 2018). On the other hand, it is also believed that chitin acts as a dietary fibre, which implies a high fibre content in insects because this is structurally similar to cellulose (Mlcek *et al.*, 2014).

Edible insects can also be excellent sources of some minerals, such as magnesium (Mg), calcium (Ca), iron (Fe), zinc (Zn), phosphorus (P) and potassium (K). Levels of Mg highlight in Orthoptera order, having *A. domesticus* an amount of 55.1 mg/Kg of ingested portion. Levels of Ca, Fe, P and K are relatively high compared to other edible insect species (Payne *et al.*, 2016), and Ca, Fe and Zn are even higher than the levels recorded for chicken, pork, and beef (Udomsil *et al.*, 2019). The abundance in Fe and Zn is particularly important due to the deficiencies of these two elements in developing countries (Govorushko, 2019; Müller & Krawinkel, 2005).

Regarding vitamin content, studies are scarce, but it is known so far that insects are a good source of B complex vitamins in general and of vitamins D, E, K and C (Azzollini *et al.*, 2018; Mlcek *et al.*, 2014). The house cricket stands out mainly for its richness in folic acid of 0.49 mg/100 g (Rumpold & Schlüter, 2013) and for its high input of riboflavin and vitamin B12 (Finke, 2002), in relation to other insects.

1.3.2.2. Economic and environmental sustainability aspects

Rearing of insects is associated with a lower ecological footprint due to several reasons (Irungu *et al.*, 2018), such as the fact that its production results in lower GHG and ammonia emissions (figure 6), being in particular the emissions of the house cricket 100 times lower than conventional livestock production (Halloran *et al.*, 2017), this one requires the use of less or no agricultural area (Garino *et al.*, 2019), with its dimensions even allowing vertical insect rearing (Tao & Li, 2018), and they still need less water because they can cover the water demand of their organism only with the moisture of the food given to them (Apolo-Arévalo & Lannacone, 2015); the production of *A. domesticus* requires approximately 1 L of water per kilo, whereas, for example, the

production of chicken requires 7.3 L of water for the production of 1 kilo of animal mass
(Halloran *et al.*, 2017) (figure 7).

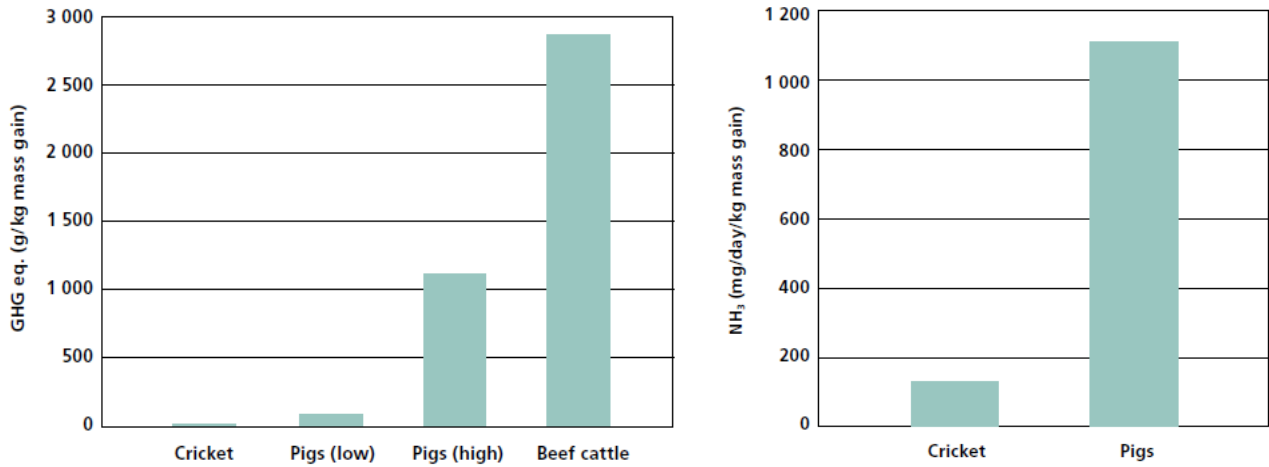


Figure 6. Quantity of GHG (left) and ammonia (NH₃) (right) produced per kg of mass gained, for cricket, pigs, and beef cattle. Adapted from: Van Huis *et al.* (2013).

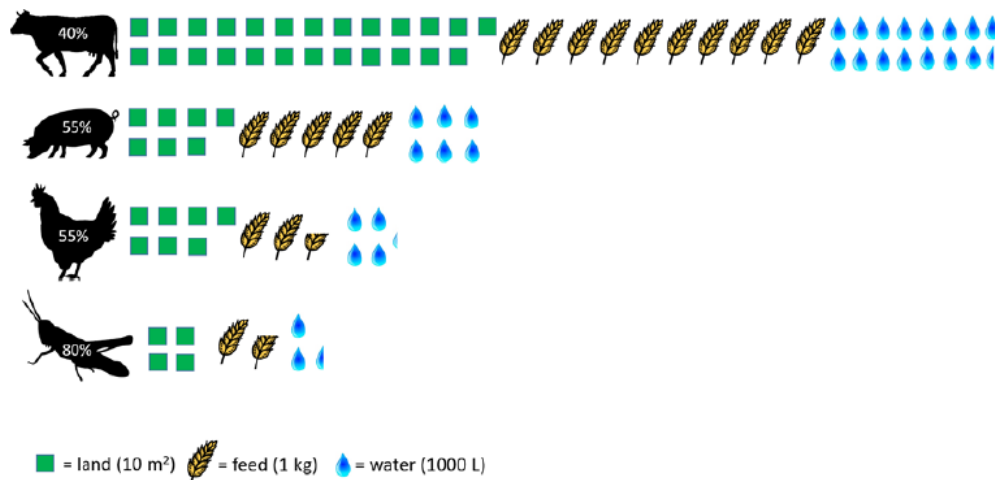


Figure 7. Amount of land, feed and water required to produce 1 kg of animal mass and percentage of animal that is edible. The following are represented: house cricket, chicken, pig, and beef cattle (from bottom to top). Adapted from: Dobermann *et al.* (2017).

Producing insects is also a very efficient process and of relatively fast economic return, since they have high reproduction rates, short life cycles, reaching adult status in a few days or weeks (Govorushko, 2019), and a high feed conversion efficiency, since being poikilothermic animals, and for the regulation of body temperature, they do not expend

energy, thus reducing the production cost (Ferri *et al.*, 2019; Smetana *et al.*, 2019). The conversion rate of *A. domestica* is 2.1, which implies that to produce 1 kg of edible food is needed 2.1 kg of dry feed. This value is very good, considering that to produce 1 kg of edible beef, pig and poultry are required 25, 9.1 and 4.5 kg of feed, respectively (Fernandez-Cassi *et al.*, 2019). In addition, the edible portion in pork is around 55%, whereas that of the house cricket is almost 80% (Van Huis *et al.*, 2013) (figure 7).

On the other hand, it is also important to note that insects do not require the consumption of expensive food sources (Bessa *et al.*, 2017). In the case of the house cricket it has already been shown that feeding, for example with carrots or chicken feed, did not result in significant differences in their feed conversion rate, weight gain and development time (Veenenbos & Oonincx, 2017).

Some insect species are able to convert organic waste into biomass rich in proteins and suitable for use in animal feed (Mlcek *et al.*, 2014). This capacity is very useful, because 1/3 of all food produced in the world is wasted and products made with insects, not consumed by humans, can even be used as animal feed (Gustavsson *et al.*, 2011). FAO has estimated that insects have as much potential as fishmeal and can be used as feed for livestock and aquaculture as well as in the pet industry, since animal feed is considered the most expensive aspect of production (Ferri *et al.*, 2019).

The increasing use of insects for food or animal feed could further reduce the use of pesticides, as this would reduce the economic burden on farmers, who have to ensure the safety of their production in relation to insect pests (Ribeiro *et al.*, 2018). All these mentioned advantages of insect rearing reduce the environmental and economic costs of such productions, which is essential to meet the demands of world population growth (Smetana *et al.*, 2019).

1.3.3. Food security

When studying alternative food sources, it is very important to have access to potential risks (Ribeiro *et al.*, 2018). The presence of hazardous substances in insects comes from two major sources: production of substances by the insects themselves or contamination from the feed used (Van der Spiegel *et al.*, 2013). Thus, due to the possibility of absorption of compounds in the feed, the substrate used is considered one of the most important factors that has an impact on the incidence and amounts of these hazards (Ferri *et al.*, 2019).

According to European Food Safety Authority (EFSA), the main hazards to the food safety of insects are divided into biological (bacteria, viruses, parasites, fungi, prions) and chemical (heavy metals, toxins, veterinary drugs and hormones, among others) hazards, and are also considered allergenicity hazards (EFSA, 2015).

1.3.3.1. Biological hazards

Bacteria

Insects are a good environment for microbial growth, given their high humidity and nutrient content (Amadi & Kiin-Kabari, 2016). As possible risks, two different types of microorganisms should be considered: the intrinsic microbiota of the insect itself and the external microbiota, present in food, substrate, or waste (Van Huis *et al.*, 2013).

One of the main problems detected was the presence of bacterial spores, including in *Acheta domesticus*, as it is one of the insects with the greatest contact with the soil; however, the microbiological content of this species can be removed by boiling, followed by roasting, so that the spores do not germinate (Klunder *et al.*, 2012). The presence of pathogenic species such as *Listeria monocytogenes*, *Clostridium perfringens*, *Salmonella* and *Vibrio* has also been studied, and none of them have been detected in insects so far (Caparros Megido *et al.*, 2017).

In accordance with EFSA (2015), pathogenic bacteria could appear in the case of unprocessed insects, dependent on the substrate utilized and production conditions. Still, the presence of these organisms is lower compared to other unprocessed animal protein sources, because although they may be present in the substrates, their active replication does not occur in the intestinal tract of insects and is therefore safe for humans (Mezes, 2018). The insect pathogenic bacteria themselves are also harmless to humans as these two hosts are phylogenetically different (Schlüter *et al.*, 2017).

However, in order to reduce possible microbiological contamination, it is necessary to explore insects with decent hygiene practices well implemented and appropriate pre-consumption processing, but always taking care not to eliminate parts of the microbiota that perform significant physiological responsibilities in insects (Ferri *et al.*, 2019).

Virus

Most of the viruses present in insects are specific to the family or species and are only pathogenic for invertebrates, not being passed to humans or other vertebrates, having only negative consequences reflected on insect production capacity (Ferri *et al.*, 2019).

Some viruses that are present in insects even have taxonomically close viruses in vertebrates, but these cannot replicate in insects and are therefore not transmitted by them. There is also a possibility of insects being vectors of viruses that infect vertebrates, but once again there is no evidence for their occurrence in insects used for human food or animal feed (EFSA, 2015). Nevertheless, as stated by EFSA (2015), there is indication that vertebrate viruses could survive in substrates for insect production and there is a risk of transmission, which can be moderated by the use of a suitable substrate and efficient processing and preservation methods.

Parasites

Insects can act as temporary or intermediaries hosts of the parasites, which poses a risk to humans when the insects are eaten raw or inadequately cooked (Ferri *et al.*, 2019). They can thus operate as vectors for some parasitosis, such as Chagas disease (*Trypanosoma cruzi*), which causes the death of more than 10000 people per year, according to the WHO (2010). This disease is usually related with transmission in rural zones of Latin America, where inadequate conditions lead to association with infected vectors; cases have also been reported that relate the disease to accidental consumption of insects or contaminated food (Pereira *et al.*, 2010).

Even so, there is no information on the existence of parasites specifically bred for human and animal consumption. Therefore, to ensure that there are no potential risks of parasitosis, it is necessary to have a production system where the conditions are controlled, with a closed farming environment so that possible parasites do not complete their life cycle due to the absence of the necessary hosts and also the application of appropriate processing techniques before consumption (EFSA, 2015).

Fungi

Insects transport and can be sensitive to fungi pathogenic to the insects themselves, which infect them by means of specific toxins that fungi produce, causing mortality of the insects, but these are normally safe for humans and other vertebrates. Once more, hygienic measures throughout the production chain can reduce the occurrence of fungi

that can potentially be a risk to humans and animals present in insects bred specifically for food and feed or introduced during farming, processing or storage (EFSA, 2015).

Prions

Insects have no genes that encode prions or prion-related proteins, so prions are not expected to be expressed in insects. Similarly, insects are also unlikely to function as biological vectors and prion amplifiers since mammalian prions are unable to replicate in insects. Anyway, several studies suggest that insects can function as mechanical vectors of prions in substrates (EFSA, 2015). In this way, insects fed on substrates with proteins of human and animal origin should be evaluated to control the risk of prions and the substrate should be submitted to treatments before its use (Ferri *et al.*, 2019).

1.3.3.2. Chemical hazards

The insect species, methods of production and especially the type of substrate chosen as feed are among the factors that most interfere with the accumulation of contaminants in insects, and these can be natural or artificial (EFSA, 2015; Ferri *et al.*, 2019).

Information on the presence of contaminants in insects is scarce, but it is known that insects can accumulate heavy metals, specifically cadmium, with substrates being in fact the main reporting route. Equally, toxins can be accumulated from the substrate or synthesised naturally by the insect itself, but there is currently no record of any insect listed for consumption in the European Union (EU) that has the capacity to produce toxic substances (EFSA, 2015). It should also be mentioned that residues of veterinary drugs just as other substances such as hormones if applied in agriculture can result in residues and these when present in substrates can also end up in insects (Belluco *et al.*, 2013).

A study of insects reared in Europe showed that the levels of various hazardous chemical compounds are usually equal to or lower than the levels recorded in typically animal feed (fish, meat, eggs), thus meeting the levels imposed by EU (Poma *et al.*, 2017).

1.3.3.3 Allergens

Insects and insect products can cause allergic reactions by biting, inhalation, or contact (Barre *et al.*, 2014). To be able to identify potential risk groups when analysing the possibility of allergenicity of new food sources, it is imperative to study the taxonomic relationships with already known allergenic sources (Ribeiro *et al.*, 2018).

The risk of allergy due to insect consumption may arise from possible cross-reactivity reactions with other arthropods, in particular crustaceans, which are one of the largest sources of food allergy in the West (Lopata *et al.*, 2010). Edible insects and crustaceans are in fact very close taxonomically and cross-reactivity is a real possibility, which means that people with allergic reactions to crustaceans may be sensitive to insects and, with repeat exposures to them, it can lead to the development of an allergic reaction (Fernandez-Cassi *et al.*, 2019; Ribeiro *et al.*, 2018). In fact, cross-reactivity between edible insects and other arthropods has already been studied, and the molecules tropomyosin and arginine kinase, with cross-reactivity between other arthropods, have been identified as allergens of insects too (Ribeiro *et al.*, 2018). This allergenicity is easily avoidable by adequately labelling insect and insect products to guarantee the safety of allergic consumers (EFSA, 2015).

It has also been suggested by some research that chitin could cause allergic reactions, together with its derivative, chitosan. In fact, these are not allergens, but have immune modulatory properties, which might have effects on the manifestation of allergic reactions to other allergens (EFSA, 2015).

1.3.4. Legislation

In 2015, EFSA issued a document on behalf of the European Commission indicating the risks associated with the consumption of insects and concluded that the microbiological risks are similar or lower compared to other protein sources (Finke *et al.*, 2015). Based on this information, whole insects and their parts were considered as Novel Food in accordance with Regulation No 2015/2283 which entered into force on 11 December 2015 and became completely applicable on 1 January 2018 in the EU. In line with Article 1 of Regulation No 258/97, Novel Foods are foods which have not been used in a significant degree for human consumption in the EU before the entry into force of this Regulation on 15 May 1997 (Raheem *et al.*, 2019).

EFSA also proposed a list of potential insect species to be used as food in the EU, which includes the house cricket, considered to be one of the most promising insects as it is native to many European countries (Fernandez-Cassi *et al.*, 2019; Ribeiro *et al.*, 2018).

With respect to the application of insects for animal feed, the EU has authorised the production and placing on the market of products intended for aquaculture, formulated with insect meal, through Regulation No 2017/893 amending Regulation No 999/2001. This amendment adds insects as a source of protein for the formulation of fish feed and

sets the conditions for their production and use. The house cricket is mentioned again as a species with potential, but this time for animal feed (Van Raamsdonk *et al.*, 2017).

1.3.5. Consumer acceptance

The attitude towards a food is influenced by the qualities of that food, by the environment where the consumer is inserted and by the characteristics of the consumer himself (Hartmann & Siegrist, 2016).

In the case of insects, they will be attractive to consumers who are constantly looking for new food experiences and will be rejected by more conservative consumers in their choices (Hartmann & Siegrist, 2016). This rejection by new or unknown food sources can be defined as food neophobia, the "fear of tasting new food" (Melgar-Lalanne *et al.*, 2019). Neophobia is related to disgust, one of the main reactions that consumers have to the idea of consuming insects, being this behaviour associated with the ingestion of substances that may potentially endanger the health of consumers. Thus, as insects are often related to something dangerous and unhygienic, consumers treat them as something unsafe to consume (Dobermann *et al.*, 2017; Melgar-Lalanne *et al.*, 2019).

Gender, age, health status and budget are other aspects that have an impact on preferences for insect consumption. According to some studies, gender effects preferences related to appearance, being women more conventional than men in their choices and more reluctant to try new products. Regarding age, young adults seem to be those who show a more open mind about insect consumption (Melgar-Lalanne *et al.*, 2019). Furthermore, it is important to consider that in the case of Western societies, insects have not been seen as food for a long time, becoming the change in mentality in relation to entomophagy a slow and complicated process (Govorushko, 2019).

A major barrier to consumer acceptance is the negative expectations regarding the sensory properties of insects, especially the taste that tends to be associated with something bad when the food has never been tasted before by the individual (Melgar-Lalanne *et al.*, 2019). It is therefore important that the first experiences are pleasant, otherwise consumers are likely to assume that all insects and products with insects will be equally negative from a sensory point of view (Hartmann & Siegrist, 2016).

As stated in the section Entomophagy in the West one way to make the consumption of insects appealing to provide a positive experience has been its incorporation into various types of products (Azzollini *et al.*, 2018). However, it is important to pay attention to the

selected product to avoid rejection by consumers and it is also essential that the product obtained has a pleasant taste, since this is one of the main factors of food choices and most consumers are not willing to give up of products that they consider tasty for products with nutritional or environmental advantages (Horgen & Brownell, 2002).

Knowledge of entomophagy or previous experience of insect consumption influences the consumption of insect products, as well as the positive experience of consuming insect products where insects are not visible influences the willingness to taste whole insects (Hartmann *et al.*, 2015; Hartmann & Siegrist, 2016). Based on the study of Le Goff and Delarue (2017), consumers initially reject the idea of tasting a product containing insects, but after the first bite, they accept it. All this is based on the theory that consumers' aversion is an acquired attitude and can be overcome by continuous exposure to insects and insect products (Deroy *et al.*, 2015).

Nevertheless, the increased willingness to experiment and taste insects does not translate into increased willingness to buy them and introduce them into the daily diet, since regular consumption is based on other factors (Tan *et al.*, 2016). Thus, a good strategy has been the incorporation of insects in expanded snacks, and the consumption of this ones among the main meals, because this type of products is very popular globally (Melgar-Lalanne *et al.*, 2019). This can be an alternative to lower negative perceptions of entomophagy and rise its acceptability, and a good way to make these novel foods in foods normally consumed by Westerners (Azzollini *et al.*, 2018).

Among the various insects, crickets stand out between the best sellers, with the market for cricket-based products increasingly rising commercial supply and diversification due to their high protein levels and well-balanced amino acid profile. These have not only attracted the attention of adults and young adults, with products such as protein and energy bars, granola, pasta, snacks as chips, cake and cricket nuts, but also children, with some products already very well accepted, formulated specially for this age group, such as cookies and chocolate chips (Lamsal *et al.*, 2019; Melgar-Lalanne *et al.*, 2019).

1.4. Extrusion

Extrusion technology is a continuous process, that use high temperatures in a short time, thus belonging to the HTST equipment family (Lucas *et al.*, 2018). It was first applied in food processing in 1870, but only in 1930, the production of breakfast cereals was applied, which had an enormous adherence, so the extrusion became introduced by

various bakeries (Egal & Oldewage-Theron, 2019). Since then, this process has become well known and has been adopted in several food processing industries (figure 8), such as pasta, baby foods, instant soups, beverages, bread, meat analogues and cheese, among others, and its use continues to grow (Gumul *et al.*, 2018; Wang *et al.*, 2019). This has a huge potential in developing new food products, in particular snacks of various types (Cuj-Laines *et al.*, 2018), which are the most produced by extrusion and the most successful type of products (Agathian *et al.*, 2015).

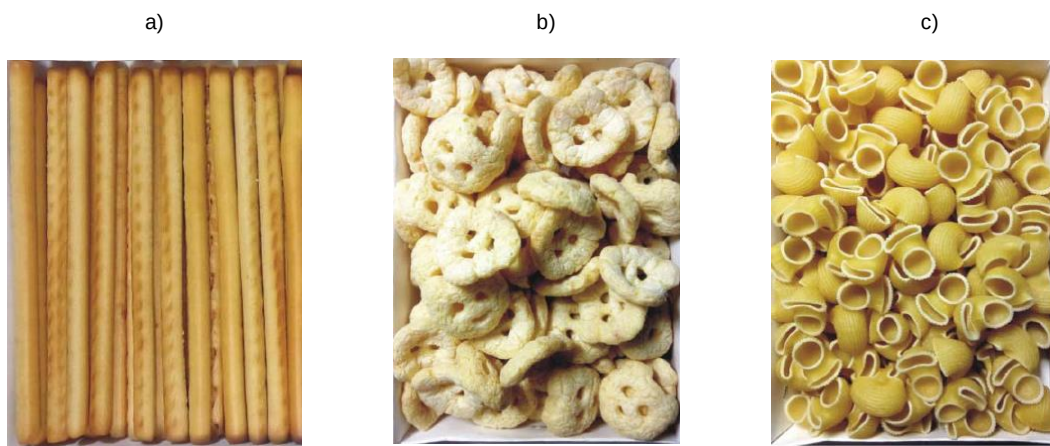


Figure 8. Some extruded products such as milk stick biscuits (a), wheat flower chips (b) and pasta (c), made with different raw materials. Source: Offiah *et al.* (2018).

During extrusion, the raw materials, under high pressure and mechanical shear, are modified by a combination of unit operations (mixing, shearing, kneading, cooking, and shaping) simultaneously (Dalbhagat *et al.*, 2019; Pensamiento-Nino *et al.*, 2018). Therefore, extrusion is more profitable than traditional cooking systems, due to the occurrence of multiple processes in a single machine, allowing to achieve high productivity in one step and decreasing production costs (Teba *et al.*, 2017). Moreover, the production by extrusion is environmentally friendly (Irungu *et al.*, 2018) and a quite promising process, since it generates products with a huge variety of shapes, sizes, flavours, and textures (Rathod & Annapure, 2017).

Based on the type of extruder construction and the method of operation, extrusion can be categorized as hot and cold (Dalbhagat *et al.*, 2019). Hot extrusion consists of using temperatures above 70°C reached by pre-conditioning or heat transferred across the extruder barrel. Conversely, cold extrusion only uses the heat created in the process itself and occurs at temperatures below 70°C (Mishra *et al.*, 2012).

Regarding the type of construction, the extruders can be classified based on the transport mechanism, in piston, roller or screw (Offiah *et al.*, 2018). Screw extruders are the most usual and are divided into single screw extruders and twin-screw extruders (Alam *et al.*, 2016). These are made up of five major parts: pre-conditioning system, feeding system, the screw, the barrel and a specific opening at the end of the barrel, known as die (figure 9) (or instead of this one it can have a cutting mechanism) (Navale *et al.*, 2015). Thus, raw materials are introduced into the extruder from a hopper and are pushed to flow along this barrel, carried by the screw (Do Carmo *et al.*, 2019) and, due to the high temperatures and pressures, the materials are transformed into a viscoelastic fluid until being forced out through the die (Azzollini *et al.*, 2018).

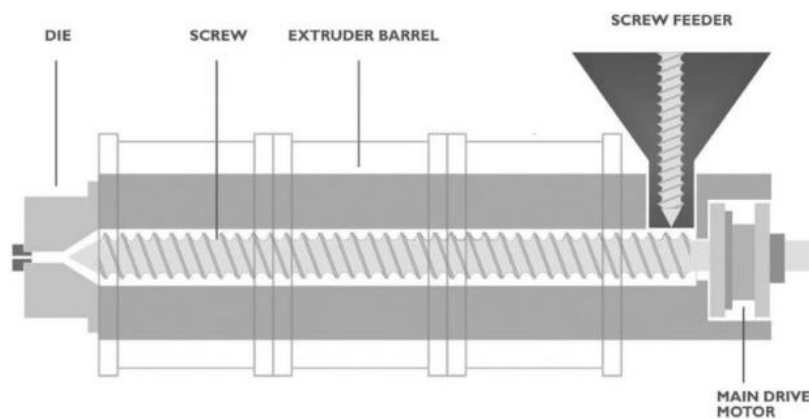


Figure 9. Scheme of the main parts of a screw extruder. Adapted from: Navale *et al.* (2015).

Single screw extruder

The single screw extruder consists of a single rotating screw which is located inside a metal barrel whose internal surface has helical or axial grooves, that help to transport the materials (Dalbhagat *et al.*, 2019). This type of extruder works according to three stages: conveying, swelling and melting/degradation. The first two stages occur in the temperature cooling zone, and the last stage, which corresponds to the most important one since it is when the starch granules are gelatinized, takes place in the third region of the extruder. There is a longer residence time of the materials in this heating zone, in relation to the cooling zone where the first two stages succeed each other, because the flow of the mass is changed when passing to this stage, as there is more mixing and, therefore, it needs to stay longer in this zone (Alam *et al.*, 2016; Shah *et al.*, 2019). Single

screw extruders are the most used in the food industry mainly for the processing of less complex ingredients, besides being an equipment with a lower cost (Offiah *et al.*, 2018).

Twin-screw extruder

The twin-screw extruder consists of two screws rotating in a single barrel, whose interior surface is smooth. For this type of extruder four different layouts are possible, according to the position and rotational direction of the screws, thus featuring more efficient displacement characteristics (Steel *et al.*, 2012). In these, inside the extruder barrel, there is a mixture of all ingredients from the two screws before they all pass to the rest of the extruder areas. The twin-screw extruders also provide better flexibility for controlling extrusion variables. Although they have some advantages over single screw extruders, mainly the fact that more complex raw material formulations can be handled, they are little used in the food industry, not only because they are more complicated, but above all for the high costs (Dalbhagat *et al.*, 2019).

1.4.1. Extrusion parameters

Extrusion parameters such as feed moisture, feed particle size, feed rate, barrel temperature profile, speed and configuration of the screw, and die opening (size and geometry) are very important, since slight variations in these have an influence on the properties of the extruded product being produced (Ding *et al.*, 2006; Rathod & Annapure, 2017). Therefore, an adequate selection and control of these processing parameters is necessary to produce attractive extruded products with the desired characteristics (Poliszko *et al.*, 2019).

The effect of these primary parameters together with the ingredient properties of the raw materials influence the process responses, known as secondary extrusion parameters which are product temperature, specific mechanical energy, die pressure and torque (Dalbhagat *et al.*, 2019). These allow the extrusion process to be described or compared when distinct operating conditions are used (Meng *et al.*, 2010).

Product temperature is determined in the die head from the variation between the barrel temperature and the die temperature, being typically higher than the barrel temperature. Its increase is due to the tension between the surface of the barrel and the granules of the material used as they are transported across the screw (Dalbhagat *et al.*, 2019).

The specific mechanical energy corresponds to the energy required to produce 1 kg of extruded material (Patil *et al.*, 2005). It consists of the ratio between the total mechanical energy obtained from the transport of the material by the rotation of the screw and the mass flow from the feeding zone to the extruder (Kantrong *et al.*, 2018).

The pressure is given by the resistance offered by the material to flow through the die, whose geometry has a significant effect on this parameter. Generally, in this area there is an increase in pressure caused by the accumulation of material in the die opening (Dalbhagat *et al.*, 2019).

Lastly, the torque corresponds to the energy required to turn the screw, and is related to the speed of the screw, as well as the degree of filling of the barrel and the viscosity of the materials in the screw barrel. It thus evaluates the impact of extrusion processing, since it is associated with the energy consumption of the extruder (Iwe *et al.*, 2001).

1.4.2. Raw materials and modifications in main components

Extruded products are usually made from cereals (corn, wheat, and rice) and their respective starches (Arribas *et al.*, 2019). These are usually the most used raw materials, since they have excellent expansion capacities due to their high starch content (Joshi *et al.*, 2014), are highly available and have a low cost (Ramos *et al.*, 2017). However, products made from these tend to have low levels of protein, given their limited levels of essential amino acids, and also low levels of other equally important nutrients (Joshi *et al.*, 2014), so that the products obtained are insufficient to meet the needs of a growing number of health-conscious consumers (Azzollini *et al.*, 2018).

Corn is one of the most used cereals in extrusion, being part of the most commercial extruded products, such as snacks made with corn (Thakur *et al.*, 2017), because it generates products very tasty with great porosity and a crunchy texture (Coutinho *et al.*, 2013). Several studies have shown that corn extruded products have good properties, having only some variations from each other due to the different processing conditions used (Gumusay *et al.*, 2019). This has been demonstrated for several years, including Martínez *et al.* (1996) developed extrudates whose main ingredient was maize from a single-screw extruder and showed that the final extruded snack obtained had all the characteristics desired by the consumer, with high expansion and low density.

Besides starch, the second most used raw material is protein, which has been increasingly used to enrich extruded formulations, thus making it possible to make

extruded products with high nutritional value available to consumers (Obradovic *et al.*, 2014). Still, the proportion of starch in the mixture is generally higher to improve the spread of the final product and to meet the preferences of consumers (Adem *et al.*, in press), because starch-rich extrudates are generally more expanded than those rich in protein, which usually have a medium to low expansion (Philipp *et al.*, 2017b).

These are the basic components of a typical mixture used to extrude, but this one can include other components such as lipids, fibres and in some cases, minerals and vitamins, and all of them will undergo various transformations during the process that will be reflected in the quality of the final extruded product (Navale *et al.*, 2015).

1.4.2.1. Starch

Starch is a carbohydrate composed of a chain of glucose molecules and consisting of two polymers: amylose and amylopectin, which are responsible for the physicochemical properties (Oladiran & Emmambux, 2020). The structure of the final product is mainly provided by starch (Navale *et al.*, 2015).

The native form of starch is not the most suitable, so it is modified by extrusion, going through several transformations to acquire starch products with the desired attributes (Bravo-Núñez & Gómez, 2019; Sharma *et al.*, 2015). In the extruder, the starch suffers several states that begin with the melting, which consists in the melting of its granules at high temperatures, so that they become softer; the continuous action of the temperature together with the shearing action, just as water content, break the organized molecular structure of its granules, corresponding this state to the fragmentation; and, finally, gelatinization occurs, which makes the starch granules digestible (Navale *et al.*, 2015).

Starch gelatinization is one of the most important transformations that take place during extrusion, as this greatly influences the properties of the extruded products obtained, for example leading to improved water absorption, which causes the starch granules to break down and consequently rise viscosity (Mościcki & van Zuilichem, 2011); generates loss of starch crystallinity, which modifies solubility in water; the formation of air cells due to the steam pressure of heated water, causes the starch material to expand and adopt a porous structure at the exit of the die due to quick vaporization (Alcázar-Alay & Meireles, 2015). It is known that the final product has various quality attributes, and thus starch plays a key role in establishing some of them, namely viscosity, moisture, expansion, texture, taste, and shelf life (Offiah *et al.*, 2018).

1.4.2.2. Proteins

Denaturation is the main change suffered by proteins through extrusion process. Proteins such as starch are dispersed from their native bodies as they form a continuous viscoelastic dough (Navale *et al.*, 2015), so denaturation consists of the unfolding of the structured native status of proteins to one partially unstructured. This transformation is prompted mainly by the high temperatures of extrusion, being often a precondition for achieving the desired characteristics of the final product (De Graaf, 2000).

As a result of denaturation, there is a change in the conformation of proteins that makes them less soluble (Steel *et al.*, 2012). Still, this transformation does not imply a decrease in protein digestibility, it may even increase (Wang *et al.*, 2020), and it can lead to the loss of biological activity of proteins with enzymatic action, and their inactivation reduces the risk of adverse reactions mediated by enzymes (Alam *et al.*, 2019; Offiah *et al.*, 2018).

But not all changes that occur in proteins are positive. Taking into account that essential amino acids cannot be synthesized by organism, but only obtained by the diet, they deserve special attention, mainly lysine in this case, since in cereals it corresponds to the most limiting amino acid (Navale *et al.*, 2015). Thus, it tends to decrease during extrusion, due to the Maillard reaction (Yovchev *et al.*, 2017), a reaction that occurs between amino acids or proteins and sugars and that leads to the reduction in protein quality (Steel *et al.*, 2012). Even so, the fact of extrusion be a process that occurs in a short period of time, make the undesirable effects on proteins and amino acids smaller than would be expected if another type of processing was used (Obradovic *et al.*, 2014).

1.4.2.3. Lipids

During extrusion, lipids can act as lubricants or plasticizers, decreasing the friction throughout the process and therefore influencing the attributes of the final product. Thus, lipids create amylose-lipid complexes responsible for modifying the physicochemical parameters of the products, such as water solubility index, bulk density, and expansion. But the production of these complexes will depend on the type of starch and lipids present in the blend for the formulation (Steel *et al.*, 2012), and cereals with high levels of lipids are not appropriate, since a content of lipids higher than 3% can have unwanted effects, as the case of reduced product expansion (Navale *et al.*, 2015).

Inactivation by extrusion of enzymes such as lipase and lipoxidase allows not to be induced the instant oxidation of lipids. But although deterioration is not immediately

apparent because of the extrusion process, during storage the exposure to that oxidation increases, since the moisture content of the extruded products is low and they have a large surface area caused by expansion (Yadav *et al.*, 2018).

1.4.2.4. Fibre

Fibres can be classified according to their solubility in water into soluble and insoluble and both have health benefits. There can even be a redistribution of insoluble fibres to soluble fibres, as a consequence of the changes in their qualities that occur during extrusion (Offiah *et al.*, 2018), such as modifications in solubility of fibre, granulometry and chemical structure (Navale *et al.*, 2015). Fibre is a component that interferes with the expansion of the final product and also with the thickness of the cell wall, when combined with protein (Beck *et al.*, 2018).

1.4.2.5. Minerals and vitamins

There are certain inhibiting factors such as tannins and phytates that create complexes with minerals and prevent their absorption, but the extrusion process allows their destruction from the product, which is an advantage since it makes it possible to improve the absorption of minerals (Dalbhagat *et al.*, 2019). In fact, according to the literature, extrusion has leads to increased iron levels in many cases studied (Zarzycki *et al.*, 2015), probably due to the screw and barrel wearing during extrusion (Yadav *et al.*, 2018).

Regarding vitamins, it is known that there are heat sensitive vitamins and therefore, in treatments whose temperatures used are 100°C or above, there is loss of these type of vitamins, particularly those soluble in water, as is the case of vitamin C (Offiah *et al.*, 2018). However, that losses are usually minimal compared to other food processes since extrusion occurs in a short time (Obradovic *et al.*, 2014). Still, manufacturers have been adopted the practice of fortification of extruded products with micronutrients after extrusion by spraying (Offiah *et al.*, 2018).

1.4.3. Characterization of extruded products

Extrusion conditions and the composition of the raw materials are the main determinants of the chemical and structural transformations that occur in extrusion, which will affect

the characteristics of the food materials melted in the extruder and, thus, the quality of extrudates obtained (Meng *et al.*, 2010). The final product is going to reflect all these changes that happen throughout the extrusion process, that can be seen from its properties, which allow evaluating the work of the extruder, the ability of the final product to be used and some of them are also important to consumer acceptance (Patil *et al.*, 2005). Therefore, for the development and production of new food products, in addition to the compositional assessment, it is essential to evaluate the physicochemical properties, such as water content and activity, indexes of water absorption and solubility, expansion, bulk density, porosity, and also colour and texture properties, that contribute differently to the final quality of the extruded products (Lucas *et al.*, 2018).

Water content corresponds to the total amount of water contained in a food, but water activity is associated with the preservation of food. The latter can be defined as the relationship, in equal condition, between the pressure of pure water and that of a solution. It determines the free water present in the matter that will participate in the reactions, reacting with microorganisms, being therefore important to determine the validity of the products (Gumusay *et al.*, 2019).

Two other relevant properties to be determined are the indexes of absorption and solubility in water, as they allow predicting the future behaviour of extruded products. The first indicates the portion of water absorbed by the extrudate when immersed in water (Alam *et al.*, 2019) and it is used by many authors to measure the volume taken by the starch after swelling in water excess, being then considered a starch gelatinization index (Lucas *et al.*, 2018). The water solubility index is widely utilized to determine the level of starch conversion during extrusion from the quantity of soluble polysaccharides freed from this component. It shows the amount of water solubilized components released during extrusion that can cause molecular damage and is subsequently used as an index of molecular degradation (Gumusay *et al.*, 2019; Ondo *et al.*, 2013).

Expansion occurs due to the momentary exit by the tight die of the molten mass by passing from a high pressure (from the barrel) to the atmospheric pressure, which generates a pressure drop, resulting from the rapid release of water, that is responsible for the extension of the product (Navarro-Cortez *et al.*, 2018) (figure 10). It is a parameter associated with the microstructure and consequently with crunchiness, being a decisive factor both for quality of extrudates and consumer acceptance (Lucas *et al.*, 2018).

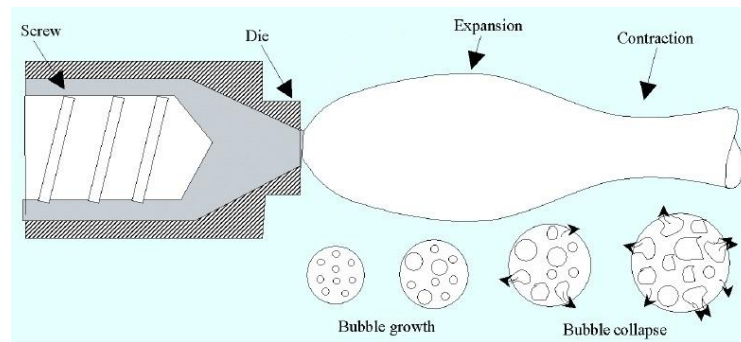


Figure 10. Scheme of how expansion occurs. Adapted from: Moraru and Kokini (2003).

Bulk density is also related to microstructure, being an essential parameter for product wrapping, since it considers both pores and voids developed through extrusion (Irungu *et al.*, 2018), indicating the porosity of the product, and therefore it gives good information on how to conceive the design and packaging material (Rathod & Annapure, 2017).

Colour and texture are important characteristics of extruded snacks, especially for the consumer, that improving the quality of food products. Colour parameter, in particular, is directly related to acceptability, being a decisive attribute that influences consumer at the time of purchase (Do Carmo *et al.*, 2019). Texture is one of the attributes that most motivates the acquisition of a certain product, so for this to be the one intended by consumers, the most indicated method for its evaluation is the use of the human senses during ingestion. However, over time it was perceived that the difficulty of comparing results from different studies could generate several errors and other tests started to be used, as puncture analyses, whose results can then be associated with sensory perceptions (Philipp *et al.*, 2017a), such as crunchiness, which stands out as the textural property most desired by consumers (Alam *et al.*, 2016; Jakubczyk *et al.*, 2017).

1.4.4. Development of promising healthy extruded products

Extrusion technology has undoubtedly been one of the preferred technologies, as it has few disadvantages, such as the initial investment of the equipment and the appropriate selection of extrusion conditions, being the advantages in relation to these disadvantages outweigh (Egal & Oldewage-Theron, 2019). In addition, as mentioned above, the snacks produced by extrusion are among the most sought-after today, even though these generally do not contain the necessary nutritional requirements (Obradovic *et al.*, 2014). There are several studies that address the incorporation of various sources

of protein in snacks, but the use of insects for extruding is relatively recent and still little investigated (Alam *et al.*, 2019).

The processing of insect products from extrusion would be a good strategy on the part of the food industry, from various points of view. On the one hand, the production of extruded snacks with insects could lead to increased acceptance of this protein source (Azzollini *et al.*, 2018) and this addition to extruded snacks would make the final product more nutritious (Philipp *et al.*, 2017a), therefore responding to consumers' demands for healthier snacks. On the other hand, the fact that the product produced is a snack would increase its approval, since these products are in demand not only for their taste and textual characteristics, but also because they are convenient and above all economical products (Alam *et al.*, 2016).

Moreover, the extrusion process itself allows for the conservation of the natural colours and flavours of food (Pensamiento-Nino *et al.*, 2018), and also for the slight degradation of nutrients (Lucas *et al.*, 2018). Consequently, this would combine the nutritional benefits of a protein source, in the case of insects, with the features sought in snacks, from the extrusion technology (Arribas *et al.*, 2019).

2. Objectives

Insects, as alternative sources of protein, have been one of the bets to solve present and future food insecurity, and their incorporation in snacks familiar to consumers has been suggested as a strategy to increase their acceptability. Taking this into consideration, the main objective of this work is the development of snacks enriched with insect meal from the extrusion technology, where it is intended to investigate the effect of different levels of incorporation of house cricket / *Acheta domestica* and two extrusion temperatures on the quality of the final extruded snacks.

In an initial phase of this study, it is proposed to formulate mixtures based on maize flour and house cricket flour in different mass ratios, and then each of these formulations will be extruded at two different temperatures. Thus, with different snacks obtained, among the specific objectives are the evaluation of the secondary extrusion variables, pressure and torque, and their impact on the extrudates, as well as the analysis of the physicochemical, compositional, textural and optical properties of the snacks produced; and also the determination of the amount of protein that is absorbed after consumption, and the overall *in vitro* digestibility of the snacks.

In the end of this work, it is planned to obtain a snack that presents the attributes most desired by the consumer, according to the literature, but also presents improved nutritional properties compared to the snacks available on the market, by adding insects, in order to meet the current consumer demands. Therefore, specific objectives also include the selection of the “ideal snack” from among all extrudates produced, by the choice of the one that showed improvements in its attributes and high protein contents over the standard snack made only with corn that already has all the characteristics appreciated.

3. Material and methods

3.1. Raw materials and formulations

Corn grits supplied by Maicerías Españolas S.L. (Valencia, Spain) and *Acheta domestica* powder by Entoma Foods (Valencia, Spain) were used for the formulation of extruded products. Their nutritional composition is represented in table 3. Four extrusion blends mixtures of corn flour and house cricket powder were prepared in mass ratios of 100:0, 95:5, 90:10 and 85:15, mixed manually.

Table 3. Nutritional composition of corn grits (Maicerías Españolas S.L.) and *A. domestica* powder (Entoma Foods).

Component (g/100g)	Corn grits	<i>A. domestica</i> powder
Energy	1671 kJ / 399 Kcal	2197 kJ / 525 Kcal
Total Fat	3.86	30.7
Saturated Fat	0	12.4
Total Carbohydrate	76.85	1
Dietary fibre	7.3	4.1
Total sugars	0	0.8
Protein	6.93	60
Salt	0	0.8

3.2. Extrusion processing

Extrusion was carried out with a single-screw laboratory extruder (Kompaktextruder KE 19/25; Brabender, Duisburg, Germany) with a barrel diameter of 19 mm and a length: diameter of 25:1 (figure 11). The extruder was conducted at 3:1 compression ratio and laden with prepared samples at a constant dosing speed of 18 rpm (feed rate range, 1.58 kg/h). The screw was rotated continuously at 150 rpm. Two temperature conditions were used in sections 1-4: 25, 70, 160 and 165°C (figure 12a), also 25, 70, 170 and 175°C (figure 12b), respectively; the temperatures of the sections 1-3 are temperatures to condition the samples to the desired final temperatures of the last section (165 and 175°C), that were variables under study. The diameter of the die used was 3 mm. Extruder and Winext software (Brabender, Duisburg, Germany) was used to monitor

screw speed, barrel temperatures, pressure, and motor torque. Calculated specific mechanical energy was from 888 to 923 kJ/kg, for temperatures of 165 and 175°C, respectively. Then, extrudates were cooled at ambient temperature and sealed in plastic bags for further analysis.



Figure 11. Single screw laboratory extruder turned off (left) and during the extrusion of corn and house cricket snacks (right).

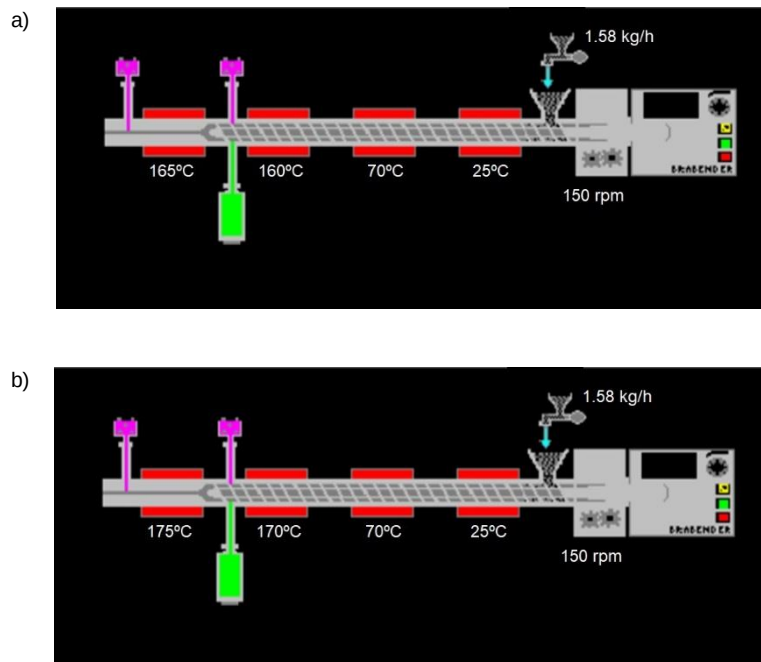


Figure 12. Scheme of conditions used in the extruder to reach final extrusion temperatures of 165°C (a) and 175°C (b).

Temperature and percentage of *A. domestica* are the two factors under study in the present work. Each of the mixtures with 0, 5, 10 and 15% of incorporated house cricket were extruded at 165 and 175°C, which consists of eight different treatments (table 4).

Table 4. Treatment code corresponding to each extrusion temperature (T) to which each formulation with different percentage of *A. domestica* (AD) has been submitted.

Treatment	T (°C)	AD (%)
165-0	165	0
165-5	165	5
165-10	165	10
165-15	165	15
175-0	175	0
175-5	175	5
175-10	175	10
175-15	175	15

3.3. Analysis

3.3.1. Water content and water activity

The determination of water content of samples ($g_{\text{water}}/100g$ sample) were made by drying in a vacuum oven at 105°C until reaching a constant weight, both for mixtures (before extrusion) (x_w^M) and for extruded samples (x_w^E), with and without *A. domestica* powder (Horwitz & Latimer, 2005). Samples were analysed in triplicate and were weighed before and after going to the vacuum oven. The water content was calculated using the following equation (where, M_s is the mass (g) of the sample and M_{DS} is the mass of dry sample):

$$x_w = \frac{M_s - M_{DS}}{M_s}$$

Due to the extrusion process, water loss (W_L) of the samples was also calculated by the difference between the water content of the mixture and the water content of the extrudate, for the same formulation.

Water activity (a_w) of the extruded samples was determined by the AquaLab PRE LabFerrer equipment (Pullman, USA).

3.3.2. Water absorption index, water solubility index and swelling index

The method of Singh and Smith (1997) was the one used to determine water absorption index (WAI) and water solubility index (WSI). First, the extrudates were milled to an average size of 180 – 250 μm . It was dispersed a sample of 2.5 g of each extrudate material in 25 g of distilled water and a rod was utilized to mix everything to avoid and break any lumps. Each of the prepared samples was then subjected to stirring for 30 minutes with magnetic stirrer. The dispersions were washed in to tared 50 mL centrifuge tubes, made up to 32.5 g and were centrifuged at 3000 g for 10 minutes. To determination of dissolved solids in the supernatant, this was decanted, and the sediment was weighted. WAI and WSI were calculated according the equations:

$$\text{WAI} = \frac{\text{weight of sediment}}{\text{weight of dry solids}}$$

$$\text{WSI (\%)} = \left(\frac{\text{weight of dissolved solids in supernatant}}{\text{weight of dry solids}} \right) \times 100$$

The swelling index (SWE) was measured by the bed volume technique. It was weighed 5 g of each sample and these were transferred to a calibrate cylinder to which 50 mL of distilled was added. The bed volume was recorded after cylinders had been left to stand undisturbed at ambient temperature for 18 hours. SWE was expressed as (Robertson *et al.*, 2000):

$$\text{SWE} = \frac{\text{volume of sample swollen (mL)}}{\text{initial weight of the sample (g)}}$$

All samples were analysed in triplicate.

3.3.3. Hygroscopicity

Around 0.5 g of each extrudate was placed on a petri dish and then each one of them in an airtight plastic container with a saturated solution of Na₂SO₄ (sodium sulphate) (81% relative humidity) at the bottom, at 25°C. After 7 days, each sample was weighed and hygroscopicity (Hg) was expressed as g of water gained per 100 g of dry solids (Cai & Corke, 2000). Samples were analysed in triplicate.

3.3.4. Expansion index

It was used an electronic Vernier calliper (Comecta, S.A., Spain) to measure the diameter of extruded (D_e) products, having been measured 20 pieces of each sample. This was used to obtain the surface expansion index of die (SEI), according to the following equation, where D_d represents the die diameter (Patil *et al.*, 2005):

$$SEI = \frac{D_e^2}{D_d^2}$$

3.3.5. Bulk density and porosity

It was cut 3 cm of each extruded product for the determination of the real (ρ) and bulk (ρ_b) densities. The real density was determined using a helium pycnometer (AccPyc 1330, Micromeritics, Norcross, USA) (figure 13). For bulk density, measurements were taken of each sample 15 times, where height and diameter of cylinders were measured with an electronic Vernier calliper (Comecta S.A., Spain) and samples were weighed with a precision scale (± 0.001 g) (Mettler Toledo, Switzerland).



Figure 13. Helium pycnometer (AccPyc 1330, Micromeritics, Norcross, USA).

Real and bulk densities were used to calculate the porosity (ε), from the equation:

$$\varepsilon = \frac{(\rho - \rho_b)}{\rho}$$

Porosity was also determined from image analysis. After extrusion process, photographs of each extruded sample of the various treatments were taken with the same light and at the same distance. Software ImageJ (Abramoff *et al.*, 2004) was used to treat the photographs and to analyse the total pore area of the cut sections of that images to obtain porosity.

3.3.6. Crude protein

Dumas method in a Leco CN628 Elemental Analyser (Leco Corporation, St. Joseph, MI, USA) was used to determine the crude protein (CP) (nitrogen x 6.25), according to official method 990.03 of AOAC International (2002).

3.3.7. Texture

Texture of extrudates was measured using a TA-TXT2 Texture Analyser (Stable Micro Systems Ltd, Godalming, UK) (figure 14) and the software Texture Exponent (version 6.1.12.0). A puncture test was performed, which consists of the force needed to push a probe or a cylindrical puncturing device through an extrudate (Desrumaux *et al.*, 1998).



Figure 14. Texture analyser TA-TXT2 (Stable Micro Systems Ltd, Godalming, UK).

It was used a cylinder of 2 mm in diameter and the crosshead speed was maintained at 0.6 mm/s. Measurements were taken 10 times. The software made a record and then analysis of the force – time curve and gave an area under the curve, corresponding to the work (S) done for a given time of displacement of the puncturing device. The force-drop, relative to each peak represents the local resistance of cell walls, having the number of peaks also been recorded (N_0) (figure 15).

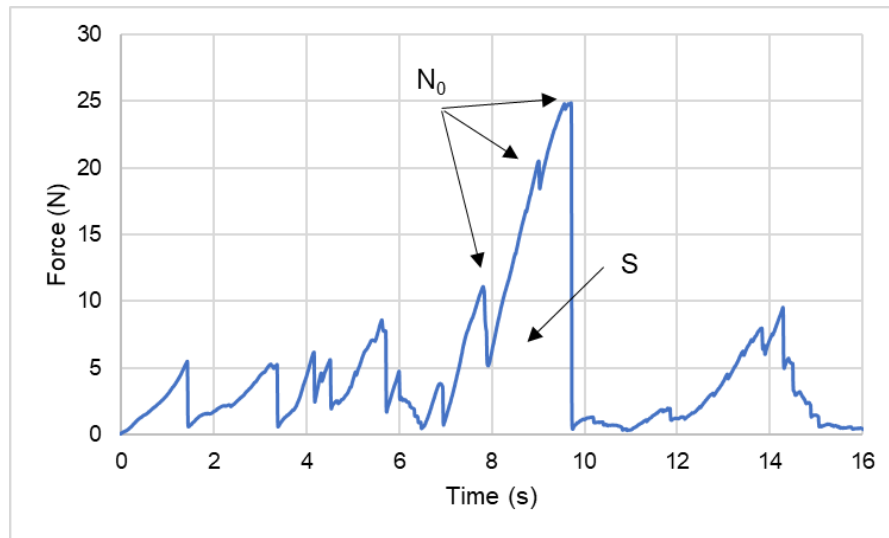


Figure 15. A typical force-time curve of extruded products for texture measurements. S represents the area under the curve and N_0 the number of peaks.

These parameters were used to calculate the average puncturing force (F_p), the average specific force of structural ruptures (F_s), the spatial frequency of ruptures (N_{sr}) and the crispness work (W_c), from the equations below, where d corresponds to the probe travel distance (Bouvier *et al.*, 1997):

$$F_p = \frac{S}{t}$$

$$F_s = \text{sum of } \frac{F}{N_0}$$

$$N_{sr} = \frac{N_0}{d}$$

$$W_c = \frac{F_p}{N_{sr}}$$

3.3.8. Optical properties

The determination of colour coordinates of $CIE^*L^*a^*b^*$ (L^* , lightness; a^* , greenness/redness; and b^* , blueness/yellowness) and translucency of the samples were made from a surface reflectance spectra obtained between 400 and 700 nm, when measuring on white and black backgrounds, using a spectrophotometer considering a standard light source D65 and a standard observer 10° (Minolta spectrophotometer CM-3600d, Japan). These measurements were taken 10 times on each sample.

Kubelka-Munk theory for multiple scattering to the reflection spectra was applied to determine translucency of the samples. This theory takes on that the light flux that passes through the sample is related to the ratio of absorbed scattered light (Hutchings, 1999; Talens *et al.*, 2002). Spectral distribution of Kubelka-Munk coefficient (ratio between light absorption (K) and scattering (S)) was achieved as follows:

$$\frac{K}{S} = \frac{(1 - R_{\infty})^2}{2 \cdot R_{\infty}}$$

$$R_{\infty} = a - b$$

$$a = \frac{1}{2} \left(R + \frac{R_o - R + R_g}{R_o \cdot R_g} \right)$$

$$b = (a^2 - 1)^{1/2}$$

Where, a and b used to determine the K/S coefficient are not the same as a^* and b^* , which correspond to the colour coordinates of $CIE^*L^*a^*b^*$ colour space. In the equations presented, R and R_o are the reflectance of the sample on a white and black backgrounds, respectively; R_g is the reflectance of the white background; and R_{∞} is the reflectance of an infinitely thick layer of the material. In the case of translucent samples, the values of R and R_o differ from each other and to obtain the $CIE^*L^*a^*b^*$ colour coordinates, R_{∞} was calculated (Hutchings, 1999). When no translucency was observed in the samples, there was no difference between the values of R and R_o , being R used to obtain the $CIE^*L^*a^*b^*$ colour values, directly from the equipment used for colour measurement.

Hue, colour shade (h_{ab}^*) and chroma, colour intensity (C_{ab}^*) were calculated from values of a^* and b^* as (Wrolstad *et al.*, 2005):

$$h_{ab}^* = \arctan \left(\frac{b^*}{a^*} \right)$$

$$C_{ab}^* = \sqrt{(a^{*2} + b^{*2})}$$

The total colour difference (ΔE) of the extrudates with 5, 10 and 15% of house cricket powder were calculated in relation to the control sample (0% of insect meal):

$$\Delta E = \sqrt{(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})}$$

3.3.9. *In vitro* digestibility and protein bioaccessibility

For the evaluation of the *in vitro* digestibility of extrudates was used the standardized static *in vitro* digestion method, suitable for food (international consensus) proposed by Minekus *et al.* (2014).

Four steps have been followed: oral phase, where the sample was mixed with simulate salivary fluid (SSF) (1:1) and amylase at pH 7, to simulate the chewing through the mincing of this mixture with a stick blender during 2 minutes; gastric phase, where the formed oral bolus was mixed with simulate gastric fluid (SGF) (1:1) and pepsin at pH 3, during 2 hours; intestinal phase, where gastric chyme was mixed with simulate intestinal fluid (SIF) (1:1) and enzymes at pH 7, during 2 hours to simulate what happens in the small intestine. In the gastric and intestinal phases, constant stirring during the 2 hours was guaranteed from magnetic stirrers (figure 16). At the end of these phases to ensure that the digestion stopped completely, protease inhibitor was added. The fourth step consisted of a centrifugation at 4500 rpm for 30 minutes and a filtration, filtering through a 1 μ m glass-fibre membrane. Analysis were repeat in duplicate. Gastric, intestinal, and *in vitro* digestion final samples were collected according to Minekus *et al.* (2014) recommendation.

To calculate the *in vitro* digestibility (IVD (%)) was used the equation (Batista *et al.*, 2017):

$$\text{IVD (\%)} = \left(\frac{\text{inicial mass} - \text{undigested mass}}{\text{inicial mass}} \right) \times 100$$

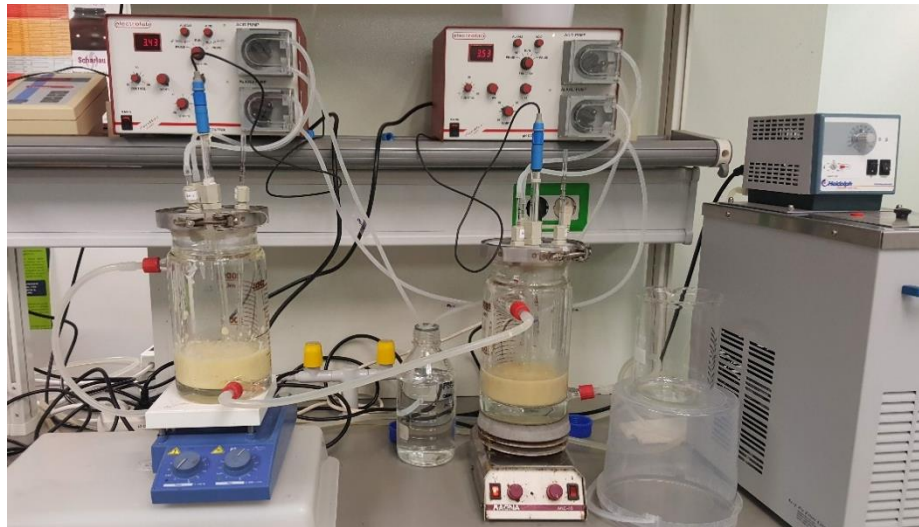


Figure 16. Gastric phase of *in vitro* digestibility.

For the determination of the protein content after digestion, the filtrate was frozen at 45°C and after 24 hours it was lyophilized. From the freeze-dried samples the protein content was determined as previously indicated. Protein bioaccessibility was determined using the equation proposed by Khouzam *et al.* (2011) and Sahuquillo *et al.* (2003):

$$\text{Bioaccessibility} = \left(\frac{A}{B} \right) \times 100$$

Where, A is the concentration of the CP in the bio-accessible fraction after *in vitro* digestion and B is the concentration of the CP in the sample before digestion. CP present in tap water and the reagents were also analysed and corrected in the final bio-accessible fraction.

Labour limitation: For the *in vitro* digestion analysis 75 g of each sample was required. In the absence of this amount, it would have been expected that more extrudates would have been produced for digestibility testing. However, in the course of this work, a breakdown occurred in the pressure probe of the extruder which prevented its correct functioning, so that samples from treatments 165-5, 165-10, 165-15 and 175-15 which did not reach 75 g could not be produced again and due to the covid-19 pandemic it was not possible to wait for the repair of the extruder probe to continue this part of the work. Consequently, *in vitro* digestibility analyses were only performed for control samples

extruded at both temperatures (165 and 175°C) and for samples with 5 and 10% of house cricket incorporated, extruded at 175°C.

3.3.10. Statistical analysis

Two-way ANOVAs (analysis of variance) were used to study the effect of the factors (temperature and percentage of *A. domesticus*) and of their interaction on the various product parameters. When significant effects were identified, differences between factor levels were evaluated using the Least Significant Difference (LSD) *post-hoc* test. All tests were applied with a confidence level of 95% ($p < 0.05$), using Statgraphics Centurion XVII Software, version 17.2.04.

Data were presented as mean $\pm$ standard deviation in three different tables for each parameter: the first, relative to the interaction between the factors under study; the second relative to the effect of extrusion temperature; and the third relative to the effect of the level of *A. domesticus*. The homogeneous groups established by the LSD test ($p < 0.05$) were indicated from the same superscript letter inside the columns in the tables where significant differences were observed. In the case of significant differences noticed both in relation to the interaction between the variables and their individual analysis, the homogeneous groups were only placed in the interaction table, since it soon relates the two factors. For the significant differences found in relation to temperature, no letters were assigned to homogeneous groups, since there are only two groups (165 and 175°C), so one is automatically different from the other.

Furthermore, using the same statistical software mentioned, a correlation analysis among physicochemical and compositional parameters and between these and the textural properties, with a 95% confidence level ($p < 0.05$), was carried out.

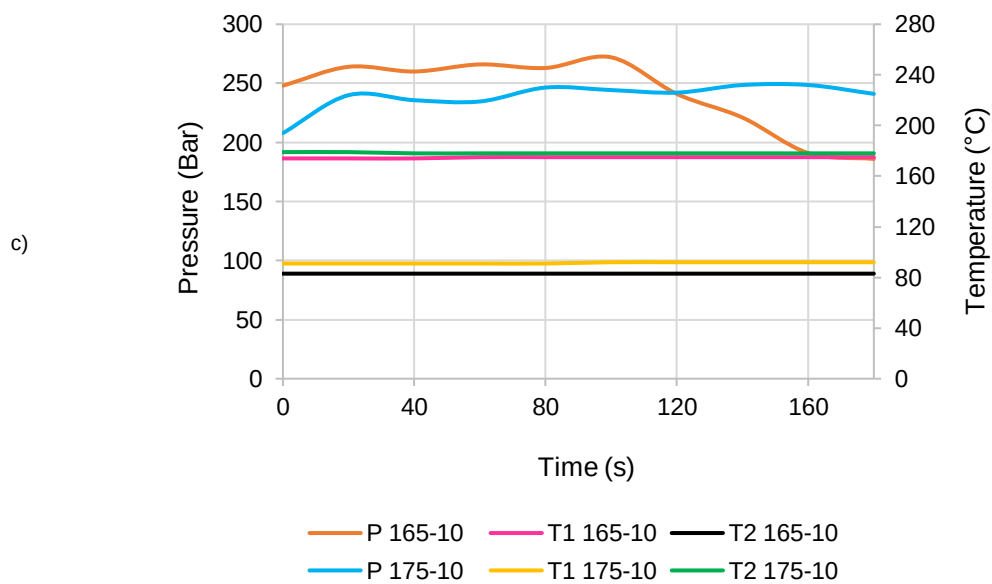
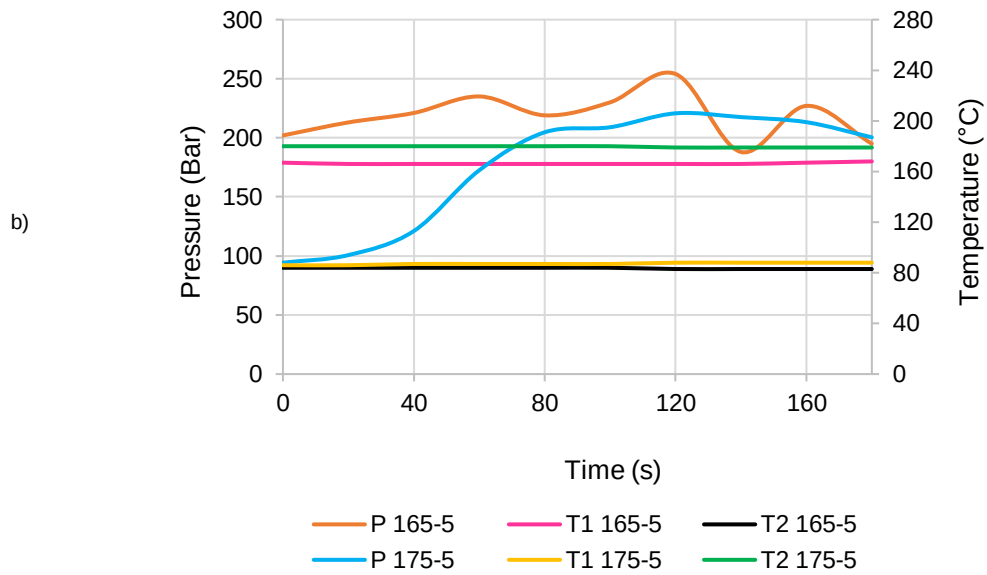
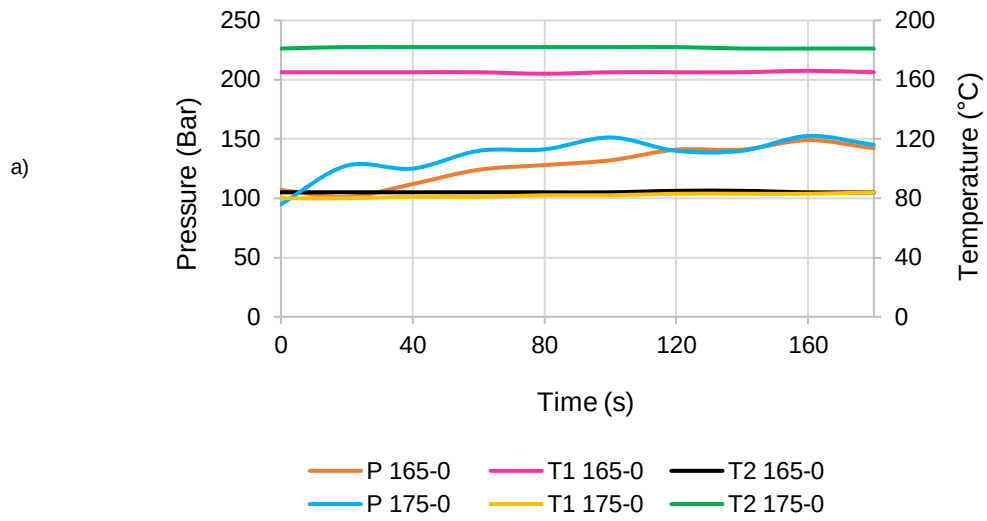
4. Results and discussion

4.1. Extrusion process responses

Pressure and torque are important secondary variables of extrusion that allow to describe the way the processing was performed, hence the importance of their determination. These contribute to the quality of the final product, since their performance was affected by the factors under study, temperature and percentage of *A. domesticus*.

Figure 17 shows the results obtained from the various extruder probes for each of the treatments. The two temperature probes (T1 and T2) recorded the temperature changes from the barrel until the desired temperatures of 165 and 175°C were obtained. With the increase in the incorporation of house cricket it was found that there were no drastic variations in the conditioning temperature until the final extrusion temperatures were reached.

The pressure was recorded from the extruder's pressure probe and the results obtained are also in figure 17. It was verified that this was increasing as the samples enriched with house cricket were placed in the extruder, even though there was a decrease in pressure from treatments 165-10 and 175-10 (figure 17c) to treatments 165-15 and 175-15 (figure 17d), respectively, when the samples with 10% incorporation were switched to samples with 15%, regardless of temperature. Other authors also observed this increase in pressure initially manifested with the increase of the level of incorporation of raw materials (Singh & Smith, 1997), but with the use of different materials from those used in the present work. In addition, it was found that the pressure of the extruded products at 165°C was generally higher and according to studies that have reached the same results, the decrease of pressure with the increase in temperature, probably occurred due to the degradation of gelatinized starch granules and this caused a reduction in viscosity, so that such a large pressure was not necessary for the mass to flow (Altan *et al.*, 2008; Meng *et al.*, 2010; Singh *et al.*, 2007).



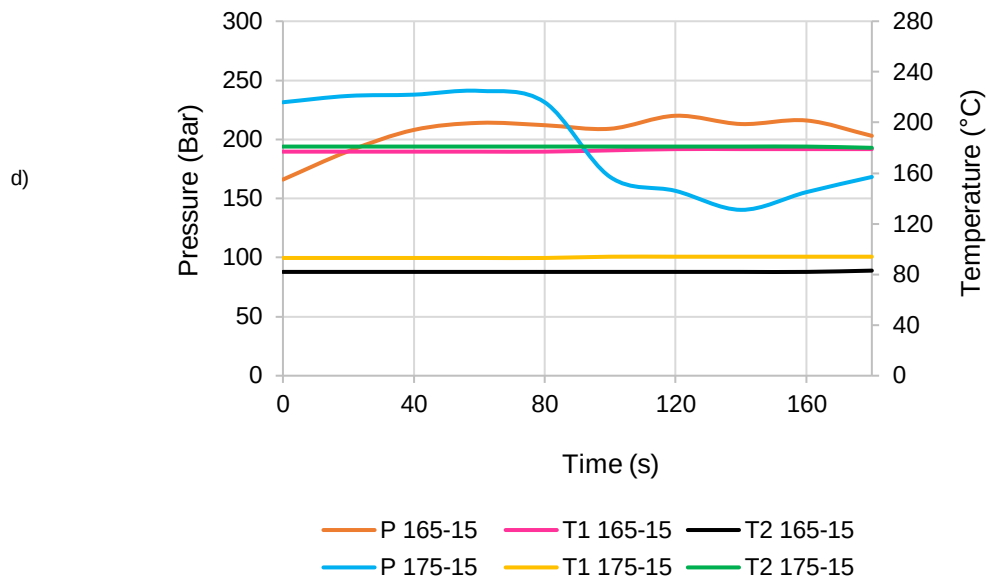


Figure 17. Variation of temperature and pressure over the processing time recorded by the temperature (T1 and T2) and pressure (P) probes of the extruder for each treatment: 165-0 and 175-0 (a); 165-5 and 175-5 (b); 165-10 and 175-10 (c); 165-15 and 175-15 (d).

Regarding torque, its values were acquired from the digital display on the monitor panel of the extruder and are shown in table 5. There were several increases and decreases in torque values, both in the extruded samples at 165°C and in the extruded samples at 175°C, so there does not seem to have been any influence from the incorporation of house cricket in these values. On the contrary, it was observed that the treatments at 165°C had, in general, higher torques than the treatments at 175°C, irrespectively of the incorporation level. These results are in accordance with the literature (Karunanithy & Muthukumarappan, 2012; Li *et al.*, 2020; Meng *et al.*, 2010; Thakur *et al.*, 2017), since any variable that interferes with viscosity will affect torque; thus, the increase in temperature contributes to the softening of the raw material, which leads to a fluent material, due to the decrease in viscosity, so it will be necessary less driving force (Karunanithy & Muthukumarappan, 2012).

The changes suffered by secondary variables of extrusion were reflected in the final product, since they may have influenced several characteristics of the extrudates obtained. Based on some studies (Cortes *et al.*, 2014; Day & Swanson, 2013), a high torque, for example, contributes to a lower absorption of water by the starch and proteins, as well as, together with a high pressure, it leads to a decrease in the extension capacities of the extruded products and, consequently affects their expansion. On the

other hand, the use of high controlled pressures is favourable for grater retention of nutrients in accordance with Alam *et al.* (2016) and Delgado-Nieblas *et al.* (2018).

Table 5. Mean values of torque obtained for each treatment.

Treatment	Torque (%)
165-0	28.3
165-5	24.0
165-10	26.7
165-15	25.0
175-0	23.5
175-5	24.8
175-10	25.5
175-15	24.8

4.2. Physicochemical and compositional properties of extrudates

4.2.1. Water content and water activity

Mean values and respective standard deviations of water content of the mixtures formulated with different percentages of *A. domesticus* are showed in table 6, noting that there were no significant differences ($p > 0.05$) between the samples. These have only been studied for the level of incorporation, since they concern mixtures prepared before extrusion, so they did not undergo any processing.

Table 6. Mean values and standard deviations of water content of the mixture (x_w^M) formulated with different levels of incorporation of *A. domesticus* (AD). The p -value obtained from ANOVA for AD is indicated.

AD (%)	x_w^M (g _{water} /100g)
0	11.262 ± 1.098
5	11.3 ± 0.8
10	11.3 ± 0.7
15	11.1 ± 0.7
p -value	0.973

The results obtained for water content of the extrudates, extrusion water loss and water activity of the extrudates are represented in table 7, as mean values and standard deviations for each treatment. It was verified that none of these parameters presented significant differences ($p > 0.05$) among the various treatments, thus there was no interaction between temperature and percentage of *A. domestica*, and for this reason there was no combined effect of the studied factors under these properties.

Table 7. Mean values and standard deviations of extrudate water content (x_w^E), extrusion water loss (W_L) and extrudate water activity (a_w) of extruded products obtained for each treatment. The p -value achieved from ANOVA for the interaction between T and AD on each parameter is indicated.

Treatment	x_w^E (g _{water} /100g)	W_L (g _{water} /100g)	a_w
165-0	8.9 ± 0.2	4.2 ± 0.3	0.396 ± 0.004
165-5	7.44 ± 1.07	5.7 ± 1.2	0.3610 ± 0.0113
165-10	6.67 ± 0.07	6.35 ± 0.13	0.3495 ± 0.0106
165-15	5.8 ± 0.7	7.1 ± 0.8	0.34 ± 0.02
175-0	6.6 ± 0.2	4.4 ± 0.3	0.35 ± 0.03
175-5	5.9 ± 0.2	5.5 ± 0.2	0.35 ± 0.03
175-10	4.70 ± 0.06	7.0 ± 0.2	0.33 ± 0.03
175-15	4.8 ± 0.3	6.7 ± 0.4	0.32 ± 0.03
p -value	0.179	0.415	0.703

For water content of the extrudates, it was observed from the values obtained for the various treatments, a similar behaviour of samples extruded at 165 and 175°C. Hence, although there was no effect of the interaction between the factors, these ones affected x_w^E individually. From a more detailed analysis of the influence of each one, it was found the existence of significant differences ($p < 0.05$) for x_w^E between the samples regarding to temperature (table 8), with the extruded at 175°C presenting the lower values and, regarding to the percentage of *A. domestica* (table 9), with the extruded samples with an incorporation of 15% of house cricket presenting the lowest values.

Overall, the values of x_w^E decreased with the increase of the level of *A. domestica*, and these results were already observed by other authors, such as Asghari-Pour *et al.* (2018) and Yang *et al.* (2020), but with the incorporation of date kernel flour and polydextrose, respectively. The reduction of water content with the increase in extrusion temperature

is also corroborated by other studies (Rani *et al.*, 2020; Sacchetti *et al.*, 2004), and it happened mainly because the high temperatures lead to an improvement in moisture evaporation. On the whole, the water content values found for all samples indicate that the extruded products obtained are sufficiently stable for storage (Rani *et al.*, 2020).

Table 8. Mean values and standard deviations of extrudate water content (x_w^E), extrusion water loss (W_L) and extrudate water activity (a_w) of extruded products obtained at different temperature (T). The *p*-value achieved from ANOVA for the effect of T on each parameter is indicated and significant parameters are highlighted in bold ($p < 0.05$).

T (°C)	x_w^E (g _{water} /100g)	W_L (g _{water} /100g)	a_w
165	7.2 ± 1.3	5.8 ± 1.3	0.36 ± 0.03
175	5.5 ± 0.8	5.911 ± 1.109	0.34 ± 0.03
<i>p</i> -value	0.000	0.744	0.074

Table 9. Mean values and standard deviations of extrudate water content (x_w^E), extrusion water loss (W_L) and extrudate water activity (a_w) of extruded products with different levels of *A. domestica* (AD). The *p*-value achieved from ANOVA for the effect of AD on each parameter is indicated and significant parameters are highlighted in bold ($p < 0.05$).

AD (%)	x_w^E (g _{water} /100g)	W_L (g _{water} /100g)	a_w
0	7.7 ± 1.3 ^c	4.3 ± 0.3 ^a	0.37 ± 0.03
5	6.661 ± 1.099 ^b	5.6 ± 0.8 ^b	0.36 ± 0.02
10	5.68 ± 1.08 ^a	6.7 ± 0.4 ^c	0.34 ± 0.02
15	5.3 ± 0.7 ^a	6.9 ± 0.6 ^c	0.33 ± 0.02
<i>p</i> -value	0.000	0.000	0.099

The same letter in superscript within the columns indicates homogeneous groups according to the LSD test ($p < 0.05$).

Besides the analysis of the water content, it is also essential to study the water loss since it is important to understand whether the processing conditions affect the final moisture of the extruded snack. Water loss corresponds to the water released from the extrudate when it comes out of the die (Mazlan *et al.*, 2019) and depends on the characteristics of the materials, but also on the steam pressure inside the air cells. It portrays the degree of gelatinization of the starch: when this is not transformed it has less capacity to form the film, conferring less extensibility and consequently the residual water gets stuck inside the structure instead of escaping (Karkle *et al.*, 2012). Therefore, the greater the water loss, the better the degree of gelatinization.

As already mentioned, there was no influence of the interaction between the two studied factors for water loss, being observed that this presented a similar behaviour with the increase of insect incorporation for both temperatures (table 7). The temperature by itself also did not affect W_L (table 8), but there were significant differences ($p < 0.05$) with the incorporation of house cricket (table 9), with the extruded ones with 15% of *A. domestica* presenting the highest values for water loss. There are studies where the opposite was observed, with the W_L values decreasing with the level of raw material under study incorporated in the extrudates (Igual *et al.*, 2020; Karkle *et al.*, 2012), so this one has an added value, since high values of water loss mean a good gelatinization of starch, which is one of the main advantages of processing by extrusion, due to the reasons already stated in the Introduction.

For water activity, it was possible to see from the values of table 9 a small decrease as the percentage of insect meal incorporation increases, with the control samples showing the highest values in relation to the samples with a 15% incorporation of house cricket. However, just as for the interaction between the factors (table 7), statistically there were no significant differences in relation to temperature (table 8) and to the percentage of *A. domestica* (table 9) individually, similar results to that obtained by Alam *et al.* (2019), so none of the factors had an effect on a_w . Moreover, it is important to mention that a product with water activity values below 0.6, as with all samples obtained in this work, present a longer shelf life (Gumusay *et al.*, 2019). In particular, the water activity of samples with insect powder was very good, being this trend of low values supported by other works where it was verified that the formulations with insects presented a lower a_w , even if it was only slightly less than the control (Garcia-Segovia *et al.*, 2020; Igual *et al.*, 2020; Tao *et al.*, 2017).

4.2.2. Water absorption index, water solubility index, swelling index and hygroscopicity

Most of the products obtained by extrusion are consumed as snacks or function as a basic ingredient of various beverages. It is therefore important to study water absorption index and water solubility index, which show the physicochemical changes that occur in polymers due to extrusion (Patil *et al.*, 2005), thus being able to evaluate the functional characteristics of food and perceive how extrudates interact with water (Alam *et al.*, 2016).

From table 10, it was observed that there were no significant differences ($p > 0.05$) for the indexes of water absorption and water solubility, so there was no interaction between

the studied factors and, consequently they did not have a joint effect on these parameters. Nevertheless, both for absorption and solubility it was verified different trends among treatments, with constant increases and decreases with the incorporation of *A. domestica* for both temperatures. Treatments at 175°C were the only ones that showed a continuous decrease behaviour in WSI values from treatment 175-0 to treatment 175-15.

Table 10. Mean values and standard deviations of water absorption index (WAI), water solubility index (WSI), swelling index (SWE) and hygroscopicity (Hg) of extruded products obtained for each treatment. The *p*-value achieved from ANOVA for the interaction between T and AD is indicated and significant parameters are highlighted in bold (*p* < 0.05).

Treatment	WAI (g _{sediment} /g _{dry solid})	WSI (%)	SWE (mL _{swollen} /g _{dry solid})	Hg (g _{water} /100g _{dry solid})
165-0	6.4 ± 0.5	9.8 ± 1.4	3.5 ± 0.2 ^b	18.4 ± 0.3
165-5	6.2 ± 0.2	4.5 ± 0.5	5.40 ± 0.09 ^{cd}	18.3 ± 0.2
165-10	5.37 ± 0.09	4.9 ± 0.3	5.8 ± 0.7 ^d	18.6 ± 0.8
165-15	5.675 ± 0.105	3.83 ± 0.08	5.6 ± 0.2 ^{cd}	18.5 ± 0.3
175-0	6.0 ± 0.7	11.7 ± 0.4	2.7 ± 0.7 ^a	20.4 ± 0.5
175-5	6.18 ± 0.13	6.4 ± 0.3	5.9 ± 0.2 ^d	21.03 ± 0.03
175-10	5.46 ± 0.06	4.35 ± 0.03	5.0 ± 0.2 ^c	21.4 ± 0.2
175-15	5.9 ± 0.3	3.5 ± 0.4	5.2 ± 0.4 ^{cd}	21.33 ± 0.09
<i>p</i> -value	0.299	0.105	0.049	0.219

The same letter in superscript within the columns indicates homogeneous groups according to the LSD test (*p* < 0.05).

The temperature effect, in an isolated way, also did not affect the indexes in analysis (table 11), although several authors have observed that this factor usually has a remarkable influence, whether with higher WAI and WSI values for extruded at high temperatures or the opposite (Hagenimana *et al.*, 2006; Joshi *et al.*, 2014; Kothakota *et al.*, 2013; Pensamiento-Nino *et al.*, 2018; Sharma & Gujral, 2013), but there is always an effect of this factor. In this work, the lack of influence of the temperature on the samples may be due to the fact that the temperatures used had only a difference of 10°C, so it was not enough to observe any kind of effect, unlike the mentioned studies where the difference between the temperatures used was quite large.

Table 11. Mean values and standard deviations of water absorption index (WAI), water solubility index (WSI), swelling index (SWE) and hygroscopicity (Hg) of extruded products obtained at different temperature (T). The *p*-value achieved from ANOVA for the effect of T on each parameter is indicated and significant parameters are highlighted in bold (*p* < 0.05).

T (°C)	WAI (g _{sediment} /g _{dry solid})	WSI (%)	SWE (mL _{swollen} /g _{dry solid})	Hg (g _{water} /100g _{dry solid})
165	5.9 ± 0.5	5.8 ± 2.6	5.09 ± 1.02	18.5 ± 0.4
175	5.9 ± 0.4	6 ± 3	4.7 ± 1.3	21.0 ± 0.5
<i>p</i> -value	0.748	0.233	0.028	0.000

Regarding the percentage of *A. domestica*, there were significant differences (*p* < 0.05) for WAI (table 12) and, in overall, there was a decrease in the values with the level of incorporation, having only been a small increase from the samples with 10% to the samples with 15%. It is advantageous for an extruded product to have low water absorption, as this means that the final snack will be crunchier. These observations are in accordance with other works where different types of raw materials were used (Asghari-Pour *et al.*, 2018; Sharma *et al.*, 2015; Yağcı & Göğüş, 2008) and even studies where, as in this work, products were enriched with insects (Alam *et al.*, 2019; Cuj-Laines *et al.*, 2018). The results suggest that with the increasing incorporation of house cricket, there is a decrease in starch content in the composition of the blends, as the amount of cornmeal integrated is lower and therefore the amount of water to be absorbed by the starch is also lower. Not only is the amount of starch inferior, but the interaction among the hydrophilic groups of proteins and starch will also make it more difficult the smallest quantity of starch to bind to water (Sumargo *et al.*, 2016).

During extrusion, the presence of *A. domestica* powder caused less solubilization of the matrix components. Significant differences (*p* < 0.05) were also observed in relation to this factor for solubility, with the decrease in WSI values relative to the control and with the samples with an incorporation of 15% of house cricket presenting the lowest values (table 12), regardless of the temperature to which they were submitted. In other research about extruded products, this decrease in WSI values was also noted (Gumusay *et al.*, 2019; Navarro-Cortez *et al.*, 2018; Rashid *et al.*, 2015; Yang *et al.*, 2008), with the incorporation of different raw materials. Hence, considering these results and based on the objective of using WSI, it was found that the presence of *A. domestica* in mixtures decreases the risk of potential molecular damage made by particles solubilized in water, which is a desirable characteristic, unlike the high solubility (Navarro-Cortez *et al.*, 2018).

Table 12. Mean values and standard deviations of water absorption index (WAI), water solubility index (WSI), swelling index (SWE) and hygroscopicity (Hg) of extruded products with different levels of *A. domestica* (AD). The *p*-value achieved from ANOVA for the effect of AD on each parameter is indicated and significant parameters are highlighted in bold ($p < 0.05$).

AD (%)	WAI (g _{sediment} /g _{dry solid})	WSI (%)	SWE (mL _{swollen} /g _{dry solid})	Hg (g _{water} /100g _{dry solid})
0	6.2 ± 0.6 ^c	10.2 ± 1.6 ^c	3.1 ± 0.6	19.4 ± 1.2
5	6.19 ± 0.15 ^{bc}	5.48177 ± 1.11009 ^b	5.6 ± 0.3	19.7 ± 1.5
10	5.41 ± 0.06 ^a	4.6 ± 0.3 ^{ab}	5.4 ± 0.6	20.0 ± 1.6
15	5.8 ± 0.3 ^{ab}	3.7 ± 0.3 ^a	5.4 ± 0.4	19.9 ± 1.5
<i>p</i> -value	0.002	0.000	0.000	0.053

The same letter in superscript within the columns indicates homogeneous groups according to the LSD test ($p < 0.05$).

Swelling index is also an important hydration property, very similar to the water retention capacity and although it is associated with the various components of the extrudates, the fibre is the component that has the highest capacity to swell in the stomach, contributing to increased satiety the greater this swelling is (Chen *et al.*, 2018). Thus, SWE can be defined, according to the conditions used, as the volume that a certain known weight of fibre occupies (Robertson *et al.*, 2000).

Results obtained for swelling showed the existence of significant differences ($p < 0.05$) between the various treatments (table 10), which means that there was an interaction among the factors under study that influenced SWE. Swelling presented different behaviours with the incorporation of house cricket: at 165°C there was an increase of its values from treatments 165-0 to 165-10 and a small decrease from the latter mentioned to 165-15; at 175°C there was an increase from control treatment to 175-5, a decrease from this one to 175-10 and again an increase up to treatment 175-15.

The two factors also affected the level of swelling, when analysed individually, and significant differences ($p < 0.05$) were also observed. Those extruded at 165°C showed the greatest SWE values for temperature (table 11). It was also verified an increase in SWE values with the increase in the percentage of *A. domestica* (table 12), only with a slight decrease of the samples with 5% of incorporation to that of 10%, but still control samples presented the lowest swelling, independently of temperature. Similar findings were obtained in the study by Tabibloghmany *et al.* (2020), where it was also observed

an increase in the SWE values of the control samples for those with the raw material under study incorporated, which in this particular work was soy hull.

Hygroscopicity is the capacity of materials absorb moisture from environments with high relative humidity and is an important parameter to consider during product storage (Hassan *et al.*, 2018; Rodriguez-Hernandez *et al.*, 2005).

From the analysis of tables 10 and 12 respectively, it was possible to observe that there was no interaction between the factors in study that influenced the Hg levels and that the incorporation of *A. domesticus* also had no influence, at individual level. Even so, in a study carried out by Garcia-Segovia *et al.* (2020) where different protein sources were used, it was found that the extruded ones enriched with 5% of insects, besides being the least hygroscopic, showed advantageous Hg values compared to the control samples. This partly corroborates the results obtained in the present study, since the samples with 5% incorporated house cricket presented similar Hg values to the control samples (table 12). On the contrary, the temperature alone affected Hg, and significant differences ($p < 0.05$) were observed, with the samples extruded at 165°C being statistically less hygroscopic than those extruded at 175°C (table 11).

4.2.3. Expansion index, diameter, bulk density, and porosity

Expansion index, diameter, bulk density and porosity are properties related to each other, since they allow to measure the expansion of extruded snacks (Do Carmo *et al.*, 2019) and are the result of the viscoelastic characteristics of melting, which depends on the attributes of the raw materials (Azzollini *et al.*, 2018).

Figure 18 shows the results of the expansion of extruded products obtained at different temperature and percentage of *A. domesticus*. SEI presented significant differences ($p < 0.05$) between the samples, so there was an interaction between the studied factors that influenced this property. It was noticed a similar behaviour for the various samples with the different levels of incorporation, for both temperatures, being observed a decrease of the expansion index with the increase of percentage of incorporated house cricket and with the rise of the temperature.

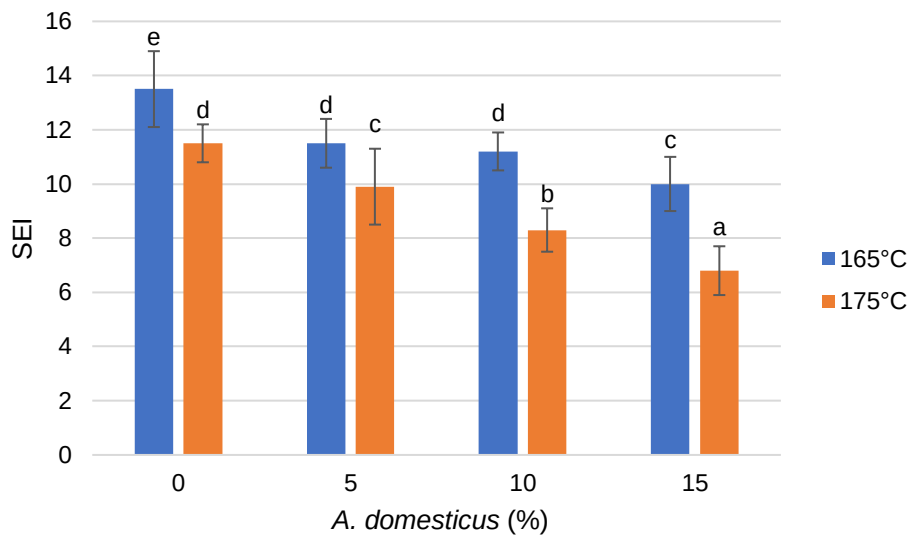


Figure 18. Mean values and standard deviations of expansion index (SEI) of extruded products obtained at different temperature and levels of *A. domestica*. Bars with different letters are significantly different ($p < 0.05$).

Although the samples behave in the same way at any of the temperatures, individual factor analyses also had an influence, given the existence of significant differences ($p < 0.05$) between the samples, on each one. Temperature alone showed that the extruded ones at 165°C presented notoriously higher values of SEI, in relation to the extruded ones at 175°C (table 13). Relatively to the percentage of *A. domestica*, a decrease in expansion was observed with the increase in the level of house cricket, with control samples presenting the highest values of SEI and with higher incorporation samples (15%) presenting the lowest values (table 14).

Table 13. Mean values and standard deviations of expansion index (SEI), diameter (D_e), bulk density (ρ_b) and porosity (ϵ) of extruded products obtained at different temperature (T). The p -value achieved from ANOVA for the effect of T on each parameter is indicated and significant parameters are highlighted in bold ($p < 0.05$).

T (°C)	SEI	D_e (mm)	ρ_b (g/cm ³)	ϵ (%)
165	11.6 ± 1.6	10.2 ± 0.7	0.126 ± 0.09	90.2 ± 1.3
175	9.1 ± 2.0	9.01 ± 1.02	0.17 ± 0.04	87.3 ± 1.7
<i>p</i> -value	0.000	0.000	0.000	0.000

Table 14. Mean values and standard deviations of expansion index (SEI), diameter (D_e), bulk density (ρ_b) and porosity (ϵ) of extruded products with different levels of *A. domestica* (AD). The *p*-value achieved from ANOVA for the effect of AD on each parameter is indicated and significant parameters are highlighted in bold ($p < 0.05$).

AD (%)	SEI	D_e (mm)	ρ_b (g/cm ³)	ϵ (%)
0	12.5 ± 1.5	10.6 ± 0.6	0.123 ± 0.008	90.3 ± 1.8
5	10.7 ± 1.4	9.8 ± 0.7	0.138 ± 0.009	88.8 ± 0.7
10	9.8 ± 1.6	9.3 ± 0.8	0.16 ± 0.02	88.3 ± 0.8
15	8.4 ± 1.9	8.64 ± 0.99	0.17 ± 0.06	87.7 ± 3.6
<i>p</i> -value	0.000	0.000	0.000	0.002

The loss in expansion with the increase in temperature was a comportment already observed in other studies (Altan *et al.*, 2008; Li *et al.*, 2020; Thakur *et al.*, 2017; Wang *et al.*, 2019; Yang *et al.*, 2020) and probably due to the fact that the evaporation of water at high temperatures is extremely brutal. This leads to an extreme breakdown of the molecules on starch, weakening its structure and consequently reducing expansion (Alam *et al.*, 2016; Navarro-Cortez *et al.*, 2018; Shirazi *et al.*, 2020), being thus starch an important player in the expansion at the exit of the die (Joshi *et al.*, 2014; Navarro-Cortez *et al.*, 2018).

The decrease in expansion with the increase in the percentage of incorporation has also been reported by other works in which different protein sources were used (Basilio-Atencio *et al.*, 2020; Horvat & Schuchmann, 2013; Sumargo *et al.*, 2016; Yu *et al.*, 2013) and even in studies which, as in this one, were used insects (Azzollini *et al.*, 2018; Cuj-Laines *et al.*, 2018; Irungu *et al.*, 2018). This limitation of expansion occurs because, during extrusion, proteins create a network from covalent interactions, that affects the distribution of water in the matrix, modifying the extension characteristics, since protein and starch will compete for water, what leads to a delay in the gelatinization of starch and, therefore, to less expansion (Kumar *et al.*, 2018; Ramos *et al.*, 2017).

Some authors have also indicated that the fibre present in the extrudates is responsible for the weak expansion, since it prevents the air molecules from expanding (Agathian *et al.*, 2015). Furthermore, the lipid concentration reduces the viscosity of the melt and prevents the starch hydration, necessary not only for its gelatinization, but also for its expansion (Ilo *et al.*, 2000). The low expansion may also arise because the greater the incorporation of house cricket in mixtures, the lower the starch content, which is the component that generates most of the expansion of extruded products (Adem *et al.*, in

press; Wojtowicz & Moscicki, 2014), due to its viscoelastic characteristics resulting from the existence of hydrogen bonds among amylose and amylopectin (Boff Zortea-Guidolin *et al.*, 2017).

Since it is necessary to determine diameter of the extrudates to obtain the expansion, it would be expected that this one behave in the same way as the SEI, as these parameters are dependent on each other (Patil *et al.*, 2005). Therefore, as for expansion, significant differences were observed ($p < 0.05$) with respect to the interaction of the studied factors, so these affected D_e (figure 19). There was a similar behaviour of the various samples with the different incorporation levels, for the two temperatures, having been observed a decrease in diameter of the extrudates with the increase in the level of house cricket and with the rise in temperature. Samples were also significant ($p < 0.05$) in relation to the two factors individually. Temperature analysis showed that the samples extruded at 175°C were those with lowest values of D_e (table 13), which is corroborated by other studies (Lazou & Krokida, 2010). But, according to some authors, the main reason for the decrease in diameter of extruded snacks was the addition of insect flour to maize flour (Garcia-Segovia *et al.*, 2020), as it was verified in the present study when there was a gradual decrease in the diameter of the extrudates, with the increase in the percentage of incorporation of *A. domesticus* (table 14).

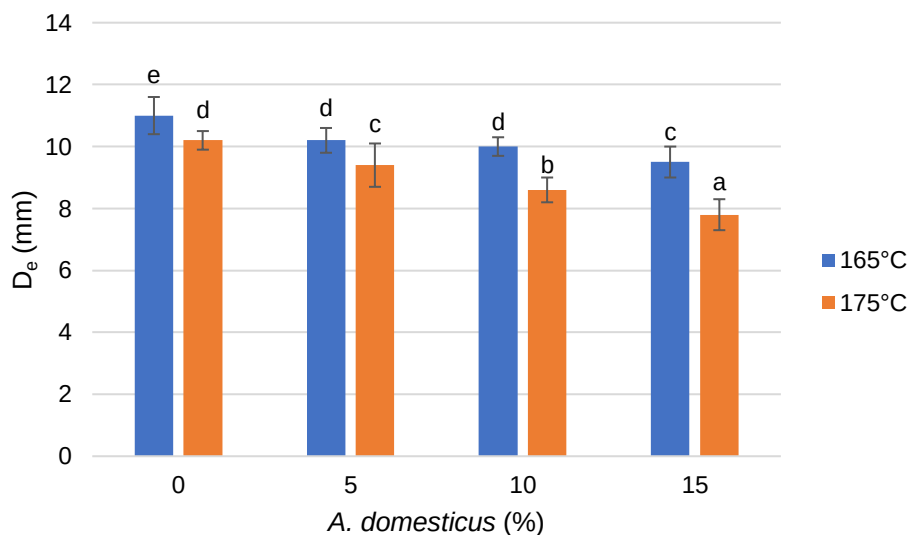


Figure 19. Mean values and standard deviations of diameter (D_e) of extruded products obtained at different temperature and levels of *A. domesticus*. Bars with different letters are significantly different ($p < 0.05$).

Unlike the index of sectional expansion that only considers the expansion perpendicular to the extrusion flow, bulk density considers the expansion in all directions (Altan *et al.*, 2008). Bulk density behaved differently with the incorporation of *A. domesticus* for the two temperatures, due mainly to variation in one sample group, thus there was an interaction among the two factors that affected this property, which was observed by the existence of significant differences ($p < 0.05$) between the samples. At 165°C there was an increase from the samples with 0% of house cricket until samples with 10% and then a slight decrease from this latter mentioned to the samples with 15%; at 175°C there was always been an increase in the ρ_b values with the increase in the percentage of *A. domesticus* from the control samples to the samples with 15% incorporation (figure 20).

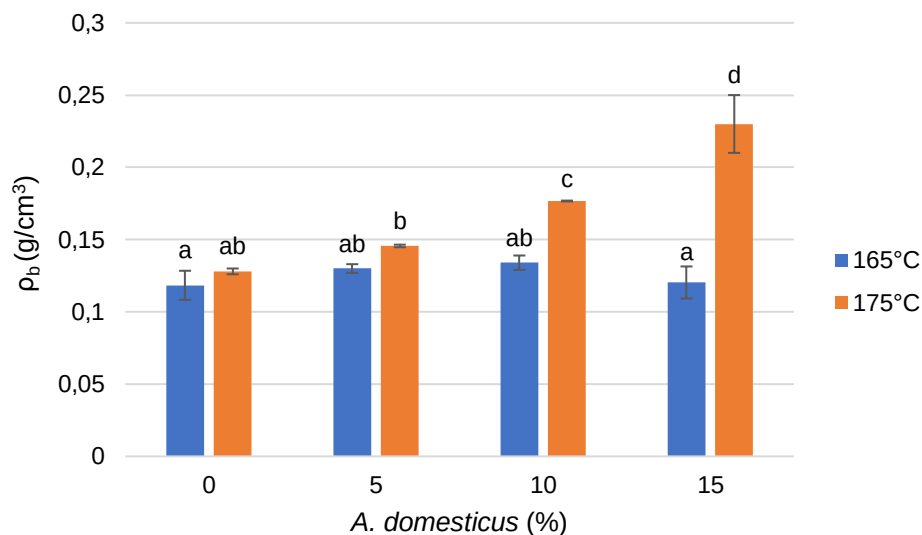


Figure 20. Mean values and standard deviations of bulk density (ρ_b) of extruded products obtained at different temperature and levels of *A. domesticus*. Bars with different letters are significantly different ($p < 0.05$).

Based on the individual analyses of each of the factors studied, where it was also observed significant differences between the samples ($p < 0.05$), it was found that at 165°C the bulk density was lower, compared to the samples extruded at 175°C (table 13), and there was a progressive increase of this one with the increase of the level of incorporation of *A. domesticus* (table 14), with the control samples showing the lowest ρ_b and 15% samples the higher. These same results, completely contrary to those of the expansion, were obtained in other studies both regarding the temperature factor (Yağcı & Göğüş, 2008; Yang *et al.*, 2020) and the percentage of incorporation of the raw material under study in such works (Asghari-Pour *et al.*, 2018; Bhat *et al.*, 2019; Irungu *et al.*, 2018; Yu *et al.*, 2013).

During the entire extrusion process, including the exit from the die, several pockets of air or cells are formed inside the extruded material, which provide the product a porous expanded structure (Kumar *et al.*, 2018), with the number and size of pores varying (Rathod & Annapure, 2016). This volume of air known as porosity may be used to characterize the properties of expansion of extruded products (Yağcı & Göğüş, 2008).

Such as bulk density, for porosity a little different behaviour was obtained with the incorporation of house cricket powder for both temperatures, as there were significant differences ($p < 0.05$) between the samples, so once again there was an effect of the interaction of the two factors under study, but this time for porosity. This way, samples containing 15% of house cricket extruded at 165°C were the exception in ϵ and p_b to the behaviour observed in those for the remaining samples. At 165°C there was a decrease in porosity from the control samples to the samples with an incorporation of 10%, and from this point on there seems to have been a sudden increase in porosity with the level of incorporation from 10 to 15%; and at 175°C there was a gradual decrease in the values of ϵ with the percentage of *A. domestica* from samples with 0% to the ones with 15% (figure 21).

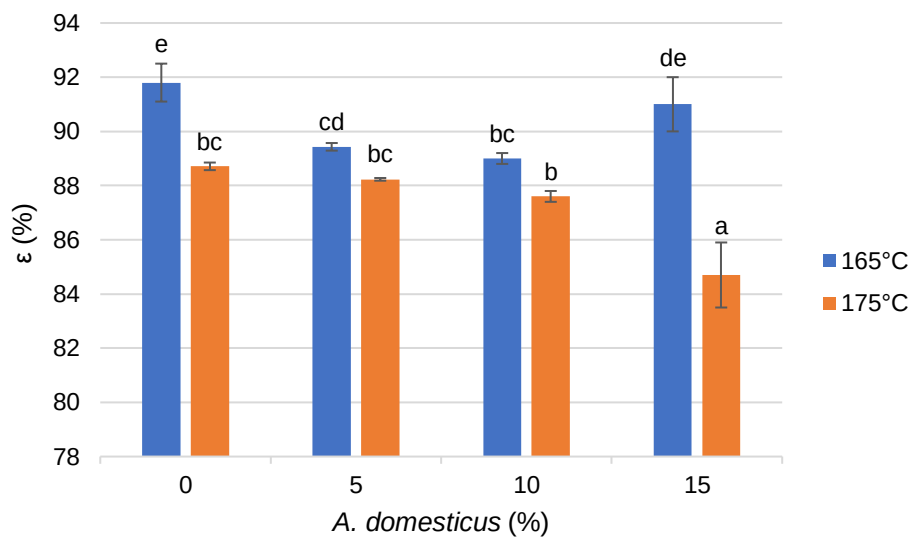


Figure 21. Mean values and standard deviations of porosity (ϵ) of extruded products obtained at different temperature and levels of *A. domestica*. Bars with different letters are significantly different ($p < 0.05$).

In relation to individual factors, significant differences ($p < 0.05$) were also observed. A decrease in porosity values was noted with the increase in the extrusion temperature (table 13) and, with the increase in the incorporation of *A. domestica* in the mixtures

(table 14), being the samples extruded at 175°C with 15% incorporation less porous and the control samples, especially the ones extruded at 165°C, more porous. Similar findings have already been recorded by other authors, with porosity values decreasing when samples were extruded at higher temperatures (Asghari-Pour *et al.*, 2018; Rathod & Annapure, 2016), possibly due to the rise of starch dextrinization (Yağcı & Göğüş, 2008). Besides that, numerous studies have also demonstrated the decrease of ϵ with increased enrichment of the product (Asghari-Pour *et al.*, 2018; Azzollini *et al.*, 2018; Yağcı & Göğüş, 2008; Yang *et al.*, 2020), even if the type of raw material used to incorporate in the extruded products was different.

For a better analysis of the extruded structure, porosity was also determined from a two-dimensional perspective. Thus, the radial and longitudinal sections of an extrudate with an incorporation of 0, 5, 10 and 15% of *A. domestica* obtained at both 165 and 175°C was photographed (figure 22).

Photographs of the radial section were those used to determine the total pore area of that specific cut section and data obtained from image analysis are represented in figure 23. It has been observed that there was a decrease in the number of pores in the area of the cut section with the increase of the level of incorporated house cricket and with the increase of the temperature, with the exception of the sample with 15% of *A. domestica* extruded at 165°C, as previously verified by the porosity determination from the equation.

Overall, with the increase in each of the factors under study there was a decrease in the number of pores, which corresponds to a lower porosity and, consequently, a more compact structure, proven both by the analysis of porosity measured from the equation and from the image analysis. This same conclusion was reached by Shirazi *et al.* (2020), who also performed both of the aforementioned analyses. The increase in the compactness of the structure with the temperature and with the percentage of incorporation of house cricket can also be observed in figure 22, especially in the photographs of the longitudinal section.

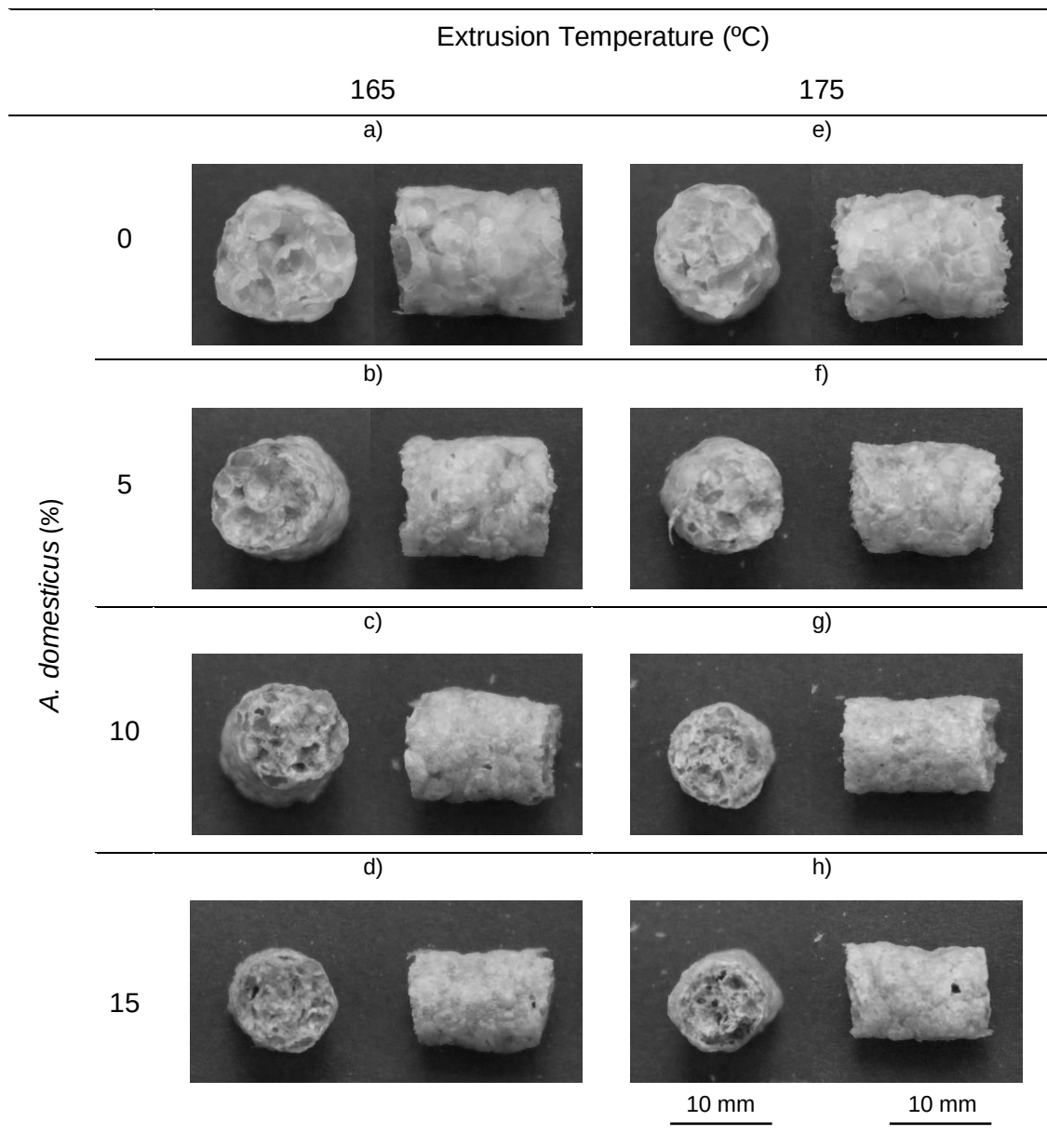


Figure 22. Radial (left) and longitudinal (right) cross sections of samples extruded at 165°C with 0 (a), 5 (b), 10 (c) and 15% (d) of *A. domestica* and samples extruded at 175°C with 0 (e), 5 (f), 10 (g) and 15% (h) of *A. domestica*.

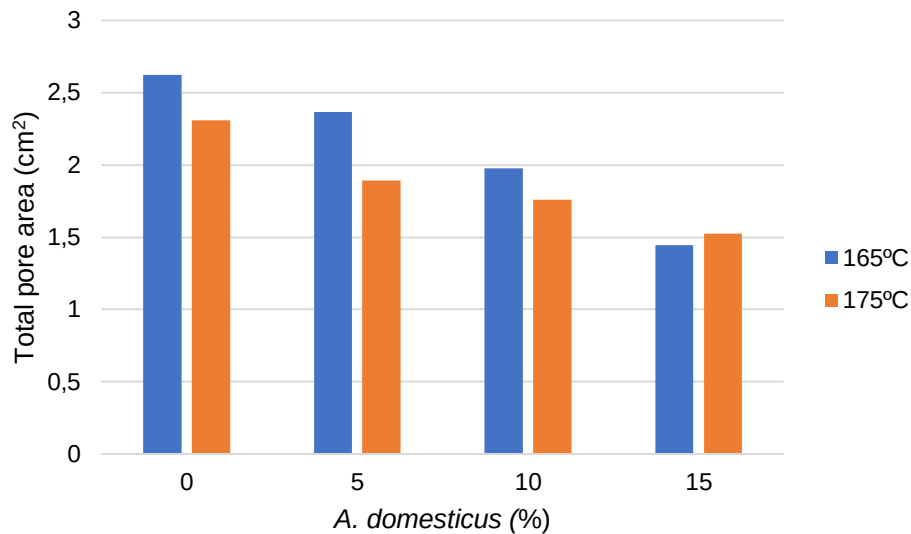


Figure 23. Total pore area for samples with 0, 5, 10 and 15% of *A. domesticus*, extruded at 165 and 175°C. Data were obtained directly from ImageJ software (Abramoff *et al.*, 2004).

The ideal and expected attribute for extruded snacks consists of products with a low bulk density combined with high expansion index and porosity (Asghari-Pour *et al.*, 2018), which is obtained when the volume of extrudates increases, but its weight decreases (Navarro-Cortez *et al.*, 2018). Products with these characteristics, which are the desirable, present a light and puffy structure (Sharma & Gujral, 2013), because they are composed of thin walls and large cells (Ramos *et al.*, 2017).

4.2.4. Crude protein

Treatments presented significant differences ($p < 0.05$) for crude protein, which means that there was an interaction between temperature and percentage of *A. domesticus* that influenced this parameter (table 15). With the increase in the addition of house cricket there has been an increasing rise in the protein values of extruded products at both temperatures. Temperature alone has not affected the values of CP (table 16), result which has also been found in other studies (Pelembé *et al.*, 2002). On the contrary, samples showed significant differences ($p < 0.05$) in relation to the level of incorporated *A. domesticus* when analysed individually (table 17), having been observed an increase of crude protein with the increase in the percentage of insect meal in the mixture, being samples with 15% those with the highest protein content.

Table 15. Mean values and standard deviations of crude protein (CP) of extruded products obtained for each treatment. The *p*-value achieved from ANOVA for the interaction between T and AD is indicated in bold, being treatments significant for a *p* < 0.05.

Treatment	CP (mg/100g)
165-0	6.441 ± 0.005 ^b
165-5	8.574 ± 0.006 ^d
165-10	10.27 ± 0.04 ^f
165-15	11.095 ± 0.013 ^h
175-0	6.29 ± 0.07 ^a
175-5	8.46 ± 0.04 ^c
175-10	9.83 ± 0.04 ^e
175-15	11.85 ± 0.03 ^g
<i>p</i> -value	0.000

The same letter in superscript within the column indicates homogeneous groups according to the LSD test (*p* < 0.05).

Table 16. Mean values and standard deviations of crude protein (CP) of extruded products obtained at different temperature (T). The *p*-value achieved from ANOVA for the effect of T is indicated.

T (°C)	CP (mg/100g)
165	9.1 ± 1.9
175	9.1 ± 2.2
<i>p</i> -value	0.466

Table 17. Mean values and standard deviations of crude protein (CP) of extruded products with different levels of *A. domestica* (AD). The *p*-value achieved from ANOVA for the effect of AD is indicated in bold, being samples significant for a *p* < 0.05.

AD (%)	CP (mg/100g)
0	6.368 ± 0.095
5	8.52 ± 0.07
10	10.1 ± 0.3
15	11.5 ± 0.4
<i>p</i> -value	0.000

These findings were expected on the basis of other studies, since by increasing the percentage of embedded insect powder, which itself has more protein than other meals, the protein content would increase, with a consequent reduction of content of starch (Azzollini *et al.*, 2018; Igual *et al.*, 2020). This way, the considerable increase in the amount of protein in the mixtures led to notable increases in these components in the final extruded snacks, so the nutritional profile of these products improved considerably by the addition of insects.

In general, all extruded snacks obtained are nutritionally more advantageous than the majority of most popular snacks available on the market, especially those for children, that have only about 2.7 – 5.5% of protein (Lucas *et al.*, 2018), since the snacks in this study had higher levels than these. Furthermore, the samples extruded at 175°C with an incorporation of 15% of house cricket showed CP values of 11.85 (table 15), which corresponds to 12.13% of the total energy value, enough for snacks with this level of incorporation produced under these extrusion temperature to be considered as a "source of protein", according to the European food Regulation No 1924/2006.

4.2.5. Relationship among physicochemical and compositional properties

All the characteristic parameters of extrudates seem to be associated, since the analysis of the results showed that the effect of some on the products obtained often accentuated or attenuated the effect of others. Therefore, in order to understand how these relate to each other, Pearson correlation analyses between the studied physicochemical and compositional parameters were performed, being the obtained coefficients represented in table 18.

Water content has a significant impact on almost all parameters, except those related to water absorption capacity, solubility and swelling. The positive correlation coefficient between x_w^E and a_w ($p < 0.01$) was highlighted as one of the major correlations, since there is a great relationship between these two properties related to the water in a food, already noted by other works (Basilio-Atencio *et al.*, 2020). The water content parameter is strongly related to expansion properties, since according to Do Carmo *et al.* (2019), the higher the water content, the lower is the resistance to bubble grow, which can be observed by the positive correlations of x_w^E with SEI and D_e ($p < 0.01$) and also with ϵ ($p < 0.05$) and the negative ones with ρ_b ($p < 0.05$), so that more expanded porous snacks will also be wetter. These correlations mentioned were already obtained by other authors (Garcia-Segovia *et al.*, 2020; Horvat & Schuchmann, 2013). Similarly, as x_w^E is correlated

with a_w , this one also presented relationships with all the parameters associated with the structure of the material, showing positive correlations with SEI and D_e ($p < 0.01$), with ϵ ($p < 0.05$) and negative with ρ_b ($p < 0.1$).

The x_w^E correlated negatively with Hg ($p < 0.05$), as in the study by Do Carmo *et al.* (2019), since the capacity of a food to absorb water from the environment is low when this food already has a large amount of water in its constitution.

Negative correlations were found between CP with x_w^E ($p < 0.1$) and a_w ($p < 0.05$), which means that the increase in the percentage of *A. domestica* incorporated in the mixture rises the protein content of the final extruded products and reduces the values of the water related properties mentioned. This is something desired by the consumers, since wetter snacks with low water activity present more crunchy textures and longer shelf life, besides of being rich in protein, that meets the growing demands for healthy snacks. These correlations corroborate the obtained by other authors (Igual *et al.*, 2020).

Water absorption index showed only a weak correlation with water activity ($p < 0.1$) and the solubility index also showed less strong correlations with expansion and diameter ($p < 0.1$), and the latter mentioned correlation was also found in the study of Seth *et al.* (2015). However, WSI showed strong significant negative correlations with CP ($p < 0.01$), since the greater incorporation of *A. domestica* in the mixture leads to the increase in protein content, being the amount of starch present smaller and therefore there are fewer molecules that are released from this component, which protects the product, because there is less risk of molecular damage. Conversely, CP correlated positively with SWE ($p < 0.05$), so the rise in protein content by increased incorporation of insect meal, means the rise of fibre as well, a relevant component for the greater swelling of extruded products and thus for a greater sense of satiety after its consumption. Thus, as expected, the WSI correlated negatively with the SWE ($p < 0.01$), as the increase in one led to the decrease in the other or the opposite. Other research has reached all these same correlations (Garcia-Segovia *et al.*, 2020; Igual *et al.*, 2020).

Hygroscopicity showed strong correlations with all parameters related to expansion ($p < 0.05$), with less hygroscopic materials being when expansion, diameter and porosity are larger and bulk density is smaller and more hygroscopic when the reverse occurs. In this way, products that are less expanded and porous when in an environment with a humid atmosphere will absorb this humidity more easily than products that are more expanded and porous (Garcia-Segovia *et al.*, 2020).

As provided, the expansion properties are all significantly correlated to each other ($p < 0.01$). The behaviour of these properties was similar for all the parameters studied: when SEI and D_e led to an increase in the values of a given parameter, ϵ also increased these values, while ρ_b led to their decrease, or the contrary happened. All these correlations confirm that found by other authors (Agathian *et al.*, 2015; Garcia-Segovia *et al.*, 2020; Zarzycki *et al.*, 2015).

In this way, expansion varies in the same reason as the porosity, and snacks with a huge expansion have a higher porosity. However, expansion and bulk density have an inverse relationship, so the increase of one implies the decrease of the other, being this effect attributed to the viscosity of the mixture (Do Carmo *et al.*, 2019). The ratio between these two parameters describes the degree of puffiness suffered by the extrudates as they leave the extruder (Bhat *et al.*, 2019). So, since porosity varies according to the expansion, it also has the opposite effect of the bulk density (Asghari-Pour *et al.*, 2018).

It is worth mentioning the higher correlation found in these analyses for SEI and D_e ($p < 0.01$), since it is only possible to determine the expansion by determining the diameter. SEI and D_e also showed negative correlations ($p < 0.05$) with CP, since the increase in protein content led to a decrease in these two properties, for the reasons explained in the results section on expansion. This negative linear relationship has already been observed in other studies (Igual *et al.*, 2020).

Table 18. Pearson correlation coefficients among studied physicochemical and compositional parameters of extruded products.

	a_w	WAI	WSI	SWE	Hg	SEI	D_e	ρ_b	ϵ	CP
x_w^E	0.969***	0.607	0.563	- 0.415	- 0.755**	0.943***	0.926***	- 0.721**	0.780**	- 0.700*
a_w		0.667*	0.609	- 0.433	- 0.618	0.912***	0.891***	- 0.673*	0.763**	- 0.763**
WAI			0.488	- 0.382	- 0.148	0.441	0.411	- 0.205	0.278	- 0.612
WSI				- 0.884***	- 0.040	0.646*	0.634*	- 0.465	0.393	- 0.911***
SWE					- 0.041	- 0.443	- 0.421	0.224	- 0.239	0.756**
Hg						- 0.737**	- 0.739**	0.736**	- 0.790**	0.165
SEI							0.998***	- 0.886***	0.869***	- 0.770**
D_e								- 0.907***	0.876***	- 0.763**
ρ_b									- 0.926***	0.589
ϵ										- 0.528

*** Correlation is significant at the level 0.01.

** Correlation is significant at the level 0.05.

* Correlation is significant at the level 0.1.

x_w^E : Extrudate water content; a_w : Water activity; WAI: Water absorption index; WSI: Water solubility index; SWE: Swelling index; Hg: Hygroscopicity; SEI: Expansion index; D_e : Diameter; ρ_b : Bulk density; ϵ : Porosity; CP: Crude protein.

4.3. Textural properties of extrudates

The walls of the extruded products can fracture when a certain force is applied, as was the case, from the puncture test in the present work. The force-time curves resulting from the implementation of this force were obtained from the Exponent software and are represented in figure 24 by a replica of each treatment. It was observed an increase in force, when the product was compressed, until the first moment of fracture. When the fracture stops, there was a drop in force, so each fall follows a fracture event. Therefore, the peaks present in each curve correspond to the fracture of walls filled with air bubbles and the height of each one differs according to the structural properties of the respective extruded (Philipp *et al.*, 2017a). From these curves were obtained the different texture parameters studied.

Crispness work is the area under the curve per peak of fracture and is the measure of the work necessary to rupture a pore or group of pores. W_c can be interpreted as the sensory criterion of fracturability (Azzollini *et al.*, 2018), described as the force applied on the first bite to fracture the sample (Cheng *et al.*, 2007). The degree of fracturability corresponds to the horizontal force required to move the food from the same site where it was applied the vertical force (Rodriguez-Vidal *et al.*, 2017).

From table 19 it was observed significant differences ($p < 0.05$) for the various treatments, so there was an interaction between the studied factors that together affected crispiness work. This property behaved similarly with the level of incorporation for the two temperatures: there was a decrease in W_c values for both temperatures, with the exception of the change from treatment 175-10 to 175-15, where there was an increase in crispness work, and the samples with an incorporation of 15% ended up with a W_c value equal to that of the corresponding control samples extruded at 175°C of 0.45. Besides that, at 165°C the decrease in W_c was accentuated, unlike in the group of extruded samples at 175°C where no significant differences were noted ($p > 0.05$).

Temperature (table 20) and percentage of *A. domesticus* (table 21) also influenced, in an individual way, the crispiness work of extruded materials, as significant differences were found between the samples ($p < 0.05$) with respect to both factors. As a result, the samples extruded at temperatures of 175°C showed higher W_c values and in relation to the level of incorporation of house cricket, control samples were the ones that showed the highest crispness work, whereas the samples with an incorporation of 15% presented the lowest values.

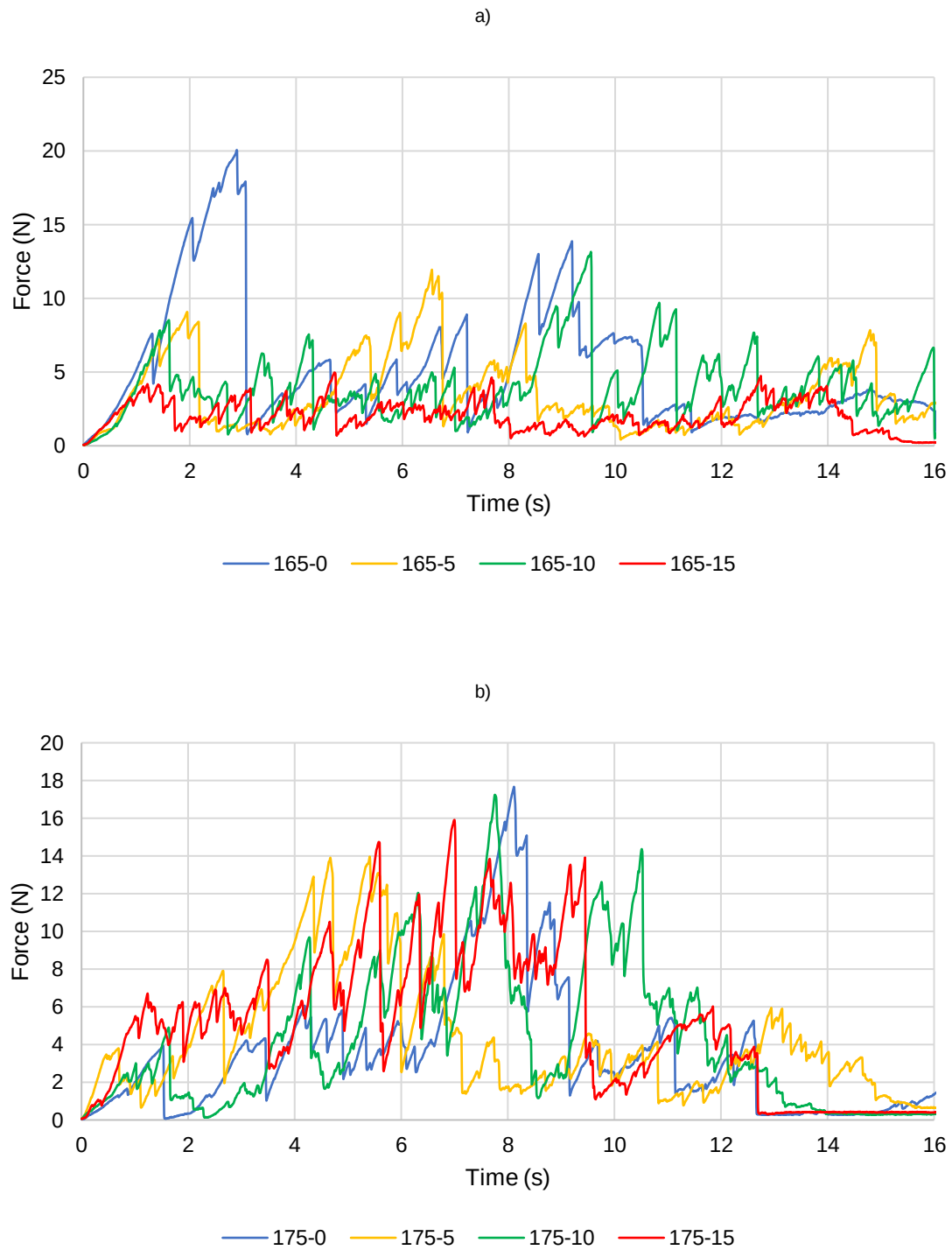


Figure 24. Force-time curves of each treatment: all formulations, 0, 5, 10 and 15% of *A. domesticus* extruded at 165°C (a) and at 175°C (b).

Table 19. Mean values and standard deviations of texture parameters of extruded products obtained for each treatment. The *p-value* achieved from ANOVA for the interaction between T and AD on each parameter is indicated and significant parameters are highlighted in bold ($p < 0.05$).

Treatment	W_c (N.mm)	F_p (N)	F_s (N)	N_{sr} (mm ⁻¹)	N_0
165-0	0.7 ± 0.2^d	4.1 ± 1.6^{cd}	4.7 ± 0.8^c	6.59 ± 0.98	78 ± 12^a
165-5	0.38 ± 0.14^c	3.3 ± 0.5^b	4.3 ± 0.8^{bc}	8 ± 2	97 ± 11^{bc}
165-10	0.27 ± 0.07^b	3.2 ± 0.6^{bc}	3.7 ± 0.6^b	11.8 ± 1.2	120 ± 14^d
165-15	0.13 ± 0.04^a	1.8 ± 0.5^a	2.2 ± 0.5^a	13.9 ± 1.7	130 ± 14^d
175-0	0.45 ± 0.13^c	3.5 ± 1.2^{bcd}	3.9 ± 1.2^{bc}	7.5 ± 1.9	76 ± 8^{ab}
175-5	0.425 ± 0.109^c	3.7 ± 0.7^{cd}	4.4 ± 0.4^c	9.43 ± 1.08	93 ± 9^{bc}
175-10	0.36 ± 0.07^{bc}	4.2 ± 0.5^d	5.0 ± 0.7^c	11.8 ± 1.3	115 ± 12^{cd}
175-15	0.45 ± 0.09^c	5.7 ± 0.7^e	6.0 ± 0.7^d	12.3 ± 0.9	90 ± 11^{bc}
<i>p-value</i>	0.000	0.000	0.000	0.357	0.016

The same letter in superscript within the columns indicates homogeneous groups according to the LSD test ($p < 0.05$).

Table 20. Mean values and standard deviations of texture parameters of extruded products obtained at different temperature (T). The *p-value* achieved from ANOVA for the effect of T on each parameter is indicated and significant parameters are highlighted in bold ($p < 0.05$).

T (°C)	W_c (N.mm)	F_p (N)	F_s (N)	N_{sr} (mm ⁻¹)	N_0
165	0.4 ± 0.2	3.0 ± 1.3	3.7 ± 1.4	9.9 ± 3.5	104.3 ± 28.3
175	0.424 ± 0.104	4.4 ± 1.4	5.0 ± 1.3	10.5 ± 2.4	94.6 ± 16.7
<i>p-value</i>	0.025	0.000	0.000	0.203	0.017

Table 21. Mean values and standard deviations of texture parameters of extruded products with different levels of *A. domesticus* (AD). The *p-value* achieved from ANOVA for the effect of AD on each parameter is indicated and significant parameters are highlighted in bold ($p < 0.05$).

AD (%)	W_c (N.mm)	F_p (N)	F_s (N)	N_{sr} (mm ⁻¹)	N_0
0	0.6 ± 0.2	3.9 ± 1.7	4.6 ± 1.6	7.1 ± 1.8^a	78.9 ± 17.8
5	0.41 ± 0.12	3.62 ± 1.06	4.40 ± 1.12	9.0 ± 1.9^b	94.8 ± 15.3
10	0.32 ± 0.08	3.7 ± 0.7	4.4 ± 0.9	11.8 ± 1.5^c	115.9 ± 16.7
15	0.29 ± 0.18	3.7 ± 2.2	4.1 ± 2.2	13.0 ± 2.5^c	108.3 ± 28.3
<i>p-value</i>	0.000	0.807	0.485	0.000	0.000

The same letter in superscript within the columns indicates homogeneous groups according to the LSD test ($p < 0.05$).

These results are in line with those obtained by other studies where it was found that the W_c increased with the increase in temperature (Brennan *et al.*, 2011). Furthermore, the conduct verified with the level of incorporation of *A. domestica* corroborates that obtained by Cheng *et al.* (2007) and Rodriguez-Vidal *et al.* (2017), where it was observed that the extrudates with greater incorporation of the raw material under study in these works, different from that used in this study, also showed less crispiness work, being therefore less resistant to fracture, unlike the control samples.

The average puncturing force and the average specific force of structural ruptures are properties that are usually associated with the sensory attribute of hardness during chewing, which is defined as the force applied to crush a substance among the molar teeth (for solids) (Patil *et al.*, 2005; Rodriguez-Vidal *et al.*, 2017). Hence, it can be expressed as the maximum force needed to make a probe penetrate over an extruded material (Kumar *et al.*, 2018).

For the two forces, significant differences ($p < 0.05$) were observed for the various treatments (table 19), so there was an interaction between the factors studied that influenced these properties. The behaviour of the samples was the opposite with the increased incorporation of *A. domestica* for both temperatures: at 165°C there was a decrease in F_p and F_s with the incorporation of house cricket and at 175°C there was an increase in these values. Based on this, treatments 165-0 and 165-5 were the ones with higher hardness, in other words, at 165°C the control samples and those with lower incorporation (5%) were the hardest, whereas at 175°C were the samples with higher incorporation of insect meal (15%), corresponding to the treatment 175-15.

According to studies, it would be expected that the extrudates with higher incorporation of the raw material under study were harder (Asghari-Pour *et al.*, 2018; Azzollini *et al.*, 2018; Kumar *et al.*, 2019; Seth *et al.*, 2015), as happened at 175°C, because when protein-rich products are added to starch-based products the density tends to increase, that consequently leads to hardness (Chakraborty *et al.*, 2020), which is assigned to a stricter network of a product low expanded (Philipp *et al.*, 2017b). However, the fall in hardness with the increase in incorporation at 165°C, is an advantage as it means that snacks with more protein can be less hard than snacks without any incorporation since hardness is an undesirable characteristic. Several studies where incorporated insects were even used in extruded products obtained these same results (Cuj-Laines *et al.*, 2018; Smetana *et al.*, 2019).

Temperature by itself also affected these forces, as it would be expected taking into account the results obtained for the interaction of the factors, which was observed by the existence of significant differences ($p < 0.05$) between the samples (table 20). Thus, the extruded at 175°C presented higher values than the extruded at 165°C being therefore considered harder, as also other authors concluded (Asghari-Pour *et al.*, 2018; Basilio-Atencio *et al.*, 2020; Rathod & Annapure, 2016; Yang *et al.*, 2020). The percentage of enrichment with *A. domestica* alone had no effect on the extruded samples (table 21).

Spatial frequency of ruptures indicates the number of fracture episodes that occur during puncture (Azzollini *et al.*, 2018). It was found that the interaction between temperature and percentage of *A. domestica* had no influence on N_{sr} (table 19), as well as temperature, when analysed individually (table 20). Conversely, it was observed significant differences ($p < 0.05$) for this parameter when analysed only in relation to the percentage of incorporation (table 21). Therefore, regardless of temperature, an increase in N_{sr} values was noticed with the increase in the percentage of house cricket, being the samples with the highest incorporation (15%) the ones with the highest values. These results do not seem to agree with those of other studies, where the samples with insects always had the lowest N_{sr} values (Garcia-Segovia *et al.*, 2020; Igual *et al.*, 2020).

Some authors suggested that the spatial frequency of ruptures may be related to the pore size of extruded snacks, having a positive relation between the two (Azzollini *et al.*, 2018). Moreover, according to Chanvrier *et al.* (2014) this behaviour can be associated with the propagation of interface fractures, because the large pores have a bigger interfacial area giving more peaks. In this light, based on the results obtained, the control samples would have smaller pores and the samples with an incorporation of 15% larger pores.

Regarding N_0 , which corresponds to the number of fractures throughout the test (Igual *et al.*, 2020), significant differences ($p < 0.05$) were observed among the treatments, so there was an interaction between the studied factors that affected N_0 . With the increase in the percentage of *A. domestica*, both at 165 and 175°C there was an increase in the N_0 values, with the exception of treatment 175-15, where there was a decrease with the change of an incorporation of house cricket from 10 to 15% (table 19).

Additionally, samples presented significant differences ($p < 0.05$) in relation to temperature (table 20) when analysed individually, being the samples extruded at 175°C those that showed lower values of N_0 compared to the ones extruded at 165°C, similar results to other works (Gondek *et al.*, 2013; Lazou & Krokida, 2010). Percentage of *A. domestica* also influenced the number of fractures ($p < 0.05$), having been observed,

as mentioned for the interaction of the factors, an increase in N_0 with the rise of incorporated house cricket, except for the samples with an incorporation of 15%, but whose values were still higher than that of the control and of lower incorporation (5%) samples (table 21). This increase in the N_0 values is contrary to what has happened in other studies, such as the one of Igual *et al.* (2020), where house cricket flour has been incorporated too, which is sensory advantageous, as will be explained below.

Parameters N_{sr} and N_0 are usually associated with the crunchiness of extruded snacks. Some previous works have shown that typically the addition of protein to mixtures leads to the formation of compacted bubbles with a dense structure, responsible for the lower crunchiness of the extrudates, which would be given by low values of N_{sr} and N_0 (Beck *et al.*, 2018; Jakubczyk *et al.*, 2017; Sharif *et al.*, 2014). Therefore, a greater incorporation of the raw material would lead to less crunchy extrudates, as was the case in the study of Lazou and Krokida (2010), where lentil flour has been added to the extruded products. Moreover, in studies where it was obtained crunchy extruded snacks, they were often harder, which is an unwanted feature (Philipp *et al.*, 2017a). However, in the present study the opposite has occurred, with the higher incorporation extrudates showing higher values of N_{sr} and N_0 , thus being more crunchy; this is an advantage, especially in the higher incorporation extruded samples at 165°C which, in addition to being more crunchy, had a low hardness, since these are two of the attributes most desired by the consumer.

4.3.1. Relationship among texture and physicochemical and compositional properties

Correlations between textural parameters and physicochemical and compositional parameters were also studied (table 22). It was observed several positive correlations between W_c and water related properties of the samples, namely a_w ($p < 0.1$), WAI ($p < 0.05$) and WSI ($p < 0.1$), which showed that more free water present and absorbed by the snacks, as well as greater solubility in water, also led to greater fracture resistance by these products (Garcia-Segovia *et al.*, 2020). In contrast, with the increase in protein content this fracture resistance was lower according to Pearson's negative correlation ($p < 0.1$) between W_c and CP.

Although not very strong, it is relevant to mention the correlations between F_p and Hg ($p < 0.1$), since samples with a high hygroscopicity showed a harder texture, because according to Patil *et al.* (2005) as the extruded products are quite hygroscopic, their texture is disturbed when they are subjected to wet atmospheres, as it happened with

their expansion characteristics. Therefore, there were also positive correlations of F_p and F_s with ρ_b ($p < 0.05$) and negative with ϵ ($p < 0.05$ for F_p and $p < 0.1$ for F_s), so samples with high bulk density and low porosity showed greater hardness, due to a greater compaction of the extruded products. Many studies have reached the same results by relating hardness to ρ_b (Meng *et al.*, 2010; Navarro-Cortez *et al.*, 2018; Oliveira *et al.*, 2017) and with ϵ (Azzollini *et al.*, 2018; Chanvrier *et al.*, 2013; Chanvrier *et al.*, 2014). Consequently, the parameters of expansion and texture are related to each other and several authors even state that the expansion itself in the die is what defines the structure of the extrudates, and then the final texture of the snack (Bouvier *et al.*, 1997).

Table 22. Pearson correlation coefficients between texture parameters of extruded products and physicochemical and composition parameters.

	W_c	F_p	F_s	N_{sr}	N_0
x_w^E	0.507	- 0.260	- 0.194	- 0.742**	- 0.419
a_w	0.629*	- 0.163	- 0.082	- 0.790**	- 0.483
WAI	0.728**	0.191	0.253	- 0.768**	- 0.778**
WSI	0.625*	- 0.015	- 0.015	- 0.781**	- 0.732**
SWE	- 0.621	- 0.161	- 0.139	0.659*	0.708**
Hg	0.132	0.629*	0.599	0.175	- 0.210
SEI	0.342	- 0.475	- 0.404	- 0.701*	- 0.296
D_e	0.298	- 0.516	- 0.441	- 0.682*	- 0.262
ρ_b	0.071	0.818**	0.752**	0.411	- 0.0556
ϵ	0.056	- 0.709**	- 0.650*	- 0.373	0.068
CP	- 0.657*	0.088	0.001	0.926***	0.704*

*** Correlation is significant at the level 0.01.

** Correlation is significant at the level 0.05.

* Correlation is significant at the level 0.1.

W_c : Crispness work; F_p : Average puncturing force; F_s : Average specific force of structural ruptures; N_{sr} : Spatial frequency of ruptures; N_0 : Number of peaks; x_w^E : Extrudate water content; a_w : Water activity; WAI: Water absorption index; WSI: Water solubility index; SWE: Swelling index; Hg: Hygroscopicity; SEI: Expansion index; D_e : Diameter; ρ_b : Bulk density; ϵ : Porosity; CP: Crude protein.

Texture parameters related to crunchiness (N_{sr} and N_0) showed negative correlations with WAI and WSI ($p < 0.05$) and N_{sr} also showed negative correlations ($p < 0.05$) with

x_w^E and a_w , which allowed verify that snacks with these well-developed water-related characteristics are less crunchy (Garcia-Segovia *et al.*, 2020; van der Sman *et al.*, 2018). According to Shirazi *et al.* (2020), the crunchiness of snacks is related to the thickness of the walls and with expansion, so that thinner walls with good expansion characteristics lead to softer extrudates. This was proven in this study by the existence of negative correlations between N_{sr} with SEI and D_e ($p < 0.1$), so that less expanded extrudates are generally crunchier. In contrast, N_{sr} and N_0 exhibited positive correlations with SWE ($p < 0.1$ and $p < 0.05$, respectively) and CP ($p < 0.01$ for N_{sr} and $p < 0.1$ for N_0), due to the increased incorporation of house cricket, which also led to the formation of crunchy snacks.

In sum, all these correlations found confirm the results obtained for each of the parameters individually and show how they relate to each other and how dependence on each other affects the quality characteristics of the final product. Based on this it is thus possible to select that considered "ideal snacks", by choosing those with the best and most desired properties.

4.4. Optical properties of extrudates

The reflection spectra of the extruded products for the different treatments are shown in figure 25. All formulations of both temperatures have a maximum reflectance between 550 and 560 nm, which corresponds to the yellow tonality. The samples containing house cricket incorporated did not showed differences in the measurements taken on white and black backgrounds, so the colour coordinates were obtained directly from the equipment used for colour measurement. In contrast, the control samples, showed differences in those measurements and were considered translucent, so the determination of the colour coordinates was made from those reflection spectra for the extruded controls, in the visible range.

In addition, based on the same spectra, the spectral distribution of the Kubelka-Munk (K/S) coefficients were analysed to quantify the translucency of the extruded controls, considering absorption (K) and scattering (S). Hence, the control samples presented relatively high K/S values (figure 26), with no differences between for both temperatures. These results are comparable to those obtained by other authors (Garcia-Segovia *et al.*, 2020), which allow to say that these samples are very transparent, since the translucency is higher the higher the K/S values are (Talens *et al.*, 2002).

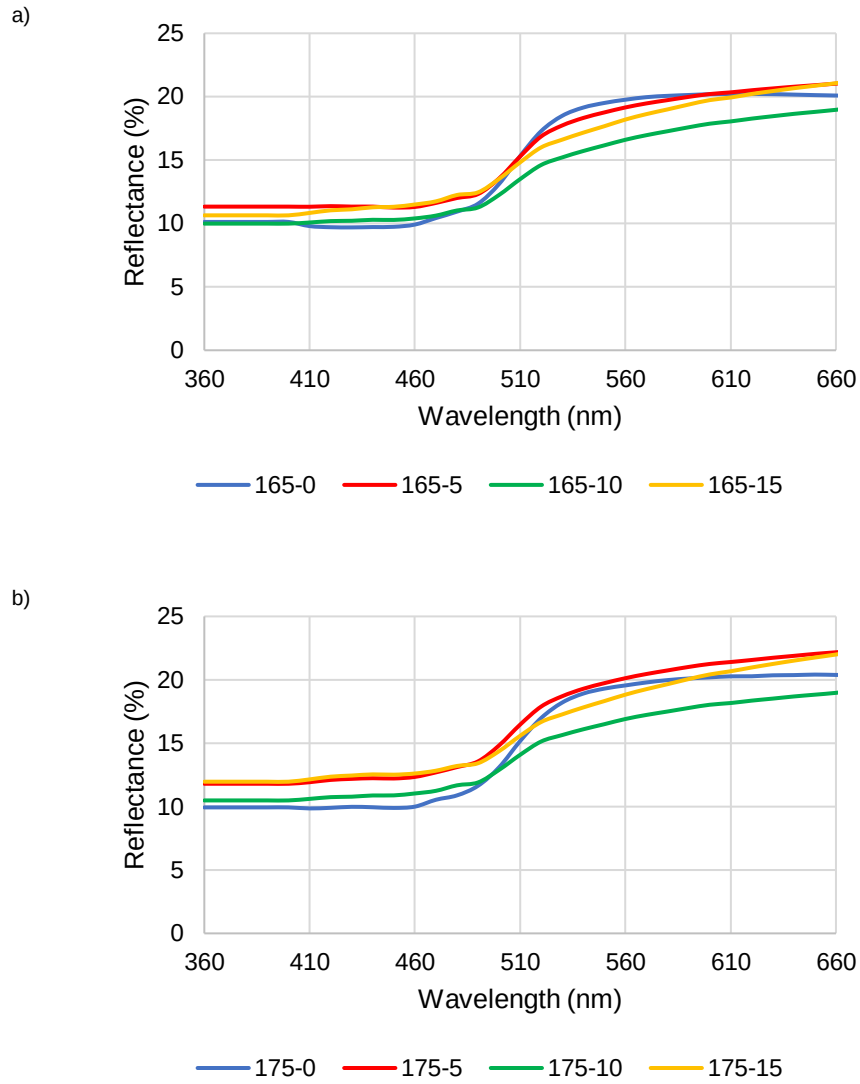


Figure 25. Reflection spectrum for each treatment: all formulations, 0, 5, 10 and 15% of *A. domesticus* extruded at 165°C (a) and at 175°C (b).

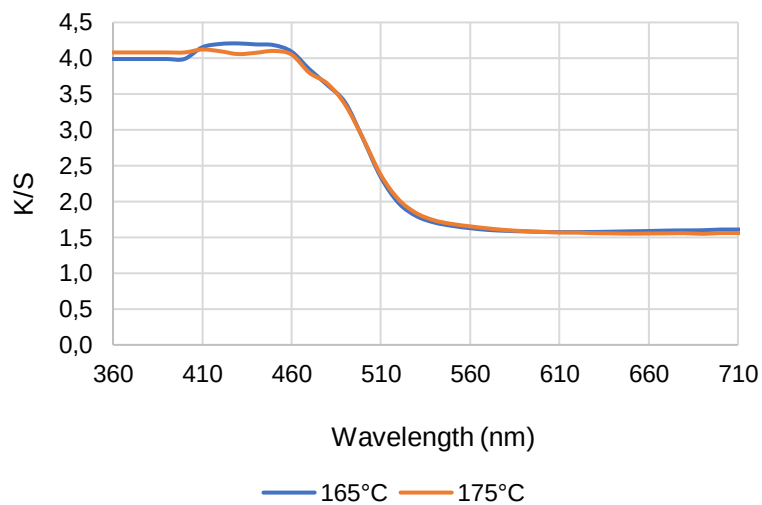


Figure 26. Spectra distribution of the Kubelka-Munk coefficients (K/S) of control extrudates at 165 and 175°C.

Among the $CIE^*L^*a^*b^*$ colour coordinates only a^* showed significant differences ($p < 0.05$) for the various treatments (table 23), and there have then been an interaction between the temperature and percentage of *A. domestica* affecting this coordinate. It was possible to verify an increase in values of a^* with the increase in the incorporation of house cricket, similar behaviour for both temperatures. On the contrary, the L^* and b^* coordinates were not influenced by this interaction. Figures 27a) and 27b) represent the variations of the a^* coordinate in relation to the L^* and b^* coordinates, respectively, where it was seen that, in fact, the range of values between which L^* and b^* varied for the various treatments was not so varied, unlike the range of a^* . The temperature factor alone had no effect on these colour coordinates (table 24). Regarding the percentage of *A. domestica* it was observed significant differences ($p < 0.05$) for the three coordinates (table 25). There was an increase in the values of a^* , as already mentioned, and a decrease in both L^* and b^* values, with the increase in the level of incorporated house cricket.

Table 23. Mean values and standard deviations of colour coordinates (L^* , a^* , b^* , C^*_{ab} and h^*_{ab}) and total colour difference (ΔE) of extruded products obtained for each treatment. The p -value achieved from ANOVA for the interaction between T and AD on each parameter is indicated and significant parameters are highlighted in bold ($p < 0.05$).

Treatment	L^*	a^*	b^*	C^*_{ab}	h^*_{ab}	ΔE
165-0	49 ± 4	0.3 ± 0.4 ^a	18 ± 2	18 ± 2	88.9 ± 1.3 ^e	
165-5	48 ± 7	1.8 ± 0.3 ^b	17 ± 5	17 ± 5	83.7 ± 2.8 ^d	7 ± 4
165-10	45 ± 6	2.3 ± 0.6 ^c	16 ± 3	16 ± 3	81.9 ± 1.4 ^{bc}	9 ± 3
165-15	45 ± 7	2.7 ± 0.4 ^d	16.5 ± 2.4	17 ± 2	80.8 ± 1.2 ^b	7 ± 3
175-0	51 ± 4	0.6 ± 0.4 ^a	18 ± 3	18 ± 3	88.1 ± 1.4 ^e	
175-5	51 ± 8	1.6 ± 0.3 ^b	17 ± 5	17 ± 5	83.8 ± 3.7 ^d	10 ± 5
175-10	47 ± 8	1.9 ± 0.4 ^b	15 ± 3	15 ± 3	82.97 ± 1.12 ^{cd}	11 ± 5
175-15	47 ± 5	3.0 ± 0.6 ^e	13.7 ± 3.7	14 ± 4	77.0 ± 3.3 ^a	8 ± 3
p -value	0.960	0.001	0.300	0.340	0.000	0.610

The same letter in superscript within the columns indicates homogeneous groups according to the LSD test ($p < 0.05$).

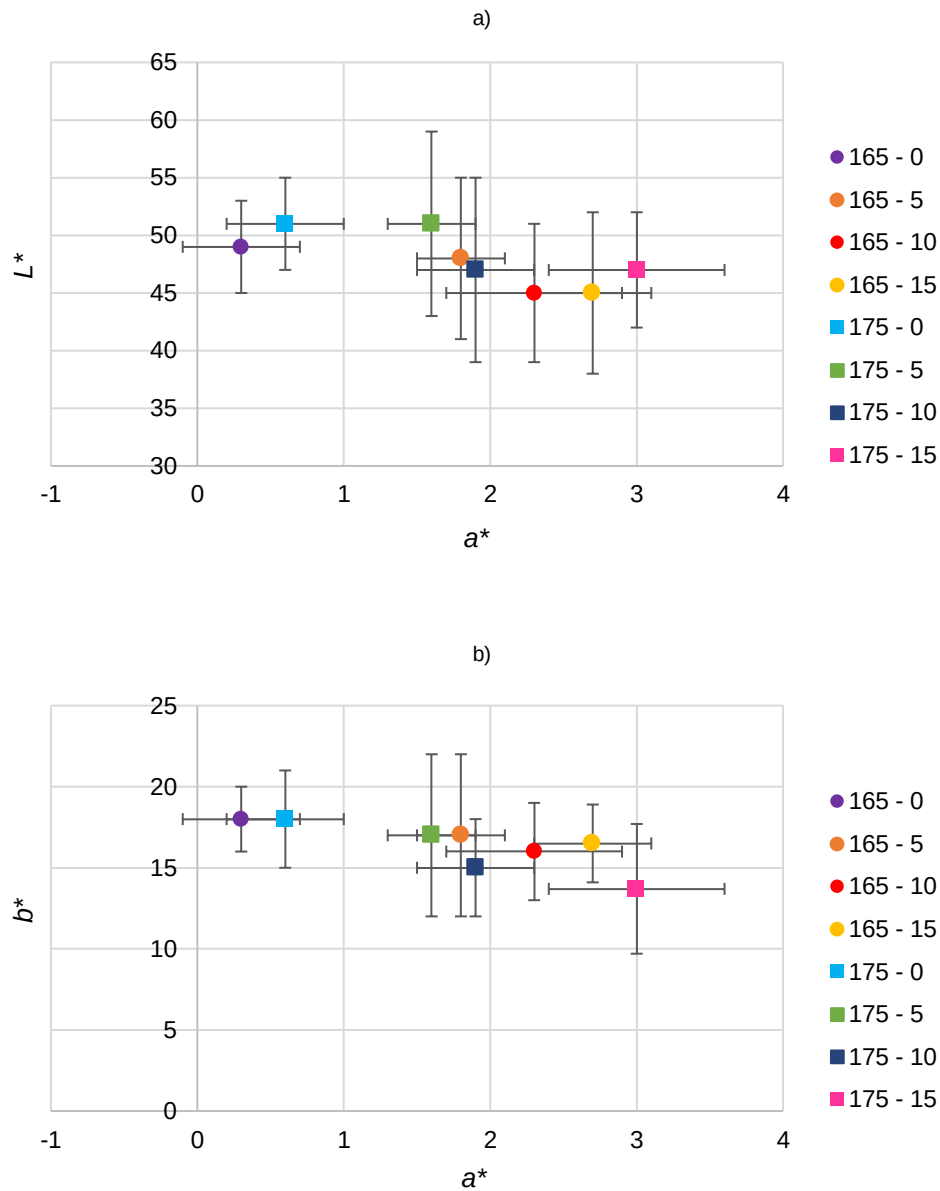


Figure 27. Variation of colour coordinate a^* in relation to the coordinate L^* for each treatment (a) and variation of colour coordinate a^* in relation to the coordinate b^* for each treatment (b).

Table 24. Mean values and standard deviations of colour coordinates (L^* , a^* , b^* , C^*_{ab} and h^*_{ab}) and total colour difference (ΔE) of extruded products obtained at different temperature (T). The p -value achieved from ANOVA for the effect of T on each parameter is indicated.

T (°C)	L^*	a^*	b^*	C^*_{ab}	h^*_{ab}	ΔE
165	46.7 ± 6.0	2.0 ± 0.9	16.8 ± 3.6	16.9 ± 3.5	83.1 ± 3.2	7.6 ± 3.6
175	48.9 ± 7.0	1.9 ± 0.9	15.7 ± 3.9	15.8 ± 3.8	82.3 ± 4.6	9.3 ± 4.4
p -value	0.060	0.591	0.163	0.158	0.090	0.052

Table 25. Mean values and standard deviations of colour coordinates (L^* , a^* , b^* , C^*_{ab} and h^*_{ab}) and total colour difference (ΔE) of extruded products with different levels of *A. domestica* (AD). The p -value achieved from ANOVA for the effect of AD on each parameter is indicated and significant parameters are highlighted in bold ($p < 0.05$).

AD (%)	L^*	a^*	b^*	C^*_{ab}	h^*_{ab}	ΔE
0	50.5 ± 4.0^c	0.5 ± 0.4	18.1 ± 2.5^c	18.1 ± 2.5^c	88.5 ± 1.5	
5	49.6 ± 7.2^c	1.7 ± 0.3	16.9 ± 4.9^{bc}	17.1 ± 4.7^{bc}	83.8 ± 3.3	8.4 ± 4.6
10	45.9 ± 6.2^a	2.1 ± 0.6	15.6 ± 2.9^{ab}	15.8 ± 2.9^{ab}	82.4 ± 1.4	9.7 ± 4.2
15	46.2 ± 6.0^b	2.8 ± 0.6	15.1 ± 3.4^a	15.4 ± 3.3^a	78.9 ± 3.1	7.3 ± 3.0
p -value	0.016	0.000	0.009	0.017	0.000	0.074

The same letter in superscript within the columns indicates homogeneous groups according to the LSD test ($p < 0.05$).

Therefore, the samples with incorporated *A. domestica* showed lower values of L^* , which means a lower lightness, compared to the control samples, values of a^* higher corresponding to redder shades and lower values of b^* associated to fewer shades of yellowish, which were reflected in a global brownish colouration of the final snacks obtained, especially in samples with 15% of house cricket (figure 28). This decrease in L^* and b^* values and increase in a^* values are similar results to those already observed both in studies where insects were used (Alam *et al.*, 2019; Smetana *et al.*, 2019; Tao *et al.*, 2017) and in others where the raw materials incorporated were different (Alam *et al.*, 2019; Asghari-Pour *et al.*, 2018; Bhat *et al.*, 2019; Chakraborty *et al.*, 2020; Sumargo *et al.*, 2016).



Figure 28. Extrudates obtained at 165°C for each of the formulations with 0, 5, 10 and 15% of *A. domestica* (from left to right respectively).

Insect meal itself has a darker colour, unlike cornmeal which has a yellow colour (Alam *et al.*, 2019; Bhat *et al.*, 2019). Thus, a decrease in brightness could be expected with the increase in the incorporation of *A. domestica* powder, since according to Goes *et al.* (2015) brightness decreases with the increase in protein content. In addition, the high temperatures used in the extrusion process triggered the Maillard reaction, a non-enzymatic browning reaction (Cuj-Laines *et al.*, 2018). This, after several steps, led to the production of melanoidins (brown pigments) that contributed to the reduction in L^* values and consequently to the darkening of the final extruded product (Bhat *et al.*, 2019; Sharma & Gujral, 2013).

Values of h^*_{ab} were influenced by the interaction between the studied factors since significant differences ($p < 0.05$) were found among the various treatments, unlike values of C^*_{ab} (table 23). The behaviour of tone coordinate with the increase in the incorporation of *A. domestica* was the same for the two temperatures, with a decrease in its values. Temperature factor alone had no effect on h^*_{ab} and C^*_{ab} (table 24). However, significant differences ($p < 0.05$) were observed for tone and chroma in relation to the percentage of *A. domestica*, with a decrease in the values of both with the increase in the level of incorporated house cricket, corresponding the highest values to the control samples and the lowest to the samples with an incorporation of 15% (table 25). The same occurred in the study by Igual *et al.* (2020).

Chroma indicates the purity or intensity of a colour and can be described from the amount of grey it contains, the greyer it is, the less saturated the colour is (Wrolstad *et al.*, 2005). In this work, samples with higher incorporation of house cricket were found to be less pure, which means that they had a less saturated colour and therefore less bright, since with the increase in the percentage of *A. domestica* the C^*_{ab} values decreased. Regarding the tone that corresponds to the angle of the chromatic diagram in relation to a^* and b^* , it was observed that all the values obtained were in the range of 0-90°, so the opening of the angle decreased, being the tone of the samples between red-purple (Obradovic *et al.*, 2015).

The study of the change in colour before and after extrusion is relevant because it allows to understand the impact that this type of processing cooking has on the product (Alam *et al.*, 2016). Thus, regarding the total colour difference, no significant differences ($p > 0.05$) were found between treatments in relation to the effect of the interaction of the studied factors (table 23), nor for the analysis of each of these factors alone (tables 24 and 25). Anyway, it was possible to verify through the obtained values a similar trend of the samples with the increase of the incorporation of *A. domestica* for both

temperatures, observing a rise in the values of ΔE of the extrudates with 5% of incorporation for those of 10% and a decrease in the values of ΔE of these extrudates for those of 15%. It is also important to mention that since ΔE was over three for all samples, it is considered that the colour of the extruded products is humanly perceptible by consumers, which means that the colour differences present are evident to the human eye (Gross & Moser, 1977; Martinez-Cervera *et al.*, 2011). These results corroborate those of other studies (Do Carmo *et al.*, 2019).

4.5. *In vitro* digestibility and protein bioaccessibility of extrudates

In vitro digestibility portrays the chemical enzymatic catalysis that happens in the digestive tract (Batista *et al.*, 2017), hence the importance of its realization, because it allows understanding of what extent the extruded are completely digested, especially those with insects, since they have non-digestible components (Marono *et al.*, 2015).

According to the literature, formulations containing only maize are those considered to have ideal characteristics (Martínez *et al.*, 1996), including their digestibility indexes. Table 26 shows the mean values and their standard deviations for both control samples, extruded at 165 and 175°C. Significant differences were found between the samples ($p < 0.05$), so that the components of the extruded at lower temperatures were more digestible than those of the extruded at high temperatures, thus there was an influence of temperature. Regarding the samples extruded at 175°C with 0, 5 and 10% of *A. domestica*, significant differences ($p < 0.05$) were observed (table 27), increasing digestibility with the level of embedded house cricket. Other studies corroborate these results, with an increase in IVD when adding increased sources of protein, as the case of the study by Uribe-Wandurraga *et al.* (2020), where microalgae were used.

Table 26. Mean values and standard deviations of *in vitro* digestibility (IVD) and protein bioaccessibility of extruded control products obtained at different temperature (T). The *p-value* achieved from ANOVA for the effect of T on each parameter is indicated and significant parameters are highlighted in bold ($p < 0.05$).

T (°C)	IVD (%)	Bioaccessibility (%)
165	94.2 ± 0.2	49.5 ± 0.4
175	85.0 ± 0.6	69.4 ± 3.6
<i>p-value</i>	0.000	0.008

Table 27. Mean values and standard deviations of *in vitro* digestibility (IVP) and protein bioaccessibility of extruded products with different levels of *A. domestica* (AD) at 175°C. The *p*-value achieved from ANOVA for the effect of AD on each parameter is indicated and significant parameters are highlighted in bold ($p < 0.05$).

AD (%)	IVD (%)	Bioaccessibility (%)
0	85.0 ± 0.6 ^a	69.4 ± 3.6 ^b
5	88.96 ± 0.98 ^b	63.13 ± 0.14 ^{ab}
10	93.0 ± 0.2 ^c	59.0755 ± 0.0115 ^a
<i>p</i> -value	0.000	0.035

The same letter in superscript within the columns indicates homogeneous groups according to the LSD test ($p < 0.05$).

Although it was not possible to perform these analyses in the remaining samples, due to the limitation of the work explained in methodology section, a tendency was observed for the samples extruded at lower temperatures. These seem to have IVD values higher than those extruded at higher temperatures, since the control samples extruded at 165°C presented an IVD value higher than all the others, even those containing embedded house cricket which were extruded at 175°C. This leads to the belief that samples with incorporated cricket extruded at 165°C would also have digestion values higher than those of the samples extruded at 175°C, regardless of the percentage of *A. domestica* incorporation, being thus more digestible.

Protein bioaccessibility, which consists of the protein fraction liberated during *in vitro* digestion and becomes accessible for absorption (Fernández-García *et al.*, 2009), was also determined. Significant differences ($p < 0.05$) were found between control samples in relation to temperature, having been the samples extruded at 175°C those that showed the highest protein bioaccessibility values (table 26). The studies by Joshi *et al.* (2014) and Day and Swanson (2013) arrived at the same results, where they were found that the increase in barrel temperature improved protein bioaccessibility.

In relation to the percentage of *A. domestica* incorporated in the samples extruded at 175°C, significant differences were observed ($p < 0.05$), with the control samples showing the highest values (table 27), thus indicating a decrease in protein bioaccessibility with the increase in the level of incorporated house cricket.

According to several works, extrusion alone improves protein bioaccessibility (Day & Swanson, 2013). However, when it comes to the incorporation of insects, lower values have been reported in the literature than would be expected given that this source is rich

in protein, as in the studies of Marono *et al.* (2015) and Yi *et al.* (2016), where the levels of bioaccessibility were 54 and 66%, respectively. These values are lower than those obtained in this study, but no extruded products have been developed in this specific works, which suggests that extrusion may even have partly contributed to improving bioaccessibility. According to Sánchez-Muros *et al.* (2014), who stated that protein bioaccessibility for various species of insects ranged from 45 to 66.9%, study where again there was no production of extrudates, these levels are lower than those obtained for other protein sources. The severe conditions of the digestion itself can be one of the factors that disturb bioaccessibility (Tonyali *et al.*, 2020), but the values obtained are possibly due to sclerotized amino acids or due to the presence of chitin, that is neither absorbed nor degraded by the intestine, which hinders protein bioaccessibility (Azzollini *et al.*, 2018; Marono *et al.*, 2015). Therefore, when one intends to use insect meal for food production, estimating the chitin content becomes especially important.

5. Conclusion

The development of this investigation has made it possible to produce a new food product that meets consumers' demand for practical, tasty and nutritious products and also serves as a starting point to ensure present and future food insecurity, being all the production from technology to raw materials more ecological. The results obtained in this work not only add scientific knowledge but could also be useful to the food industry for the production of other products extruded with insects.

Analyses performed on the snacks allow to notice that the addition of cricket flour to extruded products and the temperature at which they were submitted affected the responses of extrusion, namely the pressure and torque, which had also an influence together with the factors under study on the quality characteristics of the final products.

Regarding the physicochemical properties, the snacks obtained with 15% of *A. domestica* presented the best characteristics of some properties associated with the water of the extrudates (x_w^E , W_L , a_w , WAI, WSI, SWE), regardless of temperature, and showed the highest protein content, so insects enhanced the nutritional profile of extrudates. In addition, snacks with 15% of incorporated house cricket extruded at 175°C permitted the use of the allegation “source of protein”.

In contrast, in relation to the structure, snacks with an incorporation of 5% were those that exhibited good properties, characterized by high values of expansion and porosity and low values of bulk density, especially those extruded at 165°C. The presence of lipids is among the reasons for the reduction of the expansion with the level of incorporation of house cricket in the mixtures, so the removal of lipid extraction from the insects would be something to consider in the future, in order to improve not only the expansion, but also the texture of the extrudates. Snacks with the lowest insect incorporation also showed the best hygroscopicity characteristics and displayed a yellow colouration and less brownish tones, similar to that of the control sample and to the tones that are most likely to please consumers.

The influence of extrusion temperature and percentage of *A. domestica* on the texture properties led to snacks with an incorporation of 10 and 15% extruded at 165°C being considered the ones with the best attributes, since they presented the least hardness, neither too much nor too little resistance to fracture and the greater spatial frequency of ruptures, what in sensory terms means the greater crunchiness.

Considering that for testing the influence of temperature on *in vitro* digestibility only the control samples were analysed, it can only be stated that digestibility of control samples extruded at 165°C were higher. For level of incorporation of *A. domesticus* it was found that the higher the level of incorporated house cricket, the higher were the values of digestibility. The opposite occurred with protein bioaccessibility, so snacks of higher incorporation manifested lower values, possibly due to the presence of chitin, an undigestible component, being its removal something to consider in future works. It would also be essential to consider the evaluation of *in vitro* digestibility, as well as protein bioaccessibility in the remaining snacks where in this work could not be realised.

Therefore, it can be concluded regarding the two factors under study that the percentage of *A. domesticus* incorporation affected more properties positively and negatively compared to the extrusion temperature whose effects were smaller. Consequently, taking into account that one of the objectives of this work was to increase the protein content of extruded snacks, but in order to maintain their typical characteristics and appreciated by consumers, which was compared to the maize snacks representing the control sample, it is recommended to use percentages of 5 and 10% of house cricket in mixtures for the formulation of snacks and that their extrusion be conducted at 165°C. It is also important for the design of good quality extruded snacks, that more than assessing to their properties individually, being considered the relationships between all properties, and more than analysing only the protein content, it was important to make evaluations of the levels of other nutrients. Finally, with the extruded snacks obtained, something to think about would be performing sensory analysis tests, in order to understand how consumers would accept these snacks.

6. References

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7. Appendix

Appendix I. Paper submitted in Journal of Insects as Food and Feed

Journal of Insects as Food and Feed

Effect of house cricket (*Acheta domestica*) content and extrusion temperature on extrudate snack properties

--Manuscript Draft--

Manuscript Number:	
Full Title:	Effect of house cricket (<i>Acheta domestica</i>) content and extrusion temperature on extrudate snack properties
Article Type:	Research article
Keywords:	Insects; extrudates; expansion; texture; in vitro digestibility
Corresponding Author:	Marta Igual, PhD SPAIN
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	
Corresponding Author's Secondary Institution:	
First Author:	Liliana Ribeiro
First Author Secondary Information:	
Order of Authors:	Liliana Ribeiro Luís Cunha Purificación García-Segovia Javier Martínez Martínez Monzó, PhD Marta Igual
Order of Authors Secondary Information:	
Abstract:	The demand for healthy, satiating, and convenient products is growing, with ready-to-eat expanded snacks being the preferred. These are produced by extrusion, which is a versatile and efficient technology, with a huge potential in the development of new food products. In addition, to satisfy consumer requests it is interesting to add alternative protein sources to these products, such as insects that not only have nutritional benefits, but also ecological advantages. The aim of this work is to evaluate the effect of extrusion temperature and enrichment with house cricket (<i>Acheta domestica</i>) on properties of extruded snacks. Extrudates were produced with a single-screw extruder at two different barrel temperatures (165°C and 175°C), being formulated with corn flour and house cricket powder in mass ratios 100:0, 95:5, 90:10 and 85:15. Water content, water activity, expansion, porosity, water absorption and water solubility indices, swelling index, hygroscopicity, colour, texture, protein content, in vitro digestibility and protein bioaccessibility were measured. The results suggest that the incorporation of edible insects in extruded snacks can be a good alternative to the currently available snacks on the market, since those not only maintain the appropriate physicochemical characteristics, especially when formulated at low temperatures, but they also improve the protein content, being then recommended the use of percentages of 5% and 10% of house cricket for the formulation of snacks and that their extrusion be done at 165°C.