clean technologies

MDPI

Article

Encapsulated EVOO Improves Food Safety and Shelf Life of Refrigerated Pre-Cooked Chicken Nuggets

Marta Barón-Yusty [1], Ginés Benito Martínez-Hernández [2,*], María Ros-Chumillas [1], Laura Navarro-Segura [1] and Antonio López-Gómez [1,*]

[1] Food Safety and Refrigeration Engineering Group, Department of Agricultural Engineering, Universidad Politécnica de Cartagena, 30203 Cartagena, Spain; marta.baron@edu.upct.es (M.B.-Y.); Mayroschu@gmail.com (M.R.-C.); laura.navarro@upct.es (L.N.-S.)

[2] Biotechnological Processes Technology and Engineering Lab, Campus Muralla del Mar, Instituto de Biotecnología Vegetal, Universidad Politécnica de Cartagena, 30202 Cartagena, Spain

* Correspondence: ginesbenito.martinez@upct.es (G.B.M.-H.); antonio.lopez@upct.es (A.L.-G.); Tel.: +34-9-6832-5585 (G.B.M.-H.); +34-9-6832-5516 (A.L.-G.)

Abstract: (1) Background: New clean technologies are needed to reduce the high frying oil waste in the food industry of fried breaded products, together with the obtention of healthier (less fat content) and safer (less microbial growth and acrylamide formation) breaded products; (2) Methods: This study proposes the new technology consisting of incorporation of encapsulated extra virgin olive oil (EVOO) (α-cyclodextrin: EVOO ratio, 1:2.6) in the breadcrumbs (corn breadcrumbs:encapsulated oil ratio, 2:1) for breading chicken nuggets combined with oil-free pre-cooking (baking 150 °C/5 min) and cooking (baking 180 °C/13 min). As controls, a conventional deep-fat frying (180 °C/30 s) and new technology but without encapsulated EVOO were used; (3) Results: Fat content of baked chicken nuggets with the new technology was reduced by 88%, while no sensory differences were scored compared with conventional deep-fat frying. Furthermore, acrylamide formation was reduced by >55% with the new technology. During storage (4 °C) of pre-cooked chicken nuggets of new technology, microbial growth was reduced by 1.4 log units lower compared with deep-fat frying method; (4) Conclusions: the proposed new technology, based on encapsulated EVOO+oil-free pre-cooking/cooking, allows to obtain chicken nuggets that are healthier, safer, and have a longer shelf-life, while frying oil waste is avoided.

Keywords: α-cyclodextrin; olive oil; inclusion complex; prebiotic; frying; quality; food safety; acrylamide; fat content

check for updates

Citation: Barón-Yusty, M.; Martínez-Hernández, G.B.; Ros-Chumillas, M.; Navarro-Segura, L.; López-Gómez, A. Encapsulated EVOO Improves Food Safety and Shelf Life of Refrigerated Pre-Cooked Chicken Nuggets. *Clean Technol.* **2022**, *4*, 53–66. https://doi.org/10.3390/cleantechnol4010005

Academic Editor: Alírio E. Rodrigues

Received: 22 December 2021
Accepted: 19 January 2022
Published: 2 February 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Deep-fat frying, or immersion frying, is a cooking procedure known since ancient times. This conventional cooking procedure is common in the industries of ready-to-eat/cook fried food, which gives typical sensory characteristics such as a bright golden color, crunchy crust, and distinctive flavor [1]. Deep-fat frying is carried out in a bath of edible fat or hot oil at a suitable temperature (≈180 °C) for a short time (≈30 s) [2]. This intense heat treatment also ensures a high microbial inactivation extending the product shelf life and food safety. Usually, commercialized fried breaded products are sold as pre-cooked products to reduce the preparation time for consumers, although pre-cooking by deep frying is one of the most troublesome and problematic parts of the manufacture of breaded foods, due to oil-wastes management [3]. However, the high-fat content, and the hazard of acrylamide formation (a carcinogen compound that is formed during cooking over 120 °C), together with the increase in consumer's health awareness is jeopardizing consumer's demand for fried foods. Furthermore, waste cooking oil pertains to the family of used vegetable oils, which are considered waste that is dangerous for the environment [4]. Thus, proper handling of waste cooking oils is mandatory in many countries [5]. In that

sense, new clean technologies for the processing of fried breaded products are needed to reduce or avoid cooking oil waste, which also will lead to products with lower fat content and minimal acrylamide formation.

Among alternative industrial processes to conventional deep-fat frying, Fiszman et al. [6] patented a clean technology for breaded fried foods that incorporates methylcellulose in its formula and substitutes the pre-frying operation for a hot water bath (≈ 60–90 °C) for 1–45 s; thus, reducing oil waste and fat uptake of the product. We also patented an innovative breading formulation that led to fat uptake by the product lower than 50% [7].

Microencapsulation is a clean technology that consists of trapping components (core or active) into a secondary material (encapsulant, wall material, carrier, or cover), producing small solid particles (1–500 μm in size) [8]. Lipid microencapsulation has been proposed to retard oil oxidation, masking undesirable tastes, aromas, and/or colors, and providing protection to the sensitive lipid components against environmental factors [9,10]. Although microencapsulation is widely used in the pharmaceutical and agrochemical industries, in the food industry is still challenging due to the high costs of this technique. However, encapsulation of components forming inclusion complexes with cyclodextrins (CDs) is a good alternative with excellent encapsulation efficiency, low cost and controlled release of the encapsulated component [11]. Among encapsulation techniques, inclusion complex formation by the kneading method (also known as physical mixture) is faster (≈45 min), simpler and less expensive than freeze-drying (≈120 h) or spray drying, which needs the use of complex machinery (freeze-drier or spray-drier) [12].

Cyclodextrins (CDs) are natural cyclic oligosaccharides starch derivates obtained by enzymatic degradation that lack taste, odor, and calories. Structurally, CDs comprise 6, 7, or 8 glucopyranose units (α-, β-, and γ-CDs, respectively) cyclically linked by glycosidic bonds α (1–4) [13]. They have a truncated cone shape with a conical cavity, with their diameters increasing as the number of glucose units rises [13]. CDs can be used in a wide spectrum of applications within the food industry, due to their ability to form inclusion complexes by hosting, in their hydrophobic and lipophilic internal cavity, a variety of food compounds, being lipophiles the encapsulated substances mainly used in this industry. The EU authorized α-CD and γ-CD as novel food ingredients in 2008 (Regulation (EC) No. 258/97), and β-CD (E-459) as a food additive (Regulation (EC) No. 1333/2008 following Annex II and III). The Codex Alimentarius of JECFA (Joint FAO/WHO Expert Committee on Food Additives) includes, when used as food supplements, α-, β-, and γ-CD as E-457, E-459, and E-458, respectively [14]. The Codex also includes the Acceptable Daily Intake (ADI) for β-CD of 5 mg/kg per day (re-evaluated without modifications in 2016), although α-CD and γ-CD still do not have a specific ADI due to JECFA's favorable toxicological data [15]. α-CD is considered as a prebiotic and also has other health-promoting properties like a promoting effect on sugar and fat metabolism [16,17]. α-CD is very stable under common food processing treatments with thermal degradation occurring at 297 °C [18,19]. In addition, Hadaruga et al. [20] found that cyclodextrin-oleic acid inclusion complexes were very stable at high temperatures (studied temperature range of 50–150 °C), showing oleic acid:α-CD better thermal stability than oleic acid:β-CD inclusion complex. Thus, α-CD inclusion complexes of olive oil might provide a high protection during common cooking treatments.

This study proposes a new clean technology based on the inclusion of encapsulated (within α-CD) extra virgin olive oil (EVOO) in the breadcrumbs for breading chicken nuggets, followed by pre-cooking and cooking with oil-free oven treatments (hot air oven). The encapsulation of an olive leaf extract was successfully made within β-CD [21]. Nevertheless, the inclusion complex of EVOO within αCD, which is of high interest due to the high dietary fiber and prebiotic nature of α-CD [22], has not been previously studied.

2. Materials and Methods

2.1. Materials

Extra virgin olive oil (EVOO) (hojiblanca and arbequina olive varieties; the rest of the provided characteristics are shown in Supplementary Material Table S1) was purchased in a local supermarket (Cartagena, Spain). αCD was obtained from Bioencapsulation and iPackaging S.L. (Fuente-Álamo, Murcia, Spain). Chicken discs (minced chicken meat discs; diameter ≈ 5 cm, ≈ 1 cm thickness; ≈ 8 g) were supplied frozen by the company Fripozo S.A. (Murcia, Spain). Xanthan gum was provided by Doscadesa 2000 S.L. (Molina de Segura, Spain). Corn breadcrumbs were also supplied by Fripozo S.A. All microbial analysis materials were acquired from Scharlau Chemie (Barcelona, Spain).

A domestic deep-fat fryer (2-L volume, model Professional 2; Taurus, Oliana, Spain) was used for the conventional pre-cooking process (deep-fat frying) using sunflower oil as commonly done in these industries. A domestic oil-free fryer (0.8-kg capacity, model FX100015; Tefal, Rumilly, France) was used for the oil-free pre-cooking process (hot air precooking). See Figure 1 for a full description of pre-cooking/cooking treatments.

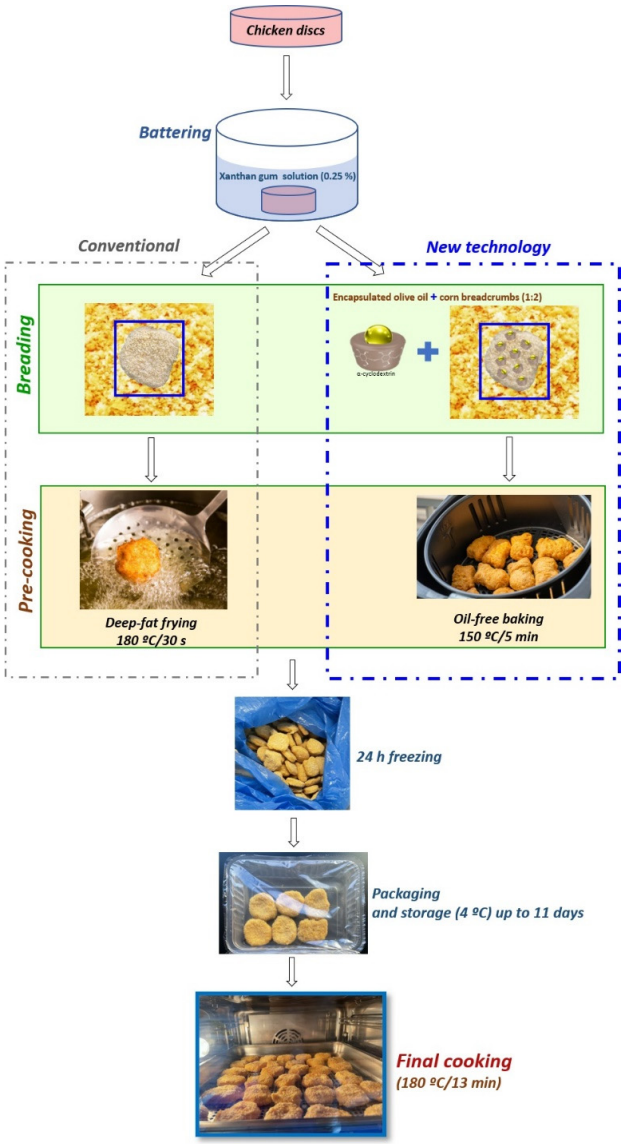


Figure 1. Flowchart production of chicken nuggets with the conventional method and the proposed new technology.

2.2. Preparation of the EVOO−αCD Inclusion Complex and Characterization

Encapsulation of EVOO was made within αCD by the kneading method according to previous literature [21,23]. For it, EVOO and αCD were manually mixed in a mortar for 45 min and finally maintained in a vacuum desiccator at room temperature until used. Different EVOO:αCD ratios were studied: 1:1.8 weight *w*:*w* (equivalent to equimolar ratio of oleuropein:αCD), 1:3.4 *w*:*w* (equivalent to equimolar ratio of oleic acid:αCD), and 1:2.6 *w*:*w* (intermediate option). The 1:2.6 *w*:*w* ratio was finally selected as described in Section 3.1.

The EVOO-αCD inclusion complex formation and thermal stability was verified by Differential Scanning Calorimetry (DSC). A Mettler−Toledo DSC instrument (model 822E; Schwerzenbach, Switzerland) was used. Briefly, samples (2 mg) were placed in aluminum pans (40 µL). Then, the specimens were heated, under a nitrogen atmosphere (flow rate of 50 mL min^{-1}), from 25 to 230 °C with a heating rate of 10 °C min^{-1}.

2.3. Chicken Nugget Preparation, Cooking Treatments, Packaging, and Storage Conditions

Chicken nugget preparation, cooking treatments and packaging were conducted in a laminar flow cabinet (ISO 5; equivalent to 100 FED STD 209E class) inside a cleanroom (ISO 7; equivalent to 10,000 FED STD 209E class) at 8 °C.

Chicken discs were allowed to defrost for 3 h at room temperature before batter and breading processes. The batter process was conducted by manual immersion (one by one) of defrosted chicken discs in a 0.25% xanthan gum solution, as previously optimized by our group for breaded products [7]. Subsequently, coated discs were manually breaded (one by one on both sides of the chicken disc) over a corn breadcrumb bead. For samples including the EVOO-αCD inclusion complex (pre-cooked with the oil-free process; see Figure 1), a corn breadcrumb:EVOOαCD inclusion complex mix (2:1, *w*:*w*) was used. Such breadcrumb:EVOOαCD mix rate was selected based on the maximum EVOO:αCD quantity that was still visually accepted (Figure 2).

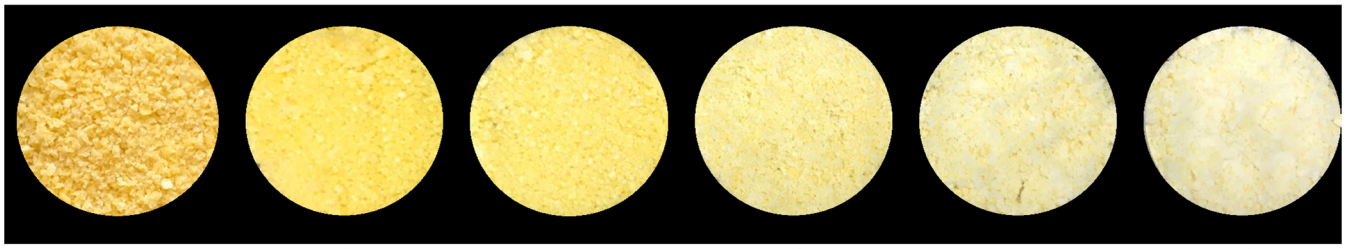


Figure 2. Different breadcrumb:EVOOαCD mixes: (**a**) breadcrumb without EVOO:αCD addition; (**b**) 3:1 weight *w*:*w*, (**c**) 2:1 *w*:*w*, (**d**) 1:1 *w*:*w*, (**e**) 1:2 *w*:*w*; and (**f**) 1:3 *w*:*w*.

Prepared chicken nuggets were pre-cooked by either oil-free (150 °C, 5 min) or deep-fat frying (180 °C, 30 s) (see Figure 1). The cooking parameters of both pre-cooking methods were selected based on preliminary tests selecting those with the best sensory attributes (color, texture, and core juiciness). Samples were allowed to cool down at room temperature (15 min) over a perforated plastic tray, and frozen at −20 °C for 24 h in a domestic freezer. Then, frozen nuggets were packaged (6 nuggets per tray) in a 750-mL plastic tray under a modified atmosphere (70% N_2, 30% CO_2) with a Cryovac® EOP616B film (39 µm thickness; Cryovac; Fuenlabrada, Spain) (gas/water transmission rates of film: O_2, 7.00 cm^3 m^{-2} day^{-1} atm^{-1}; CO_2, 25.00 cm^3 m^{-2} day^{-1} atm^{-1}; N_2, 0.50 cm^3 m^{-2} day^{-1} atm^{-1}; water, 10.00 g m^{-2} day^{-1}) using a semiautomated packaging device (model Efaman; Efabin, Murcia, Spain). These packages containing pre-cooked nuggets were stored at 4 °C with sampling times at 1, 3, 5, 8, and 11 days. At each sampling time, a final cooking step was conducted (see Figure 1) consisting of baking (after removal of samples from packages) at 180 °C for 13 min in a domestic oven (model HBC36P753; Bosch, Gerlingen, Germany) with the forced-air fan switched on. The cooking parameters of this cooking method were selected based on preliminary tests (color, texture, and core juiciness).

Sensory analyses of cooked samples (after the final cooking step) were performed at storage days 1, 3, 5, 8, and 11. Microbial analyses of pre-cooked samples (before the final cooking step) were performed at storage days 1, 3, 5, 8, and 11. The rest of the studied parameters (fat, color, and acrylamide) were only analyzed on 14-days stored samples before and after the final cooking step. Color was not measured on intermediate days (1, 3, 5, and 8) since no high color differences among samples were observed. Fat and acrylamide contents were not measured on intermediate days (1, 3, 5, and 8) since their contents were not expected to change during refrigerated storage.

2.4. Analyses and Determinations

2.4.1. Fat Content

Total fat extraction was performed by the widely known Soxhlet exhaustive extraction technique. Briefly, one nugget piece was placed into a 33 mm × 80 mm porous cellulose thimble (Whatman 10350240) covered with cotton for sample transfer avoidance. The sample was then placed into the Soxhlet apparatus extraction chamber equipped with a condenser and a distillation flask containing 200 mL of *n*-hexane. Nugget fat was extracted for 4 h at 69 °C. Then, the extraction solvent was removed by a vacuum rotary evaporator at 40 °C. Finally, the weight of the extracted fat was measured. Total fat content was expressed as grams of fat per 100 g of product.

2.4.2. Color

The color of pre-cooked samples stored for 11 days at 4 °C was evaluated before and after the final cooking step. The external color of samples was determined using a colorimeter (Chroma Meter CR−400; Konica Minolta, Tokyo, Japan) at illuminant D65 and 2° observer, and with a viewing aperture of 8 mm. Three measurements were made per sample, which was automatically averaged by the device. Hue angle and Chroma index were from a^* and b^* CIE color parameters [24].

2.4.3. Sensory Analyses

Sensory analyses were performed according to international standards and previous literature on chicken nuggets [25,26]. Sensory tests were conducted in a standard room [27] equipped with ten individual taste booths. The panel consisted of six assessors (four women and two men, aged 24–63 years old) who were familiarized with fried breaded products. The quality attributes scored were appearance/color, aroma, texture, flavor, and overall quality, which were evaluated using a five-point numerical rating scale, where a score of 5 indicated the sample was excellent, 4 = very good, 3 = good and at the limit of marketability, 2 = fair and the limit of usability, and 1 = poor and inedible.

2.4.4. Acrylamide Contents

Acrylamide content was analyzed in accordance with the supplier of the extraction kits as follows. Samples were ground (mill IKA A11 Basic; Staufen, Germany) with liquid nitrogen. To 1 g of the milled sample was added 1 mL of an internal D3-acrylamide (isotopically labelled acrylamide) standard (0.04 ppm) (Sigma-Aldrich). Then, 5 mL of hexane, 9 mL of water, and 10 mL of acetonitrile are added. Subsequently, the content of one DisQuE quecher (containing 6 g $MgSO_4$ and 1.5 g sodium acetate) (Waters) was added and vortex for 1 min. The tube was then centrifuged at 22,000× *g* for 15 min, and the upper phase (hexane) was discarded. Then, 2 mL of the intermediate phase was purified using SPE columns (Oasis® MCX 3 cc, 60 mg, LP extraction; Waters, Milford MA, USA). The purified extract was then evaporated under N_2 until dryness. The sample was finally resuspended in 1 mL of acidified water (1% formic acid), vortexed, and filtered at 0.22 µm (nylon syringe filter) prior to UHPLC-MS analyses.

Acrylamide extracts were analyzed using a UHPLC coupled to a triple Quadrupole MS System (Agilent 6420 Series; Agilent, Santa Clara CA, USA). Chromatographic analyses were carried out onto a UHPLC Acquity UPLC HSS C18 SB column (100Å, 1.8 µm,

2.1 mm × 100 mm, 1/pkg; Waters). Mobile phases were water (1% formic acid) (A) and methanol (B). The flow rate was 0.2 mL min^{-1} with a linear gradient eluent starting with 1% B to reach 5% B at 4 min, 90% B at 4.5 min, 90% B at 6 min, 1% B at 6.5 min, and 1% B at 7.5 min. The temperature was maintained at 30 °C and injection volume of 5 µL. Mass spectrometry was performed using an ion trap detector equipped with electrospray ionization (ESI) system at a collision energy of 10 eV for the 72.2 > 55.1 transition, and nitrogen was used as the nebulizing gas.

2.4.5. Microbial Analyses

Mesophiles, psychrophiles, enterobacteria, lactic acid bacteria, *Pseudomonas* spp. Yeasts, and molds loads were analyzed. Briefly, 2 nuggets (≈16 g) were mixed with 160 mL of buffered peptone water and then homogenized for 1 min using a stomacher (Colewort Stomacher 400 Lab, Seward Medical, London, UK). Viable counts were based on counts by 10−fold serial dilutions in buffered peptone water. Then, aliquots (1 mL) of the microbial dilutions were pour-plated into Plate Count Agar (mesophiles and psychrophiles), Violet Red Bile Dextrose Agar (enterobacteria), De Man Rogosa and Sharpe Agar (lactic acid bacteria), Cetrimide Agar (*Pseudomonas* spp.) and Rose Bengal Agar (yeasts and molds). Mesophiles, psychrophiles, enterobacteria, lactic acid bacteria, *Pseudomonas* spp., yeasts and molds were incubated at 31 °C (48 h), 4 °C (7 days), 37 °C (24 h), 37 °C (48 h), 37 °C (48 h), 5 days (22 °C), and 7 days (22 °C), respectively. Results were expressed as log colony forming units (CFU) g^{-1}. Each of the three biological replicates was analyzed in duplicate (technical replicate).

2.5. Statistical Analyses

Data were analyzed with a unidirectional analysis of variance (ANOVA) computed in R studio. Tukey HSD test at a 95% confidence level was assessed (statistical significance $p = 0.05$). Results were expressed as mean ± standard deviation.

3. Results and Discussion

3.1. Preparation and Characterization of the EVOO:αCD Inclusion Complex

The inclusion complex EVOO:αCD at the 1:1.8 *w*:*w* ratio showed a creamy texture (Figure 3a), which led to a sticky paste after mixing with corn breadcrumbs. This sticky paste would make impossible a correct nugget breading process in the industry due to the difficulties of handling. A subsequent lyophilization process, which would lead to a powder texture, was discarded since microencapsulation by lyophilization is very expensive and less efficient (long lyophilization processes) being rarely used in the food industry. On the other side, the inclusion complex EVOO:αCD at 1:3.4 *w*:*w* ratio displayed a whiter color (Figure 3c) that led to nuggets with a duller orange color, which would lead to potential consumer rejection. Then, intermediate EVOO:αCD ratios (1:1.8–1:3.4) were assayed (data not shown), and the EVOO:αCD ratio 1:2.6 (*w*:*w*) was selected based on its best organoleptic perception (color and texture) (Figure 3b).

The inclusion complex formation between EVOO within αCD at the technologically-efficient 1:2.6 (*w*:*w*) ratio was also confirmed by DSC. Endothermic peaks were observed at 76, 86, and 180 °C for the DSC thermogram of αCD, which may represent the moisture bound with different levels of energy of interaction: weak (at the surface), strong (in the cavity), and very strong (monolayer moisture) [28]. The inclusion complex of guest molecules within cyclodextrins may be confirmed by DSC by the replacement of water by guests due to inclusion complexation within the cyclodextrin molecule. Indeed, the sharp endothermic peak of αCD at 180 °C was broadened and minimized (Figure 4), probably owed to such water replacement by guests (EVOO) within the αCD molecules, being expected with a high EVOO encapsulation efficiency. Similarly, minimization of the endothermic peak at 180 °C of βCD was observed by Mourtzinos et al. [21] when the EVOO:βCD inclusion complex (1:4.1, *w*:*w*) was formed, being also confirmed by these authors by nuclear magnetic resonance.

Figure 3. EVOO:αCD inclusion complexes at different ratios: (**a**) EVOO:αCD at 1:1.8 weight *w*:*w*; (**b**) 1:2.6 *w*:*w*, and (**c**) 1:3.4 *w*:*w*.

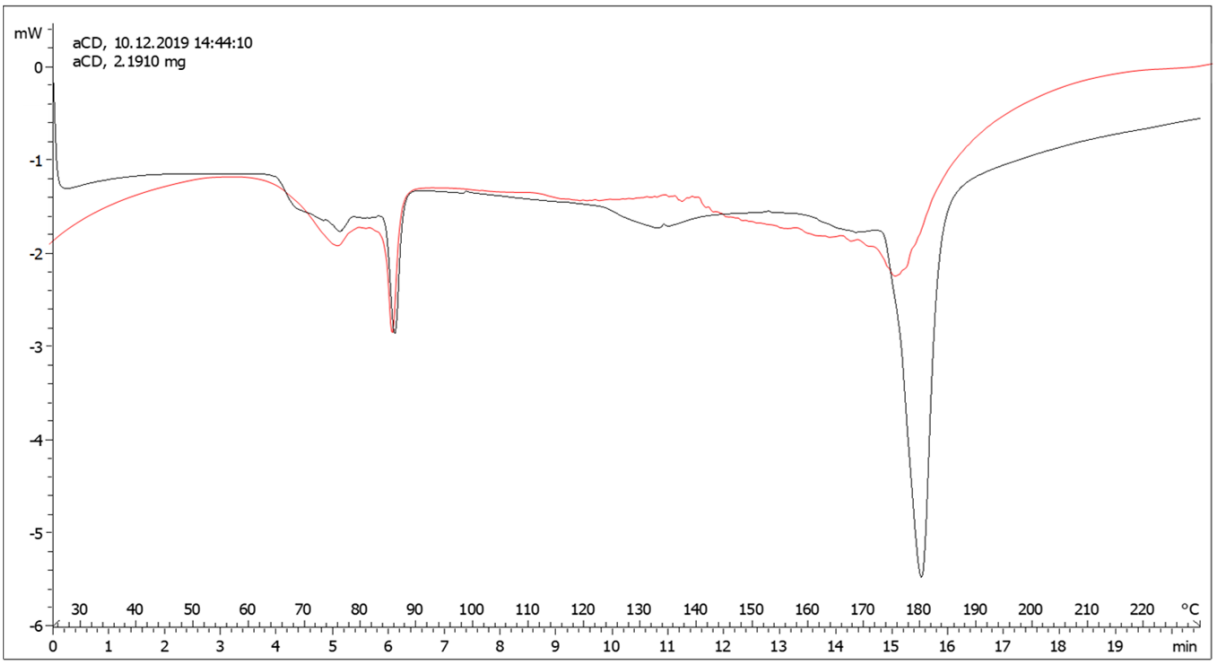


Figure 4. DSC thermograms of α-CD (black line) and EVOO-αCD inclusion complex (red line).

3.2. Fat Content

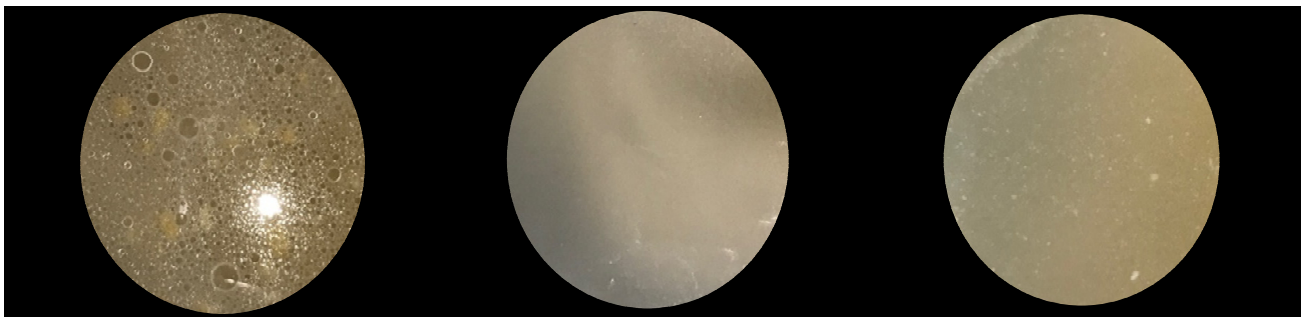
Fat content is an important quality parameter of fried breaded products, which is directly perceived by the consumer as a greasy texture in the mouth. Several theories have been made to explain the complexity of oil uptake in fried foods. Several investigations show that some of the oil is taken up during frying (water replacement), while others suggest a correlation between oil degradation and oil uptake (surfactant theory). Overall, these mechanisms remark the strong dependence of oil absorption on microstructure and surface characteristics of the fried products [29]. As expected, fat uptake of samples pre-cooked with deep-fat frying was higher than the used oil-free pre-cooking, regardless ($p > 0.05$) of EVOO-αCD addition (Table 1). The same trends were also observed after the final cooking process. Hence, the fat content of nuggets incorporating EVOO-αCD and pre-cooked with the oil-free process was reduced by 88% when compared with the conventional deep-fat frying pre-cooking.

Table 1. Fat content (g 100 g^{-1}) and colour parameters of chicken nuggets pre-cooked with different methods. Different letters denote significant differences ($p < 0.05$) among samples of the same row.

Parameter	Deep-Fat Pre-Cooking		Oil-Free Pre-Cooking		New Technology *	
	Pre-Cooked	Cooked	Pre-Cooked	Cooked	Pre-Cooked	Cooked
Fat content	19.9 ± 7.6 ^{a}	17.9 ± 3.5 ^{a}	0.92 ± 0.52 ^{b}	1.33 ± 0.60 ^{b}	2.29 ± 0.49 ^{b}	4.37 ± 1.42 ^{b}
L *	57.6 ± 3.9 ^{c}	52.9 ± 6.3 ^{d}	68.2 ± 2.6 ^{b}	65.6 ± 2.6 ^{b}	75 ± 1.6 ^{a}	68.5 ± 2.3 ^{b}
a *	4.43 ± 2.04 ^{b}	7.58 ± 2.15 ^{a}	1.10 ± 0.63 ^{cd}	2.32 ± 0.84 ^{c}	0.21 ± 0.56 ^{d}	1.70 ± 0.90 ^{d}
b *	42.3 ± 3.1 ^{c}	42.4 ± 5.5 ^{c}	46.5 ± 2.5 ^{b}	50.5 ± 1.6 ^{a}	40.9 ± 2.6 ^{c}	49.8 ± 1.7 ^{ab}
Hue	1.46 ± 0.05 ^{a}	1.39 ± 0.06 ^{a}	1.55 ± 0.01 ^{a}	1.52 ± 0.01 ^{a}	0.51 ± 1.54 ^{b}	1.54 ± 0.01 ^{a}
Chroma	42.6 ± 2.9 ^{d}	43.2 ± 5.3 ^{de}	46.5 ± 2.5 ^{bce}	50.6 ± 1.56 ^{a}	42.8 ± 3.45 ^{cd}	49.9 ± 1.65 ^{ab}

* New technology refers to chicken nuggets including EVOO-αCD within the breading process followed by oil-free pre-cooking.

Furthermore, samples were homogenized in water (using stomacher as described in Section 2.4.5) to visually evaluate the EVOO release from samples after cooking (Figure 5). As observed, EVOO release with the new technology after pre-cooking and cooking was controlled with fewer EVOO drops of small diameter, compared with the high content of larger oil drops of prepared samples with the deep-fat precooking.



(**a**) (**b**) (**c**)

Figure 5. EVOO release from nugget samples prepared with: (**a**) deep-fat precooking, (**b**) oil-free precooking, and (**c**) new technology, followed by final cooking step.

It has been proposed that calories of fat per serving should be below 35% [29], which means a 42% reduction of fat content in a fried breaded chicken-based product, according to the Spanish Food Composition Database (BEDCA). In that sense, the proposed new technology in this study almost doubled the proposed fat reduction by BEDCA.

Several studies and technological innovations have been conducted to reduce the fat content of a wide range of fried products. In particular, functional ingredients (proteins or non-protein hydrocolloids) are incorporated into the batter and/or breading or applied as a post-breading dip aimed to retard oil absorption [29]. Nevertheless, most technological solutions have not achieved fat content reductions higher than 40%. Exceptionally, only two patents achieved fat content reductions of fried breaded products by 50% [7,30]. In that sense, the proposed new technology in this study allows reducing the fat content by approximately 90%, with the consequent higher consumer acceptance (better sensory perception and healthy aspects) and waste reduction of fats (used during conventional deep-fat frying) from the related industries.

3.3. Colour

Colour changes during the frying of breaded products is the first quality parameter evaluated by consumers, being critical in the acceptance of the product since it is associated with the taste [31]. Among measured colour parameters, *L* * (luminosity) is a critical parameter in the frying industry and is usually used as a quality control factor [32]. Accordingly, *L* * values of pre-cooked nuggets was reduced after cooking, together with *b* * increment (Table 1). Browning reactions during cooking also have a high impact on red-green chromaticity (*a*), which was incremented (Table 1).

Focusing on pre-cooked samples (without final cooking step), the conventional deep-fat frying pre-cooking led to product browning (reduction of luminosity (L *)), which reduced the typical yellow colour of our corn breadcrumbs nuggets showing deep-fat fried pre-cooked samples lower yellowness (b) than oil-free pre-cooked samples (without encapsulated EVOO) (Table 1). The decrease in luminosity during deep-fat frying is a characteristic consequence that has been reported for many fried products, including chicken nuggets, due to non-enzymatic browning reactions like caramelization and Maillard kinetic reactions (for temperatures higher than 100 °C) that are temperature-dependent together with other intrinsic food factors (such as water activity, pH and chemical composition) [33]. Furthermore, deep-fat frying pre-cooking increased sample redness parameter (a), when compared to oil-free pre-cooked samples (Table 1), being the increment of this parameter (a) not desirable during frying of food products in general [34] because of the potential consumer rejection. On the other side, the addition of EVOO-αCD to nuggets increased luminosity (L *) and reduced yellowness (b) when comparing oil-free pre-cooking and new technology. Nevertheless, the last trends (observed in both pre-cooked and cooked samples) were only significant among pre-cooked samples. The observed increased luminosity might be due to: (1) the added αCD (white colour), and/or (2) protection of EVOO within the α-CD inclusion complex against lipid peroxidation during the thermal treatment. Chroma index, which reflects colour saturation, increased after deep-fat frying pre-cooking compared with oil-free pre-cooked samples (Table 1). It is important to minimize Chroma increments during frying of breaded products since it reflects colour purity or saturation and thus could be a good indicator of consumer acceptance [34]. Attending to Hue, which reflects the colour tone, no significant differences were observed among both pre-cooking processes, while EVOO-αCD slightly reduced Hue.

Attending to the final cooking step, oil-free pre-cooked samples showed lower browning, higher yellow colour and reduced redness, with higher L^*, higher b^* and lower a^*, respectively, regardless of EVOO-αCD addition, compared with deep-fat frying pre-cooked samples (Table 1). Chroma of oil-free cooked samples also was higher compared with deep-fat frying cooking, regardless of EVOO-αCD addition. No significant Hue changes were observed for any of the cooking treatments and EVOO-αCD addition.

In conclusion, oil-free pre-cooking and cooking processes combined with EVOO-αCD within the breading of chicken nuggets led to samples with higher luminosity, higher yellowness, and reduced redness, which was correlated with better appearance scores of samples as observed in the sensory analyses section. Our findings are in accordance with literature about frying products since the golden yellow colour in a fried product indicates to consumers the use of: (1) fresh raw materials, (2) fresh frying oil, and (3) the use of a suitable frying technique. However, dark brown colors of fried products are associated by consumers with: (1) poor raw materials, (2) a highly reused frying oil, and (3) excessively long frying [1].

3.4. Sensory Analyses

The sensory analyses (appearance, aroma, texture, and flavor) of pre-cooked samples during refrigerated storage are shown in Figure 6. As observed, no sensory differences ($p > 0.05$) were found among the new technology (EVOO-αCD plus oil-free pre-cooking) and the conventional deep-fat frying, with even better flavor scores for the new technology.

Attending to appearance, no significant differences ($p > 0.05$) were perceived in the colour among treatments. However, colour differences between the new technology and the conventional were detected in the colour measurements shown in the previous Section 3.3. It may be explained since the consumer acceptance of a fried product based on its colour/appearance is highly influenced by the particular consumer preferences and their perception of the binomial color: quality [1].

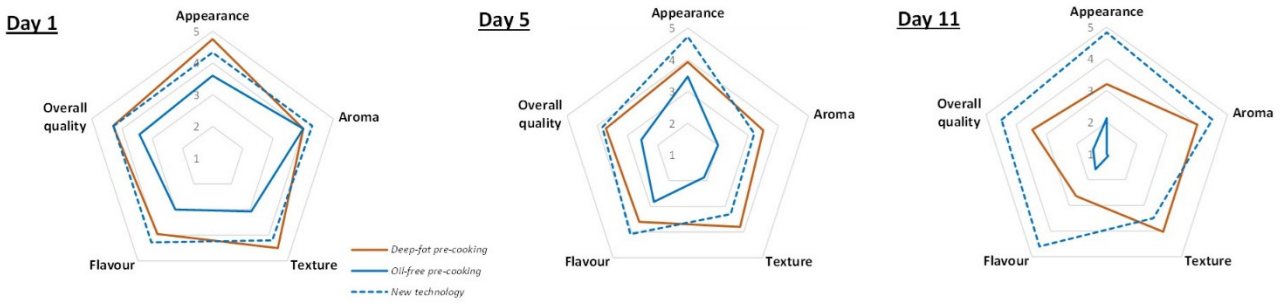


Figure 6. Sensory analyses of pre-cooked (different methods) chicken nuggets stored up to 11 days at 4 °C and cooked before sensory analysis.

Texture is another sensory parameter that highly influences the consumer acceptance of a fried product [35]. Particularly for texture, a crunchy crust and juicy core are expected by the consumer of these types of products [2]. No significant texture differences were found between the new technology and the conventional deep-fat frying, although control oil-free pre-cooking treatment (without EVOO-αCD) was evaluated with a lower texture score than those treatments. In that sense, the new technology has a similar crunchy crust and juicy core compared with conventional deep-fat frying.

Flavour was better scored for the new technology compared with the conventional deep-fat frying. In particular, panelists commented that the greasy and boggy perceptions of samples produced with the conventional deep-fat frying were not perceived for the new technology.

A previous study that proposed the elimination of the pre-cooking step of fried breaded squids by adding a cellulose derivative revealed that consumers poorly scored these samples due to a low fat perception, although the product was gaining acceptance after 7 days of sensory analyses [36]. Furthermore, vacuum-frying produced chicken nuggets showed normal organoleptic characteristics but oil uptake was not significantly reduced [33]. To the contrary, the chicken nuggets of our study produced with the new technology were better scored by the panelists since the undesirable sensory perceptions of a typical fried breaded product with conventional deep-fat frying were eliminated.

3.5. Acrylamide Content

The acrylamide contents of control samples before and after the final cooking step, for the different pre-cooking treatments, are shown in Figure 7. The acrylamide content of samples after the oil-free pre-cooking was very low, ca. 10 μg kg^{-1}. The addition of the EVOO-αCD within the breading did not affect the acrylamide contents of samples. Nevertheless, the deep-fat frying pre-cooking increased ($p < 0.05$) the acrylamide contents of samples by 56%. Furthermore, the final oil-free step did not increase ($p > 0.05$) the acrylamide contents of any of the treatments.

The hereby observed low acrylamide contents of nuggets are in accordance with previous studies [36]. In particular, Eerola et al. reported no detectable levels of acrylamide contents in chicken nuggets purchased from local retail shops and fast-food restaurants (cooking treatments not described) [36]. Acrylamide, which is formed during cooking processes at high temperatures (>120 °C), is considered a carcinogen (Group 2A) by the International Agency on Research in Cancer [37]. In addition, the toxicological studies of acrylamide also consider it as neurotoxic, genotoxic, carcinogenic, and affecting reproduction [38]. Hence, pre-cooking/cooking processes that minimize acrylamide formation are needed and are being proposed in literature as new cooking technologies with lower cooking temperatures, such as vacuum frying [39]. Furthermore, the controlled EVOO release from the inclusion complex would limit the available amount of lipids that could be oxidized and, consequently, of reactive species from Maillard process that can interact with asparagine and form acrylamide. Our study shows for the first time how the proposed clean technology based on oil-free pre-cooking+cooking reduced acrylamide formation

in chicken nuggets. Furthermore, the addition of the EVOO-αCD within the breading of nuggets did not affect ($p > 0.05$) the acrylamide formation of samples.

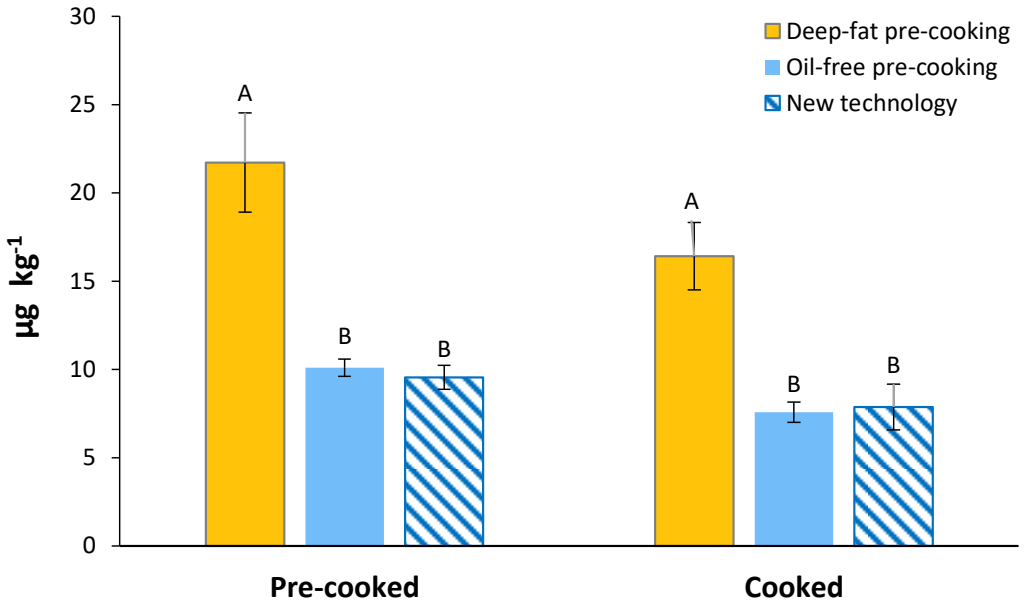


Figure 7. Acrylamide contents (ug kg^{-1}) of pre-cooked (different methods) chicken nuggets stored up to 11 days at 4 °C (n = 3 ± SD). Different letters denote significant differences ($p < 0.05$) among pre-cooked or cooked.

3.6. Microbial Analyses

Pre-cooked foods, such as pre-cooked chicken nuggets, are products sensitive to microbial growth, mainly because they are manipulated after their heat treatment to be marketed as chilled or frozen products [2,40]. Furthermore, the nature of the used ingredients is crucial to identify the microbial risk of the product to be processed. For that sense, the microbiological loads of the used ingredients were analyzed with the following counts: chicken cores (3 log CFU g^{-1}) > non-precooked nugget (3.1 log CFU g^{-1}) > EVOO-αCD inclusion complex (2.3 log CFU g^{-1}) > batter compound (< 1 log CFU g^{-1}) > αCD (< 1 log CFU g^{-1}) > corn breadcrumbs (< 1 log CFU g^{-1}). As expected, uncooked fresh meat was the ingredient with the highest microbial counts. The high microbial loads of meat are of high concern during the preparation of ready-to-cook products that are marketed as refrigerated or frozen products. In particular, poultry is prone to be colonized by several pathogens like *Salmonella* and *Campylobacter* spp., while others like *Arcobacter* and *Helicobacter* spp. and, occasionally, verotoxigenic *Escherichia coli*, have been also found in poultry [41].

An adequate pre-cooking/cooking of meat products is essential to ensure food safety. The pre-cooking methods hereby studied, either oil-free or deep-fat frying, was enough to reduce microbial loads close to the detection limit (1 log CFU g^{-1}), showing similar results to the samples cooked with the final cooking step. Nevertheless, it is essential to monitor the microbial loads of samples during the subsequent refrigerated storage of these ready-to-cook products. In general, microbial growth of pre-cooked samples stored under refrigeration was very low for all the monitored microbial groups showing only mesophiles had a remarkable growth (Figure 8). In particular, mesophilic growth for deep-fat frying pre-cooking was lower than oil-free pre-cooking (without EVOO-αCD) with 2.1 lower log units at day 11. Nevertheless, samples including EVOO-αCD showed lower microbial growth with 3.5 and 1.5 lower log units than deep-fat frying and oil-free pre-cooking (without EVOO-αCD), respectively, after 11 days at 4 °C. In any case, microbial loads of samples did not exceed the permissible level of microbial standards (6 log CFU g^{-1}) in cooked meat products as reported by Jay [42].

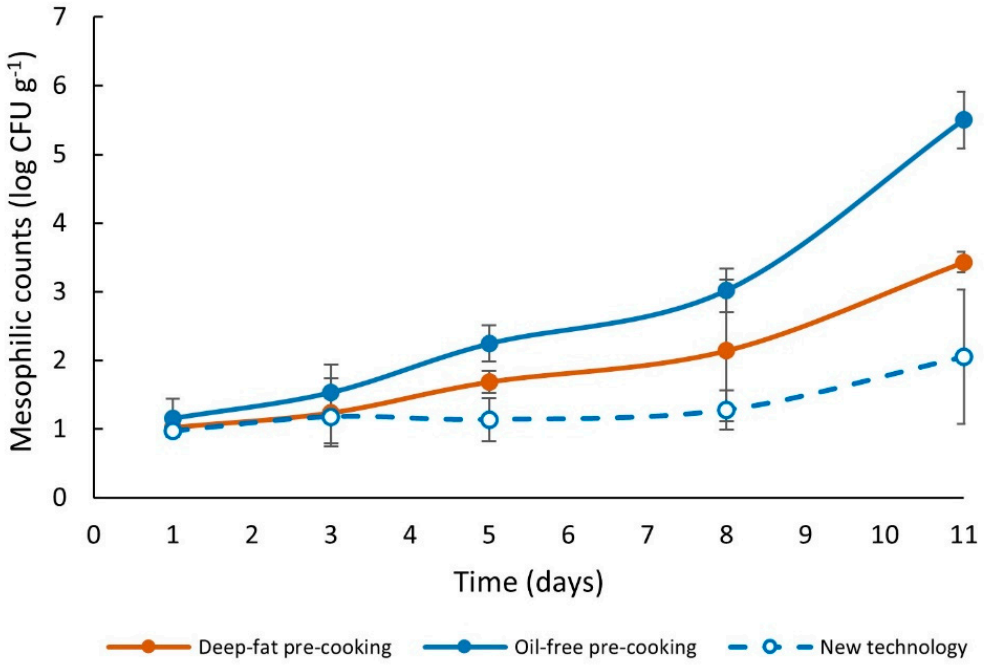


Figure 8. Mesophilic loads (log CFU g^{-1}) of pre-cooked (different methods) chicken nuggets stored up to 11 days at 4 °C (n = 3 ± SD).

A possible explanation for the lower microbial growth in samples processed with the new technology could be the high antimicrobial activity of EVOO, which once encapsulated within cyclodextrins is more water soluble leading to a higher microbial control. Such enhanced antimicrobial effects of compounds encapsulated in inclusion complexes with cyclodextrins have been attributed to greater water solubility, and their controlled release from these complexes during product storage [43]. Nevertheless, the enhanced antimicrobial effect of encapsulated EVOO to control microbial growth in breaded meat products has not been addressed yet. On the other side, the addition of other antimicrobial compounds within the formulation of chicken nuggets has been studied by some researchers. For example, Shahrezaee et al. [44] controlled mesophilic growth of chicken nuggets during refrigerated storage when samples were prepared including *Aloe vera* powder (2.5–3.5%) into the minced meat preparation. Other technologies, like modified atmosphere packaging (60% N_2, 40% CO_2, and O_2 < 1%) better controlled microbial growth in chicken nuggets with 1 log unit lower loads than samples under atmospheric composition after 21 days of storage at 4 °C [40].

4. Conclusions

The proposed new clean technology, which consists of encapsulated extra virgin olive oil+oil-free pre-cooking/cooking, for the production of chicken nuggets implies an oil reduction of 88%, compared to the conventional production procedure. This clean technology will also contribute to the product food safety since the microbial growth is better controlled (with a shelf-life longer than 11 days) during refrigeration storage of commercialized pre-cooked nuggets, while acrylamide formation is also highly reduced. Hence, chicken nuggets produced with this technology would lead to a potential consumer acceptance since sensory analyses do not show differences compared with the conventional production method. In future studies, such technology may be also validated for other breaded products such as croquettes and other meat preparations, with the potential benefit to provide the consumer with more healthy and safe products, together with less fat waste production by the related industries with this clean technology.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/cleantechnol4010005/s1: Table S1.

Author Contributions: Conceptualization, A.L.-G.; methodology, A.L.-G., M.R.-C. and G.B.M.-H.; validation, A.L.-G.; formal analysis, M.B.-Y. and G.B.M.-H.; investigation, all authors; resources, A.L.-G.; data curation, G.B.M.-H.; writing—original draft preparation, G.B.M.-H. and M.B.-Y.; writing—review and editing, G.B.M.-H. and A.L.-G.; visualization, G.B.M.-H.; supervision, A.L.-G.; project administration, A.L.-G.; funding acquisition, A.L.-G. All authors have read and agreed to the published version of the manuscript.

Funding: This research has received funding from the Centre for the Development of Industrial Technology (CDTI, Madrid, Spain) through the project "New Process Technologies for Healthy Breaded Products (Ref. IDI-20190627)".

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Acknowledgments: We give thanks to Elisa Orcajada Hermosilla for her technical assistance.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Rossell, J.B. *Frying: Improving Quality*; CRC Press: Boca Raton, FL, USA, 2001; ISBN 9781855735569.
2. Soto-Jover, S.; Boluda-Aguilar, M.; Esnoz-Nicuesa, A.; Iguaz-Gainza, A.; López-Gómez, A. Texture, oil adsorption and safety of the european style croquettes manufactured at industrial scale. *Food Eng. Rev.* **2015**, *8*, 181–200. [CrossRef]
3. Fiszman, S.M.; Salvador, A. Recent developments in coating batters. *Trends Food Sci. Technol.* **2003**, *14*, 399–407. [CrossRef]
4. European Commission. Commission Decision of 3 May 2000 replacing Decision 94/3/EC establishing a list of wastes pursuant to Article 1(a) of Council Directive 75/442/EEC on waste and Council Decision 94/904/EC establishing a list of hazardous waste pursuant to Article 1(4) of Council Directive 91/689/EEC on hazardous waste (notified under document number C(2000) 1147) (Text with EEA relevance) (2000/532/EC). *Off. J. Eur. Communities* **2000**, *6*, 3–24.
5. Mannu, A.; Garroni, S.; Porras, J.I.; Mele, A. Available technologies and materials for waste cooking oil recycling. *Processes* **2020**, *8*, 366. [CrossRef]
6. Fiszman, S.M.; Salvador, A.; Sanz, T.; Lluch, M.A.; Castellano, J.V.; Camps, J.L.; Gamero, M. Proceso para la Preparación de un Alimento Rebozado y Congelado. Patent Application No. 200,201,276, 2002.
7. López-Gómez, A.; Soto-Jover, S.; Boluda-Aguilar, M. Composition and Method of Making Breaded Foods with Low Oil Absorption during Frying. Spanish Patent 2 440 092 B1, 14 August 2014.
8. do Amaral, P.H.R.; Andrade, P.L.; de Conto, L.C. Microencapsulation and its uses in food science and technology: A Review. In *Microencapsulation—Processes, Technologies and Industrial Applications*; Salaün, F., Ed.; IntechOpen: London, UK, 2019; ISBN 978-1-83881-870-8.
9. Velasco, J.; Dobarganes, C.; Márquez-Ruiz, G. Variables affecting lipid oxidation in dried microencapsulated oils. *Grasas y Aceites* **2003**, *54*, 304–314. [CrossRef]
10. Sobel, R.; Versic, R.; Gaonkar, A.G. Introduction to microencapsulation and controlled delivery in foods. In *Microencapsulation in the Food Industry*; Academic Press: Cambridge, MA, USA, 2014; pp. 3–12. [CrossRef]
11. Pereira, A.G.; Carpena, M.; Oliveira, P.G.; Mejuto, J.C.; Prieto, M.A.; Gandara, J.S. Main applications of cyclodextrins in the food industry as the compounds of choice to form host–guest complexes. *Int. J. Mol. Sci.* **2021**, *22*, 1339. [CrossRef]
12. Ozdemir, N.; Pola, C.C.; Teixeira, B.N.; Hill, L.E.; Bayrak, A.; Gomes, C.L. Preparation of black pepper oleoresin inclusion complexes based on beta-cyclodextrin for antioxidant and antimicrobial delivery applications using kneading and freeze drying methods: A comparative study. *LWT* **2018**, *91*, 439–445. [CrossRef]
13. Szejtli, J. Utilization of cyclodextrins in industrial products and processes. *J. Mater. Chem.* **1997**, *7*, 575–587. [CrossRef]
14. Matencio, A.; Navarro-Orcajada, S.; García-Carmona, F.; López-Nicolás, J.M. Applications of cyclodextrins in food science. A review. *Trends Food Sci. Technol.* **2020**, *104*, 132–143. [CrossRef]
15. Mortensen, A.; Aguilar, F.; Crebelli, R.; Di Domenico, A.; Dusemund, B.; Frutos, M.J.; Galtier, P.; Gott, D.; Gundert-Remy, U.; Leblanc, J.; et al. Re-evaluation of β-cyclodextrin (E 459) as a food additive. *EFSA J.* **2016**, *14*, e04628. [CrossRef]
16. Furune, T.; Terao, K. Complexation of ingredients in foods by alpha-cyclodextrin to improve their functions. In *Functionality of Cyclodextrins in Encapsulation for Food Applications*; Ho, T.M., Yoshii, H., Terao, K., Bhandari, B.R., Eds.; Springer: Cham, Switzerland, 2021; pp. 277–297. [CrossRef]

17. EFSA. Scientific Opinion on the substantiation of health claims related to alpha cyclodextrin and reduction of post prandial glycaemic responses (ID 2926, further assessment) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. *EFSA J.* **2012**, *10*, 2713. [CrossRef]
18. Trotta, F.; Zanetti, M.; Camino, G. Thermal degradation of cyclodextrins. *Polym. Degrad. Stab.* **2000**, *69*, 373–379. [CrossRef]
19. Hădărugă, N.G.; Bandur, G.N.; David, I.; Hădărugă, D.I. A review on thermal analyses of cyclodextrins and cyclodextrin complexes. *Environ. Chem. Lett.* **2019**, *17*, 349–373. [CrossRef]
20. Hadaruga, D.I.; Hadaruga, N.G.; Hermenean, A.; Rivis, A.; Paslaru, V.; Codina, G. Bionanomaterials: Thermal stability of the oleic acid/α- and β-cyclodextrin complexes. *Rev. Chim.* **2008**, *59*, 994–998. [CrossRef]
21. Mourtzinos, I.; Salta, F.; Yannakopoulou, K.; Chiou, A.; Karathanos, V.T. Encapsulation of olive leaf extract in β-cyclodextrin. *J. Agric. Food Chem.* **2007**, *55*, 8088–8094. [CrossRef]
22. Chikamoto, K.; Terao, K. Alpha-cyclodextrin functions as a dietary fiber. In *Functionality of Cyclodextrins in Encapsulation for Food Applications*; Ho, T.M., Yoshii, H., Terao, K., Bhandari, B.R., Eds.; Springer: Cham, Switzerland, 2021; pp. 255–276.
23. Manolikar, M.; Sawant, M. Study of solubility of isoproturon by its complexation with β-cyclodextrin. *Chemosphere* **2003**, *51*, 811–816. [CrossRef]
24. Pathare, P.B.; Opara, U.L.; Al-Said, F.A.-J. Colour measurement and analysis in fresh and processed foods: A review. *Food Bioprocess. Technol.* **2013**, *6*, 36–60. [CrossRef]
25. ASTM. *Physical Requirement Guidelines for Sensory Evaluation Laboratories*; Eggert, J., Zook, K., Eds.; ASTM International: Philadelphia, PA, USA, 1986; ISBN 0803109245.
26. Jiménez-Martín, E.; Pérez-Palacios, T.; Carrascal, J.R.; Rojas, T.A. Enrichment of chicken nuggets with microencapsulated omega-3 fish oil: Effect of frozen storage time on oxidative stability and sensory quality. *Food Bioprocess. Technol.* **2016**, *9*, 285–297. [CrossRef]
27. International Organization for Standardization. *Sensory Análisis—General Guidance for the Design of Test Rooms*; International Organization for Standardization: Geneva, Switzerland, 2007.
28. Ho, B.T.; Joyce, D.C.; Bhandari, B.R. Encapsulation of ethylene gas into α-cyclodextrin and characterisation of the inclusion complexes. *Food Chem.* **2011**, *127*, 572–580. [CrossRef]
29. Brannan, R.G.; Mah, E.; Schott, M.; Yuan, S.; Casher, K.L.; Myers, A.; Herrick, C. Influence of ingredients that reduce oil absorption during immersion frying of battered and breaded foods. *Eur. J. Lipid Sci. Technol.* **2014**, *116*, 240–254. [CrossRef]
30. Gerrish, T.; Higgins, C.; Kresl, K. Method of Making Battered and Breaded Food Compositions Using Calcium Pectins. U.S. Patent US5601861A, 11 February 1997.
31. Purlis, E. Browning development in bakery products—A review. *J. Food Eng.* **2010**, *99*, 239–249. [CrossRef]
32. Mariscal, M.; Bouchon, P. Comparison between atmospheric and vacuum frying of apple slices. *Food Chem.* **2008**, *107*, 1561–1569. [CrossRef]
33. Teruel, M.R.; García-Segovia, P.; Martínez-Monzó, J.; Linares, M.B.; Garrido, M.D. Use of vacuum-frying in chicken nugget processing. *Innov. Food Sci. Emerg. Technol.* **2014**, *26*, 482–489. [CrossRef]
34. Ngadi, M.; Li, Y.; Oluka, S. Quality changes in chicken nuggets fried in oils with different degrees of hydrogenatation. *LWT Food Sci. Technol.* **2007**, *40*, 1784–1791. [CrossRef]
35. Mallikarjunan, P.K.; Ngadi, M.O.; Chinnan, M.S. *Breaded Fried Foods*; CRC Press: Boca Raton, FL, USA, 2010; p. 179.
36. Salvador, A.; Hough, G.; Fiszman, S.M. Acceptability of batter-coated squid rings prepared without industrial pre-frying. *Eur. Food Res. Technol.* **2005**, *221*, 36–40. [CrossRef]
37. Carere, A. Genotoxicity and carcinogenicity of acrylamide: A critical review. *Ann. Ist Super Sanita* **2006**, *42*, 144–155.
38. Matoso, V.; Bargi-Souza, P.; Ivanski, F.; Romano, M.A.; Romano, R.M. Acrylamide: A review about its toxic effects in the light of Developmental Origin of Health and Disease (DOHaD) concept. *Food Chem.* **2019**, *283*, 422–430. [CrossRef]
39. Mariotti-Celis, M.S.; Cortés, P.; Dueik, V.; Bouchon, P.; Pedreschi, F. Application of vacuum frying as a furan and acrylamide mitigation technology in potato chips. *Food Bioprocess. Technol.* **2017**, *10*, 2092–2099. [CrossRef]
40. Yavas, E.; Bilgin, B. Effect of calcium lactate, sodium diacetate and sodium chloride mixture on the microbiological, chemical and sensory properties of chicken nuggets stored in refrigeration and under modified atmospheres. *Int. J. Poult. Sci.* **2010**, *9*, 66–71. [CrossRef]
41. Corry, J.E.L.; Atabay, H.I. Poultry as a source of Campylobacter and related organisms. *Symp. Ser. Soc. Appl. Microbiol.* **2001**, *90*, 96S–114S. [CrossRef] [PubMed]
42. Jay, J.M. *Modern Food Microbiology*; Chapman & Hall: London, UK, 1996; ISBN 0412076918.
43. Zhu, Y.; Li, C.; Cui, H.; Lin, L. Encapsulation strategies to enhance the antibacterial properties of essential oils in food system. *Food Control* **2021**, *123*, 107856. [CrossRef]
44. Shahrezaee, M.; Soleimanian-Zad, S.; Soltanizadeh, N.; Akbari-Alavijeh, S. Use of *Aloe vera* gel powder to enhance the shelf life of chicken nugget during refrigeration storage. *LWT* **2018**, *95*, 380–386. [CrossRef]