

New Food Processing Technologies and Food Safety

Guest Editors: Latiful Bari, Alexandru Grumezescu, Dike O. Ukuku, Gargi Dey, and Tatsuro Miyaji





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and Food Safety**

Guest Editors: Kazuo Toda, Jorge L. Zeredo, Sae Uchida,
and Vitaly Napadow



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Editorial

New Food Processing Technologies and Food Safety

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The microflora of foods is very significant to food producers, processors, and consumers and the food manufacturers including distributors are responding to consumers' demand for food products that are safe, fresher, and convenient for use. In some cases foods may be improperly processed and/or contaminated with spoilage bacteria or human bacterial pathogens. Knowledge of the levels of bacteria in food systems before and after processing, as well as the impact of storage time and temperature on microbial populations of minimally processed foods, should provide guidance to the food industry, regulatory agencies, and consumers in implementing HACCP plans and good manufacturing practices (GMP). Consumers' demand for fresh, healthy nutritious foods has triggered food industries to look for alternative technologies that can produce higher quality food, ensure safety and reasonable cost for the consumer, and at the same time improve food safety by reducing or eliminating foodborne bacterial pathogens. Similarly, the alternative novel processing technologies might be used as tools to tailor foods with added or enhanced functional and nutritional values, to lower carbon footprint and substantially reduce water volumes used in heat-transfer processes. Some of these new processing concepts include advanced thermal and nonthermal technology that uses mechanical, electrical, and electromagnetic energy and hurdle (combined-applications) approaches. The different fundamental principles and the performance capabilities of some novel technologies and processes differ from traditional processing in terms of the

types of food categories, microbial efficacy and destruction models, and desired and undesired effects on food quality and their economic and environmental impact were published in this issue. This special issue addresses 7 such new food processing technologies, including high pressure processing (HPP), electrical impedance spectroscopy (EIS), ultrasound, low water activity food, vacuum frying, innovative transduction process to supply safe fresh snack, and false labeling prevention technique.

High hydrostatic pressure processing (HPP) has been successfully applied to heat-sensitive drinks and solid foods such as jams and jellies, fruit juices, ham, cooked ready-to-eat meat products, and seafood products such as oysters. In this special issue, H. Ogihara et al. showed the effectiveness of 400 and 500 MPa of high hydrostatic pressure processing on raw beef liver which effectively kills pathogens and thus is safe for consumption, but this treatment also changes the texture of the raw beef liver. On the other hand, low HPP treated liver tissue became firmer, which changed to pale color that was considered unsuitable for raw consumption. In another study, O. A. Ijabadeniyi and Y. Pillay investigated the microbial safety of low water activity food and reported that certain low water activity foods may present a public health risk, despite fungal contamination. The spore-forming bacterium can be osmotolerant at both reduced and elevated temperatures. Thus they suggested that combination of other nonthermal treatments could be useful in controlling the safety and quality of low water activity foods.

Electrical impedance spectroscopy (EIS) is an effective analytical technique to assess quality and safety of food and has been shown in wide application in food industries. In this special issue, X. Zhao et al. developed online EIS detection methods to replace traditional methods which save time, cost, and manpower and ensure quality grading of meat and fish. In another study, A. B. Oyediji et al. described the impact of frying on texture and color of cassava root slices chips and suggested that vacuum frying at 118°C and 8 min could best preserve the quality attributes of cassava root slices chips. This simple but new technical piece of information could improve the product quality and consumers acceptance. In addition, L. M. Carrillo-Lopez et al. in their review article reported that the applications of ultrasound in food produce acoustic cavitations, which modifies the physical, chemical, and functional properties of food. The combination of ultrasound and sanitizing agent can improve the microbial reduction in foods and, thereby, retain their quality. However, they concluded that more research is still needed before applying this technology to a wider range of industrial sectors.

In another study, P.-Y. Chen described a dynamic mathematical model through which food adulteration or false labeling could be effectively prevented. He also discussed how the supply-demand factors in the food market are influenced by the administrative means that the sanitary inspectors have used to prevent false labeling of food. Furthermore, P.-Y. Chen constructed an innovative transduction process over the existing methods to assist snack food dealers or microfood enterprises in developing their food channels to supply safe fresh snack foods. This study also found that food safety assurance and providing sufficient nutrition information were the most essential topics that can influence consumers' decision-making regarding purchase of fresh snack foods.

Therefore, all the papers published in this special issue represent exciting, innovative, and applicable technologies in food safety and quality of various food, as well as emerging future research topics, in this multidisciplinary field. We hope that this special issue would attract major attention of the peers.

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Review Article

Electrical Impedance Spectroscopy for Quality Assessment of Meat and Fish: A Review on Basic Principles, Measurement Methods, and Recent Advances

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Electrical impedance spectroscopy (EIS), as an effective analytical technique for electrochemical system, has shown a wide application for food quality and safety assessment recently. Individual differences of livestock cause high variation in quality of raw meat and fish and their commercialized products. Therefore, in order to obtain the definite quality information and ensure the quality of each product, a fast and on-line detection technology is demanded to be developed to monitor product processing. EIS has advantages of being fast, nondestructive, inexpensive, and easily implemented and shows potential to develop on-line detecting instrument to replace traditional methods to realize time, cost, skilled persons saving and further quality grading. This review outlines the fundamental theories and two common measurement methods of EIS applied to biological tissue, summarizes its application specifically for quality assessment of meat and fish, and discusses challenges and future trends of EIS technology applied for meat and fish quality assessment.

1. Introduction

Electrical impedance spectroscopy (EIS) is a method to analyze electrical properties of materials and systems by inducing alternating electrical signals at different frequencies into them and measuring the responding signals [1]. A function of impedance according to frequencies is established and further correlated with physical parameters or properties of materials and systems, for the aim of analysis and evaluation. EIS was originally applied in research on electrochemical system, and, from 1920s, it began to be used for biological systems. Until now EIS has had an extensive application in biological research. According to the biological objects, application of EIS can be divided into three aspects, that is, electrical impedance tomography in medical imaging [2–4], quality and safety assessment in food industry, and

phytophysiology in agronomy [5–7]. Research objects and targets of EIS applied in food are abundant and extensive, including for fruits, such as study on dry matter content of durian [8] and ripening of banana [9], for vegetables, such as changes in potato and spinach tissues during or after heating [10, 11] and moisture content of carrot slices during drying [12], for meat, such as quality evaluation of pork meat during storage [13] and investigation of beef meat behavior during ageing [14], for chicken, such as discrimination of fresh and frozen-thawed chicken breast muscles [15], for fish, such as salt and moisture content determination of salted rainbow trout [16] and freshness estimation of carp [17], for dairy products, such as real-time detection of bovine milk adulteration [18], and moreover for determination of the additives content in natural juices [19], fermentation process of bread dough [20], and quality assessment of cooking oil [21].

TABLE 1: Comparison of four new technologies.

Technologies	Fast	Nondestructive	Easily implemented	Inexpensive
Near infrared spectroscopy	√	√	√	×
Hyperspectral image	√	√	√	×
Electronic nose technology	√	√	×	×
EIS	√	√	√	√

Nowadays customers pay more attentions on quality attributes of meat products, such as appearance, flavor, and nutrients. However due to individual differences of livestock, there is a broad-ranging variability in quality of the raw meat, which also causes high variation in the commercialized end products. Customers and manufacturers expect that products can be further graded according to quality, in order to gain more benefit. Thus there is a strong demand for manufacturers to assess the quality of every product on-line to monitor processing, in order to obtain reliable and definite quality information for customers and further processing manufacturers [22]. It also can help to determine the best destination for meat carcasses and to reduce economic losses for industries and customers [23]. There are similar requirements for fish industry as well.

Traditional methods for quality assessments of both meat and fish are precise but destructive, time-consuming, complicated for experiments, and requiring skilled operators. Hence quality information for every individual product cannot be easily collected on-line and nondestructively. In addition to EIS, other new alternative technologies for on-line quality detection also have been investigated, such as near infrared spectroscopy [24–26], hyperspectral image [27–29], and electronic nose technology [30–32]. The comparison of the four technologies was showed in Table 1. All of the technologies are fast, nondestructive, and suitable for development of on-line detecting instrument. However, near infrared spectroscopy and hyperspectral image demand expensive equipment, and electronic nose technology requires specific environmental conditions for measurement [33]. Compared with other new technologies, EIS shows outstanding advantages of being inexpensive and low requirement to the operation [14, 17].

The review mainly introduced principles of EIS applied to biological tissue and summarized its application for quality assessment of meat and fish. Specifically, the article consisted of four sections: (i) the fundamental theories and principles, (ii) two common measurement methods and corresponding probes with diverse configurations, (iii) the latest development in application for quality assessments of meat and fish, and (iv) challenges and future trends of using EIS for quality assessment of meat and fish.

2. Three Important Fundamental Theories

EIS began to be tested on biological system in the 1920s [34, 35]. Some classic theories about principles of EIS applied to biological system were proposed in later study. They were Fricke model, an equivalent circuit diagram of biological tissue [36–38], Schwan's dispersion theory, which proposed

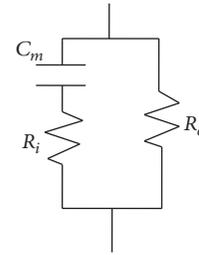


FIGURE 1: Fricke model.

three main dispersions occurring in biological tissue [14, 39], and Cole-Cole equation, an empirical formula describing the dispersions [40].

2.1. Fricke Equivalent Circuit Model. Fricke model [36–38], shown in Figure 1, is an elementary and excellent method to simulate biological system at microscopic level by electronic components [17, 41, 42]. It considers biological tissue as a homogeneous suspension of cells in an ionized liquid medium and simulates biological tissue components, such as membranes, intracellular (ICF) and extracellular fluids (ECF), with passive electrical elements, such as resistor and capacitor, connected in series and in parallel.

The model consists of three elements (R_e , R_i , C_m), so it is also called three-element circuit model. The three elements R_e , R_i , and C_m represent resistance of ECF, resistance of ICF, and capacitance of membrane, respectively. Na^+ and Cl^- ions exist in ECF. In ICF, major cation is K^+ , while major anions are phosphate and proteins. Therefore, ICF and ECF can be regarded as electrolytes. Cell membrane performs similar to capacitance. At low frequencies, the current cannot pass through the cells membrane because of its high resistance, while, at higher frequencies, the current passes through ECF, membrane, and ICF. Parameters of the three electrical elements depend on ions concentration and mobility during metabolism of cells, which reflect on physicochemical properties of biological tissue [41]. The model has been widely used in cells, microorganisms' suspensions in a liquid medium, and homogeneous media [41, 42].

2.2. Schwan's Dispersion Theory. A phenomenon that electrical parameters have dramatical changes in a certain frequency range is called frequency scattering, referred to as dispersion, which is due to corresponding relaxation phenomena [43]. Schwan [39] proposed that there are three main dispersions (α , β , γ) in biological system occurring at three frequency

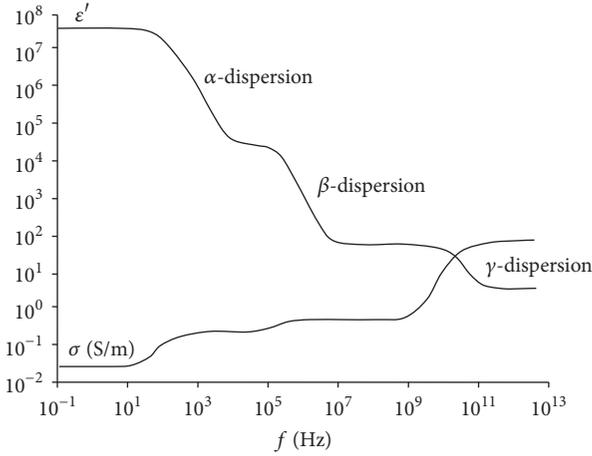


FIGURE 2: Three dispersions in biological system [σ (S/m) represents electric conductivity and ϵ' represents dielectric constant] [23].

bands (Figure 2). The α -dispersion, from few Hz to few KHz, is caused by the polarization phenomena in the electrical double layer of the tissue and is a relaxation of “nonpermanent” dipoles, which is formed during free counterions laterally moving along the insulating cell membrane or large molecules [14]. The α -dispersion has been extensively studied in biomedical applications on monitoring tissue or organ vitality for transplantation [41].

The β -dispersion, at radio frequencies ranging from few KHz to MHz, is mainly due to the Maxwell-Wagner effect, which is related to interface polarization occurring in systems where the electric current passes at the interface between two different materials. The β -dispersion is associated with the dielectric properties of the cell membranes, as well as the interactions between membranes and the extracellular or intracellular electrolytes [14]. The β -dispersion is directly associated with the cell membranes behavior and could serve in study of meat ageing based on membrane integrity, as oxidation of the phospholipid membrane layers and lysis occurring during ageing make the membrane porous, which causes insulating properties of membrane to decrease [14, 44].

The γ -dispersion, at microwave frequencies above 100 MHz, is mainly a result of the permanent dipole relaxation of small molecules, mainly water molecules in biological tissues [14, 23].

In general, the α - and β -dispersions are more relevant to the cells states compared with γ -dispersion and commonly used in impedance measurements for study on biological tissues.

2.3. The Cole-Cole Theory. The Cole-Cole equation (shown in (1)) derived based on a considerable amount of experimental data can be used to fit the dispersion processes [40, 45]. In (1), Z^* is complex impedance. R_0 and R_∞ are the impedances at the “static” and “infinite frequency.” $\omega = 2\pi f$. τ is a characteristic constant which may be called the relaxation time. α is a dimensionless exponent parameter, which is a constant reflecting distribution in dispersion and is used for

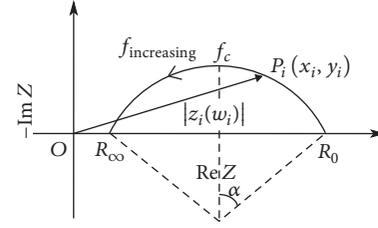


FIGURE 3: The Cole-Cole plot [45].

correcting the nonstrict capacitive behavior of membranes in biological tissues caused by dielectric losses [14, 41]:

$$Z^* = R_\infty + \frac{(R_0 - R_\infty)}{1 + (i\omega\tau)^\alpha} = z_{Re} + iz_{Im}. \quad (1)$$

As shown in Figure 3, locus of (1) in complex plane is an arc with its center below the horizontal axis, which is consistent with the measured bioimpedance data plotted in the complex impedance plane. This is called Cole-Cole plot [40].

Moreover, there is another similar equation described by Foster and Schwan [46] and shown as (2) [47], where $f_c = 1/2\pi\tau$ (shown in Figure 3) is the characteristic frequency, at which the imaginary part of impedance is the largest in absolute value among other frequencies [47].

$$Z^* = R_\infty + \frac{(R_0 - R_\infty)}{1 + (i(f/f_c))^\alpha} = z_{Re} + iz_{Im}. \quad (2)$$

Furthermore, the Cole-Cole equation can be related to Fricke model by following equations shown in (3) [41, 45]. Electric parameters in the fitting equations reflect physical and chemical properties of biological tissues. Prediction models for quality parameters are established with the electric parameter data as input, which is the principle of EIS applied to quality assessment of food.

$$\tau = (R_i + R_e) C_m,$$

$$R_0 = R_e, \quad (3)$$

$$R_\infty = \frac{R_e R_i}{R_e + R_i}.$$

2.4. Progress of Equivalent Circuit Model. EIS technology is able to deduce elementary reactions steps and extract the kinetic parameters characterizing the reaction steps of a total electrochemical reaction system, by using equivalent circuits or functions derived from the known mechanism [48]. However, for biological tissues, although Fricke model and Cole-Cole equation have made some contributions to theoretical analysis, there are still a lot of complicated unknown reactions and transformations in biological system needed to be discovered and explored. Therefore, equivalent circuit model plays an important role in EIS analysis, and more effective ones are still expected to be developed for application in food quality detection.

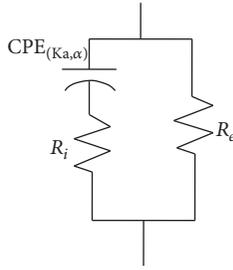


FIGURE 4: Modified Fricke model.

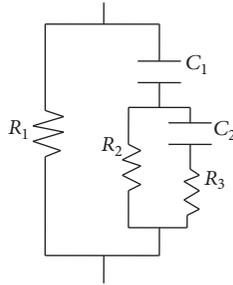


FIGURE 5: Equivalent circuit model proposed by Zhang et al. [51].

In (1), when α is 1, the equation mathematically describes the Fricke model. However, it was observed that the Fricke model was not accurate enough to fit the experimental results [14, 40], as cell membrane performs different with capacitance. According to Cole-Cole equation, a constant phase element (CPE), which is used to describe cell membrane with modified capacitive performance, is defined as $Z_{\text{CPE}} = 1/K_a(j \cdot \omega)^\alpha$. Guermazi et al. [14] used a modified Fricke model (shown in Figure 4) to investigate the ageing state of beef meat during 14 days. Results indicated that the modified Fricke model led to a good fitting performance with the expected behavior of muscle during ageing.

Fricke model and modified Fricke model are the most classic equivalent circuit models for biological tissue and are still used in recent research on animal or plant tissue, such as estimating freshness of carp [17], preliminary analysis to predict breast cancer [49], detecting phosphorus nutrition level for *Solanum lycopersicum* [7], and changes of potato tissues during drying [50].

Additionally, there is also an equivalent circuit model used to simulate plant tissue. As shown in Figure 5, the model was developed by Zhang et al. (1990) [51] during study on parenchymatous plant tissues. It takes into account cell wall, vacuole, and tonoplast. In Figure 5, the components R_1 , R_2 , and R_3 represent the cell wall resistance, cytoplasm resistance, and vacuole resistance. C_1 and C_2 represent plasma membrane capacitance and tonoplast capacitance, respectively.

Although the model contains more detailed cell structure, the fitting performance of the model to the measured impedance data was worse than that of the modified Fricke model according to research of Wu et al. [52] on eggplant pulp. Harker and Maindonald [53] also used the model to

study nectarine during ripening. The results showed that the model was not able to predict all of the changes during ripening, but it helped to improve the understanding of nectarine ripening.

Recently, a new electrical model of a biological cell with nucleus was developed and simulated [54, 55]. The simulation results of the model were compared well with published data, which indicated the promising utilization to serve as an aid to further understand behavior of cells over frequency range.

3. Bipolar and Tetrapolar Measurement Methods

3.1. Bipolar Measurement Method. The mathematical principle of electrical impedance measurement is Ohm's law, $Z = U/I$. Thus the most fundamental measuring method is using two electrodes to induce the current (I) and to measure the voltage (V). However, in this bipolar measurement method, because of the existence of electrode polarization, the measured impedance consists of two parts, that is, impedance of measured object and parasitic capacitive impedance on the interface of the electrode-sample ohmic contacts [22, 56]. The parasitic impedance decreases with frequency increasing; thus it can be ignored when frequency is high in bipolar measurement system [17]. Material and configuration of electrodes have an impact on electric field loaded on the tested biological tissue and are important factors in EIS measurements [57, 58].

Five common configurations of two-electrode probes used in research of meat are introduced as follows, and the corresponding schematic diagrams are shown in Figure 6. Sun et al. [17] estimated freshness of carp based on EIS morphological characteristic with a pair of platinum (Pt) electrodes (Figure 6(a)). Masot et al. [57] used a coaxial needle electrode (Figure 6(b)) in measurement to predict salt content in cured ham or pork loin. The electrode was made of a hollow needle and an isolated wire inside the needle, which were both made of stainless steel. A dielectric material, an epoxy resin, was between them. The needle is used for electromyography in medical applications. Damez et al. [59] used a cylindrical probe (Figure 6(c)) to measure electrical anisotropy of meat to assess ageing state. The probe was made of 20 stainless steel needles electrodes equally arranged on a periphery with 8 cm in diameter. Each pair of electrodes that are diametrically opposite was a bipolar system. The ten electrodes pairs were able to measure impedances at radial directions. Ćurić et al. [16] tested the applicability of needle-type multielectrode array (Figure 6(d)) in evaluating salt and moisture content of structurally heterogeneous fish samples. The needle-type multielectrode array arranged in bipolar configuration was comprised of two rows of 6 parallel, electrically connected, and gold-plated needles and was considered able for more accurate measurements for electrical properties. Guermazi et al. [14] measured the impedance of beef meat during ageing period with circular penetrating multielectrodes (Figure 6(e)). The circular penetrating probe consisted of one central electrode and eight surrounding electrodes. All electrodes were made of gold-plated steel. The

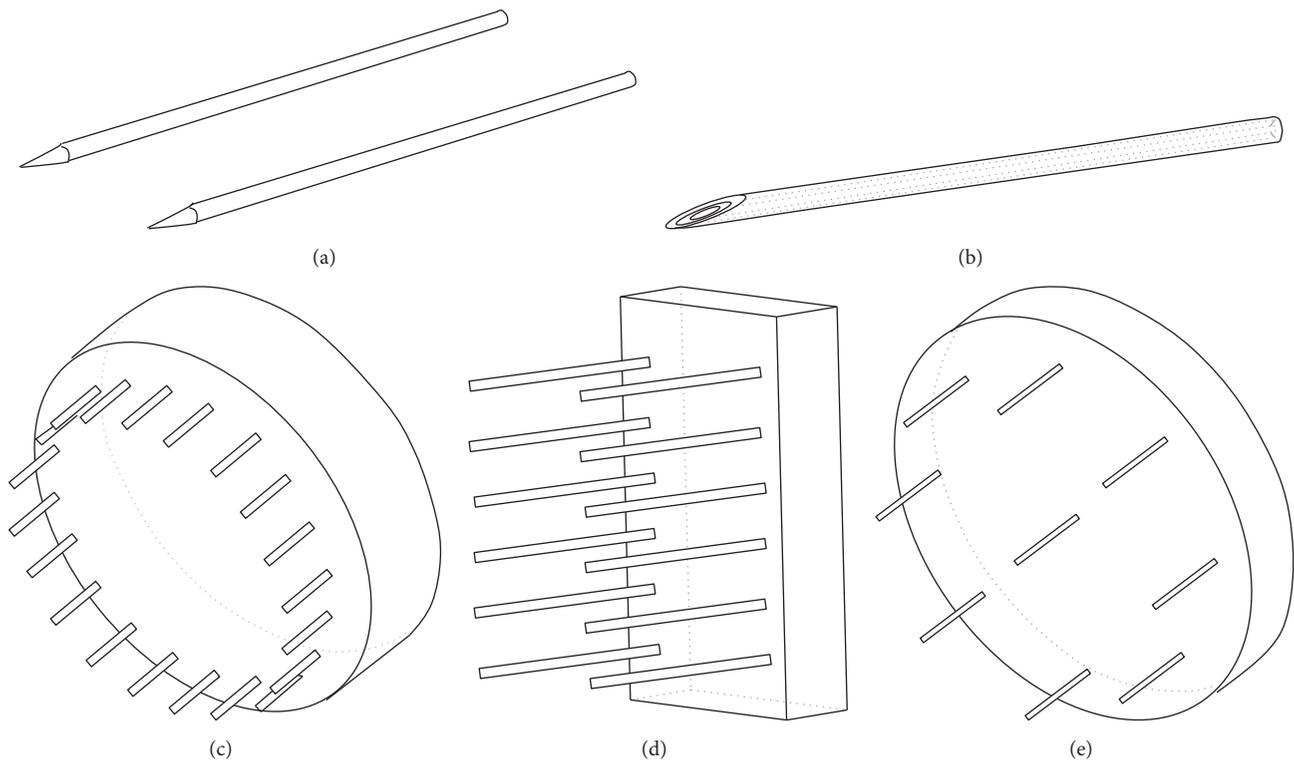


FIGURE 6: Schematic diagrams of five configurations of bipolar probe.

excitation signal was delivered from the inner electrode to the meat and the surrounding electrodes were grounded. This provided information about the distribution of the electric field in meat independent of anisotropy effects [14]. It was found that measured data had higher reliability and the instrument was more stable, when using two rows of pairs of needles electrodes than using circular probe with needles electrodes equally arranged on a periphery [60].

Electrode distance variation technique can be used for electrode polarization correction in bipolar measurement system. Some researches thought that capacitive gap contact between electrode and sample reflected the feature of the samples [22, 56]. Based on electrode distance variation technique, Damez et al. [61] investigated contact impedance as a parameter of interest for assessment of meat ageing. The probe used in measurement composed of eight stainless needles aligned and spaced 15 mm apart ($\phi = 0.6$ mm; $L = 5$ mm). Any pair of electrodes was a bipolar system, and 28 data points were obtained from these pairs of electrodes. Some data were used to plot a figure of average impedance against distance between electrodes at several frequencies. It showed linear relation between impedance and distance, and the slope of line corresponded to impedance per unit length of the sample, while the y -intercept represented contact impedance. Contact impedance was found to be related to meat fibers strength and reflected conductive properties of the extracellular spaces [61]. Limitation of the technique is that the polarization impedance becomes large in comparison with the sample impedances at low frequencies. Therefore,

the spacing between electrodes should be as large as possible in order to increase the weight of the sample impedance [56].

3.2. Tetrapolar Measurement Method. To eliminate the electrode polarization for more accurate measurement of impedance, a tetrapolar measurement method was proposed. The parasitic voltage is caused when a current flows through the interface of electrode sample. In tetrapolar system, current inducing and voltage measurement are via two separate electrodes pairs [22]; thus current is not introduced in the voltage measurement circuit and the parasitic impedance is eliminated. However, in practical terms, electrodes with uniform specific polarization impedance (per unit surface area) over the entire electrode surface are difficult to obtain, which causes the electrode on a potential level different from the one it ought to register [56].

The simplest configuration of four-electrode probe is four metal electrodes arrayed in line. Yang et al. [45] used four stainless steel electrodes (Figure 7(a)) to measure impedance of porcine meat for moisture content prediction. Furthermore, Altmann and Pliquett [62] carried on an impedance measurement using Purdue Tetrapolar probe (PTP) (Figure 7(b)) on the *M. longissimus dorsi* in pork and beef. The configuration of PTP was a steel shaft with four ring electrodes mounted in a line along it. The electrodes were insulated from each other, and the outer pair of electrodes was used for inducing a current into system while the inner pair of electrodes was for voltage measurement. Similar to the cylindrical bipolar probe mentioned above, Damez et al. [59]

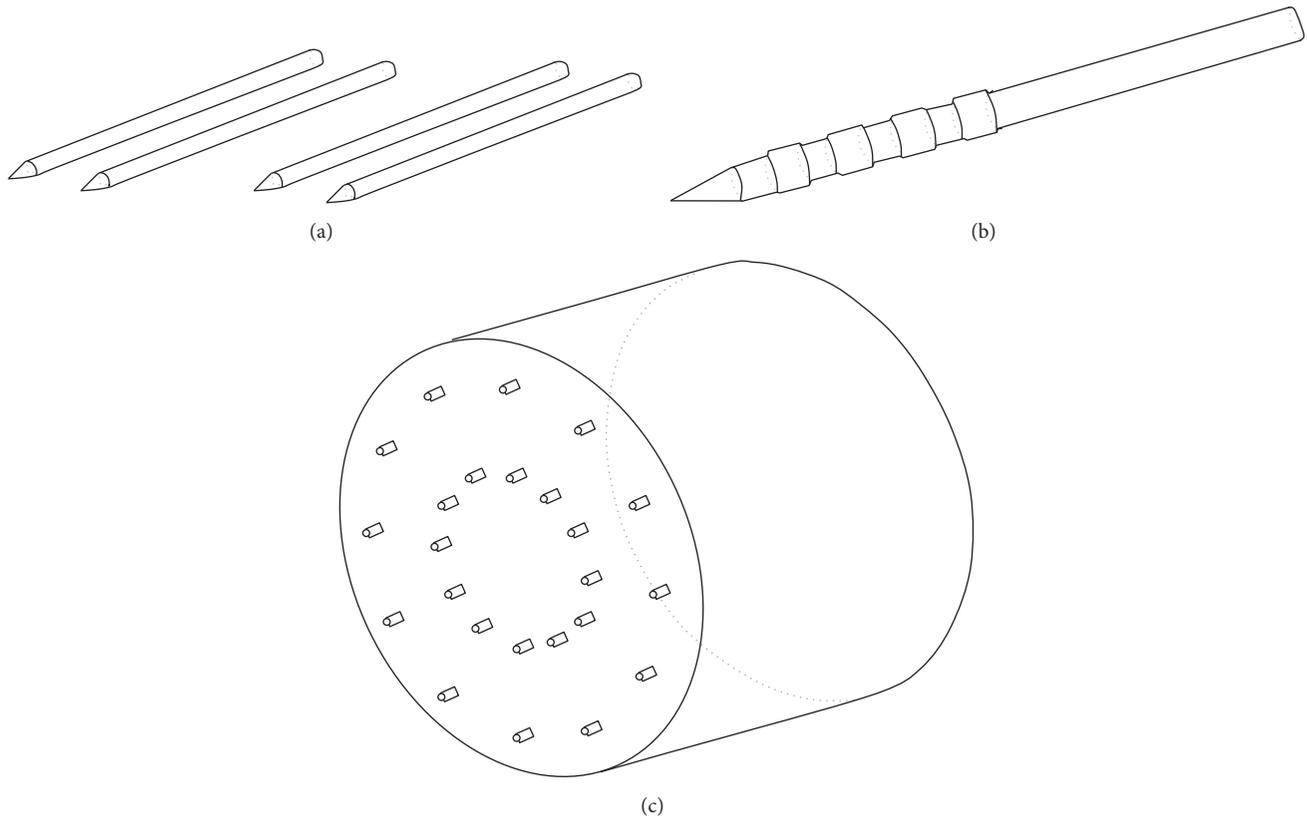


FIGURE 7: Schematic diagrams of three configurations of tetrapolar probe.

also used a cylindrical tetrapolar probe (Figure 7(c)). The probe consisted of 24 noninvasive electrodes which were equally spaced on a periphery in two concentric circles. Each four electrodes arrayed along diameter were a tetrapolar system. The advantage of this probe was that the impedances of six directions could be obtained in a single application. There is also a three-point measurement method, which is the simplification of tetrapolar method with one electrode working to induce current and to measure voltage. However, this method is rarely used in measurement for biological system and is not introduced in detail in this paper.

4. Quality Assessments of Meat and Fish

This section summarized recent development in quality assessments of meat and fish using EIS. The published research was summarized on two aspects of quality assessments: (i) physicochemical properties of raw meat and fish and (ii) chemical compositions of both raw meat and fish and their products. For physicochemical properties evaluation, four common quality issues, ageing state of beef, PSE and DFD of pork, freshness of fish, and defrosting of fish and chicken, were discussed, respectively. For chemical compositions evaluation, salt and water are the main evaluated ingredients, and the discussions are separated into two parts, meat and fish, depending on distinct features between them. Specific framework of the summary is shown in Figure 8. The general information of all studies reviewed is given in Table 2.

4.1. Physicochemical Properties. Physicochemical properties of raw meat and fish include color, pH, water-holding capacity (WHC), Warner-Bratzler shear force (WBSF), and total volatile basic nitrogen (TVB-N). These properties are the indicators for diverse quality issues, which draw attention of customers and manufacturers. EIS has been studied to realize prediction of physicochemical properties in order to solve the appropriate quality issues.

4.1.1. Ageing State: Beef. One of the most important factors of beef palatability or quality is tenderness that is closely related to meat ageing state [59]. Meat after slaughter undergoes two periods, rigor mortis and ageing. Then it is either further processed or sold in the retail markets. During ageing, connective proteins break down, causing structural changes of fragmentation of myofibrils and degradation of cytoskeletons. Well-aged meat is tender with improved flavor. Assessment of meat tenderness optimizes refrigerated storage time and the ageing state for sale [61]. Ageing results in changes in membrane and intracellular and extracellular electrolytes. These can impact electrical properties and can be reflected by impedances with increasing frequencies [83]. During post-rigor-mortis ageing, impedance of meat decreases more slowly than prerigor period [84] and there is no convincing explanation of the mechanisms.

It was found that ratio $Z_{1\text{kHz}}/Z_{100\text{kHz}}$ decreased free from the impact of fat level during ageing [85, 86]. Therefore, the ratio was suggested as an indicator for meat ageing

TABLE 2: Application of EIS for quality assessments of meat and fish.

Object	Quality indicator	Predictor variable	Frequency	Algorithm	Accuracy	Reference
<i>Ageing state: beef</i>						
Heifers	WBSF	Electrical impedance(EI), conductivity(EC)	A wide range for EI, 1 KHz for EC	CA	$R = 0.68$ (EI), and -0.65 (EC)	[63]
beef	Mechanical resistance	$Z_{1\text{ kHz}}/Z_{100\text{ kHz}}$	1,100 kHz	LRA	$0.73 \leq R^2 \leq 0.98$	[64]
beef	Mechanical resistance	Lineic impedance index (I), contact impedance (CI)	100 Hz	LRA	$R^2 = 0.79$ (I); $0.77 \leq R^2 \leq 0.95$ (CI)	[61]
Cull cows	Mechanical resistance	8 selected parameters related to complex impedance	100 Hz–1.5 MHz	LRA	$R^2 = 0.71$	[59]
Beef and veal	Time of postmortem period	R_e, R_i, C derived from the modified Fricke model	40 Hz–110 MHz	TA	— ^a	[14]
<i>PSE, DFD, and RFN: pork</i>						
Porcine muscle	pH ₄₅	Changes in phase angle	1 kHz	CA	$R = 0.73$	[65]
Pig carcass	Drip loss at 24 h	Electrical impedance	1000 Hz	PLS	$R = 0.50$, RMSEP = 2.53%	[66]
Green ham	pH ₄₅	$R_{\infty}/R_0, \alpha$, and f_c	8 kHz–1 MHz	MRA	$R^2 = 0.50$ $0.758 \leq R^2 \leq 0.992$, $0.11 \leq \text{RMSE} \leq 0.57$ for TAC;	[47]
Lean pork tenderloins	TAC, TVB-N	$Z = (Z_0 - Z_t)/Z_0$ (Z_0 , impedance at day 0; Z_t , impedance at a given storage interval)	20, 200 Hz, 2, 20, 200 kHz	NRA	$0.636 \leq R^2 \leq 0.989$, $0.29 \leq \text{RMSE} \leq 1.68$ for TVB-N	[13]
<i>Freshness: fish</i>						
Carp, herring and sea bass	Hypoxanthine content	Phase angle	2.5 kHz	LRA	$R^2 = 0.92$ for carp; 0.87 for herring; 0.86 for sea bass	[67]
Grass carps	TAC, K value, TVB-N, sensory assessment (SA)	$Q = (Z_{1\text{ kHz}} - Z_{16\text{ kHz}}) \times 100/Z_{16\text{ kHz}}$	1 kHz, 16 kHz	CA	$R = 0.943$ for TAC, 0.996 for K value, 0.951 for TVB-N, 0.968 for SA	[68]
Bighead carp	pH, texture, TVB-N, K value, drop loss, SA and TAC	$Q = (Z_{1\text{ kHz}} - Z_{20\text{ kHz}}) \times 100/Z_{20\text{ kHz}}$	1 kHz, 20 kHz	CA	$R \geq 0.919$ for pH and texture, ≥ 0.955 for TVB-N, K value, drop loss, SA and TAC	[69]
Sea bream	TVB-N	Impedance modulus and phase	1 Hz–1 MHz	PLS	$R^2 = 0.72$	[70]
Carp	Storage time (ST), SA	Morphological characteristic parameter, impedance modulus, phase	1 Hz–1 MHz	LRA	$R^2 = 0.69$ for ST, 0.66 for SA	[17]

TABLE 2: Continued.

Object	Quality indicator	Predictor variable	Frequency	Algorithm	Accuracy	Reference
<i>Defrosting: fish and chicken</i>						
Sea bass	Slow-frozen in 1 and 2 cycles, Fast-frozen in 1 and 2 cycles	Resistance, reactance, water, fat content, WHC and pH	1 Hz–1 MHz	PCA, DA	78% for total samples, 100% for the unfrozen	[71]
Salmon	Frozen and stored for 15, 30 and 60 days, frozen in 1 and 2 cycles	Impedance modulus and phase	1 Hz–1 MHz	PCA, DA	71.93%	[72]
Sea bream	Frozen and stored for 15, 30 and 60 days, frozen in 1 and 2 cycles	Impedance modulus and phase	1 Hz–1 MHz	PCA, DA	70.24%	[73]
Sea bass	Frozen for 1 month with and without temperature fluctuations (TF), frozen for 4 months with and without TF	Impedance modulus and phase	0.1–1000 kHz	ANOVA, LRA	$R^2 = 0.918$ between phase angle and protein solubility	[74]
Atlantic chub mackerel	Slow-frozen in 1 and 2 cycles, Fast-frozen in 1 and 2 cycles	Resistance, reactance	1 Hz–1 MHz	ANOVA	— ^a	[75]
Chicken breast meat	1 and 2 frozen-thawed cycles	Impedance modulus and phase	50–200 kHz	LVQNN	100% for fresh, >90% for one cycle, >88% for two cycles	[60]
Chicken breast meat	1, 2, and 3 frozen-thawed cycles	$(Z_{50\text{ Hz}} - Z_{200\text{ kHz}})/Z_{200\text{ kHz}}$, $(\text{Ph}_{50\text{ Hz}} - \text{Ph}_{200\text{ kHz}})/\text{Ph}_{200\text{ kHz}}$, chewiness, hardness, expressible loss (Z is the modulus; Ph is the phase angles)	50 Hz, 200 kHz	LVQNN	100%, 97.5%, 87.5%, and 77.5% for 0, 1, 2, and 3 cycles samples, respectively	[15]
<i>Water, fat, and salt: meat</i>						
Potted minced pork products	Water (W), lipid (L)	Impedance modulus and phase	5 kHz–2 MHz	PLS	A standard deviation of the residues of 0.66% for W and 1.08% for L	[76]
Porcine meat	Water	R_{∞}	1–250 kHz	LRA	$R^2 = 0.879$, RMSEP = 0.00566, RSD = 0.73%	[45]
Chicken breast meat	Water	Impedance module and phase	1 Hz–1 MHz	PLS	$R^2 = 0.9388$, predictive residual sum of squares of 1.9242	[58]
Green ham	Visual fatness	R_{∞} , α , conformation, ham weight	8 kHz–1 MHz	MRA	$R^2 = 0.59$	[47]
Beef and pork from different size grinds	Fat	Resistance, temperature, weight	50 kHz	— ^a	$R^2 = 0.36$, 0.60, 0.86 for beef trim, 0.95-, 0.32 cm ground; $R^2 = 0.64$, 0.66, 0.92 for pork trim, 0.95-, 0.32 cm ground	[77]

TABLE 2: Continued.

Object	Quality indicator	Predictor variable	Frequency	Algorithm	Accuracy	Reference
Pig of various breeds and cattle	Intramuscular fat	R_p , resistance at high frequencies immediately after the change of current signal; R_p , derived for the voltage extrapolated to infinite time	500 Hz–100 kHz	SFR, CA	$R = 0.34$ for total pork, $R = 0.69$ for beef	[62]
Minced pork loin	Salt	Impedance modulus and phase	100 Hz–1 MHz	PLS	$R^2 = 0.934$ $p1 = 0.985$, NR = 0.388 for chloride;	[57]
Minced pork loin	Sodium chloride, sodium nitrite, sodium nitrate content	Impedance modulus and phase	1 kHz–1 MHz	PLS	$p1 = 0.709$, NR = 1.436 for nitrite; $p1 = 0.648$, NR = 1.511 for nitrate ^b	[78]
Dry-cured hams of three qualities (deep spoilage, spoiled swollen, and unaltered)	Salt	Impedance modulus and phase	100 Hz–1 kHz	PLS	$0.72 \leq R^2 \leq 0.78$	[79]
<i>Salt and water: fish</i>						
Fresh salted Atlantic salmon	Salt, water, water phase salt (WPS)	Conductance at 1 MHz, capacitance increment ($Cap_{10\text{ MHz}} - Cap_{1\text{ MHz}}$)	1, 10 MHz	LRA	$0.822 \leq R^2 \leq 0.926$ for salt, $0.488 \leq R^2 \leq 0.534$ for water, $0.732 \leq R^2 \leq 0.890$ for WPS	[80]
Fresh salted rainbow trout	Salt, water, WPS	Impedance modulus and phase	50 kHz	LRA, MRA	$R^2 = 0.864$ for WPS, 0.844 for water, 0.853 for salt	[16]
Salmon during salting-smoking process	Salt, water, water activity (a_w)	Impedance modulus and phase	1 Hz–1 MHz	PLS	RMSEP = 0.685 for salt, 0.006 for a_w , 3.579 for water	[81]
Smoked salmon and cod products (of different brands and batches)	Salt, water, lipid, a_w	Impedance modulus and phase	1 Hz–1 MHz	PLS	$0.701 \leq R^2 \leq 0.823$ for a_w , $0.351 \leq R^2 \leq 0.640$ for lipid, $0.564 \leq R^2 \leq 0.851$ for water, $0.600 \leq R^2 \leq 0.761$ for salt	[82]

Correlation analysis: CA; Linear regression analysis: LRA; Trend analysis: TA; Multiple regression analysis: MRA; Nonlinear regression analysis: NRA; Analysis of variance: ANOVA; Learning vector quantization neural network: LVQNN; Stepwise forward regression: SFR; ^a Accuracy was not available; ^b The parameters of $p1$ (slope of the fitting line) and norm of residuals (NR) were obtained from the fitting line of predicted and measured salt content, and were used as indicators of performance of the PLS model.

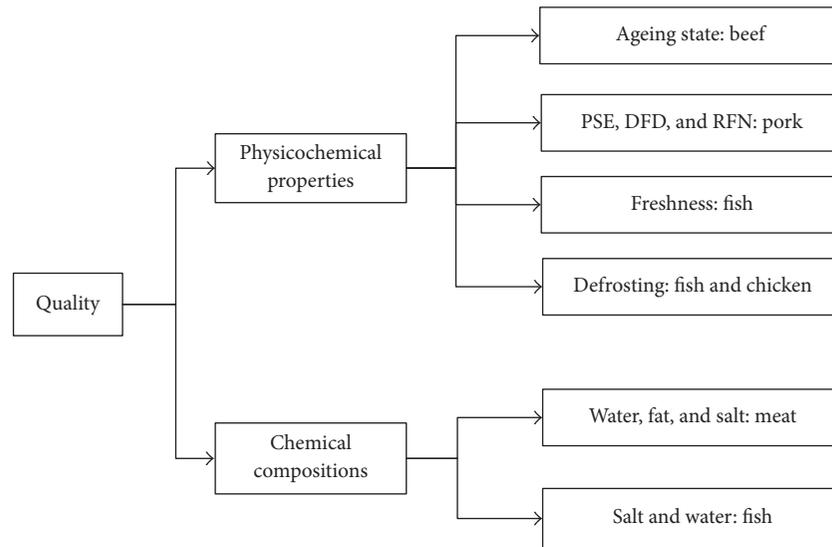


FIGURE 8: Framework of overview.

state. However, Lepetit et al. [64] found that the ratio $Z_{1\text{ kHz}}/Z_{100\text{ kHz}}$ and impedance (Z) tempted to decrease with ageing, but the slope of curves for the same muscle varied with different animals of Charolaise bulls. Thus these two parameters were not suitable for prediction of the ageing state. Meanwhile, they put forward electrical anisotropy as a new prediction parameter, since it decreased with ageing period and became almost isotropic after meat was fully aged. Based on this, Damez et al. [59] developed a circular probe, which was able to measure impedance at different directions of bovine meat. Ageing state evaluated by impedance measurement using the probe showed strong correlation with mechanical measurement for all muscles from three types ($R^2 = 0.71$). However the correlations showed great difference between the three muscle types (rectus abdominis: $R^2 = 0.83$; semimembranosus: $R^2 = 0.58$; semitendinosus: $R^2 = 0.18$). The results also showed that impedance measured parallel to muscle fibers is the minimum, while impedance measured across the muscle fibers is the maximum. Furthermore, Damez et al. [61] carried out impedance measurement only on the two directions for early assessment of beef meat ageing. The lineic impedance index was defined as the difference between the director coefficients of the regression lines of transversal and longitudinal impedance respectively against distance between electrodes. Higher correlation ($R^2 = 0.79$) based on the lineic index were obtained. Another parameter, contact impedance, was also tested. It was calculated as the y -intercept of impedance regression line against the distance, and high correlations with meat fibers strength were obtained for all tested muscle types ($0.77 \leq R^2 \leq 0.95$). Recently, Guermazi et al. [14] applied the modified Fricke model to characterize the ageing state of different muscles of beef and veal. The extracellular resistance R_e showed the highest sensitivity to ageing, with decreasing about 7% to 25% from day 6 to day 14 depending on the muscle type. These

indicated that prediction ability of same model varied among different types of muscle.

4.1.2. PSE, DFD, and RFN: Pork. Pork meat can be divided into DFD (dark, firm, and dry), PSE (pale color, soft texture, and high exudation), and RFN (red, firm, and nonexudative) based on its color, pH, and drip loss. PSE meats are unsuitable for processing and DFD meats are perishable [61]. Both of them present unfavorable appearance for consumers and thus cause economic losses for meat industry [87]. In contrast, RFN meat is desirable meat and ideal for both producers and consumers. The benefits of identification of low quality meat for industry include reducing economic losses and distributing the best destination of meat carcasses [23].

Forrest et al. [66] used complex impedance (at 1000 Hz) to predict drip loss at 24 h of pig carcass at the slaughter line. The cross-validation showed a correlation of 0.50. The moderate results were thought to be due to a lack of temperature correction. Besides, the rate of changes in impedance and phase angle had a better performance of prediction for meat qualities than the absolute magnitude of impedance, which was concluded by Whitman et al. [65]. A better result was obtained by Oliver et al. [47] studying on green ham with Cole-Cole theory. Multiple regression model based on variables of ratio (R_∞/R_0), α , and f_c showed a result of $R^2 = 0.50$ to predict pH_{45} in the *semimembranosus* (SM) region. It was also found that ratio in SM region classified the technologically normal meat ($\text{pH}_{45} > 6.10$) from the PSE meat with 88.46% accuracy.

For DFD meat, the results were found not promising for the early detection based on electrical measurements [23, 61]. Besides, the detection for PSE meat was also difficult during the rigor mortis period, because pH, temperature, and metabolic modifications, which have influence on electrical properties of meat, are rapidly evolving at this period [88].

It was also concluded that discrimination of PSE, DFD, and RFN pork meat was more valid when the final pH has been reached, based on dielectric properties [23]. These indicated that detection time is also a significant factor in meat quality prediction.

H. B. Nguyen and L. T. Nguyen [13] used a new parameter of relative changes impedance [$Z = (Z_0 - Z_t)/Z_0$, Z_0 , impedance at day 0; Z_t , impedance at a given storage interval] to evaluate TVB-N and total aerobic count (TAC) of lean pork tenderloins during storage. The results were promising, with $0.636 \leq R^2 \leq 0.989$ for TVB-N and $0.758 \leq R^2 \leq 0.992$ for TAC. This study can be used as a reference for other quality parameters prediction, such as pH and drop loss.

4.1.3. Freshness: Fish. Because of fish being highly perishable and significant impact of freshness on flavor, freshness is the primary quality attribute of fish for consumers and manufacture. EIS applied for freshness estimation of fish shows the promising results. Based on the phase angle and admittance changing with time, four stages of freshness (fresh, semifresh, semideteriorated, and deteriorated) of carp, herring, and sea bass can be easily defined. Furthermore, phase angle at 2.5 kHz had good linear relationship with hypoxanthine concentration which is used to estimate fish freshness ($R^2 \geq 0.86$ for carp, herring, and sea bass) [67]. Impedance change ratio (Q value), defined as $Q = (Z_{f1} - Z_{f2}) \times 100/Z_{f2}$, is also found to have high correlation ($R \geq 0.943$) with TAC, TVB-N, and sensory assessment (SA) of grass carps and bighead carp during storage [68, 69].

Although these impedance parameters demonstrated the ability as freshness indicators, the studies for practical application are in the preliminary stage and need to be confirmed in more situations [68]. Furthermore, it was found that impedance parameters to predict freshness of fish samples of different batches showed worse results than for the samples of same batches [70]. The reason was thought to be the difference in composition terms among the distinct batches. It was proved that growth environments and dietaries have an effect on body composition of fish [89, 90]. To eliminate the effect of different origins of fish, Sun et al. [17] defined a morphological characteristic parameter extracted from Bode plots to predict freshness. Compared with impedance module and phase angle, morphological characteristic parameter showed highest correlations ($R^2 = 0.69$). The results were not outstanding due to 20 samples obtained from 20 different retailers.

4.1.4. Defrosting: Fish and Chicken. Due to perishability of fish and chicken, freezing is the common means to slow down meat quality deterioration during postharvest handling and storage. But in freezing, frozen storage, and thawing, the processes of protein denaturation and lipid oxidation take place, which not only cause tissue injury but also affect the sensory as well as nutritional quality of the products [72, 91]. Because of this, a lot of consumers prefer fresh products regardless of price. However in many cases, the difference between frozen-thawed products and fresh ones are too small to tell based upon their visual appearance [92]. Processes of freezing/thawing and frozen storage provoke protein

denaturation and the destruction of cell membranes, which cause modification produced on the mobility of water and ion concentration and affect electrical impedance measured on fish and chicken [71, 93].

Fish samples of fresh and suffered with different freezing styles, frozen storage periods, and freezing cycles were investigated to be distinguished based on EIS at a frequency range of 1 Hz to 1 MHz [71–73]. Impedance data were analyzed with principal components analysis (PCA) and discriminant analysis (DA). Classification accuracy was above 70% (71.93% and 70.24%) for total samples [72, 73], while impedance data combined with physical and chemical parameters (water, fat content, WHC, and pH) as input had a higher accuracy of 78% [71]. The accuracies for fresh samples were up to 100%, while the results for the separation between freezing styles, storage periods, and freezing cycles were not good. This was considered caused by no significant difference between samples of different storage periods and freezing cycles in electrical impedance, which was the same as in other physicochemical parameters such as moisture content, TVB-N, K1 value (an indicator of ATP related compounds), pH, and microbial counts [72]. Moreover, it was found that reactance can be used to differentiate between different freezing styles and freezing cycles for sea bass at frequencies higher than 500 kHz [71]. And it seems that phase or reactance may be better indicator of the freezing history compared to impedance modulus or resistance [74, 75].

For chicken breast meat, the fresh samples and frozen samples with different freezing cycles were successfully distinguished (100% for fresh, >90% for one cycle, and >88% for two cycles), based on impedance magnitude and phase at frequencies from 50 to 200 kHz and a modeling method of learning vector quantization neural network (LVQNN) [60]. Furthermore, impedance modulus and phase angles measured at two frequencies (50 Hz and 200 kHz), combined with three physical properties (chewiness, hardness, and expressible loss), also showed the discrimination ability (100%, 97.5%, 87.5%, and 77.5% for the fresh samples, the one frozen-thawed cycles, two and three cycles, respectively) [15].

4.2. Chemical Compositions. There are many kinds of meat and fish products, such as meat floss, ham, bacon meat, and fish, which are deeply liked by customers in the market. The flavors of these products are affected not only by their physicochemical properties of the raw materials but also by chemical compositions in raw materials and end products as well as additives added in processes. Some of these compositions are moisture, lipid, and salt. Some research [94–97] has demonstrated that electrical properties of food are closely associated with their chemical compositions, which shows the possibility of EIS to assess component contents of food.

4.2.1. Water, Fat, and Salt Content: Meat. Prediction of water content of meat showed promising results, with EIS being applied to raw porcine meat [45] and potted minced pork products [76]. De Jesús et al. [79] studied dry-cured hams of three qualities (deep spoilage, spoiled swollen, and unaltered), and no good performance for water content prediction

was obtained. This was due to no significant differences for water content among the all dry-cured ham samples. It was discovered that the moisture content differs from part to part and, even in one single piece of meat, moisture values vary from different detecting directions. Furthermore, meat muscles with fat uniformly distributed and low fat levels may obtain better accuracy for prediction [45]. Schmidt et al. [58] predicted moisture content of skinless deboned chicken breast meat, during cooking process with different heating time. Good prediction was obtained ($R^2 = 0.9388$), which indicated EIS can also be applied to chicken for moisture prediction.

The prediction of fat content of muscle meat based on EIS is not good [47, 62]. It was considered due to the fat concentrated in clustered adipose cells and inhomogeneously distributed within muscles [62]. This can be verified by the study of Marchello et al. [77], which assessed fat content of beef and pork from different size grinds based on impedance measurement. For the beef samples with fat percentage range of 4–35%, R^2 for the trim, 0.95, and 0.32 cm ground meat were 0.36, 0.60, and 0.86, respectively. For the pork samples with fat percentage range of 7.5–35%, R^2 for the pork samples of the trim, 0.95, and 0.32 cm ground were 0.64, 0.66, and 0.92, respectively. The results showed that the smaller the grind, the higher the accuracies of the prediction. These also demonstrated that fat content assessment for minced samples showed a better performance than for block samples.

Good results were also obtained for salt content prediction of salted meat based on EIS [57, 78, 79]. It was also found that minced samples had better prediction accuracies than block samples, which indicated that salt homogeneously distributed within meat favorably impacts prediction accuracy. Furthermore, Labrador et al. [78] applied EIS to predict concentration levels of different salt types in minced meat. Precise prediction of chloride was obtained, whereas the predictions for nitrite and nitrate were moderate.

4.2.2. Salt and Water Content: Fish. Salt content and water phase salt (WPS) of fresh salted fish have good correlations with impedance parameters, even at two or single frequencies [16, 80]. These parameters include impedance modulus at 50 kHz, conductance at 1 MHz, and capacitance increment between 10 MHz and 1 MHz. Impedance modulus generally reflects changes of conductance. Conductance is thought to be related to ions capability of movement and capacitance which includes the information of the muscle state. For smoked product and during salting-smoking process, salt content prediction also showed good results based on impedance data at a frequency range of 1 Hz to 1 MHz [81, 82]. For both situations, prediction for water activity (a_w), a parameter closely related to microbial spoilage, obtained better results than for salt content. These indicated the feasibility to determine shelf life of smoked product based on EIS [82].

Studies showed different prediction performance for moisture content of fish [16, 80, 81]. In addition, it was found that prediction results for smoked products differed among brands and species, and the difference between species

was greater [82]. Species with lower lipid content had better accuracies, which is consistent with prediction for meat. Moreover, configuration of electrode is also considered as an influence factor on moisture prediction accuracy [16]. This was indirectly verified by Fuentes et al. [73], who tested two types of electrode to separate the fresh fish from the frozen. One type of electrode was able to separate, while the other one failed. However, the failed electrode was found to be efficient to separate for another species of fish [72]. Effect caused by species is considered due to the different structures and compositions of fish muscle between species, while effect of electrode is the various electrical fields loaded on muscle tissue. Guermazi et al. [14] investigated electrode configuration by using Finite Element Methods to simulate the distribution of the electric field in meat sample.

4.3. Challenges and Future Trends. Although the above review shows a promising application of EIS in quality assessment/prediction of raw meat, fish, and/or its products, several challenges remain to be addressed before it can be applied in practical manufacturing process. The challenges are mainly from two aspects, that is, electrical impedance measurement and the tested meat or fish.

Electrode polarization presents one main challenge of EIS in impedance measurement and data exploration. Forced by the electric field, dissolved free ions existing in conductive systems tend to move towards the electrode/sample interface, leading to the formation of ionic double layers. The ionic double layers exhibit capacitive impedance character, producing voltage drops. This phenomenon is electrode polarization. The resultant spectra of electrode polarization overlay the relaxation process of the interested samples, impeding the interpretation of the data. Various approaches on both description of electric double layer using equivalent circuits and measurement setup configuration compensations are studied to correct the electrode polarization. However, effect of electrode polarization depends on impedance of the samples, the measuring temperature, the structure and materials of the electrode, and even the roughness of the electrode surface. These factors make it complicated and no correction technique can satisfy ideally in practical application [56].

Some challenges from the tested meat and/or fish are caused by variety of biological tissue. Diverse species, muscle types, and origins cause different muscle structure and/or composition, which produce various measurement results. Furthermore, in one muscle, anisotropic properties of tissues adversely impact prediction accuracy. These imply that predict model and/or electrode need be specialized for different application objects, and measurements should be performed on exact location of samples as well as control electrode orientation. Complexity of biological tissue also makes the difficulty in data interpretation and exploration. Although prediction of quality indicators performed promising results, the interpretations of relationships between EIS data and status of tested meat or fish samples are ambiguities.

Further research should focus on enhancing measurement system, which includes developing optimal electrode in suitable material and configuration, controlling or correcting temperature during measurement, and optimizing

measurement setup configuration, in order to reduce effect of electrode polarization and muscle anisotropy to improve precision and stability. More effective equivalent circuit models and data processing methods need to be further studied to explore interpretation of the data with status and/or components of diverse tested meat or fish samples, in order to help extract the most important feature parameter for quality estimation accordingly and to help investigate the most suitable prediction model strategy with high precision, efficiency, and robustness for practical application.

5. Conclusions

EIS has been proved as a promising detection technology with advantages of being fast, nondestructive, inexpensive, and easily implemented and shows potential to replace traditional methods in order to save time, cost, and skilled persons. The reviewed studies above, including predictions of physicochemical properties and chemical compositions for raw meat and fish and their commercial products, illustrate that EIS has potential for application in quality assessments of meat and fish. Challenges of EIS lie on impact factors of impedance measurement, including electrode polarization, materials and structure of electrodes, and the measurement setup configuration, and lie on difficulty in data processing and interpretation for diverse and complex tested meat or fish tissue. These challenges still need to be carefully considered before moving this technology from the laboratories to the industrial real-time detection.

Conflicts of Interest

The authors declared that they have no conflict of interests.

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Research Article

Microbial Safety of Low Water Activity Foods: Study of Simulated and Durban Household Samples

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Sixty household low water activity foods were examined and a simulative study was conducted in a high sugar, low a_w almond and macadamia butter to determine the survival of *Bacillus cereus* and *Staphylococcus aureus* ATCC 25923. Results obtained from 60 low a_w samples collected at household level had some significant differences ($P \leq 0,05$) within food categories amongst the various tests. Spices had the highest number of aerobic bacteria, aerobic spore-formers, anaerobic spore-formers, and *S. aureus*. Mean aerobic colony counts for nuts and spices were 2.30 log CFU/g and 4.40 log CFU/g, respectively. Pathogens such as *Escherichia coli* and *Cronobacter sakazakii* were present in nuts, whilst *Salmonella* spp. was present in chocolates. This implies that certain low a_w foods may present a public health risk. In the simulative study, temperature and high sucrose concentrations played a significant role in the survival of *B. cereus* and *S. aureus* ATCC 25923. *B. cereus* was found to be more osmotolerant at both reduced and elevated temperatures (18°C and 25°C) in the 12% sucrose sample in both butters, whilst *S. aureus* ATCC 25923 seemed to grow better in sucrose-free samples at both temperatures in both butters. This implies that certain low a_w foods may present a public health risk. Also, *B. cereus*, being a spore-forming bacterium, can be osmotolerant at both reduced and elevated temperatures.

1. Introduction

Food safety, is a global concern with the developing countries being the worst affected. Recently over the years, a number of incidences relating to outbreaks caused by the presence of food-borne pathogens in low water activity (a_w) foods have increased [1]. According to Byrd-Bredbenner et al. [2], households were ranked as the most common setting and primary location in which food-borne illnesses took place in the United States. This has become a serious problem not only for food industries but also for the final consumer consuming these products.

These increased outbreaks have therefore sparked the interest and urgency to investigate and understand the possible causes of the presence of food-borne pathogens in low a_w foods. Low a_w foods that have drawn considerable attention to the food industries, research communities, and the public are those of spices, dry nuts, chocolates, infant and adult cereals. and milk powder, being the most common amongst many other low a_w foods [3].

Low a_w foods are derived from high moisture foods which are often subjected to dehydration or desiccation processes to achieve this, or they could be naturally low in moisture. Furthermore, the water content of such foods could also be achieved by adding solutes (salt or sugar) or freezing to control the quality of the final product. a_w present in foods is a vital element in predicting the stability and safety of a food product with respect to the rate at which deterioration reactions occur [4].

Foods with low a_w contributes to the extended shelf life of the food product which is often desirable to the consumer. However, over the years, this has led to consumer perceptions that such low a_w foods were not of concern from a microbiological or food safety perspective and were safe for human consumption [2].

The main reason for this perception was that microorganisms were not able to thrive and proliferate in dry conditions. However, many studies done by researchers in literature have found that microorganisms have the capability of surviving in low a_w foods [1, 5]. The increased number of food-borne

related illness outbreaks recently, associated with low a_w foods, has portrayed that even though microorganisms cannot proliferate in such foods, there is a likelihood for them to be persistent for extended periods of time [1, 5, 6]. Depending on the type of microorganism, they can cause illness even with their low infectious dose or subsequent temperature abuse that permits the growth of these microorganisms [5]. Furthermore, literature has also demonstrated that Gram-negative bacteria exhibited more sensitive behaviour to low a_w than Gram-positive bacteria. The minimum a_w for all microbial growth is 0,60 and spoilage of foods would not be of a microbiological nature below this value [7].

The main pathogens of concern in literature were those of *Salmonella* spp., *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Cronobacter sakazakii*, *Staphylococcus aureus*, and *Clostridium botulinum* [1, 8]. A crucial step that is often omitted is an additional heating step which has the ability to control or kill potentially contaminated food-borne bacteria. Consumers often consume such foods without additional heating which is the possible cause for food-borne illness outbreaks.

Although food-borne diseases and illnesses have been on the increase in recent times, they mainly have been reported in the developed countries where food safety and quality are a priority. Rarely are such cases reported in Africa or in other developing countries and limited research has been done in Africa to combat this problem. This could be due to a lack of financial resources to conduct such research or a lack of knowledge amongst these developing communities about food safety in general.

The purpose of this study is to determine the prevalence of food-borne pathogens in household samples and to conduct a simulative study in low a_w , high sugar almond and macadamia butters. The samples collected were spices, milk chocolates, nuts, milk powder, and infant and adult cereals. The pathogens of concern in this study are those mentioned above. Since fungal growth has been widely studied in low a_w foods, this study aims to focus on the bacterial growth in such foods. Furthermore, limited research has been done in Africa in this regard and, therefore, the purpose of this research is also to enlighten African communities about food safety of low a_w foods.

2. Materials and Methods

2.1. Collection and Storage of Samples. For the first objective, commercial samples of six different categories were collected from ten Durban homes using sterile Ldpe Ziplock bags and stored at -18°C to prevent the growth of bacteria. At the time of collection, the samples were collected at 25°C . The following samples collected were as follows:

- (1) Adult cereal
- (2) Infant cereal
- (3) Milk powder
- (4) Spices
- (5) Nuts
- (6) Milk chocolate

For the second objective, sugar, almond, and macadamia butters were purchased from a retail food store to conduct the simulative study. These samples were stored at room temperature after the test organisms (*B. cereus* and *S. aureus*) have been confirmed to be absent in them.

2.2. Bacterial Analysis of the Samples. All the sixty samples collected were examined for the presence of *L. monocytogenes*, *S. aureus*, *Salmonella* spp., *E. coli* O157:H7, *C. sakazakii*, and *C. botulinum* using conventional methods. Before microbial analysis was conducted, a_w of each sample was checked using a Novasina AG Labswift a_w meter. Two strains were used for the simulative study on almond and macadamia butters. *S. aureus* ATCC 25923 was purchased from Anatech and a laboratory strain of *B. cereus* was used individually to inoculate the butters to determine its survival over a period of time in a certain sugar concentration.

2.2.1. Conventional Methods

C. sakazakii. 10 ml of the macerated solution was added to 90 ml of buffered peptone water (BPW) and was incubated at 37°C for 24 hours after which 1 ml was introduced into Lauryl Sulphate Tryptose Broth and incubated at 37°C for 24 hours. After incubation, a loopful was streaked onto *Enterobacter sakazakii* Agar plates and incubated at 44°C for 24 hours according to the method by Nokwanda and Ijabadeniyi [9].

E. coli. A modified method by MFHPB [10] was used for this study where one gram of food sample was inoculated into 10 mL double strength of Lauryl Sulphate Tryptose (LST) Broth and incubated for 24 hours. After 24 hours, a 1 ml aliquot was transferred into 10 ml of Brilliant Green Lactose Broth (BGLB) and incubated at 35°C for 24 hours. After 24 hours, 1 ml aliquot was transferred into *Escherichia coli* (EC) Broth tubes in a water bath at 45°C . This was transferred into Eosin Methylene Blue (EMB) Agar, which was incubated in a water bath at 45°C . After 24 hours, EMB plate was incubated at 35°C for 24 hours.

S. aureus. A dilution series was done on all samples. They were prepared using BPW. 0,1 ml of the dilution was thereafter spread-plated onto Baird-Parker Agar with egg-yolk tellurite emulsion and incubated at 35°C for 24 hours according to the method by ISO [11].

Salmonella. The method by SABS [12] was used for this experiment. 25 g of the food sample was preenriched with 225 ml of BPW and was incubated at 35°C for 16 to 20 hours (preenriched sample). 0,1 ml of the preenriched sample was then transferred into a test tube containing 10 ml of Rappaport-Vassiliadis Enrichment Broth (RVB) and another 10 ml of preenriched medium was transferred into a bottle containing 100 ml of sterile Selenite Cystine Medium (SCM). RVB was incubated at 42°C for 24 hours and SCM at 35°C for 48 hours. The culture obtained from RVB after incubation for 24 hours and the culture obtained from SCM after incubation for 48 hours were then inoculated onto the surface of *Salmonella* HiCrome Agar and XLD Agar to obtain

isolated colonies. All of these plates were incubated at 35°C for 24 hours and thereafter examined for growth.

L. monocytogenes. The method by Ijabadeniyi and Naidoo [13] was used for this experiment. Half-strength and full-strength Fraser Listeria Selective Enrichment (FLSE) Broth were prepared as stipulated by the manufacturer. 1 ml of the test sample was inoculated into 9 ml of half-strength FLSE and incubated at 35°C for 24 hours. 0,1 ml of the culture obtained in the previous step was then inoculated into a test tube containing 10 ml of full-strength FLSE and incubated at 35°C for 48 hours. The culture obtained from the half-strength FLSE incubated for 24 hours was streaked onto Oxford Listeria Selective Agar to observe growth of colonies and incubated microaerobically at 35°C for 24 hours. The plates were reincubated if growth was slight or if no colonies were observed for a further 24 hours. The same procedure was followed for the culture obtained from the full-strength FLSE.

Aerobic and Anaerobic Spore-Formers Count. The test sample was prepared by adding 25 g of sample to 225 ml of BPW. 20 ml of this 10^{-1} dilution was then pipetted into two test tubes, whereby one test tube served as a control. These two test tubes were then placed into a water bath with a thermometer placed in the control test tube. These test tubes were held at 75°C for 20 minutes. A dilution series was done and thereafter 0.1 ml of the sample will be pipetted into Petri dishes and pour-plated with Trypticase Soy Agar. Both aerobic and anaerobic plates will be incubated at 35°C for 48 hours. The anaerobic plates will be placed into anaerobic jars with Anaerocult according to the method of MFLP [14].

Aerobic Colony Count. The test sample was prepared in the same way that they were prepared for aerobic and anaerobic spore-formers count. A tenfold dilution series was done in duplicate. 0,1 ml of the dilutions was spread-plated onto Nutrient Agar and incubated for 37°C for 24 hours according to the method of ISO [15].

2.2.2. Simulative Study

Preparation of Inoculum. A modified method by Nummer et al. [16] was used. Two strains were used for this simulative study: a laboratory strain of *B. cereus* and *S. aureus* ATCC 25923. *B. cereus* cells were received as a lawn of cells on TSA and *S. aureus* ATCC 25923, which was received in the form of a swab, was swabbed directly onto Baird-Parker Agar. Baird-Parker Agar plates were incubated at 37°C for 24 hours. After incubation, the cells from the respective plates were washed with 5 ml nutrient broth and were transferred into 15 ml centrifuge tubes. A pellet was obtained by centrifugation at 2200g for 10 minutes. The pellets were then resuspended into 1 ml nutrient broth and this was used to inoculate the butters.

Inoculation of the Butters. Almond and macadamia butters were separated into 25 g samples. Sucrose was added to added in 3 g and 5 g quantities into the 25 g samples of almond and macadamia butter and mixed thoroughly. a_w of the samples were taken before the two strains were

TABLE 1: Average water activities for the low a_w food samples collected from households.

Food category	Average a_w
Infant cereal	0,352
Adult cereal	0,381
Milk powder	0,284
Nuts	0,648
Spices	0,437
Chocolates	0,427

individually inoculated. 1 ml of the two strains, *S. aureus* ATCC 25923 and *B. cereus*, were individually inoculated into each butter sample aseptically. The concentration of the cells of both strains was approximately $1,3 \times 10^7$ CFU/ml, which is approximately 7 log CFU/ml which was compared to a 0,5 McFarland standard for approximate turbidity using the method by [13].

Recovery and Enumeration of S. aureus ATCC 25923 and B. cereus. The inoculated almond and macadamia butter samples (25 g) were assayed for *S. aureus* ATCC 25923 and *B. cereus* in duplicate at 1, 7, 14, 21, and 28 days, respectively. These samples were stored at 18°C to 20°C and at 25°C. Serial dilutions were done whereby 1 g of all samples was transferred into 9 ml of nutrient broth and the method by ISO [11] was used to recover and enumerate *S. aureus* ATCC 25923 and the method by MFLP [14] was used to recover and enumerate *B. cereus*.

2.3. Statistical Analysis. One-Way Analysis of Variables (ANOVA) and Tukey's comparison test were done to determine if there were significant differences ($P \leq 0,05$) between the household samples as well as to compare the mean values for the simulative study.

3. Results and Discussion

The average a_w per food category was calculated and is depicted in Table 1. Table 2 shows the results achieved from the first objective which was to assess the quality of low a_w foods collected from Durban households.

Based on these average a_w readings (refer to Table 1), all of the samples were found to have had a_w below 0,7. a_w for samples such as nuts (0,648) and spices (0,437) corresponds with the values from literature in which such samples had a_w of <0,6 [3]. Infant cereal (0,352), adult cereal (0,381), and milk powder (0,284) a_w values also corresponded with values in the literature by [1, 17].

The samples were stored in clean air-tight containers prior to collection. Furthermore, upon collection, the food was of good quality, whereby no visible fungi or mould was present. Furthermore, all samples were still within their shelf life. As per the results obtained from household samples collected, as seen in Table 2, most of the samples had low counts. Spices had the highest counts of aerobic bacteria, aerobic spore-formers, anaerobic spore-formers, and *S. aureus* followed by nuts. This could be due to poor hygiene practices in industry

TABLE 2: Microbiological quality of low a_w activity food presented in means of ACC, ASF, AnSF, and *S. aureus* (Log CFU/g) collected at household level.

Category	ACC (Log CFU/g)	ASF (Log CFU/g)	AnSF (Log CFU/g)	<i>S. aureus</i> (Log CFU/g)
Infant cereal	2,022 ^a ± 0,83	1,90 ^{ab} ± 0,76	0,19 ^c ± 0,31	0,30 ^{cd} ± 0,34
Adult cereal	1,00 ^a ± 0,77	ND ^b	ND ^{bc}	0,19 ^{bcd} ± 0,25
Milk powder	1,30 ^a ± 0,72	0,092 ^b ± 0,29	0,096 ^{bc} ± 0,30	0,15 ^{bcd} ± 0,29
Nuts	2,30 ^a ± 2,10	0,66 ^{abcd} ± 1,40	0,97 ^{abcd} ± 2,10	0,37 ^d ± 1,20
Spices	4,40 ^a ± 0,33	3,30 ^b ± 0,40	2,70 ^{bc} ± 1,00	1,30 ^d ± 1,10
Chocolates	0,74 ^a ± 0,33	0,07 ^b ± 0,23	0,065 ^{bc} ± 0,21	0,024 ^{bcd} ± 0,075

(1) Results represented as Means ± Standard Deviation. (2) Means ($n = 60$). (3) Means with same superscript letters in rows are not significantly different ($P \geq 0,05$). (4) ACC: aerobic colony count; ASF: aerobic spore-formers count; AnSF: anaerobic spore-formers count; ND: not detected.

and prolonged exposure to air. Furthermore, spices and nuts originate from natural sources and could also have been contaminated from the natural environment as mentioned in the literature [1, 3].

There was no growth detected for aerobic and anaerobic spore-formers counts in adult cereal, indicating that these samples were well kept at household level and were not exposed to the air for prolonged periods of time. Infant cereal and milk powder exhibited a similar trend with regard to their colony counts indicating that there were no significant differences ($P > 0,05$) between these two samples. Most of the food categories apart from spices and nuts had relatively low colony counts amongst ACC, ASF, AnSF, and *S. aureus*. Chocolate samples compared to the other five food categories had the least colony counts. Whilst significant differences ($P < 0,05$) were noted amongst the different colony counts within a food category, spices and nuts were the only two categories that differed significantly from the rest of the categories in this regard.

Foods such as infant cereal, adult cereal, and milk powder have been desiccated to achieve to their desired organoleptic properties. According to [17], desiccation in turn increases the concentration of solutes in the remaining water available. The osmotic potential of water is increased by the addition of solutes. Whilst cereals contain added sugar (usually in the form of sucrose), the patterns of effect on microbial responses have exhibited similar qualities when KCl, NaCl, sucrose, and glucose were used. Glycerol as a humectant on the other hand permitted growth at lower a_w , although there were exceptions: *S. aureus*, for example, was more inhibited by glycerol than NaCl.

Presence and absence tests for pathogens such as *C. sakazakii*, *E. coli*, *L. monocytogenes*, and *Salmonella* spp. were also conducted on the collected household samples.

Whilst *C. sakazakii*, *E. coli*, *L. monocytogenes*, and *Salmonella* spp. were not recovered from infant cereals, adult cereals, milk powder, and spices. Nut samples had *C. sakazakii* and *E. coli* which were found in 60% and 100% of the samples, respectively. Chocolates, on the other hand, had *Salmonella* present in all (100%) of the samples [1, 8].

According to [7, 17], Gram-negative bacteria are more susceptible to low a_w foods than Gram-positive bacteria. Whilst Gram-negative bacteria can only survive in foods

with $a_w \geq 0,95$, Gram-positive bacteria, on the other hand, can withstand a_w in foods as low as $<0,8$. However, in the present study, *C. sakazakii*, *E. coli*, and *Salmonella* which are Gram-negative pathogens survived in low a_w foods being nuts and chocolates which had an average a_w of 0,648 and 0,427, respectively, which is well below the limit as stipulated in the literature. Gram-positive bacteria like *L. monocytogenes* was absent in these samples. Aerobic spore-formers like *B. cereus* and anaerobic spore-formers like *C. botulinum* are Gram-positive bacteria that also survived in low a_w foods in the present study. A study done by Doyle and Glass [18] showed the prevalence of *C. botulinum* in dried dairy products. This could be due to the presence of spores that these bacteria possess since they are spore-forming bacteria and, by having the presence of spores, would enhance their survival in stressed environments. According to Beuchat et al. [1], such bacteria survive at a_w ranging between 0,93 and 0,92. a_w of the foods in the present study were significantly lower than the values stipulated in the literature implying that Gram-positive aerobic and anaerobic spore-formers like *B. cereus* and *C. botulinum* have the ability to survive in foods that possess a_w lower than this range as seen in Table 1.

It would be highly acceptable to have an absence of pathogens in food samples such as infant and adult cereals and milk powder, due to the desiccation process that they undergo as well as spices being a naturally occurring low a_w food that is high in antioxidants, therefore possessing high antimicrobial activity [9]. Although these pathogens of importance were absent in these samples in the present study, according to review articles and studies conducted by [1, 3, 9], pathogens such as *C. sakazakii* were found to be present in foods such as spices and powdered infant formula and *E. coli* was found to be present in infant rice cereal.

Tables 3–6 represent the results obtained from the second objective which was a simulative study that was conducted on two different butters, almond and macadamia butter with an adjusted sugar content, stored at two different temperatures (18°C and at 25°C) for a period of 28 days.

The almond butter used for this simulative study contained no added sugar or salt. Although there were significant differences amongst the groups within pathogen for both *S. aureus* ATCC 25923 and *B. cereus* (refer to Table 3), it was noted that the growth of *S. aureus* ATCC 25923 at 18°C to

TABLE 3: Populations of *S. aureus* ATCC 25923 and *B. cereus* in almond butter at varying concentrations of sugar stored at 18°C.

Concentration of sucrose at 18°C to 20°C	Populations (Log CFU/g) over storage time (days)				
	1	7	14	21	28
<i>S. aureus</i> ATCC 25923					
0%	5,34 ^a	5,25 ^{ab}	5,23 ^{abc}	4,73 ^d	3,59 ^e
12%	5,033 ^{ns}	4,32 ^{ns}	2,72 ^{ns}	ND	ND
20%	4,72 ^a	ND	ND	ND	ND
<i>B. cereus</i>					
0%	5,033 ^a	4,74 ^b	4,53 ^c	ND	ND
12%	4,56 ^{ns}	4,14 ^{ns}	3,45 ^{ns}	3,30 ^{ns}	3,00 ^{ns}
20%	3,17 ^a	2,88 ^b	ND	ND	ND

(1) Means with same superscript letters in rows within pathogen are not significantly different ($P \geq 0.05$). (2) Means ($n = 2$). (3) ND: not detected; ns: no significance.

TABLE 4: Populations of *S. aureus* ATCC 25923 and *B. cereus* in macadamia butter at varying concentrations of sugar stored at 18°C.

Concentration of sucrose at 18°C	Populations (Log CFU/g) over storage time (days)				
	1	7	14	21	28
<i>S. aureus</i> ATCC 25923					
0%	5,29 ^a	4,94 ^{ab}	4,51 ^{bc}	3,92 ^d	3,45 ^e
12%	4,74 ^a	4,31 ^b	4,029 ^c	ND	ND
20%	4,61 ^a	ND	ND	ND	ND
<i>B. cereus</i>					
0%	4,29 ^a	3,94 ^{ab}	2,24 ^c	ND	ND
12%	5,053 ^a	5,021 ^{ab}	4,86 ^c	4,40 ^d	4,30
20%	3,64 ^a	ND	ND	ND	ND

(1) Means with same superscript letters in rows within pathogen are not significantly different ($P \geq 0.05$). (2) Means ($n = 2$). (3) ND: not detected; ns: no significance.

TABLE 5: Populations of *S. aureus* ATCC 25923 and *B. cereus* in almond butter at varying concentrations of sucrose stored at 25°C.

Concentration of sucrose at 25°C	Populations (Log CFU/g) over storage time (days)				
	1	7	14	21	28
<i>S. aureus</i> ATCC 25923					
0%	5,40 ^a	5,39 ^{ab}	5,22 ^c	4,87 ^d	4,53 ^e
12%	5,074 ^a	4,93 ^b	4,63 ^c	4,56 ^{cd}	4,00 ^e
20%	4,89 ^a	4,79 ^{ab}	4,65 ^{abc}	4,49 ^{bd}	3,15 ^{de}
<i>B. cereus</i>					
0%	4,88 ^a	4,77 ^b	4,51 ^c	ND	ND
12%	5,25 ^{ns}	5,22 ^{ns}	5,041 ^{ns}	3,77 ^{ns}	3,53 ^{ns}
20%	4,80 ^{ns}	4,76 ^{ns}	4,72 ^{ns}	3,69 ^{ns}	3,22 ^{ns}

(1) Means with same superscript letters in rows within pathogen are not significantly different ($P \geq 0.05$). (2) Means ($n = 2$). (3) ND: not detected; ns: no significance.

20°C in almond butter did not survive as well as *B. cereus* in the 12% sucrose sample. Furthermore, both strains did not survive very well in the 20% sucrose sample, as the growth of *S. aureus* ATCC 25923 had ceased by the 7th day and *B. cereus* by the 14th day. However, *S. aureus* ATCC 25923

survived very well in a sucrose-free medium, whilst *B. cereus* survived moderately well in a low sucrose content medium (12% sucrose). The growth of *B. cereus* was inhibited after the 21st day. This indicates that a high sucrose content inhibits the growth of both these bacteria at slightly lower temperatures.

TABLE 6: Populations of *S. aureus* ATCC 25923 and *B. cereus* in macadamia butter at varying concentrations of sucrose stored at 25°C.

Concentration of sucrose at 25°C	Populations (Log CFU/g) over storage time (days)				
	1	7	14	21	28
<i>S. aureus</i> ATCC 25923					
0%	5,34 ^a	5,32 ^{ab}	4,59 ^c	4,47 ^d	4,38 ^{de}
12%	5,00 ^a	4,99 ^{ab}	4,85 ^{abc}	4,67 ^{abcd}	3,15 ^e
20%	3,97 ^a	3,67 ^b	3,00 ^c	ND	ND
<i>B. cereus</i>					
0%	5,39 ^a	5,066 ^b	ND	ND	ND
12%	5,18 ^a	4,67 ^b	4,66 ^{bc}	4,19 ^d	3,45 ^e
20%	4,20 ^a	3,88 ^b	3,72 ^c	3,60 ^d	3,49 ^e

(1) Means with same superscript letters in rows within pathogen are not significantly different ($P \geq 0.05$). (2) Means ($n = 2$). (3) ND: not detected; ns: no significance.

Whilst limited studies implicate the presence of *B. cereus* and *S. aureus*, several have implicated the presence of *E. coli* and *Salmonella* [1, 8, 16, 19, 20]

The macadamia butter used in this simulative study had a carbohydrate content of 5 g of which of total sugar amounted to 3,3 g per 100 g (3,3%). A similar trend was noticed in macadamia butter stored at 18°C despite the butter having the contained added sugar. *S. aureus* ATCC 25923 grew well in the sucrose-free sample (0%) but, because of the butter already containing 3,3% sucrose, it can be deduced that *S. aureus* did not survive after 14 days at very low sucrose contents. *B. cereus*, on the other hand, survived after 14 days but ceased to grow after the 21st day in a very low sucrose medium indicating that *B. cereus* is more osmotolerant than *S. aureus* ATCC 25923. Furthermore, like almond butter, *B. cereus* grew fairly well in the 12% sugar sample. This is, in addition to the 3,3% sucrose that butter had already contained, implying that *B. cereus* grows moderately well in 12–18% sucrose medium. Both strains of bacteria did not survive very well at 20% sucrose content, implying that a high sucrose content would inhibit their growth.

Temperature seemed to have significantly influenced the growth of both bacteria. It was observed that the growth of *S. aureus* ATCC 25923 had grown fairly well in both the 12% and 20% sucrose samples. The growth of *B. cereus* had ceased after the 21st day in the sucrose-free (0%) sample but was now able to thrive in the 20% sucrose sample, implying that *B. cereus* is more osmotolerant compared to *S. aureus* ATCC 25923. However, at 25°C, *S. aureus* ATCC 25923 was able to metabolise sucrose as a carbon source than it did at a reduced temperature.

A similar trend was noticed in macadamia butter stored at 25°C. It was observed that the growth of *S. aureus* ATCC 25923 was inhibited after the 21st day in the 20% sucrose sample, whilst the growth of *B. cereus* ceased after the 7th day in the 0% sucrose sample. However, *B. cereus* grew fairly well throughout the 28 days in both the 12% and 20% sucrose samples.

From this simulative study, it can be deduced that the concentration of sugars (sucrose) and the effect of temperature affected the growth of both strains of bacteria.

Whilst *S. aureus* ATCC 25923 was able to thrive in a sample that was sucrose-free (0%), *B. cereus* proved to be more osmotolerant surviving in the 12% sucrose concentration at reduced temperatures (18°C to 20°C). Both strains did not survive very well at 20% sucrose concentration at reduced temperatures. At an elevated temperature (25°C), both *B. cereus* and *S. aureus* ATCC 25923 were able to grow at all three concentrations of sucrose in almond butter. There were no significant differences between the growth of both bacteria in both types of butter, except for 20% sucrose concentration in almond butter.

According to Finn et al. [5], bacteria have osmoprotectants which aid in bacterial survival in low a_w environments. The function of osmoprotectants is to balance the osmolarity within the bacterial cell to that of the external environment to avoid any loss of water.

In this present study, temperature and humectants (sucrose) induced a stressed environment for the bacteria to grow. According to Carlin et al. [21], despite the optimum growth limits for bacteria to thrive, bacteria have to adjust to changes in temperature. Mechanisms of modification to their lower or higher temperature growth limits show significant differences.

There could be a high possibility that, due the spore-forming ability of *B. cereus* in an unfavourable environment, the vegetative cell itself is protected from the stressed environment as opposed to *S. aureus* which is not a spore-forming bacterium and is more susceptible to stressed environments. Due to the fact that a_w of the nut butter has increased after sucrose was added, it would be possible for both strains of bacteria to survive. Carbon as a source of nutrition also plays a vital role, when present in low concentrations.

4. Conclusion

From this study, it can be concluded that low a_w food samples possessed low colony counts except, for spices and nuts which had the highest colony counts as well as aerobic spore-formers, anaerobic spore-formers, and *S. aureus* compared to the other food categories. Overall, good manufacturing practices should be followed in industry and in the home

to avoid outbreaks of food-borne illness. Future work can be conducted on fungi producing mycotoxins, which have been in the spotlight in recent years; furthermore since fungal growth has been highly associated in low a_w foods, it would be apt to conduct such work.

Disclosure

This work has been accepted for presentation at IAFP 2017 Annual Meeting in Florida, USA.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Research Article

Fresh Snack Food Channel Evaluation Model for Integrating Customers' Perception of Transaction Costs in Taiwan

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The primary purpose of this study was to explore how food dealers develop methods that facilitate transaction efficiency and how they select the optimal food channels. This study establishes a model according to the impact of transaction cost factors on consumers' decision-making regarding purchase of fresh snack foods. Using fresh snack foods in Taiwan as an example, this study employed a fuzzy analytic network process to solve decision-making problems with multiple criteria by comparing the interaction between each transaction cost factor to obtain the factor weightings as well as the weightings of the transaction costs at each decision stage. This study found that food safety assurance and providing sufficient nutrition information were the most essential topics; thus, the optimal choice for snack food producers is to develop retail outlets. This study construction process proposed is innovative and operational, and the results may provide a reference for snack food dealers or microfood enterprises to assist them in developing their food channels.

1. Introduction

The rise of the Internet has enabled the rapid flow of information and various improvements in life convenience, both of which have led to various lifestyle changes. Compared with the short supply of snack foods in earlier times, snack foods have shifted from being luxury goods to being normal goods. Snack foods are known as “finger foods” in Japan and simply “snacks” in China. In fact, snack foods have existed for a long time, although the term was introduced only in recent decades. Conventional snack foods include peanuts, melon seeds, packaged dry noodles, Ramune soda, and preserved fruit, and more recently introduced snack foods include potato chips, chocolates, chewing gum, and instant noodles. Because of the increasing trend of Western cake with coffee food culture emerging in Taiwan in recent years, as well as the substantial reduction in tariffs following Taiwan's accession into the World Trade Organization in 2002, food and beverage industries have been constantly introducing snacks, desserts, and pies with distinct national characteristics. The quality of the snack food market has been markedly affected by rapid changes in the types of snack foods that are available, their sale through convenience store sales channels (Taiwan

has the highest density of convenience stores in the world), and the introduction of the home delivery distribution model.

The qualitative effect on the snack food market is that the industries can sell their products through online platforms, which lowers the market entry threshold for leisure goods. For example, established traditional market snack industries that own stores can also expand into online channels. Following the rise of health awareness, customers have placed greater emphasis on food freshness. Home deliveries using preservation technologies such as low-temperature storage and freezers have overcome food freshness and regional restrictions, allowing snack foods to become more diverse and resulting in substantial changes in their sales patterns. Ready-to-eat or cooked snack foods can be home-delivered and eaten after simple processing or heating. Examples of famous Taiwanese snack foods include Chou's shrimp rolls from Tainan and Wanluan pork knuckle. Under the current trend of the microenterprise model, various homemade desserts and pastries can be delivered to customers with adequate freshness and sufficient time until expiry, with home delivery techniques making up for the capital and equipment deficiencies of microenterprise.

The quantitative effect on the snack food market stems from the lower transaction costs perceived by customers, which results in an increase in market size. In addition, customer-oriented market policies and high customer awareness prompt businesses to view customer access to product information and customer convenience in buying and using products as the most essential topics in product marketing. When facing the aforementioned problems, industries usually adopt approaches that improve transaction efficiency, such as additional sales branches establishment, home delivery, promoting website exposure, and providing online transaction services; or they directly absorb customers' partial transaction costs to stimulate demand. Therefore, how industries determine their resource input levels in promoting transaction efficiency (reducing customers' transaction costs) and prompt consumer purchasing behaviors is a topic worth investigating.

2. Literature Review

2.1. Transaction Cost Theory. Engel et al. [1] noted that customer purchasing decisions involve activities pertaining to obtaining, consuming, and disposing products or services, and these activities include pre- and postpurchase decisions. Blackwell et al. [2] studied customers' various purchasing factors and listed a series of activities involving obtaining, consuming, and disposing of products and services, including the processes involved in pre- and postpurchase decisions. Peter and Olson [3] showed that customers' purchasing decisions are dynamic interactions among cognitive, behavioral, and environmental outcomes throughout the process of individual life exchange. Recent customer behavior studies have focused on customers' consumption experiences and the various dynamically interacting factors affecting transactions throughout customer exchange processes. According to Williamson [4], Ronald Coase was the first to introduce the concept of transaction costs into industry and market analysis. Coase [5] posited that the price mechanism under the economic operation of specialization and exchange will have *ex ante* (such as information searching, contract negotiation, and signing costs) and *ex post* costs (costs for supervising contract execution), which are collectively known as transaction costs. Coase found that industries usually replace markets in fulfilling the function of economic coordination and can effectively save transaction costs. According to Teo and Yu [6], transaction cost theory became prominent only after Williamson [7] expanded Coase's original framework and published other relevant studies on trading and transaction costs. In measuring transaction costs, Liang and Huang [8] defined them as the cost of processes involving transaction-related activities and divided the decision-making aspect of customer purchasing behaviors into three stages: prepurchasing, purchasing, and postpurchasing processes. Accordingly, the present study was based on transaction cost theory, which stems from the decision-making process in customer behaviors.

2.2. Customer-Perceived Transaction Costs. The Internet has caused qualitative changes in the composite factors of transaction costs in recent years. For example, Degeratu et al. [9] found that information searching costs differ according to the attributes of products and that appropriate product exposure or better service can reduce customers' searching costs [10]. Earlier studies have shown that the immediacy of interactions on the Internet can lower negotiation costs during transactions. An example of this is the question and answers sections that some companies provide on their websites [11, 12], and online interaction has also been shown to promote consumer trust [13, 14]. The development of credit card and third-party payment online transaction mechanisms and tools has facilitated reducing transaction costs and promoted purchasing behaviors [15]. Zhang and Oh [16] and Workman and Cho [17] have empirically investigated the impacts of product display, trial use, or food testing on consumers; however, they have also been shown to pose a security risk and thus challenge consumer trust in online transactions [6].

The diversity of products that customers currently face in the market raises the question of how they conduct their purchasing and selection behaviors when deciding between the various brands, prices, payment methods, dealers, and distributors that are available. Liang and Huang [8] stated that when choosing between products with similar attributes or prices, customers will choose those that have lower transaction costs or purchase from suppliers that provide the highest value [6, 18–22]. Empirical studies have discussed the differences in transaction costs between online and offline shopping contexts, as well as the relationship between online shopping information and transaction costs [10, 23, 24]. Tyagi [25] verified that an increase in dealers' investments in transaction costs reduces customers' transaction costs. However, previous studies investigating the problem of waiting time cost differences in the goods obtained by different customers (meaning a different time cost for each customer) have been limited to superficial discussions of the concept. In fact, customers may adopt different purchasing decisions based on the length of waiting time, because this directly affects the remaining effective period (expiry date) of the products (or food), which is particularly pertinent for products whose quality is defined by the level of freshness. The effects of waiting time costs and product (or food) freshness on customer demand have been widely studied in the field of inventory, and various relevant models have been developed [26, 27]. However, previous customer studies and food research have rarely mentioned the perceived waiting time and food freshness costs; this might be due to the lack of operability for measuring the time cost, which varies from person to person.

2.3. Channel Decisions. With the current prevalence of Internet marketing, businesses understandably invest considerable resources into developing Internet marketing strategies. Previous studies have indicated that most dealers adopt multiple channels for product sales, whereby the level of investment for developing new customers is also lower [28, 29]. Many consumers have become multichannel users under such an environment, although enterprises may also lose customers during the shopping process, particularly those who

tend to research products online before purchasing them in a brick-and-mortar store [11]. When marketing through multiple channels, enterprises must also account for the wide variability in the composition of customers' transaction costs [30, 31]. Despite developing multiple channels being the optimal marketing strategy for enterprises, they encounter problems in balancing the amount of investment or resource allocation in different channel types [32, 33].

Suppliers' channel development simultaneously affects their production strategies and inventory costs. For example, a supplier sells its products through multiple channels, but customers' transaction decisions in choosing retail outlets or Internet purchasing may differ when transaction costs are considered [11]. Suppliers adopt the nonimmediate delivery (or even preorders) of products in response to customers' Internet orders, and this can enable them to be more responsive in adjusting their production lines, which effectively controls the level of inventory. Thus, channel decisions are particularly critical for fresh food suppliers, whereby effective channel strategies ensure that products retain adequate freshness when they reach the customers. Although previous food suppliers have used various methods to estimate product demand, the expiry dates of fresh foods mean that the increase in customers' transaction costs when food loses its freshness (or when the waiting time increases) should be considered. Therefore, the optimal means for facilitating transaction efficiency should be determined, because a lack of an operational model still leads to difficulties in production decisions [32, 34].

Current trends in the snack food market show that customers are fastidious with a greater emphasis on affordability, practicality, health, nutritional value, food hygiene, and freshness in their snack food purchases. In addition, the demand for functional, organic, and fiber-rich foods results in a richer snack food market with new food types emerging. From the perspective of Taiwan, the snack food market is the most dynamic segment of the food industry with a wide customer age range and diverse product types. In particular, there are various distributors providing access to snack foods, and their channel types can be roughly divided into discount stores; general merchandise stores; snack food chain stores; convenience stores; traditional shops and grocery stores; markets, night markets, and street vendors; and others. The development and progress of online platforms has also resulted in the emergence of Internet ordering or home delivery services as well as multichannel platforms such as TV shopping.

2.4. Fuzzy Analytic Network Process. In the present study, a type of resource allocation decision model was developed to explore the interaction between transaction cost elements under different channel types. The multiple criteria decision-making (MCDM) methods are the most suitable methods for resolving decision problems under uncertainty with multiple evaluation criteria. MCDM provides a systematic approach that helps decision-makers combine the inputs with profit/cost information and stakeholder perspectives in order to rank all the alternatives to the item. The MCDM framework contains various methods, such as the weighted sum

model (WSM), weighted product model (WPM), and the analytic hierarchy process (AHP), whereas the analytic network processes (ANP) are an extension of the AHP. The ANP can add a feedback mechanism to the hierarchical framework in the decision-making model, which can indicate the dependence between each hierarchical criterion.

Evaluators use clearly defined values for measurement in a conventional decision-making process, although it is difficult to use the same approach for evaluation and measurement in actual environments in the presence of uncertainty. The main reasons are that human thinking is subjective and ambiguous, and the various selected factors and criteria will possess similarity problems because of speech defects [35]. In other words, it is impossible to draw from the experts' complete, accurate, and reliable knowledge and simultaneously conduct alternative evaluations with respect to the various standards and similar criteria. Because of the fuzziness in decision-making, as mentioned, measuring it using a fuzzy set theory-based approach should be a feasible solution [36].

In terms of revising the aforementioned MCDM method, the fuzzy analytic network process (FANP) is one the most suitable methods for resolving decision problems under uncertainty with multiple evaluation criteria. FANP integrates ANP [37, 38] with fuzzy set theory [39]. The ANP method compiles expert opinions whereby the complex evaluation problems are systematized and stratified, after which they are subjected to pairwise comparisons by decision-makers who determine the weights between multiple evaluations. The correlations between the criteria of each level are considered, whereby a prioritized arrangement of decision-making is established for all of the evaluation criteria. To incorporate the uncertain and fuzzy characteristics of the subjective factors of human thinking, reasoning, and perception toward environmental surroundings, the ANP linguistic variable value is incorporated into the triangular fuzzy number and becomes FANP, which still operates according to fuzzy set theory. FANP has the advantage of displaying the fuzzy phenomenon of expert cognition without eliminating any unique opinions. The fuzzy intervals of the experts' collective decision-making can be used as the flexible space for decision-makers in judgments based on their personal experience.

FANP has been widely used in various fields of research in recent years. For example, some scholars have used it to develop supplier selection models (e.g., [40–44]), whereas others applied it to transportation choice decision-making problems (e.g., [45]), selection models for developing new product concepts [46], sustainable energy technology alternatives [47], hotel management system problem selection [48], and oversight problems in food management and research [49] and to determine the key indicators of green supply chain for food products [50]. MCDM has been adopted to explore the development of new product by century-old enterprises [51], improve productivity in small-scale food enterprises [52], and study site selection problems in food distribution [53]. Baviera-Puig et al. [54] used a similar approach to analyze corporate social responsibility in the food industry and how companies successfully achieved

social communication and meet the demands of the stakeholders. FANP has also been adopted to study design problems of food supply chains [55–57]. Despite the wide range of applications of FANP, information on the channel assessment problems in the literature is scarce. This may be due to the effects of different channels on customers' purchasing behaviors, which lack a specific operation model.

Based on this analysis, no specific mode of operation has been proposed for food distributors to consider the factors of consumer-perceived transaction costs and to determine the optimal level of investment for promoting transaction efficiency and developing multiple channels. To solve this type of problem, first, a method for measuring consumers' perceived transaction cost is required, particularly for measuring the time cost, which varies among individuals (e.g., food waiting times or food freshness). Chang and Chen [32] devised a demand function model incorporating the transaction costs of individual customer's purchasing decisions relative to the time costs, waiting time, and service time. They verified that the transaction costs of dealers and customers do not exhibit a zero-sum relationship. In other words, for every dollar that dealers absorb of the transaction costs, the overall transaction cost for customers is reduced by more than a dollar, which can reveal the optimal level of transaction costs for industries to absorb to facilitate transaction efficiency. Therefore, the present study adapted the transaction cost measurement method proposed by Chang and Chen [32] and simultaneously accounted for the waiting time cost, food freshness, and service level to bring research on the effects of transaction cost factors on customers' purchasing behavior of fresh snack food in line with actual circumstances. Next, FANP was combined to solve the multicriteria decision-making problem. The main purpose of this study was to explore how dealers develop methods that foster transaction efficiency and to investigate their process for selecting the optimal food channels from the perspective of the food dealers. The model construction process proposed in the present study is more innovative and operational than previous ones.

3. Pattern Construction and FANP Analysis Procedures

This study constructed a weighted model composed of transaction cost factors under different channels. The model construction and analysis steps are described as follows.

Step 1. The transaction cost dimensions and criteria in each customer decision-making dimension were constructed. Textual analysis was performed to develop the transaction cost factors in each decision-making dimension of customer purchasing behaviors, after which marketing departments and research scholars were requested to revise the content and construct the evaluation dimensions and criteria of transaction costs in each decision-making dimension; the definitions are shown in Table 1.

Step 2. Construct the ANP hierarchical structure.

Step 2.1. The ANP assumption methods are as follows by Saaty and Vargas [38]. (1) A system can be broken down into multiple classes or components, forming a network-like hierarchical structure. (2) The elements of each level in the hierarchy need not be independent. (3) The elements within each hierarchy level can be evaluated using some or all of the elements from the previous level. (4) An absolute numerical scale can be converted into a ratio scale during the evaluation of an element. (5) After pairwise comparisons have been performed, the hierarchical elements are processed in a positive reciprocal matrix. (6) The preference and intensity relations of the elements must satisfy the transitivity. (7) Considering the difficulty of complete transitivity, their degree of consistency is further tested. (8) The advantage degree of the elements can be calculated using the weighting principle.

Step 2.2. The relationship network diagram for the transaction costs of fresh snack food is constructed using the evaluation dimensions and criteria of Step 1, as shown in Figure 1.

Step 3. Construction of the fuzzy pairwise comparison matrix.

Step 3.1. The ratio scale is used as the scale for measuring the pairwise comparison matrix, whereby each evaluation factor is subjected to a pairwise comparison by using the evaluation scale to assess the relative importance of each assessment factor at the same level. This study reduced Saaty's 9-point scale to a 5-point scale, as suggested by Lai [58] (see Table 2 for definitions).

Step 3.2. Calculate the eigenvalues and eigenvectors of the comparison matrix. Assume there were N criteria (C_1, C_2, \dots, C_n) and comparison matrix $A = a_{ij}$, wherein a_{ij} represented the relative importance of criteria C_i and C_j . For all i and j , it is necessary that $a_{ii} = 1$ and $a_{ij} = 1/a_{ji}$. Using the row vector averaging proposed by Saaty for nominalization, the calculation of the approximate weighting W_i through (1) is as follows:

$$W_i = \frac{\sum_{j=1}^n (a_{ij} / \sum_{i=1}^n a_{ij})}{n}, \quad \forall i, j = 1, 2, \dots, n. \quad (1)$$

The pairwise comparison matrix A fully satisfies $a_{ik} = a_{ij} \cdot a_{jk}$, $\forall i, j, k$; thus, the approximate value of the maximal eigenvalue λ_{\max} can be obtained from the following:

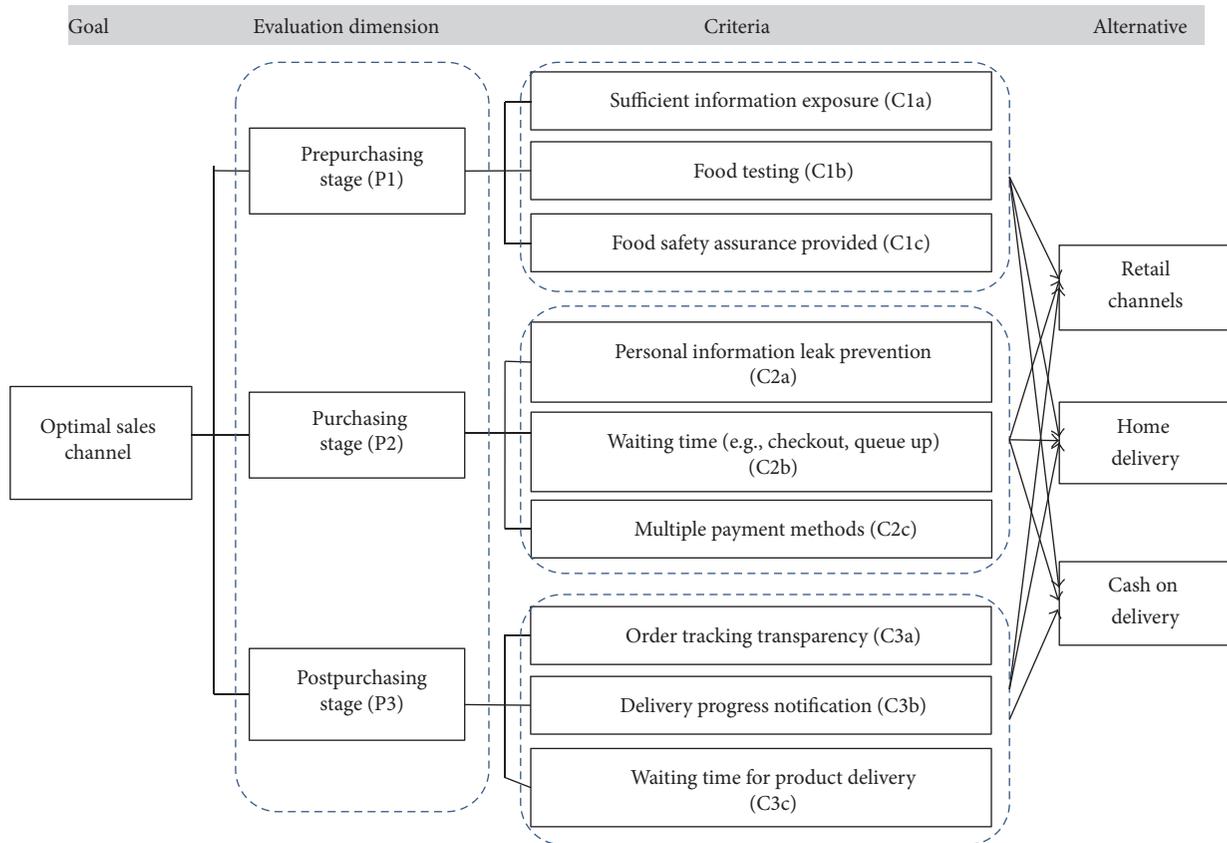
$$AW = \lambda W \lambda_{\max} = \frac{1}{n} \sum_{i=1}^n \frac{(AW)_i}{W_i}. \quad (2)$$

Step 3.3. The consistency index (C.I.) is calculated using (3), whereby each pairwise comparison matrix is calculated to have C.I. < 0.1 , thus confirming their compliance to the criteria of judgment consistency.

$$\text{C.I.} = \frac{\lambda_{\max} - n}{n - 1}. \quad (3)$$

TABLE 1: Evaluation dimensions and criteria.

Dimension	Criteria	Definition
Prepurchasing (P1)	C1a	Sufficient information exposure Level of detail and coverage of snack food information content that reaches customers
	C1b	Food testing Provide opportunities, methods, and occasions for snack food testing
	C1c	Food safety assurance provided Provide the ingredient sources, production verification, and expiry date of snack foods
Purchasing (P2)	C2a	Personal information leak prevention Minimize customer concerns regarding personal privacy during purchases
	C2b	Waiting time (e.g., checkout, queue) The time required to check customers' credentials or for the queue to be cleared when customers checkout
	C2c	Multiple payment methods Provide multiple payment methods such as cash, credit card, third-party payment, or cash on delivery
Postpurchasing (P3)	C3a	Order tracking transparency Customers can instantly monitor product delivery processes through the phone or Internet after ordering snack foods
	C3b	Delivery progress notification The manufacturers may, via message or E-mail, actively communicate delivery information or expected time of arrival of the product
	C3c	Waiting time for product delivery The waiting time before customers receive products affects their satisfaction and repurchasing intentions



indicates that the elements within the clusters possess internal dependencies

FIGURE 1: Architecture diagram of fresh snack food marketing channels.

TABLE 2: Definition of the ANP evaluation scale anchors.

Evaluation scale	Definition	Remarks
1	Equal importance	The contributions of the two compared plans are equally important
2	Moderate importance	Experience and judgment showing a slight preference for a specific plan
3	Essential strong importance	Experience and judgment showing a strong preference for a specific plan
4	Very strong importance	An extremely strong preference for a specific plan
5	Extreme importance	Sufficient evidence showing an absolute preference for a specific plan

TABLE 3: Eigenfunction of triangular fuzzy numbers.

Fuzzy numbers	Eigenfunctions
1	(1, 1, 1)
2	(1, 3, 5)
3	(3, 5, 7)
4	(5, 7, 9)
5	(7, 9, 9)

Step 3.4. Convert the decision makers' and experts' linguistic variables into triangular fuzzy numbers " \sim " to represent the subjective intensity. This improves the imbalances in the measuring scales of the ANP method to make the evaluation process smoother and define the eigenfunction of the symmetrical triangular fuzzy numbers \sim (see Table 3).

Step 3.5. According to the range analysis method by Chang [59], the triangular membership function was used to represent the evaluation value between each criteria. Let $X = \{x_1, x_2, \dots, x_n\}$ denote the attribute level set and $U = \{u_1, u_2, \dots, u_n\}$ denote the target set. The range of values for each target set attribute level g_i can be calculated and the range analysis value for m is defined as follows:

$$\widetilde{M}_{g_i}^1, \widetilde{M}_{g_i}^2, \dots, \widetilde{M}_{g_i}^n, \quad i = 1, 2, \dots, n, \quad (4)$$

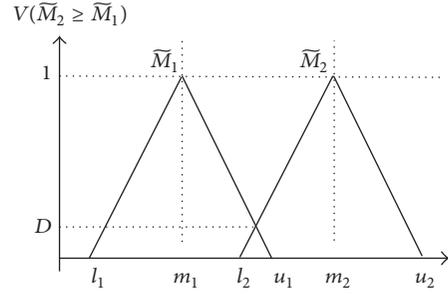
where $\widetilde{M}_{g_i}^j$ ($j = 1, 2, \dots, m$) represents the triangular fuzzy number.

Step 3.6. The fuzzy comprehensive evaluation value according to the i th target is calculated as follows:

$$\widetilde{S}_i = \sum_{j=1}^m \widetilde{M}_{g_i}^j \otimes \left[\sum_{i=1}^n \sum_{j=1}^m \widetilde{M}_{g_i}^j \right]^{-1}. \quad (5)$$

The fuzzy operation of m range analysis values is conducted to obtain $\sum_{j=1}^m \widetilde{M}_{g_i}^j$ whereby a fuzzy analysis matrix is constructed:

$$\sum_{j=1}^m \widetilde{M}_{g_i}^j = \left(\sum_{j=1}^m l_j, \sum_{j=1}^m m_j, \sum_{j=1}^m u_j \right). \quad (6)$$

FIGURE 2: Intersection graph of fuzzy numbers \widetilde{M}_1 and \widetilde{M}_2 .

Through the fuzzy operation of $\widetilde{M}_{g_i}^j$ ($j = 1, 2, \dots, m$), the evaluation value of $[\sum_{i=1}^n \sum_{j=1}^m \widetilde{M}_{g_i}^j]^{-1}$ is obtained:

$$\sum_{i=1}^n \sum_{j=1}^m \widetilde{M}_{g_i}^j = \left(\sum_{j=1}^m l_j, \sum_{j=1}^m m_j, \sum_{j=1}^m u_j \right). \quad (7)$$

The reciprocal value of the vector for (7) is further calculated with the following results:

$$\left[\sum_{i=1}^n \sum_{j=1}^m \widetilde{M}_{g_i}^j \right]^{-1} = \left(\frac{1}{\sum_{j=1}^m u_j}, \frac{1}{\sum_{j=1}^m m_j}, \frac{1}{\sum_{j=1}^m l_j} \right). \quad (8)$$

Step 3.7. The degree of possibility of $\widetilde{M}_2 = (l_2, m_2, u_2) \geq \widetilde{M}_1 = (l_1, m_1, u_1)$ is defined as follows:

$$V(\widetilde{M}_2 \geq \widetilde{M}_1) = \sup_{y \geq x} [\min(u_{\widetilde{M}_1}(x), u_{\widetilde{M}_2}(y))]. \quad (9)$$

Using the concept of triangular membership function, (9) can be rewritten as follows:

$$V(\widetilde{M}_2 \geq \widetilde{M}_1) = \text{hgt}(\widetilde{M}_1 \cap \widetilde{M}_2) = u_{\widetilde{M}_1}(d) = \begin{cases} 1, & \text{if } m_2 \geq m_1 \\ \frac{l_2 - u_1}{(m_2 - u_2) - (m_1 - l_1)}, & \text{if } l_1 < u_2 \\ 0, & \text{otherwise,} \end{cases} \quad (10)$$

where d represents the abscissa value of the intersection of the two membership functions $u_{\widetilde{M}_1}$ and $u_{\widetilde{M}_2}$, and its corresponding ordinate value D is $(\widetilde{M}_2 \geq \widetilde{M}_1)$, meaning a degree whereby the fuzzy number \widetilde{M}_2 is higher than \widetilde{M}_1 (see Figure 2); thus $V(\widetilde{M}_1 \geq \widetilde{M}_2)$ and $V(\widetilde{M}_2 \geq \widetilde{M}_1)$ must be obtained to enable the comparison of the fuzzy numbers \widetilde{M}_1 and \widetilde{M}_2 .

Step 3.8. Define \widetilde{M}_i ($i = 1, 2, \dots, k$) as the probability value of a particular convex fuzzy number that is larger than that of convex fuzzy number k :

$$V(\widetilde{M} > \widetilde{M}_1, \widetilde{M}_2, \dots, \widetilde{M}_k) = V[(\widetilde{M} > \widetilde{M}_1) \text{ and, } (\widetilde{M} > \widetilde{M}_2) \text{ and, } \dots, (\widetilde{M} > \widetilde{M}_k)] \\ = \min V[(\widetilde{M} > \widetilde{M}_i)]. \quad (11)$$

TABLE 4: Unweighted supermatrix.

Goal	Stage			Criteria									Alternatives		
	P1	P2	P3	C1a	C1b	C1c	C2a	C2b	C2c	C3a	C3b	C3c	A1	A2	A3
Goal	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000
P1	0.607	0.580	0.430	0.463	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
P2	0.338	0.097	0.206	0.086	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
P3	0.056	0.323	0.364	0.4510	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C1a	0.000	0.277	0.000	0.000	0.469	0.288	0.440	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C1b	0.000	0.265	0.000	0.000	0.220	0.222	0.084	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C1c	0.000	0.459	0.000	0.000	0.311	0.490	0.476	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C2a	0.000	0.000	0.452	0.000	0.000	0.000	0.000	0.423	0.522	0.402	0.000	0.000	0.000	0.000	0.000
C2b	0.000	0.000	0.303	0.000	0.000	0.000	0.000	0.233	0.098	0.193	0.000	0.000	0.000	0.000	0.000
C2c	0.000	0.000	0.244	0.000	0.000	0.000	0.000	0.344	0.380	0.405	0.000	0.000	0.000	0.000	0.000
C3a	0.000	0.000	0.000	0.268	0.000	0.000	0.000	0.000	0.000	0.000	0.413	0.481	0.279	0.000	0.000
C3b	0.000	0.000	0.000	0.219	0.000	0.000	0.000	0.000	0.000	0.000	0.295	0.272	0.229	0.000	0.000
C3c	0.000	0.000	0.000	0.513	0.000	0.000	0.000	0.000	0.000	0.000	0.292	0.247	0.492	0.000	0.000
A1	0.000	0.000	0.000	0.000	0.670	0.579	0.667	0.272	0.429	0.167	0.218	0.275	0.232	0.470	0.365
A2	0.000	0.000	0.000	0.000	0.112	0.264	0.116	0.242	0.285	0.410	0.331	0.288	0.292	0.095	0.351
A3	0.000	0.000	0.000	0.000	0.218	0.157	0.217	0.486	0.286	0.423	0.452	0.438	0.476	0.435	0.284

Assume

$$d(A_i) = \min V \left[\left(\tilde{S}_i > \tilde{S}_k \right) \right], \quad k = 1, 2, \dots, n; \quad k \neq i. \quad (12)$$

Thus, the weight vector is

$$W' = \left[d'(A_1), d'(A_2), \dots, d'(A_n) \right]^T, \quad (13)$$

where $A_1 (i = 1, 2, \dots, n)$ represents n elements.

Step 3.9. The normalized weight vector is obtained using the normalization operation:

$$W = \left[d(A_1), d(A_2), \dots, d(A_n) \right]^T, \quad (14)$$

where W is a nonfuzzy number.

Step 4. The preferences of experts and scholars are consolidated using the integrative method.

Step 5. The optimal method to facilitate snack food transaction efficiency can be determined by compiling the evaluation factor weights.

Step 6. The optimal channel plans for snack food dealers are selected.

4. Empirical Case Analysis

Using fresh snack foods as the research subject, this study surveyed four experts in snack food sales, marketing, and public relations and four scholars in marketing research. A supermatrix was constructed from the survey data according to the network framework in Figure 1, after which a supermatrix operation was conducted using Super Decisions software. To meet the column-stochastic principle,

the unweighted supermatrix (see Table 4) was subjected to weighting, whereby the preliminary matrix for the weighted supermatrix was obtained (see Table 5). The weighted supermatrix was minimized to obtain the relative weights for each criterion, meaning that the weighted supermatrix was multiplied by $2k + 1$ power, after which the dependencies gradually converged to yield a limiting supermatrix (see Table 6).

The analysis results revealed that in terms of the influence on customers' decision-making in fresh snack food purchases, the transaction costs were in the order of prepurchase, purchase, and postpurchase transaction costs (weights = $0.564 > 0.229 > 0.207$). In terms of each dimension of decision-making, under prepurchase transaction costs, the provision of food safety assurance had the maximal impact (weight = 0.440), followed by providing sufficient information and then food testing. At the purchase stage, protecting personal information leakage had the most notable impact (weight = 0.447), followed by multiple payment methods and waiting time. Finally, at the postpurchase stage, the waiting time for product delivery had the strongest influence (weight = 0.441), followed by order tracking transparency and delivery progress notification; the various decision-making dimensions and their transaction cost factor weights are shown in Table 7. From Table 8, in terms of the ideal sales channels for snack food dealers, retail stores were found to be the optimal mode, followed by home delivery and cash on delivery (weights = $0.438 > 0.360 > 0.202$).

5. Conclusions

Prepurchase transaction costs have the greatest effect on customers' purchasing of fresh snack foods, with "providing food safety assurance," "sufficient information exposure," and "food testing" identified as the top three criteria in the

TABLE 5: Weighted supermatrix.

Goal	Stage			Criteria									Alternatives			
	P1	P2	P3	Cl _a	Cl _b	Cl _c	C2 _a	C2 _b	C2 _c	C3 _a	C3 _b	C3 _c	A1	A2	A3	
Goal	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.500	0.500	0.500
P1	0.607	0.290	0.215	0.232	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
P2	0.338	0.048	0.103	0.043	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
P3	0.056	0.162	0.182	0.226	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Cl _a	0.000	0.138	0.000	0.000	0.235	0.144	0.220	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Cl _b	0.000	0.132	0.000	0.000	0.110	0.111	0.042	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Cl _c	0.000	0.229	0.000	0.000	0.155	0.245	0.238	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C2 _a	0.000	0.000	0.226	0.000	0.000	0.000	0.000	0.211	0.261	0.201	0.000	0.000	0.000	0.000	0.000	0.000
C2 _b	0.000	0.000	0.152	0.000	0.000	0.000	0.000	0.117	0.049	0.096	0.000	0.000	0.000	0.000	0.000	0.000
C2 _c	0.000	0.000	0.122	0.000	0.000	0.000	0.000	0.172	0.190	0.203	0.000	0.000	0.000	0.000	0.000	0.000
C3 _a	0.000	0.000	0.000	0.134	0.000	0.000	0.000	0.000	0.000	0.000	0.207	0.241	0.140	0.000	0.000	0.000
C3 _b	0.000	0.000	0.000	0.110	0.000	0.000	0.000	0.000	0.000	0.000	0.148	0.136	0.115	0.000	0.000	0.000
C3 _c	0.000	0.000	0.000	0.257	0.000	0.000	0.000	0.000	0.000	0.000	0.146	0.123	0.246	0.000	0.000	0.000
A1	0.000	0.000	0.000	0.000	0.335	0.289	0.334	0.136	0.215	0.083	0.109	0.137	0.116	0.235	0.182	0.163
A2	0.000	0.000	0.000	0.000	0.056	0.132	0.058	0.121	0.143	0.205	0.165	0.144	0.146	0.047	0.176	0.108
A3	0.000	0.000	0.000	0.000	0.109	0.078	0.109	0.243	0.143	0.212	0.226	0.219	0.238	0.218	0.142	0.229

TABLE 6: Limiting supermatrix.

Goal	Stage			Criteria									Alternatives			
	P1	P2	P3	Cl _a	Cl _b	Cl _c	C2 _a	C2 _b	C2 _c	C3 _a	C3 _b	C3 _c	A1	A2	A3	
Goal	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143
P1	0.161	0.161	0.161	0.161	0.161	0.161	0.161	0.161	0.161	0.161	0.161	0.161	0.161	0.161	0.161	0.161
P2	0.065	0.065	0.065	0.065	0.065	0.065	0.065	0.065	0.065	0.065	0.065	0.065	0.065	0.065	0.065	0.065
P3	0.059	0.059	0.059	0.059	0.059	0.059	0.059	0.059	0.059	0.059	0.059	0.059	0.059	0.059	0.059	0.059
Cl _a	0.056	0.056	0.056	0.056	0.056	0.056	0.056	0.056	0.056	0.056	0.056	0.056	0.056	0.056	0.056	0.056
Cl _b	0.034	0.034	0.034	0.034	0.034	0.034	0.034	0.034	0.034	0.034	0.034	0.034	0.034	0.034	0.034	0.034
Cl _c	0.071	0.071	0.071	0.071	0.071	0.071	0.071	0.071	0.071	0.071	0.071	0.071	0.071	0.071	0.071	0.071
C2 _a	0.029	0.029	0.029	0.029	0.029	0.029	0.029	0.029	0.029	0.029	0.029	0.029	0.029	0.029	0.029	0.029
C2 _b	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016
C2 _c	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020
C3 _a	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019
C3 _b	0.014	0.014	0.014	0.014	0.014	0.014	0.014	0.014	0.014	0.014	0.014	0.014	0.014	0.014	0.014	0.014
C3 _c	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026
A1	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125
A2	0.058	0.058	0.058	0.058	0.058	0.058	0.058	0.058	0.058	0.058	0.058	0.058	0.058	0.058	0.058	0.058
A3	0.103	0.103	0.103	0.103	0.103	0.103	0.103	0.103	0.103	0.103	0.103	0.103	0.103	0.103	0.103	0.103

overall factor ranking. Food distributors are also committed to providing food safety information and assurance on the food packaging (e.g., international Safe Quality Food and the Certified Agricultural Standards in Taiwan). Food safety incidents in 2008 (infant formula contaminated with melamine in China), 2011 (a plasticizer used as a replacement for palm oil in Taiwan), and 2013 (a series of food scandals in Taiwan) have had a marked effect on the sales, operations, and brand reputations of product manufacturers, dealers, and street vendors in Taiwan and even led to bankruptcy and problems with subsequent compensation. The food industries have adopted various approaches such as issuing press releases, holding press conferences, and even providing raw material

source verifications, product assurance, and food production records at retail stores to restore customer trust. The incidents highlight the practical importance of prepurchase transaction cost factors. The results of this study can also be verified by manufacturers' responses to food safety scandals and their practical operations.

The protection of personal information has the largest effect on customers' purchasing decisions. In practical terms, leakage of such information can be roughly divided into loss of personal information and theft of financial information. To demonstrate efforts aimed at protecting personal information, industries can reinforce customer confidence by providing written guarantees or online confidentiality

TABLE 7: Decision-making dimension and transaction cost factors weights.

Dimension	Weights	Ranking	Criteria	Weights (local)	Weights (global)	Ranking (local)	Ranking (global)
Prepurchasing	0.564	1	C1a Sufficient information exposure	0.347	0.196	2	2
			C1b Food testing	0.213	0.120	3	3
			C1c Food safety assurance provided	0.440	0.248	1	1
Purchasing	0.229	3	C2a Personal information leak prevention	0.447	0.102	1	4
			C2b Waiting time	0.246	0.056	3	8
			C2c Multiple payment methods	0.308	0.071	2	6
Postpurchasing	0.207	2	C3a Order tracking transparency	0.319	0.066	2	7
			C3b Delivery progress notification	0.240	0.050	3	9
			C3c Waiting time for product delivery	0.441	0.091	1	5

TABLE 8: Weights of sales channels.

Alternatives	Retail store	Home delivery	Cash on delivery
Weights	0.438	0.360	0.202

contracts (e.g., mobile payment). Regarding the theft of financial information, assurance can be given by displaying the electronic security mechanism being used to ensure system security and privacy (e.g., secure sockets layer encryption). Customers who are concerned about personal computer system vulnerabilities and online transaction systems naturally prefer to visit stores to purchase items in person. Despite the relative weight of postpurchase transaction costs being the lowest, the waiting time for product delivery was ranked fifth among all transaction cost factors, indicating the high value placed by food manufactures on the level of freshness when products reach their customers. Through the approaches of order notification or instant inquiry, customers can track the product delivery progress and determine whether the products are affected by adverse environmental conditions (e.g., too much time in transit) during the delivery process, which results in unsatisfactory quality. Concurrently, numerous food distributors have also attempted to incorporate multichannel cooperation into their practical operations and have maintained satisfactory food freshness with their deliveries.

The optimal sales channel for snack food dealers is retail stores. Channel decision-makers can not only display products and their complete information but also provide demonstrations and food testing. In particular, customers who are unfamiliar with a product require more information for searching and inquiring about the costs, and dealers can reduce customers' prepurchase costs the most by selling through retail store channels. Many examples of the research findings are evident in food business practices. Some food distributors or microenterprises initially sold their products online, after which they opened physical locations and developed into chain stores. Businesses that were initially brick-and-mortar stores can utilize their stores to develop online sales, thereby achieving home delivery or channels for picking up merchandizes at the specified point.

In this study, the influences of the various transaction cost factors on customers' purchasing behaviors at different stages of purchasing were modeled for discussion. The findings may provide a reference for product suppliers or microenterprise channel development, which can be expanded to marketing and inventory management applications in food industries. In terms of developing the research methods, future studies can compare the ranking of different transaction cost factors or channel assessments under different MCDM approaches, the results of which can be verified through interviews with enterprises or experts.

Conflicts of Interest

The author declares that there are no financial or other conflicts of interest.

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Research Article

Effects of High Hydrostatic Pressure Processing on the Number of Bacteria and Texture of Beef Liver

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Providing beef liver for raw consumption was banned in Japan on July 1, 2012. To lift the ban, the establishment of effective countermeasures for safe raw consumption is necessary. In this study, we examined the effects of high hydrostatic pressure processing on raw beef liver. Beef liver samples subjected to 300 MPa of pressure or higher for 10 min at 25°C became firmer and showed a paler color and were considered unsuitable for raw consumption. More than 3.0 log reductions of bacteria were seen after treatments at 400 and 500 MPa, but the treatment with lower pressure did not show enough microcidal effects for safe consumption. Histological and ultrastructural analysis revealed that high hydrostatic pressure processing increased mitochondrial swelling and reduced rough endoplasmic reticula in hepatocytes, and such changes might be related to the observed changes of texture in the treated raw beef liver.

1. Introduction

Raw meat dishes, such as “steak tartare,” “mett,” and “yukhoe,” are consumed in many countries, in Europe and Asia. In Japan, raw beef meat and liver, called “Gyu-sashi” and “Reba-sashi,” have/had sometimes been consumed. However, triggered by an outbreak of enterohemorrhagic *Escherichia coli* that occurred on April and May 2011, the Ministry of Health, Labour and Welfare of Japan set a new standard for preparing raw beef on October, 2011. The Ministry imposed a ban on serving raw beef liver at restaurants and meat shops in July, 2012, which will remain in effect until effective countermeasures for safe raw consumption are established [1]. Nevertheless, some people in Japan want the ban lifted to have access to raw beef liver dishes.

Animal offal can be highly contaminated with pathogens, such as enterohemorrhagic *E. coli*, *Campylobacter* spp., and *Salmonella* spp. [2–6]. In fact, many cases of food poisoning have been associated with meat and/or offal

consumption [7–10]. For safe consumption, meat and offal are usually cooked with heat. Heat cooking is quite effective in killing pathogens, but it changes the taste, aroma, and texture of foods. Therefore, alternative processing methods are needed to kill pathogens with minimum changes to taste, aroma, and texture, and thereby allow safe consumption of raw meat and offal.

Radiation [11, 12], high-voltage pulse [13], light pulse [14], high pressure [15], ozone [16], and other methods are known to be effective nonheat sterilization procedures. In particular, high hydrostatic pressure (HHP) processing may be potentially an effective method for reducing the risk of raw beef liver without changing taste, aroma, or texture [17, 18]. So far, a single report describing the effects of high pressure processing on the number of pathogenic bacteria in livestock offal has been found in the literature [19].

In this study, we aimed to examine the effects of HHP processing on bacterial reduction and histological changes in raw beef liver.

2. Materials and Methods

2.1. Preparation of Bacteria. A nonpathogenic *E. coli* strain, ATCC25922, was used instead of enterohemorrhagic *E. coli* O157:H7. The strain was kept at -80°C until use and passaged twice in Trypticase Soy broth (Becton Dickinson and Company, Franklin Lakes, NJ, USA) at 37°C before experiments. The culture was centrifuged at 8,000 rpm for 10 min. Cell pellets were washed and resuspended in sterilized phosphate buffered saline (PBS, pH 7.0). Bacterial cell density was adjusted to 1×10^8 or 1×10^9 colony forming units (CFU)/mL.

2.2. Preparation of Beef Liver Samples. Beef liver, which was taken on the previous day and stored under refrigeration, was purchased from Tokyo Shibaura Zouki Co., Ltd. (Tokyo, Japan), a meat broker company. The beef liver was transferred under cold conditions (using refrigerants) to the laboratory and used on the same day. The beef liver was cut into pieces of approximately $2 \text{ cm} \times 3 \text{ cm} \times 0.5 \text{ cm}$, weighing approximately 10 g. The samples were vacuum-packed in plastic bags twice and sealed using a heat sealer. To evaluate the microcidal effects of HHP treatment, $10 \mu\text{L}$ of *E. coli* suspension (in PBS), 1×10^8 CFU/mL, was injected in the sample at each of 10 regularly spaced points using micropipette with sterilized pipette tips before being packed.

2.3. HHP Treatment. Beef liver samples in plastic bags were pressurized with a water-based prototype pressurization apparatus (HPV-80C20-S; Sugino Machine Ltd., Toyama, Japan). Pressurization reached 200 MPa after approximately 60 sec, and decompression took approximately 10 sec. The initial temperature before HHP treatment was always at 25°C ; however, the temperature during HHP treatment was not monitored.

2.4. Bacteria Counting after HHP Treatment. The number of *E. coli* in PBS after HHP treatment was determined by the pour culture method in Trypticase Soy agar (TSA, Becton Dickinson and Company). The number of *E. coli* in beef liver after HHP treatment was determined using TSA and chromogenic XM-G agar (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan), a selective agar for coliforms and *E. coli*. After incubation at 37°C for 24 hr, typical colonies with blue color on XM-G agar were identified as presumptive *E. coli*. The number of bacteria in each sample was calculated from the number of colonies multiplied by the dilution factor.

2.5. Experimental Designs

2.5.1. Effects of HHP Treatment on *E. coli* in PBS. Five milliliters of polypropylene tubes (Greiner Bio-One, Kremsmünster, Austria) was filled with *E. coli* suspension in PBS (1×10^9 CFU/mL) and subjected to HHP at 200, 300, 400, and 500 MPa. The treatment was performed for 10, 20, and 30 min at each pressure at 25°C . The bacterial suspension after HHP treatment was serially diluted with sterilized saline supplemented with 0.1% peptone and inoculated on

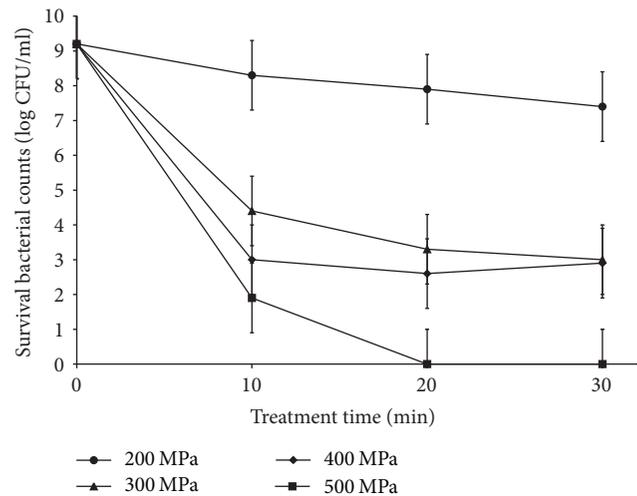


FIGURE 1: Effects of HHP treatment on *E. coli* in PBS.

TSA plates. The limit of detection for this experiment was 1 CFU/mL. The experiments were repeated 3 times.

2.5.2. Effects of HHP Treatment on *E. coli* Inoculated in Liver Samples. Liver samples inoculated with *E. coli* in plastic bags were submitted to HHP at 200, 300, 400, and 500 MPa for 10 min at 25°C . For *E. coli* colony counting, 10 g of liver sample was homogenized in 90 mL of PBS for 2 min using a Stomacher® 400T circulator (Seward Ltd., West Sussex, UK), serially diluted in PBS, and inoculated on TSA and XM-G agar plates. The limit of detection for this experiment was 10 CFU/g. The experiments were repeated 3 times.

2.5.3. Effects of HHP Treatment on Color, Histology, and Ultrastructure of Liver Samples. Liver samples treated at 200, 300, 400, and 500 MPa for 10 min at 25°C were observed macroscopically and then cut into slices. The colors of the samples were measured by colorimeter (CR-200, Minolta Co., Ltd., Osaka, Japan). Results are expressed as a combination of the values of L^* , a^* , and b^* : L^* represents a gradation from light to dark; a^* , red to green; and b^* , yellow to blue.

For light microscopy analysis, samples were fixed in 10% neutral buffered formalin and embedded in paraffin. Thin sections ($4 \mu\text{m}$) were stained with hematoxylin and eosin (HE).

For electron microscopy analysis, small pieces of liver samples were prefixed in MacDowell's and Trump's 4F:1G fixative [20] and postfixed in 1% osmium tetroxide, followed by 0.2 M phosphate buffer, and then embedded in epoxy resin. Using an electron microscope (JEM1011; JEOL Ltd., Tokyo, Japan), ultrathin sections were examined after staining with uranyl acetate and lead citrate.

3. Results

3.1. Effects of HHP Treatment on *E. coli* in PBS. The number of *E. coli* in PBS before HHP treatment was 9.0 log CFU/mL (Figure 1). No significant change was found in the number

TABLE 1: Colorimetric values of liver samples before and after HHP treatment.

Treatment	L^*	a^*	b^*
0.1 Mpa	36.7 ± 1.3	6.5 ± 0.6	2.2 ± 0.3
200 MPa	38.1 ± 1.4	6.7 ± 0.5	0.5 ± 0.4
300 MPa	$44.3 \pm 1.1^{\dagger\dagger}$	$10.1 \pm 1.0^{\dagger\dagger}$	2.2 ± 1.4
400 MPa	$47.7 \pm 1.9^{\dagger\dagger}$	$10.9 \pm 0.4^{\dagger\dagger}$	$6.2 \pm 0.7^{\dagger\dagger}$
500 MPa	$50.4 \pm 0.4^{\dagger\dagger}$	$10.1 \pm 0.2^{\dagger\dagger}$	$8.0 \pm 0.6^{\dagger\dagger}$

$^{\dagger\dagger}p < 0.01$.

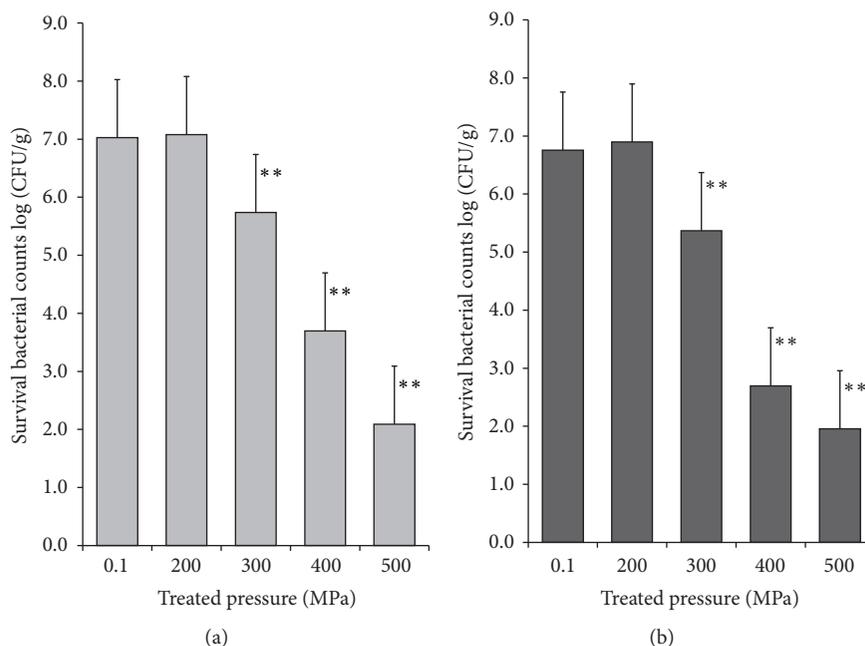


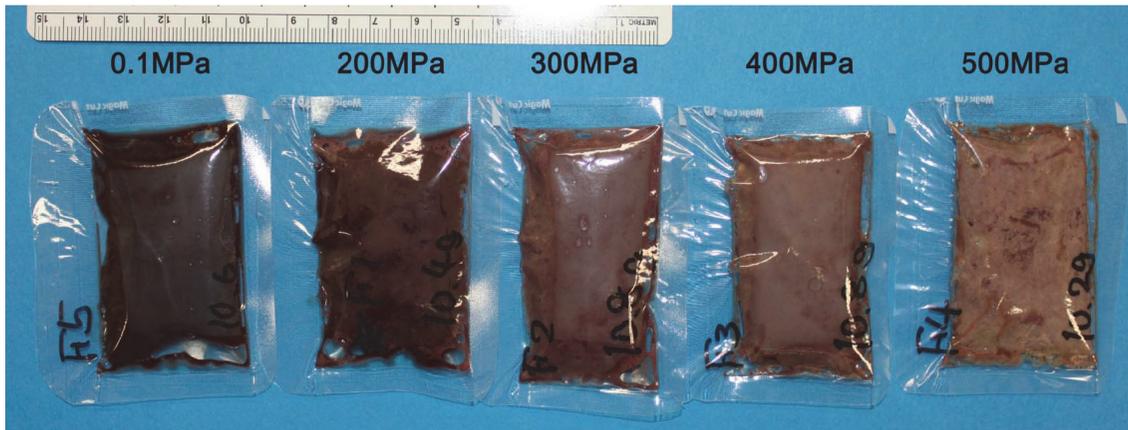
FIGURE 2: (a) The number of *E. coli* in the liver samples treated with high pressure measured (TSA plates). $^{**}p < 0.01$ compared with nontreated samples (expressed as 0.1 MPa, because of the atmospheric pressure). (b) The number of *E. coli* in the liver samples treated with high pressure measured (XM-G agar plates). $^{**}p < 0.01$ compared with nontreated samples (expressed as 0.1 MPa, because of the atmospheric pressure).

of *E. coli* after HHP treatment at 200 MPa for 10 min, but a slight decrease was observed after 20 min and a decrease of 1.0 log in cell number was seen after 30 min HHP treatment. Treatments at higher pressures and longer periods led to more rapid and significant decreases in the number of *E. coli*. Treatment at 500 MPa for 10 min led to a decrease in the number of *E. coli* to 2.5 log CFU/mL, and after 20 or 30 min the number of viable cells was below the detection limit (1 CFU/mL).

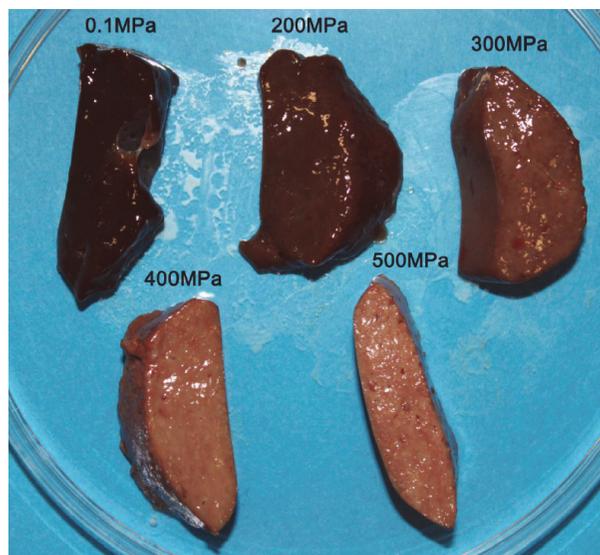
3.2. Effects of HHP Treatment on *E. coli* Inoculated in Liver Samples. Figures 2(a) and 2(b) show the number of *E. coli* in the liver samples treated with high pressure counted on TSA and XM-G agar plates, respectively. Nontreated liver samples were expressed as 0.1 MPa in the figures, because of the atmospheric pressure. The number of bacteria spontaneously contaminated in the liver was $5.4 \pm 0.7 \times 10^3$ CFU/g as viable aerobic bacteria on TSA agar plates, $7.0 \pm 3.0 \times 10^1$ CFU/g

as coliform (red colony), and $6.3 \pm 4.5 \times 10^1$ CFU/g as *E. coli* (blue colony) on XM-G agar plates, respectively. The number of *E. coli* in nontreated (*E. coli* inoculated) liver sample was approximately 7.0 log CFU/g. After treatment at 200 MPa, no significant change was observed in the number of *E. coli*. In contrast, a reduction of 1.5, 3.0, and 5.0 log CFU/g was found at 300, 400, and 500 MPa, respectively.

3.3. Effects of HHP Treatment on Color, Histology, and Ultrastructure of Liver Samples. Liver samples in plastic bags after HHP treatment are shown in Figure 3(a); cut surfaces are shown in Figure 3(b). Liver sample volumes did not change after high pressure treatment compared with nontreated samples. The color of liver samples became paler, from reddish brown to whitish brown, under high pressure treatment. Colorimetric results of liver samples are shown in Table 1. The color of liver samples treated with high pressure showed higher L^* , a^* , and b^* values. In addition,



(a)



(b)

FIGURE 3: (a) Liver samples after HHP treatment. (b) Cut surfaces of liver after HHP treatment.

liver samples submitted to high pressure showed a firmer consistency when cutting. Nontreated liver samples were expressed as 0.1 MPa in the figures and the table, because of the atmospheric pressure.

Histological analysis showed no obvious changes in lobular structures and funicular arrangements of liver cells treated with high pressure (Figure 4(a)) when compared to nontreated samples. However, hepatocytes showed a faintly eosinophilic cytoplasm containing diffusely eosinophilic granules that increased in a pressure-dependent manner (Figure 4(b)). Small eosinophilic particles were also observed in blood vessels (Figure 4(b)).

Ultrastructural analysis showed swollen mitochondria containing dense amorphous granules in hepatocytes (Figure 5(a)). The size of the amorphous granules increased with high pressure treatment (Figure 5(b)), and the rough endoplasmic reticula disappeared completely (Figure 5(c)).

4. Discussion

The Ministry of Health, Labour and Welfare of Japan has banned providing beef liver for raw consumption and requires it to be cooked at 63°C for at least 30 min, or any other heat condition (e.g., at 75°C for at least 1 min) with proven equivalent microcidal effects [21]. However, some people in Japan have been waiting for the establishment of effective countermeasures and for the ministry to lift the ban.

In European countries such as France and Germany, raw ground meat is consumed and is considered a public health concern. Black et al. [22], Hsu et al. [23], and Jiang et al. [24] reported that high pressure processing of ground meat is potentially effective in reducing the risk of ingesting pathogenic bacteria.

Uenaka et al. [19] reported that *E. coli*, *Salmonella* spp., and *Staphylococcus aureus* inoculated into beef liver are killed effectively by high pressure processing (400 MPa for 10 min

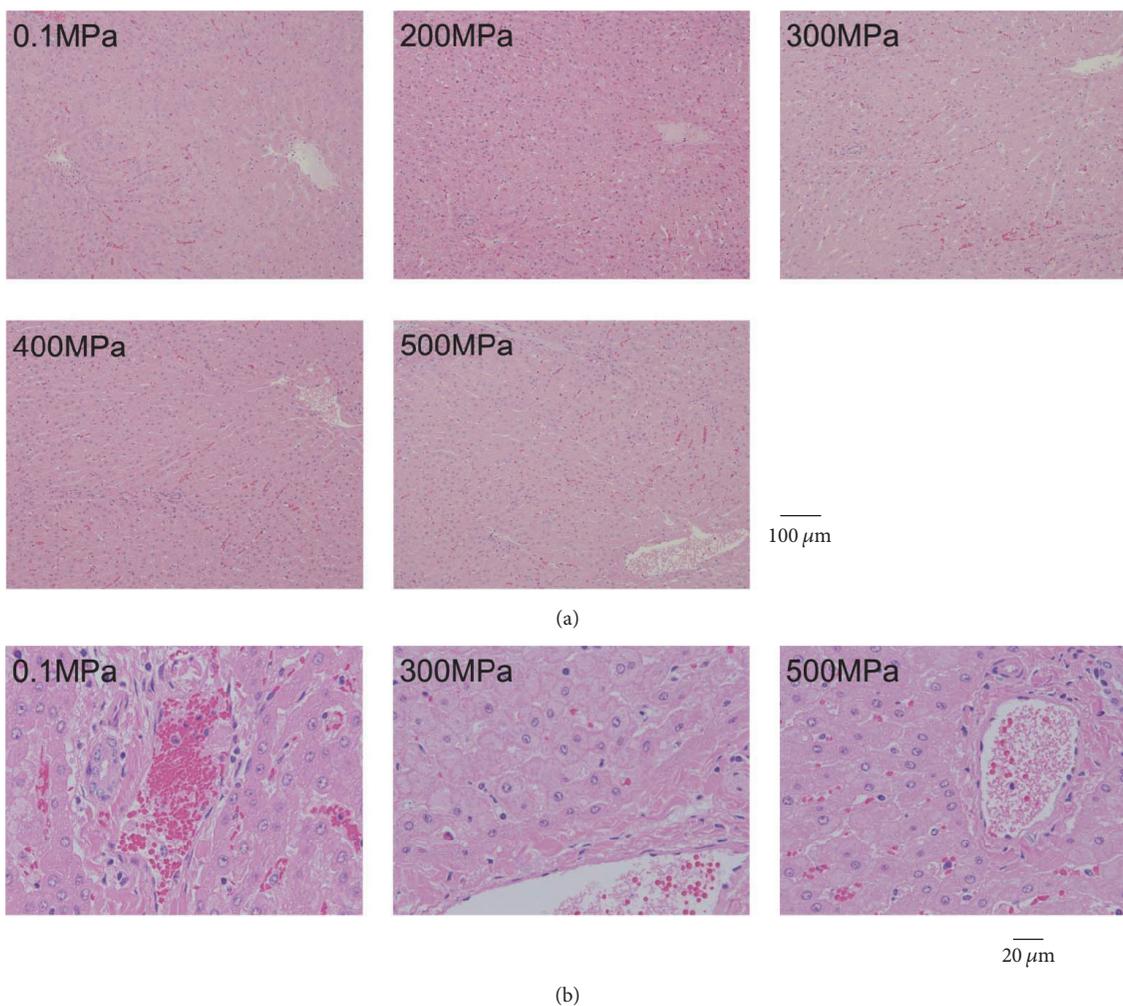


FIGURE 4: (a) Histological analysis of liver after HHP treatment (low magnification). (b) Histological analysis of liver after HHP treatment (high magnification).

6 times, or 600 MPa for 30 min once, at room temperature). They also reported that beef liver treated 6 times with 400 MPa for 10 min at room temperature showed a paler color but its taste was similar to that of untreated beef liver as evaluated by sensory tests. In this study, liver samples treated at 300 MPa or higher for 10 min also showed a paler color, and samples treated with 400 MPa or 500 MPa showed a firmer consistency and were not considered raw. No sensory tests were performed in this study. In future studies, the tenderness of treated liver samples will be measured using a rheometer.

Histological and ultrastructural changes of the raw beef liver by HHP treatment were first reported in this study. The changes were more severe in liver samples treated with higher pressure, both histologically and ultrastructurally. The eosinophilic granules seen dispersed in hepatocytes under light microscopy are thought to correspond to the dense amorphous granules observed in mitochondria under electron microscopy. Such changes are thought to underlie

changes in texture of beef liver. The small eosinophilic particles observed in blood vessels are thought to be the debris of red blood cells. The destruction of red blood cells and less eosinophilic cytoplasm of hepatocytes may be related to the paler color of liver treated with high pressure.

We conclude that HHP treatment at 300 MPa or higher for 10 min is unsuitable for raw consumption of beef liver. However, lower pressure treatment did not show enough microcidal effects for safe consumption. In future studies, combinations of shorter/longer duration, single/multiple treatment(s), and lower/higher temperature under lower pressure will be performed to find the optimal conditions to minimize health risks and keep texture of raw beef liver.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

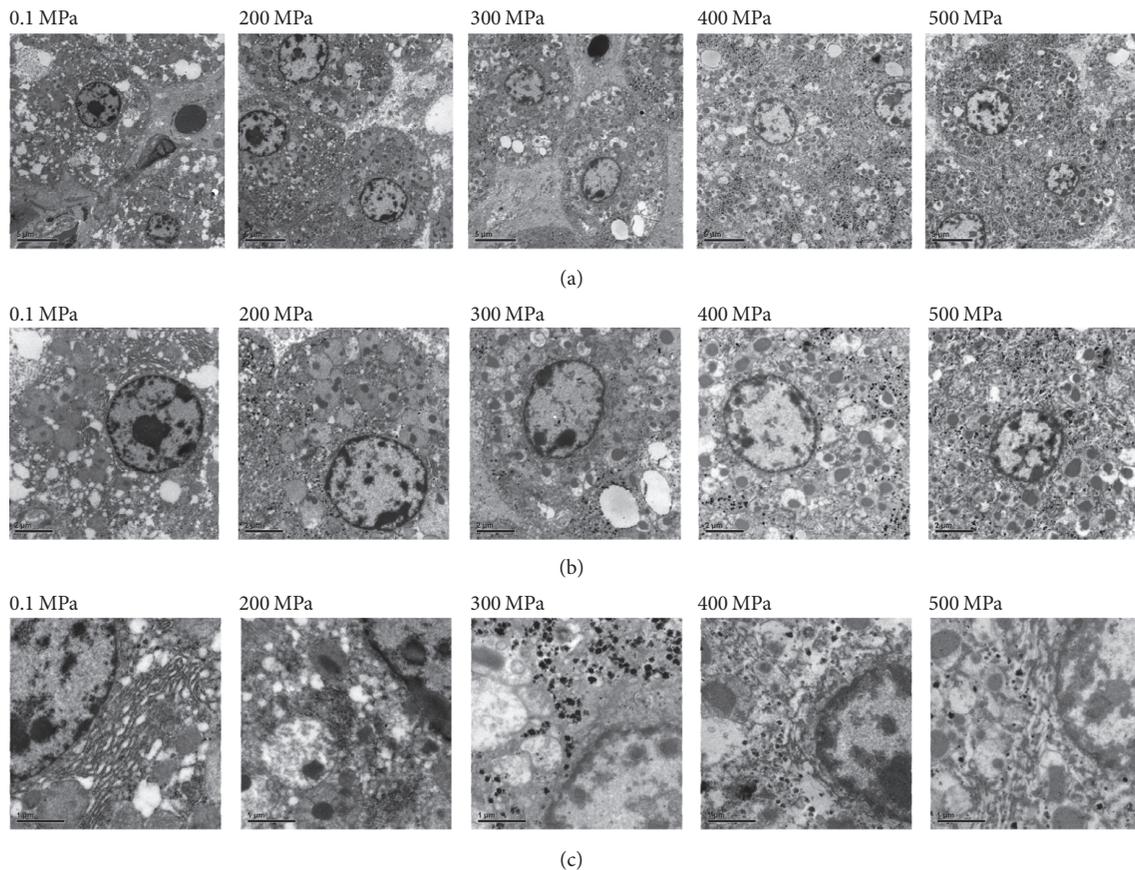


FIGURE 5: (a) Ultrastructural analysis of liver after HHP treatment (hepatocyte). (b) Ultrastructural analysis of liver after HHP treatment (mitochondria). (c) Ultrastructural analysis of liver after HHP treatment (rough endoplasmic reticulum).

Acknowledgments

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Research Article

Effect of Frying Treatments on Texture and Colour Parameters of Deep Fat Fried Yellow Fleshed Cassava Chips

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Effects of frying treatments on texture (hardness) and colour parameters (L , a , b , ΔE) during deep fat frying of yellow fleshed cassava root slices (TMS 01/1371) were investigated. Slices (dimension of 40 mm × 25 mm × 3 mm) were divided into three portions and subjected to vacuum frying (fresh slices) and atmospheric frying (fresh and predried slices) and equivalent thermal driving forces (ETDF) of 60°C, 70°C, and 80°C were maintained during frying. The quality attributes investigated were best preserved in vacuum fried chips. The overall colour change in chips fried under vacuum conditions at 118°C and 8 min was the least (21.20) compared to fresh and atmospherically predried ones (16.69 and 14.81, resp.). A sharp reduction in the breaking force was obtained for all frying treatments after 8 min and this effect was the least in vacuum fried chips. First-order kinetics modeled the changes in quality attributes for all the temperatures investigated. Rate constants k (min^{-1}) obtained for vacuum frying were almost equal to that of atmospheric frying while activation energies for hardness and colour change were 53.30 and 467.11 KJ/mol, respectively. Quality attributes studied were best preserved during vacuum frying.

1. Introduction

Frying as a complex unit operation is essentially a cooking process that has been widely used in the food industry. Traditionally, it involves the immersion of foods in frying oil chambers, mostly at temperatures above the boiling point of water, bringing about a counter-flow of water bubbles and oil on the surface of the product [1]. Texture is a quality attribute which is important in determining the acceptability of fried products as the resulting texture of fried foods is dependent on the properties of raw materials such as starch content, size of starch granules, cell wall polysaccharides, nonstarch polysaccharides, pectin substances, and the processing conditions which include frying time and temperature [2]. Hardness is a key texture parameter and it depends on physical and chemical changes such as release of intracellular materials, starch gelatinization, dehydration, crust formation, breakdown of adhesive forces between cells,

water evaporation, and tissue expansion [3]. The resulting colour of foods processed in a high heat unit operation is also a major quality attribute as it plays an important role in the perception of consumers about the foods at the point of purchase. Parameters of colour such as lightness (L), redness (a), and yellowness (b) have been widely used in recent times to evaluate colour changes between the raw food materials and final products [4].

Deep fat frying is traditionally carried out at atmospheric pressure conditions and high temperatures of up to 180°C (atmospheric frying). However, it had been proven that frying under atmospheric conditions leads to loss of desirable colour and textural characteristic of the resulting products [5]. This could be attributed to destruction of colour components of foods and excessive crust formation due to high frying temperatures. Prefrying treatments aimed at improving the resulting colour and texture of fried food products had earlier been investigated. Califano and Calvelo [6] used blanching as

a pre-frying treatment in potato chips to improve the colour and texture of the resulting products while Krokida et al. [7] used pre-drying to improve these quality attributes. Vacuum frying had been employed as a frying method aimed at preserving the quality attributes of fried foods because it is carried out at reduced atmospheric pressure conditions and hence lower frying temperatures [8].

Since frying leads to changes in quality attributes in fried food products, Hindra and Baik [9] reported that the study of kinetics of these changes during frying is of critical importance because the knowledge of kinetic parameters during the process will help to predict the final quality changes and permit improvement of product value through proper selection of processing conditions. Vitrac et al. [10] studied the kinetics of colour changes in fried chips obtained from white fleshed cassava, Nourian and Ramaswamy [2] and Nourian and Ramaswamy [4] studied the kinetics of change in texture and colour parameters during frying of potato chips, and Odenigbo et al. [11] studied the kinetics of changes in quality attributes during frying of different cultivars of potato. However, information on kinetics of changes in texture and colour parameters in deep fat fried yellow fleshed cassava chips is scarce in literature.

The objective of this research is to study the effect of changes in frying treatments on the texture and colour parameters during deep fat frying of yellow fleshed cassava chips and to specifically investigate changes in these quality attributes at each frying time for vacuum and atmospheric frying, determine kinetic parameters for these changes, and evaluate the activation energies for changes of these quality attributes with respect to each frying treatment.

2. Materials and Methods

We followed the methods of [12]. Healthy and freshly harvested YFCR (TMS 01/1371) were obtained from the International Institute of Tropical Agriculture, Ibadan, Nigeria, and palm olein used for frying was sourced from a local market in Abeokuta, Nigeria.

2.1. Sample Preparation. The yellow fleshed cassava roots were peeled, washed, and carefully sliced using very sharp stainless steel knives into a regular rectangular shape with the dimension 40 mm × 25 mm × 3 mm. Samples were divided into three portions where the first two were fried under vacuum and atmospheric conditions while the third portion was pre-dried to 75% of the initial moisture content in a cabinet drier before atmospheric frying.

2.2. Frying Experiments

2.2.1. Atmospheric Frying Experiments. Twenty (20) slices per sample were placed inside the frying basket of deep fat fryer (Bush Domestic FCO300, UK) and were covered with a metal lid in order to prevent floatation of samples in the frying oil during frying. Frying was carried out at 160°C, 170°C, and 180°C for 2 to 12 minutes. This was done for fresh slices and also for pre-dried samples. After each frying time, the samples were packaged in Ziploc packaging films for further analyses.

TABLE 1: Experimental conditions for atmospheric and vacuum frying treatments.

Frying conditions	Temperatures (°C)		
	Atmospheric	160	170
Vacuum (8.5 mmHg)	108	118	128
ETDF	60	70	80

ETDF: equivalent thermal driving force.

2.2.2. Vacuum Frying. Twenty (20) samples were placed inside the frying basket of vacuum fryer (Model: VF 30, Agrindo Cipta Mandiri, Indonesia). To achieve this, the pressure of the fryer was set at 8.5 mmHg equivalent to 48°C boiling point of water and was maintained throughout the vacuum frying experiments. Frying was carried out at 108°C, 118°C, and 128°C for 2 to 12 min. This was done for fresh slices alone. These experimental conditions are as presented in Table 1. In order to compare samples fried under both atmospheric and vacuum conditions, the concept of equivalent thermal driving force (ETDF) was used. ETDF is the difference between the frying oil temperature and the boiling point of water at the working pressure.

2.3. Analyses of Samples

2.3.1. Texture Analysis. Maximum breaking force of fried chips was determined in a Texture Analyser TA.XT (Stable Micro System, Surrey, UK) using the method described by [3].

2.3.2. Colour Analysis

Image Acquisition and Capture. As used by Mariscal and Bouchon [5], images of fried samples were captured using a colour digital camera model PowerShot A70 (Canon USA) placed on the perforated surface of a large box impervious to light with internal black surfaces. The lighting system inside the box consisted of four CIE source D65 lamps (60 cm length and 18 W) placed above the samples at 45° angle to maximize diffuse reflection responsible for colour. The angle between the camera and the lens axis and the sample was maintained around 90° to reduce gloss. Images were acquired after frying. High resolution images acquired were stored in JPEG (Joint Photographic Experts Group) format in RGB colour coordinates. Thereafter, RGB colour images were converted to lab values using Adobe Photoshop CS6 software (Adobe System Inc., San Francisco, USA), where L^* , a^* , and b^* values were obtained according to the equations below:

$$\begin{aligned}
 L^* &= \frac{L}{255} \times 100, \\
 a^* &= a \times \frac{240}{255} - 120, \\
 b^* &= b \times \frac{240}{255} - 120.
 \end{aligned} \tag{1}$$

Also, from L , a , and b values obtained, the colour change ΔE was obtained using the relation:

$$\Delta E^* = \left[(L_o^* - L^*)^2 + (a_o^* - a^*)^2 + (b_o^* - b^*)^2 \right]^{1/2}. \quad (2)$$

2.4. Kinetics of Changes in Texture and Colour Parameters. First-order equation (3) was applied in modeling the changes in the texture parameter while colour properties were modeled using modified first-order equation (4). Arrhenius graphs were also plotted and the activation energies for each frying methods were determined (5).

First-order kinetics equation:

$$\ln \frac{dP}{P} = kt. \quad (3)$$

Modified first-order equation:

$$\ln \frac{(P_t - P_{\min})}{(P_{\max} - P_{\min})} = kt. \quad (4)$$

Arrhenius equation:

$$\ln k = - \left[\frac{E_a}{RT} \right] \left[\frac{1}{T} \right] + \ln k_o, \quad (5)$$

where P is the quality attribute, k is the first-order rate constant (min^{-1}), t is the frying time, E_a is the activation energy (kJ/mol), R is gas constant ($\text{J K}^{-1} \text{mol}^{-1}$), T is temperature (Kelvin), and k_o is frequency factor.

2.5. Data Analysis. Statistical analysis was carried out on the data obtained using data fit version 9.0 (Oakdale Engineering, 2008). Experimental data obtained were fitted into the first-order kinetics model and the applicability of the model was ascertained with R^2 values obtained being close to 1. Significant differences ($p < 0.05$) were ascertained at 5% level in the quality attributes for different frying conditions using SPSS version 16.0.

3. Results and Discussions

3.1. Texture Parameter (Hardness). The textural properties of fried starch based products are influenced by gelatinization of starch, sugar content, and the strength of the cell wall [13]. In this study, the parameter of texture measured is hardness and is indicated by the breaking force (N). As presented in Figure 1, a rapid increase in the breaking force was observed at lower frying times for all frying methods used. The increase could be a result of rapid moisture loss on the surface of fried chips. During food frying, loss of surface moisture brings about dehydration of crusts, which results in increase in hardness of foods [14].

A rapid decrease in the maximum breaking force was noticed from frying times of 8–12 min. for all frying treatments (Figure 1). This sharp decrease was most prominent in predried atmospheric fried chips. Sharp decrease in breaking

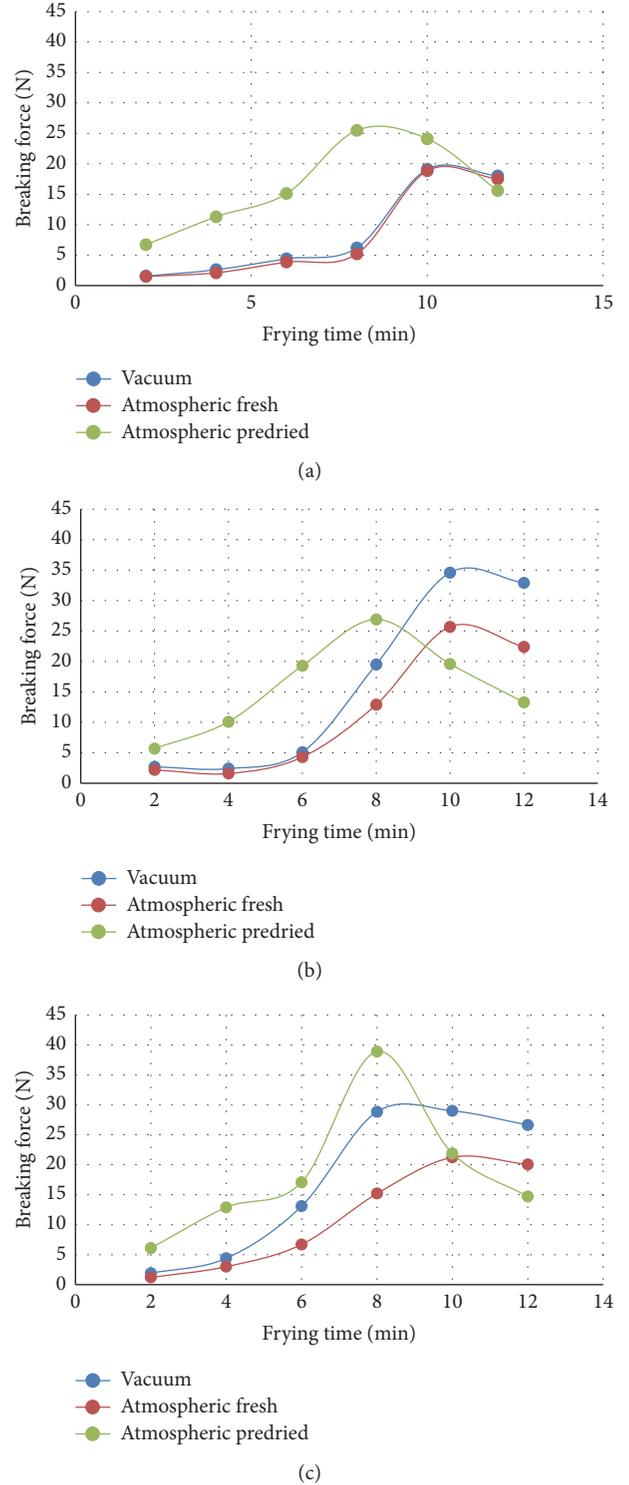


FIGURE 1: Effect of frying conditions on breaking force (N) of yellow fleshed cassava chips (YFCC) at different equivalent thermal driving forces (ETDF) of (a) 60°C, (b) 70°C, and (c) 80°C.

force had been attributed to higher extent of gelatinization of the native starch of cassava at extended frying times [15] and the highest reduction observed in predried chips could be due

TABLE 2: Kinetic parameters for quality attributes at different frying conditions.

Quality attributes	Frying treatments	Frying temp (°C)	k (min ⁻¹)	R^2	
Texture (hardness)	Vacuum	108	0.2655	0.95	
		118	0.3121	0.89	
		128	0.2807	0.83	
	Atmospheric (fresh)	160	160	0.1062	0.18
			170	0.3004	0.88
			180	0.2967	0.91
		Atmospheric (predried)	160	0.1003	0.56
			170	0.0936	0.39
			180	0.0972	0.35
	Lightness	Vacuum	108	-0.2526	0.67
			118	-0.3437	0.86
			128	-0.2085	0.95
Atmospheric (fresh)		160	160	-0.2520	0.81
			170	-0.3502	0.97
			180	-0.3446	0.90
		Atmospheric (predried)	160	-0.2219	0.93
			170	-0.3626	0.89
			180	-0.4945	0.67
Redness		Vacuum	108	0.1842	0.71
			118	0.3202	0.59
			128	0.0957	0.79
	Atmospheric (fresh)	160	160	0.1733	0.91
			170	0.1531	0.87
			180	0.2795	0.87
		Atmospheric (predried)	160	0.1319	0.88
			170	0.1997	0.84
			180	0.5698	0.83
	Yellowness	Vacuum	108	-0.1744	0.67
			118	-0.5729	0.86
			128	-0.2837	0.95
Atmospheric (fresh)		160	160	-0.3428	0.85
			170	-0.4725	0.72
			180	-0.2125	0.79
		Atmospheric (predried)	160	-0.6792	0.91
			170	-0.4841	0.97
			180	-0.5099	0.92

to the reduction in moisture content before frying, leading to a high rate of crust formation [5]. Structural degradation is a resultant effect of increased moisture loss due to increase in frying oil temperatures across the all the frying methods used. Similar trend was reported by Nourian and Ramaswamy [2].

Vacuum fried chips showed a higher rate of increase in breaking force at lower frying times. This could be due to a faster rate of surface moisture removal and microstructural changes during vacuum frying. Such higher rate of moisture removal had been earlier reported by Mariscal and Bouchon [5]. However, the rapid reduction in the breaking force from 8 min frying time was the least in vacuum fried chips. This behavior could be attributed to the reduced boiling point

of water at vacuum conditions and this brought about a slower rate of moisture removal throughout frying leading to reduced structural degradation and less crust formation compared to atmospheric frying. As such, vacuum fried chips were the crispiest and less crusty chips and hence could be more acceptable.

The kinetic parameters obtained for the texture parameter (hardness) are as presented in Table 2. Rate constants k (min⁻¹) for each of the frying temperatures and activation energies E_a (KJ/mol) for each of the frying treatments were determined. For vacuum frying temperatures (108°C, 118°C, and 128°C) investigated, rate constants obtained were 0.2655, 0.3121, and 0.2807 min⁻¹ while for atmospherically fried

TABLE 3: Activation energies for different frying experiments.

Quality attribute	Frying treatment	Activation energy (kJ/mol)
Texture (hardness)	Vacuum	53.30
	Atmospheric (fresh)	1220.57
	Atmospheric (predried)	37.47
Lightness	Vacuum	737.52
	Atmospheric (fresh)	218.65
	Atmospheric (predried)	372.17
Redness	Vacuum	587.72
	Atmospheric (fresh)	557.30
	Atmospheric (predried)	1720.70
Yellowness	Vacuum	584.06
	Atmospheric (fresh)	446.34
	Atmospheric (predried)	341.60

samples (fresh and predried) the values of 0.1062, 0.3004, and 0.2967 min^{-1} and 0.1003, 0.0936, and 0.0972 min^{-1} were obtained at 160°C , 170°C , and 180°C , respectively. The rate constants at lower vacuum frying temperatures were higher than those obtained at their equivalent temperatures for atmospheric frying and this indicates that desired hardness of fried cassava chips could be achieved at a higher rate during vacuum frying at reduced frying temperatures. Also, the lowest values of rate constants were obtained for predried, atmospherically fried samples. The values of activation energies obtained were 53.30, 1220.57, and 37.47 KJ/mol for vacuum and atmospheric frying (fresh and predried), respectively (Table 3). The low activation energy for vacuum frying indicates that frying proceeded at lower energy requirement when compared with atmospheric frying [12] and is less sensitive to temperature changes during frying [10].

3.2. Colour Parameters (Lightness, Redness, and Yellowness and Total Colour Change). The changes observed in the colour attributes of yellow fleshed cassava slices are as presented in Figures 2–5. Lightness (L^*), a critical colour parameter of fried foods, is usually used as a quality control determinant and so its adequate control is of utmost importance [5]. For all the frying treatments in this study, L^* values decreased with increase in temperature. This trend is similar to what was observed by Kumar et al. [16]. The least loss in lightness was observed during the vacuum frying of fresh slices and is greatest in fresh atmospheric fried chips. The reduction in lightness may be attributed to intense browning reaction and increase crust formation due to exposure to high temperature, especially in atmospheric fried chips. Lightness

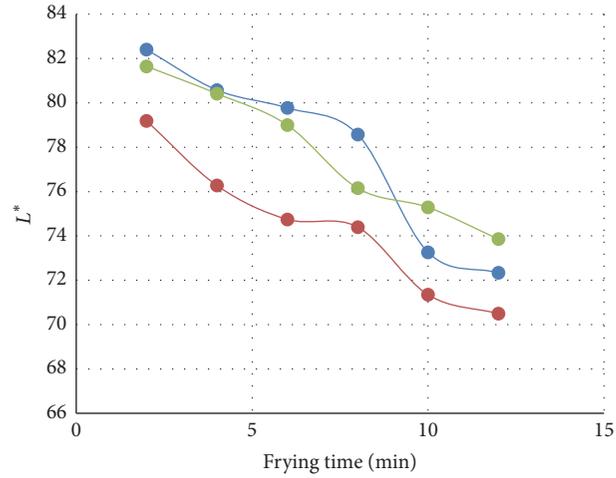
was more preserved in pretreated atmospheric fried samples compared to fresh, atmospherically fried ones. Mariscal and Bouchon [5] reported that there was no significant loss in lightness of predried and vacuum fried apple slices from 10 min frying time.

It was also observed that the rate of reduction in the values for lightness parameter at higher frying temperatures (i.e., 10 to 12 min) is slower compared to the rate observed at lower frying times across all frying methods and temperatures. Similar behavior in fried potato chips was reported by Nourian et al. [17]. Since lightness is a very important colour quality parameter, lower frying temperatures (especially at vacuum conditions) with lower boiling point of water are preferable to preserve the lightness and hence the attractiveness of fried products.

Redness is an undesirable quality factor in fried foods [7]. Increase in redness shows increased crust development, resulting in lower acceptability. The increase in redness for all frying treatments may imply that all fried chips experienced increase in browning with increased frying temperature and time. This could be due to Maillard reaction resulting from the utilization of available reducing sugars [11]. Redness was highest in fresh, atmospherically fried chips. Increase in redness with increased frying temperature was the least during vacuum frying (Figure 3). Also, frying at a reduced temperature in atmospheric condition reduced the level of redness for each frying time studied. These observations are in line with the experimental results of [11]. Redness was the least in vacuum fried chips and hence could be the most preferable by consumers.

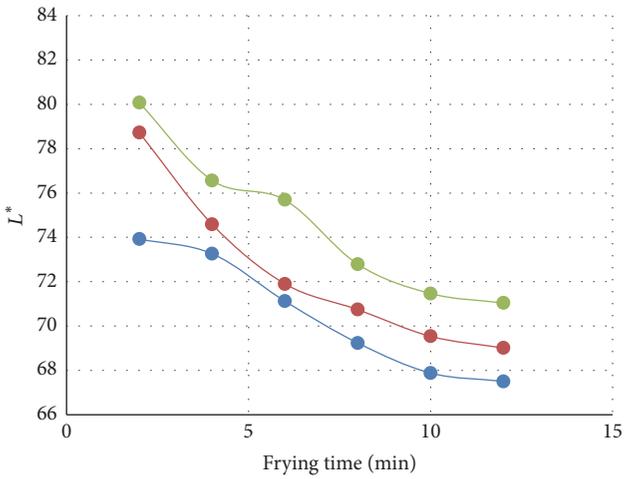
The fresh samples of cassava roots fried were yellow in colour due to fortification with carotenoids. Hence, preservation of yellowness in chips resulting from yellow fleshed cassava roots would be a desirable quality attribute of the chips and can be a major determinant for acceptance of fried yellow fleshed cassava chips. Generally, yellowness of fried chips decreased with increase in frying temperatures. It was also observed, as in lightness and redness, that yellowness had the least value for fresh atmospheric fried chips. This could be traced to the intensity of frying temperatures of untreated slices. Yellowness was the best preserved during vacuum frying and the least preserved during the atmospheric frying of fresh slices.

Vacuum fried chips had the least overall colour change compared with the atmospheric fried chips. This implies that the colour deviation of the resulting chips from the fresh slices due to processing conditions was the least in vacuum fried chips for all the frying times investigated. A similar trend had also been reported for fried apple slices by Mariscal and Bouchon [5]. Also, since the colour change is also a reflection of the extent of degradation of the total carotene content, it could further be established that vacuum fried chips had the highest total carotene retention levels. Colour changes were most pronounced in predried chips. This could be because of the initial exposure of fresh slices to drying at 80°C before frying and also due to a higher extent of surface crust formation. These findings agree with the report of Tran [18].



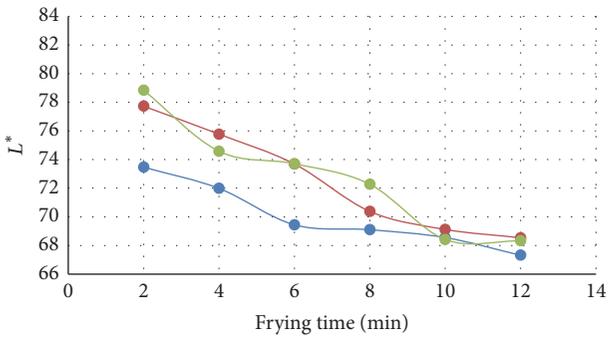
● Atmospheric fresh
● Atmospheric predried
● Vacuum

(a)



● Atmospheric fresh
● Atmospheric predried
● Vacuum

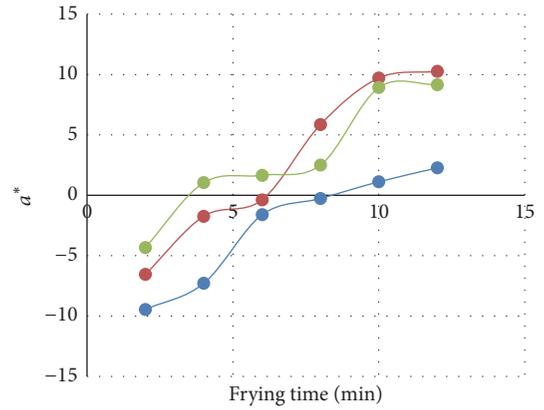
(b)



● Atmospheric fresh
● Atmospheric predried
● Vacuum

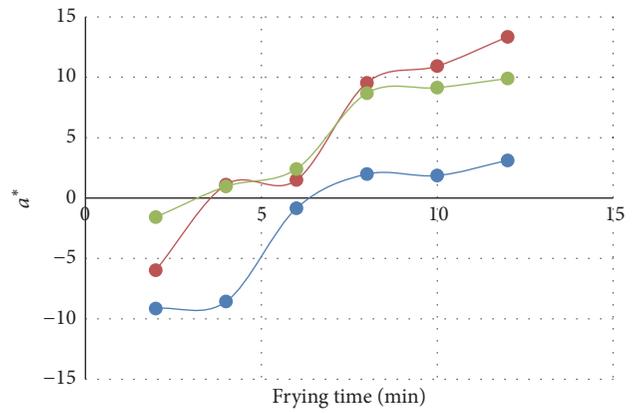
(c)

FIGURE 2: Effect of frying conditions on lightness of yellow fleshed cassava chips (YFCC) at different equivalent thermal driving forces (ETDF) of (a) 60°C, (b) 70°C, and (c) 80°C.



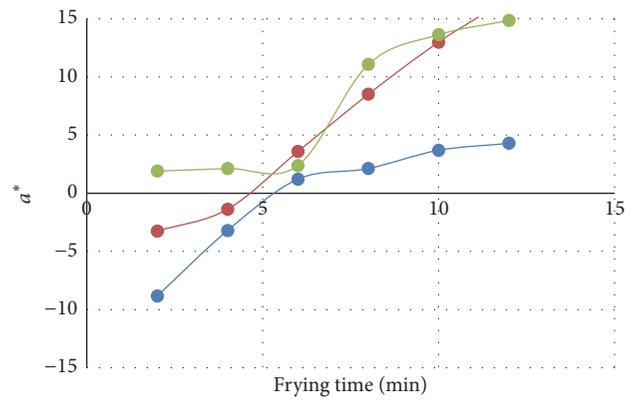
● Vacuum
● Atmospheric fresh
● Atmospheric predried

(a)



● Vacuum
● Atmospheric fresh
● Atmospheric predried

(b)



● Vacuum
● Atmospheric fresh
● Atmospheric predried

(c)

FIGURE 3: Effect of frying conditions on redness of yellow fleshed cassava chips (YFCC) at different equivalent thermal driving forces (ETDF) of (a) 60°C, (b) 70°C, and (c) 80°C.

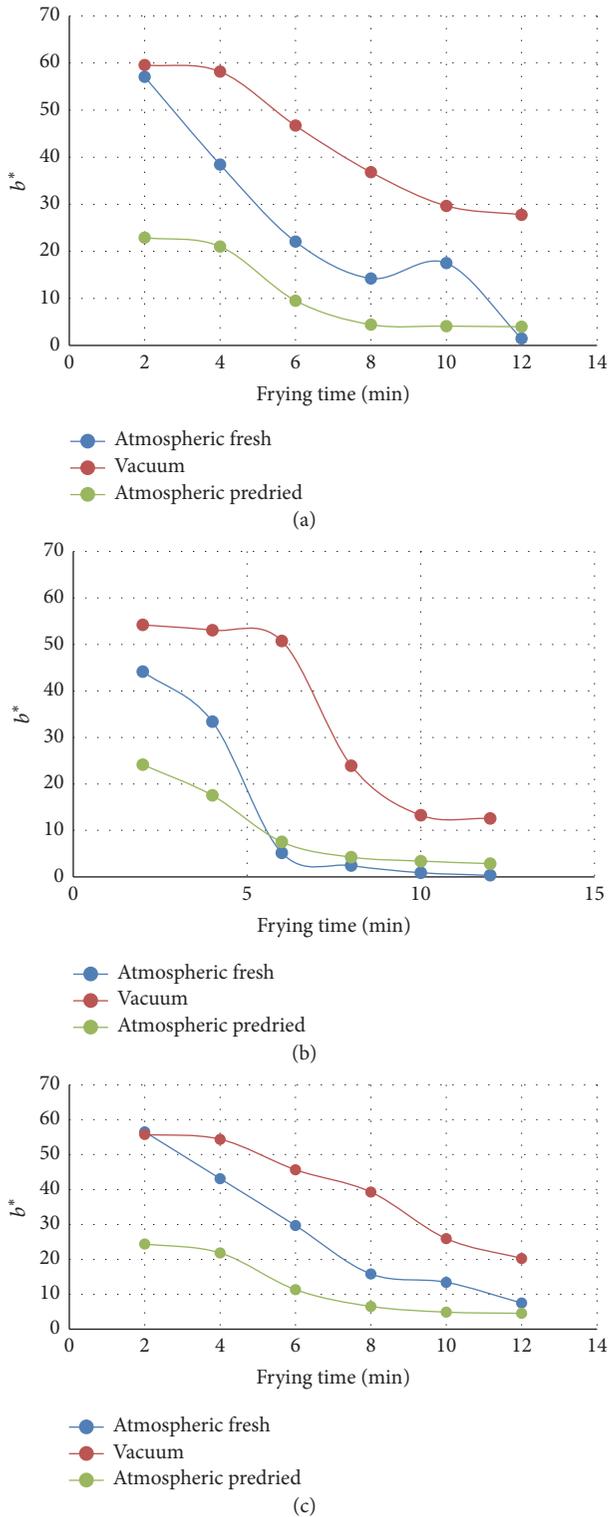


FIGURE 4: Effect of frying conditions on yellowness of yellow fleshed cassava chips (YFCC) at different equivalent thermal driving forces (ETDF) of (a) 60°C, (b) 70°C, and (c) 80°C.

As illustrated by the kinetic parameters presented in Table 2, despite the fact that vacuum frying was achieved at reduced frying temperature, the rate constants were close in

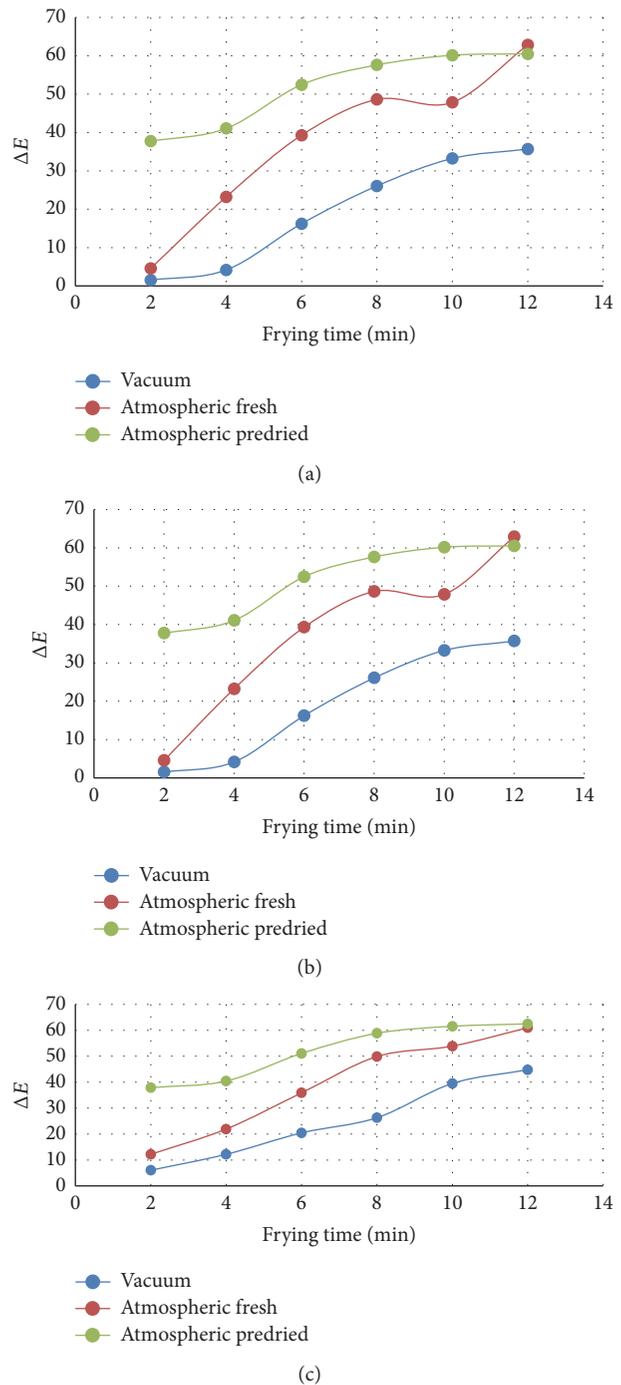


FIGURE 5: Effect of frying conditions on total colour change in yellow fleshed cassava chips at different equivalent thermal driving forces (ETDF) of (a) 60°C, (b) 70°C, and (c) 80°C.

magnitude to those obtained during atmospheric frying for the colour parameters. This suggests that vacuum frying can achieve the desired changes in the quality attributes studied at reduced frying temperatures and similar rates as atmospheric frying. The kinetic parameters for the colour attributes investigated are also presented in Table 2. The trend in the values of rate constants obtained for lightness, yellowness, and overall

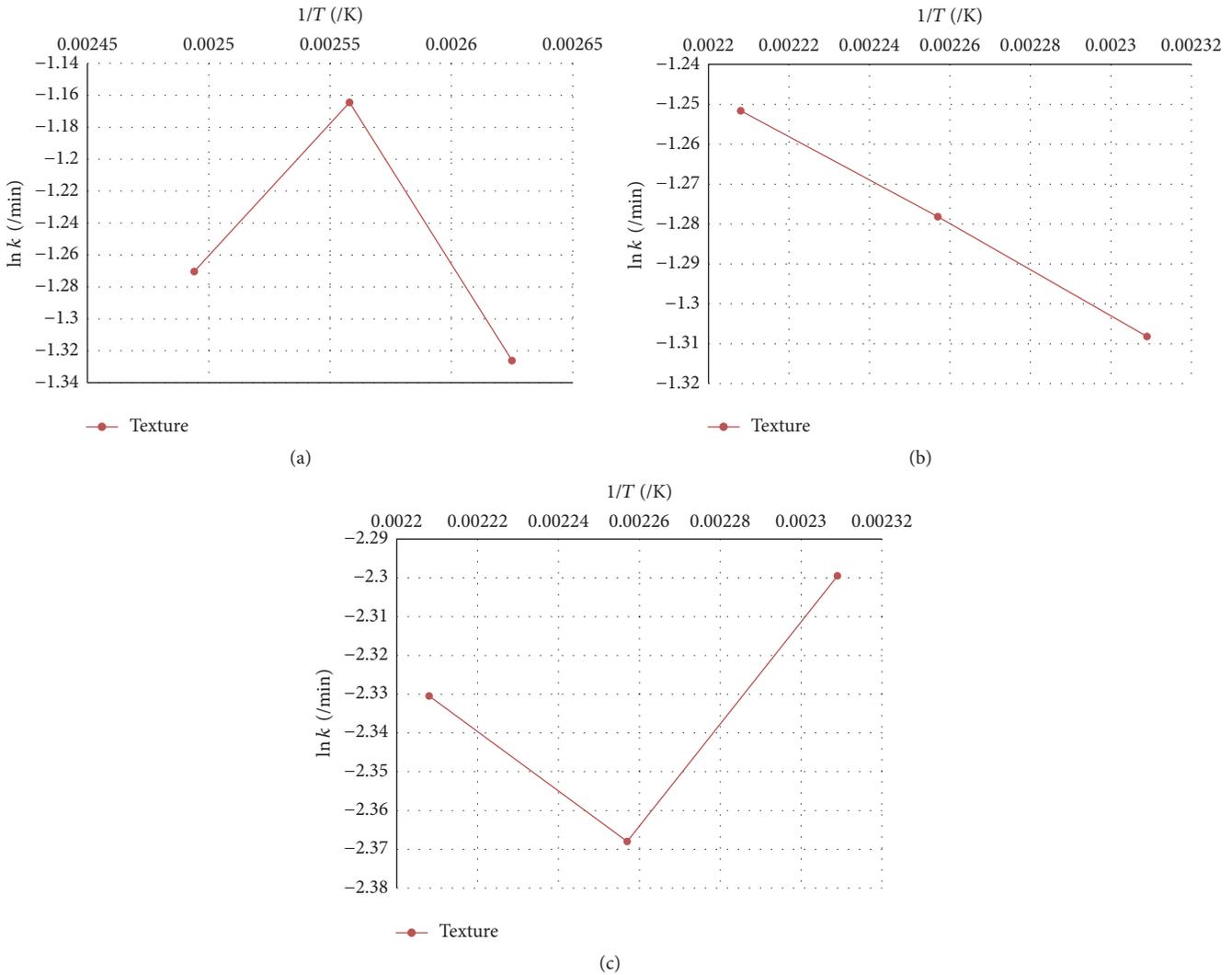


FIGURE 6: (a) Arrhenius plot relating rate constants with frying temperatures at vacuum conditions for texture (hardness) of YFCC. (b) Arrhenius plot relating rate constants with frying temperatures at atmospheric frying conditions for texture (hardness) of YFCC. (c) Arrhenius plot relating rate constants with frying temperatures at atmospheric frying conditions for texture (hardness) of predried YFCC.

colour change is because the magnitudes of these parameters generally decrease as frying temperature increases. Similar trends have been reported by Nourian et al. [17].

The plots of the temperature dependence of rate constants are as shown in Figures 6 and 7. The activation energies (E_a) for each of the quality attributes investigated were obtained from these plots and presented in Table 3. Since activation energy is the energy required to transform a reactant to the product by passing a transition level [9], it follows that the energy requirement for changes in hardness was the least during vacuum frying. However, for the colour parameters investigated, higher values of activation energies were obtained during vacuum frying compared to atmospheric frying. Vacuum frying is carried out at lower temperatures compared to atmospheric frying and this may lead to a slower rate of degradation of colour components in yellow fleshed cassava chips. Hence, higher activation energy could be required to achieve the desired change in colour attributes,

since colour change is dependent on the surface temperature of the fried chips.

4. Conclusions

It could be concluded that the texture and colour parameters investigated were most preserved during vacuum frying. Also, for each of the frying temperatures, vacuum frying proceeded at equal or higher rates, as compared with atmospheric frying. In addition, the higher magnitudes of activation energies obtained for colour parameters during vacuum frying showed that the colour parameters in yellow fleshed cassava are sensitive to high processing temperatures. Vacuum frying technology could be explored in preserving texture and colour parameters of fried chips, which are the major quality attributes that influence the decision of consumers.

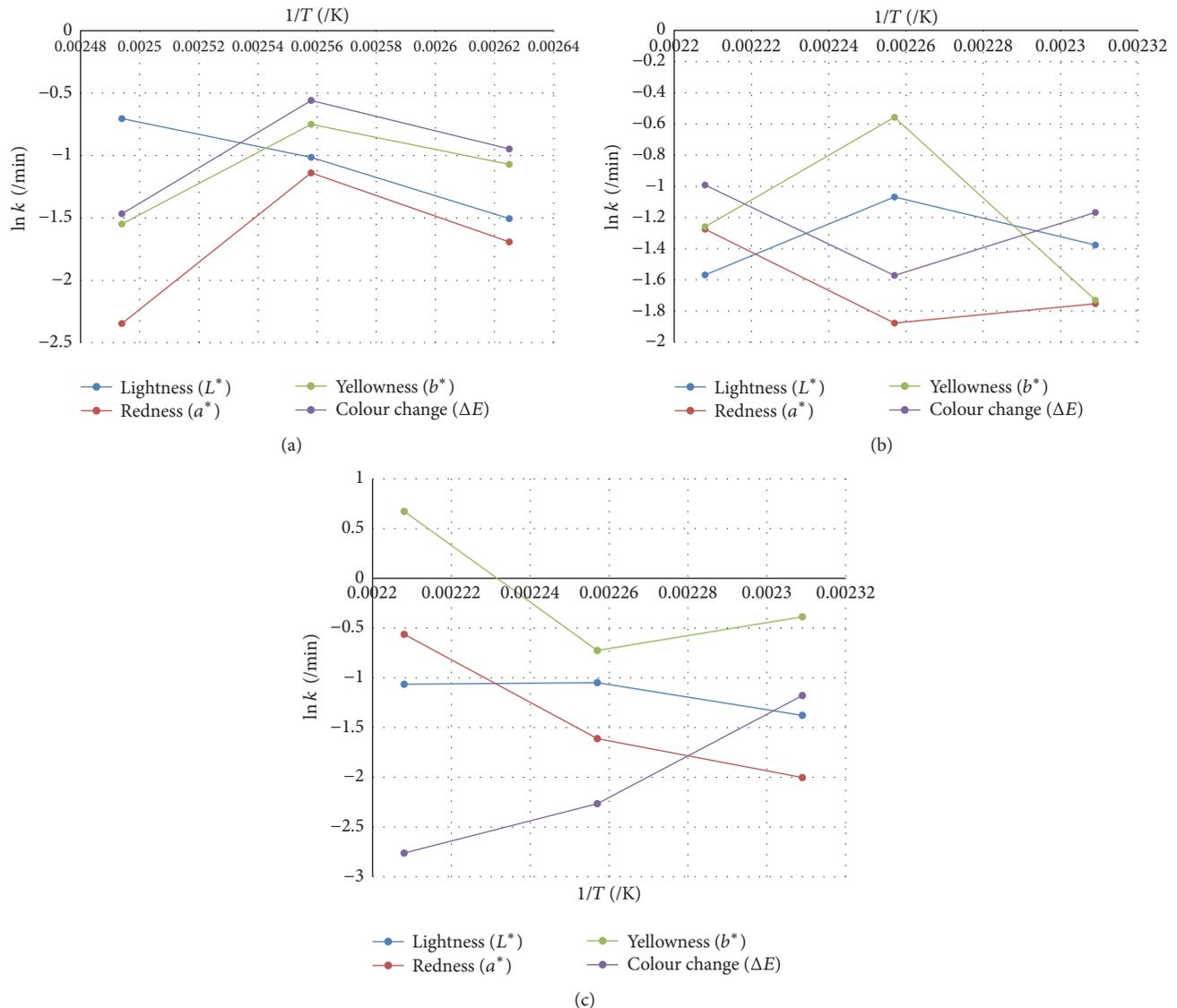


FIGURE 7: (a) Arrhenius plot of rate constant against frying temperatures at vacuum frying conditions for colour parameters. (b) Arrhenius plot of rate constants against frying temperatures at atmospheric frying conditions colour parameters (fresh slices). (c) Arrhenius plots of rate constants against frying temperature at atmospheric frying conditions for colour parameters (predried slices).

Disclosure

The abstract of this study was accepted for oral presentation at the 30th EFFoST International Conference held in Vienna, Austria.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Review Article

Modification of Food Systems by Ultrasound

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This review describes the mechanism, operation, and recent potential applications of ultrasound in various food systems, as well as the physical and chemical effects of ultrasound treatments on the conservation and modification of different groups of food. Acoustic energy has been recognized as an emerging technology with great potential for applications in the food industry. The phenomenon of acoustic cavitation, which modifies the physical, chemical, and functional properties of food, can be used to improve existing processes and to develop new ones. The combination of ultrasonic energy with a sanitizing agent can improve the effect of microbial reduction in foods and, thereby, their quality. Finally, it is concluded that the use of ultrasound in food is a very promising area of research; however, more research is still needed before applying this technology in a wider range of industrial sectors.

1. Introduction

Consumers demand foods with organoleptic and nutritional characteristics similar to those found in nature; in addition, food products should have a sufficiently long shelf life to allow their freshness during distribution and storage before consumption. This can be achieved by the use of minimal processing technologies that preserve food, reduce processing times, and improve the shelf life of food products [1] while preserving a significant degree of nutritional quality and sensory characteristics. This has led to an increasing interest in the development of emerging technologies with potential applications in the food industry, including ultrasound.

Ultrasound is acoustic energy considered a mechanical, nonionizing, nonpolluting type of energy [1], with great potential for use in production processes of high quality food products. Ultrasound produces changes in the physical, chemical, and functional properties of food products [2]; it can therefore influence the quality of various food systems, improving their productivity and performance [3].

Ultrasound is used successfully in the food industry to improve quality and process control. It is used to assess

the composition of meat, fish, and poultry products and in quality control of vegetables, cheeses, oils, breads, and cereals. Other applications include detecting adulteration of honey and protein analysis [4]. There are also several reports of the application of ultrasound in mass transfer and marination processes, meat tenderization, crystallization, freezing, drying, degasification, filtration, foam production and reduction, and emulsification, as well as homogenization and inactivation of microorganisms [1] and enzymes.

Several theories have been proposed to elucidate the mechanism of ultrasonic treatment. This review describes the theoretical foundations (mechanism and operation) of this phenomenon, as well as the effects and potential applications of ultrasound in food.

2. Basics of Acoustic Waves

2.1. Introduction. Acoustic waves are a vibrating disturbance of the environment and need an emitting source and a means of propagation to travel and transmit, unlike electromagnetic waves that can propagate in any medium, including vacuum.

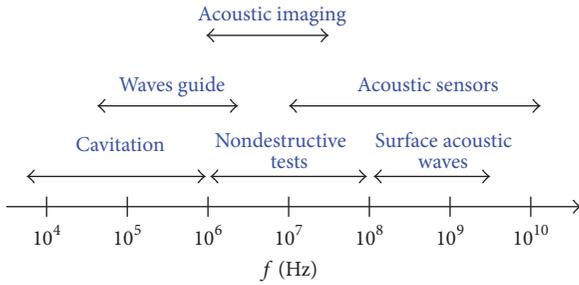


FIGURE 1: Applications of the acoustic waves.

TABLE 1: Frequency scale of the acoustic waves.

Acoustic frequency spectrum	
Name	Range, (Hz)
Infrasound	<20
Audible sound	20 < AS < 20 k
Ultrasound	20 k < US < 1 G
Hipersound	HS > 1 G

Because of this, acoustic waves are also called mechanical waves. These can be propagated in two ways, in longitudinal mode and in transverse mode. In longitudinal mode it means that the acoustic energy emitted propagates in the same direction in which the acoustic wave travels; on the contrary, in the transverse mode, the emitted acoustic energy propagates perpendicularly in the direction in which the acoustic wave travels [5].

In this context, the acoustic waves also comply with Snell's law, in optics [6]. It also presents the phenomenology of absorption, diffraction, dispersion, scattering, transmission, and reflection [7]; and this occurs through a propagation medium such as a solid or a fluid (both liquid and gaseous), as well as in a biological tissue and so on, and where the main parameter is the acoustic impedance (Rayls) of propagation medium that is given by the product of the volumetric density (kg/m^3) and the propagation velocity (m/s) of the medium [8]; it should be noted that, in solid materials, the longitudinal and transverse propagation velocity is obtained. The acoustic waves have well defined parameters such as amplitude, intensity, power, and frequency, and it is the latter that provides the working spectrum of the acoustic waves. Some of the applications that have the frequency dependent acoustic waves are described in Figure 1 [9–14].

2.2. Acoustic Waves in the Ultrasound Spectrum. Acoustic waves are divided into a field of work given by the spectrum of frequencies. Table 1 describes the most common spectrum of acoustic waves.

The spectrum of the ultrasound is given in a frequency range between 20 kHz and 1 GHz. In ultrasonic waves there are two main applications, that is, in nondestructive and destructive tests. In the first, inspection tests are performed to determine the acoustic properties of the material, as well as to determine fractures and deterioration of the same. Also,

there is the area to generate the ultrasonic waves and to detect them, made by emitting devices and acoustic detectors, which are development mainly by piezoelectric materials [15].

On the other hand, the destructive tests are used to remove tissue or matter from a very precise area, like the ultrasound used in medicine [16]. But here is another important parameter, the acoustic intensity (W/m^2), where the combination of ultrasound and the increase in acoustic intensity can generate two effects, acoustic cavitation and shock waves and modulating frequency [17].

The acoustic wave in frequency of the ultrasound in steady state generates the phenomenon known as acoustic cavitation; this happens in the compression and expansion of the waves [18, 19].

2.3. The Effect of Acoustic Cavitation. There are different tools to generate the effect of acoustic cavitation; this can be generated by means of shock waves or by steady state. There is a great field of study about this phenomenon and it is still an area of current exploration, in which the description of the phenomenon continues in the areas of sonoluminescence, sonophysics, and sonochemistry [43].

In an ultrasonic system, electric energy is transformed into vibrational energy, that is, mechanical energy, which is then transmitted into a sonicated medium. Part of the input energy is lost (turned into heat); the other part can cause cavitation. A fraction of the energy of cavitation produces chemical, physical, or biological effects [44].

The basis of many applications of ultrasound at a frequency range of 20 kHz to 1 MHz is acoustic cavitation, which occurs in regions under rapidly alternating high-amplitude pressure waves [45] and consists of the growth and collapse of gas bubbles within a liquid medium [25]. Vapor or gas bubbles are created by the change of average distance between molecules and the decrease in pressure. The bubbles grow in areas of low pressure, collapsing violently when passing to high pressure areas (Figure 2) and producing temperatures close to 5000 K, as well as pressures above 1000 atm [46] due to the release of the energy stored during expansion. However, the heat produced by the implosion of the bubbles is instantly dissipated, so there is no substantial temperature rise in the medium [45]. When the intensity increases, the size of the bubbles also increases, and thereby the energy released during collapse.

Acoustic power is the total energy radiated by the ultrasonic source per unit of time; it can be calculated from acoustic intensity and the area of the radiating surface [47]. The frequency determines the radius of resonance and the lifetime of the bubbles; the higher the frequency, the smaller the bubbles and the lower the energy released. A direct result of the high temperatures after the collapse of the bubbles is the production of chemically active radicals by the dissociation of vapors [28].

The efficiency of the cavitation mechanism depends on the frequency and intensity of the transmitted ultrasound waves, as well as on the physical properties of the observed sample. When the frequency increases, the number of formed bubbles increases, but their diameter is smaller (Figure 3), and

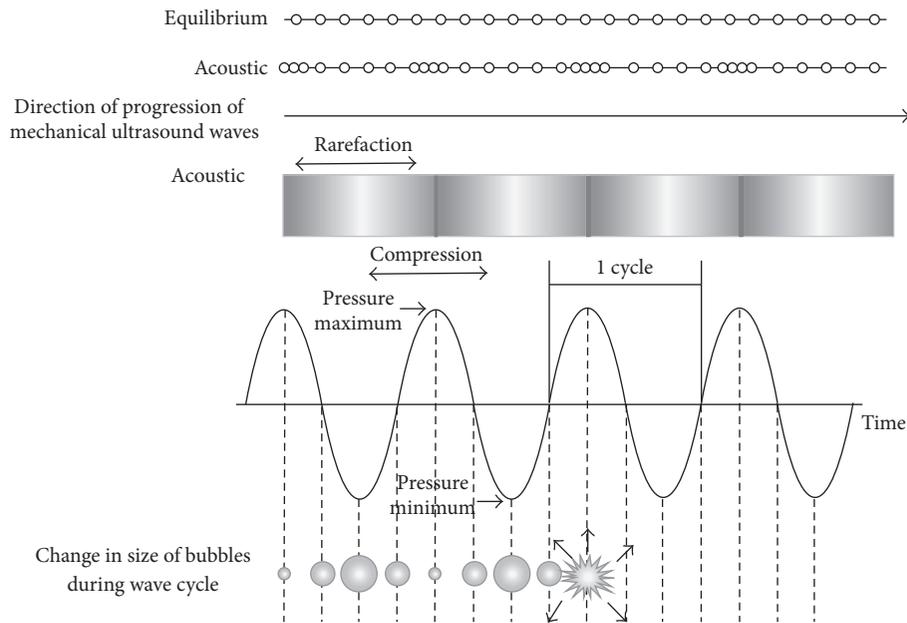


FIGURE 2: Ultrasonic cavitation [46].

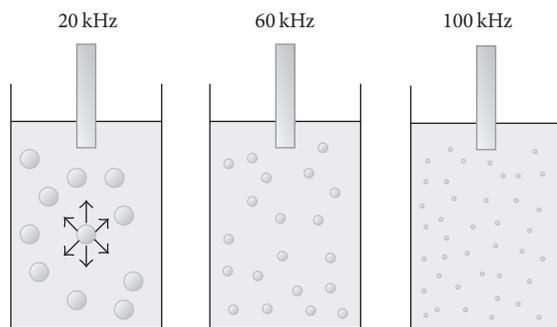


FIGURE 3: Influence of frequency on the number and diameter of cavitation bubbles [48].

the amount of energy released during implosion is minimum [48].

The propagation of ultrasound is a nonlinear phenomenon associated with various factors [49] and involving the properties of ultrasonic waves (speed, frequency, length, width, and intensity). Tsaih et al. [50] indicate that as the intensity of ultrasound increases, it generates higher acoustic pressure, which leads to a greater and more violent collapse and a consequent increase in the chemical or physical effects. Thus, this phenomenon can produce a range of effects on biological tissues and materials.

Ultrasound lies in a range of 20 kHz to 10 MHz and is divided into three categories: (1) ultrasound with high power ($>5 \text{ W cm}^{-2}$ or 10 to 1000 W cm^{-2}) and low frequency (20 to 100 kHz); (2) ultrasound with average power and intermediate frequency (100 kHz–1 MHz); and (3) ultrasound with low power ($<1 \text{ W cm}^{-2}$) and high frequency (10.01 MHz)

[51]. Ultrasound can be applied using three different methods: (a) directly to the product; (b) coupling the product to a device; (c) immersion in an ultrasonic bath [1].

3. Potential Applications of Ultrasound in Food Systems

3.1. Meat. Numerous studies have been focused on obtaining meat with better technological and sensory qualities [27]; in this regard, ultrasound has shown both positive and negative effects. The discrepancies in the results are due to intrinsic (species, age, ageing, and type of muscle) and extrinsic factors (ultrasonic systems, time, intensity, and frequency); thus, it is necessary to show a summary of the effects of ultrasound on the physicochemical characteristics and tenderness of the meat (Tables 1 and 2).

3.1.1. Effect of Ultrasound on the Chemical Characteristics of Meat. The initial pH of the meat (semimembranosus muscle) increased when applying ultrasonic treatment (2.6 MHz, 10 W cm^{-2}) before rigor mortis; but the final pH did not differ significantly [26]. Ultrasound has no influence on the pH of postrigor meat [20, 21]; but the depletion factor indicates that there is an effect on metabolism and the actin-myosin interaction [52].

The parameters CIE $L^*a^*b^*$ were not affected by treatment with ultrasound [21, 52]; the heat generated was not sufficient to induce denaturation and oxidation of the color pigments (Mb, metMb) [20]. The color measurements made by Pohlman et al. [53] in meat subjected to ultrasound (22 W cm^{-2}) indicate changes to a lighter color (lower L^*), less red (lower a^*), more yellow (higher b^*), more orange (larger hue angle), and less brightness compared to the

TABLE 2: Studies of ultrasound aimed to improve of meat quality.

Meat	Purpose	Conditions	References
Steers meat (<i>longissimus lumborum</i> and <i>semitendinosus</i>)	Impact on the ageing of meat and characteristics quality	24 kHz and 12 W cm ⁻² for 240 s	Jayasooriya et al. [20]
Beef (<i>semimembranosus</i>)	Influence of ultrasound in pH of meat, its color, and shear force	45 kHz and 2 W cm ⁻² for 2 min	Stadnik and Dolatowski [21]
Beef (<i>semitendinosus</i>)	Effects on meat quality and connective tissue collagen	40 kHz, 1500 W for 10, 20, 30, 40, 50, or 60 min	Chang et al. [22]
Poultry (breast)	Influence of ultrasound together with either a protease inhibitors cocktail	24 kHz, 12 W cm ⁻² for 4 min after 7 d of storage	Xiong et al. [23]
Holstein bulls (<i>longissimus lumborum</i>)	To develop a novel process for improving meat tenderness	20 kHz, 100 and 300 W for 10, 20, and 30 min	Barekat and Soltanizadeh [24]

control. Furthermore, Stadnik and Dolatowski [21] observed that ultrasound accelerates total color change by limiting the formation of MbO₂ and slowing the formation of metMb.

Water retention capacity is a meat quality parameter with economic importance; therefore, it is important to evaluate it in meat treated with ultrasound. The results mentioned that ultrasound increases the rates of meat exudate and water loss [22]. However, Jayasooriya et al. [20] do not mention changes in drip loss (24 kHz, 12 W cm⁻²); similarly, Smith [54] reports no effect on the water retention capacity of meat. In contrast, other authors indicate that ultrasonicated meat has an increased water-holding capacity [22, 53, 55], similar to a meat in advanced postmortem stage; they suggested an increase in the ageing rate of meat due to structural changes in myofibrillar proteins induced by ultrasound; this has been confirmed by photograms of the microstructure of these proteins [22].

Furthermore, there are reports that ultrasound causes the degradation of proteins with a molecular weight higher than 20–25 kDa and increases the activity of calpains and the release of lysosomal contents, which have a positive effect on tenderness [20].

3.1.2. Effect of Ultrasound on the Structural Components of Meat Related to Texture. The postmortem degradation of myofibrillar proteins is closely linked to structural changes that result in increased meat tenderness during the ageing process [56]. It has been proposed that acoustic cavitation induces mechanical disruption of the structure of myofibrillar proteins [22], as well as the fragmentation of collagen macromolecules and the migration of proteins, minerals, and other compounds, with a consequent acceleration of proteolysis or protein denaturation [57].

It is also possible that the application of ultrasound induces changes in the amount of ATP available in the muscle during the prerigor stage [52], accelerates the onset of rigor mortis [21], and increases the ageing rate of meat [58]. There have been experiments with the application of ultrasound to increase meat tenderness [20–22] and reduce the ageing period without compromising other quality characteristics of meat [22, 55].

Low-frequency and low-intensity ultrasound seem to be particularly suited for softening meat [21, 59]; several studies report a significant effect on reduction of the cutting force and some are presented in Table 2. Shear force has been evaluated in the following beef muscles: *longissimus lumborum* and *semitendinosus* muscles (24 kHz and 12 W/cm² for 240 s) [20], *semimembranosus* muscle (45 kHz and 2 W cm⁻² for 2 min) [21], *semitendinosus* muscle (40 kHz, 1500 W for 10, 20, 30, 40, 50, or 60 min) [22], also poultry (24 kHz, 12 W cm⁻² for 4 min after 7 d of storage) [23], and pork (2.5 to 3 W cm⁻² for 180 min) [57]. A more recent report by Barekat and Soltanizadeh [24] indicated that applying ultrasound in addition to papain to young Holstein bulls (*Longissimus lumborum*) for 10, 20, and 30 min (20 kHz, 100 and 300 W) had significant effect on tenderness.

With regard to the effect of ultrasound on the collagen structure, the results are heterogeneous. It has been reported that low-frequency ultrasound has no effect on the content of soluble collagen [60] or the content of insoluble collagen [20]; but Chang et al. [60] showed that ultrasound had a significant effect on the characteristics of collagen, especially on its thermal properties, without having any effect on the content of insoluble collagen; moreover, Chang et al. [60] reported effects on collagen solubility during cooking.

The contribution of connective tissue to meat toughness is greater than that of fat; however, in addition to fragmenting collagen, ultrasound disrupts cell membranes and promotes the formation of free radicals [61]. Consequently, it intensifies oxidation of meat due to the increase in the rate of chemical reactions [4]; however, Stadnik [62] mention that sonication can be an effective method to improve the technological properties of beef muscle without affecting lipid oxidation. Furthermore, after analyzing samples treated with ultrasound (45 kHz, 2 W/cm² for 120 s) and stored in refrigeration, Stadnik [62] reported values of 2-thiobarbituric acid reactive substances (TBAR's) that do not compromise the oxidative stability of meat.

3.1.3. Other Applications of Ultrasound in Meat. Ultrasound has been applied to meat for various purposes (Table 3). Regarding the cooking of beef, ultrasound improves cooking

TABLE 3: Application of ultrasound in meat for various purposes.

Meat	Effect	Conditions	References
Beef (<i>longissimus</i> and <i>pectoralis</i>)	Ultrasound-assisted cooking for improving cooking time, moisture retention capacity, and energy efficiency	20 kHz, 1,000 W	Pohlman et al. [53]
Pork (<i>longissimus dorsi</i>)	Influence of ultrasound on the mass transfer process during meat brining depended on the intensity applied	20 kHz, 450 W	Cárcel et al. [66]
Pork (<i>longissimus dorsi</i>)	Ultrasound-assisted meat curing obtained better distribution of the brine, reduced water loss, and caused favourable microstructural changes in meat tissue	20 kHz, 2–4 W cm ⁻²	Siró et al. [57]
Pork (<i>longissimus thoracis</i> and <i>lumborum</i>)	Ultrasound-assisted meat curing for accelerating the mass transfer achieves a 50% reduction in processing times with no adverse effects on quality in the production of wet-cured cooked hams	20 kHz, 4.2, 11, or 19 W cm ⁻² , 10, 25, or 40 min 1 W cm ⁻²	McDonnell et al. [64]
Chicken breast	Dye (methylene blue) penetration is an indication of meat permeability when using ultrasound and so it is estimate of marinating of meat	40 kHz, 22 W cm ⁻² , 15 and 30 min	Leal-Ramos et al. [63]
Hen breast	Ultrasound-assisted marination for improvement of meat tenderness, improvement efficiency, and cooking yield	24 kHz, 12 W cm ⁻² , 4 min	Xiong et al. [23]
Pork	The NaCl and moisture effective diffusivities were improved and promoted changes in meat texture	40 kHz, 37.5 W dm ⁻³ 15, 30, 45, 60, 90, and 120 min	Ozuna et al. [65]
Pork (<i>longissimus dorsi</i>)	Improving the diffusion of sodium chloride	20 kHz, 2–4 W cm ⁻²	Siró et al. [57]

time, moisture retention capacity, and energy efficiency [53]; therefore, this method could be a fast and energy-efficient way to enhance the quality attributes of cooked meat. The technology of ultrasound-assisted meat curing is proposed as an alternative to the traditional process of meat curing; its aim is to accelerate mass transfer [63] of brine into meat, preserve sensory attributes [64], obtain a better distribution of solutes [65], and reduce water loss [57].

It has been shown in solid-liquid systems that acoustic waves improve the kinetics of mass transfer [66] due to the physical disruption of tissues, which creates microchannels and causes changes in the concentration gradients and diffusion coefficients [1, 67]; longer implementation times cause denaturation of proteins [57].

During the meat curing process, the transfer of mass (saturated NaCl solution) into meat depends on the intensity thresholds (39 and 51 W cm⁻²); above them, the transfer of mass is proportional to the intensity of ultrasound [66]. Ultrasonic treatment is also reported to significantly improve salt diffusion; the diffusion coefficient increases exponentially with increasing ultrasound intensity [57]. McDonnell et al. [64] achieved a reduction of up to 50% in processing times during the production of cured hams, without adverse effects on the quality; they inferred that ultrasound has potential advantages for the processing of other meat products.

However, Smith [54] applied low-intensity ultrasound in chicken marination and reported lower water absorption,

less drip loss, lower cooking yield, and no significant effects on hardness. Contrary to this, Ozuna et al. [65] observed greater tenderness in pork meat marinated with ultrasound. The results are not yet conclusive, and further studies are needed on the effects of ultrasound on meat properties before recommending the use of ultrasound at industrial scale. Although numerous techniques are used for cooking meat other than conventional heating, the flavor of the meat and the consumption of energy are sometimes far from optimal. It can be concluded that power ultrasound has an important effect on the texture and maturation of meat from various species by weakening myofibrillar and connective tissues and reducing cooking losses without affecting other quality parameters. Moreover, salting with ultrasound could be a surface phenomenon that can accelerate mass transfer and extract proteins. The benefits of ultrasound on mass transfer are very convincing and industrial implementation could be very close.

3.2. Dairy. Ultrasonic processing is among promising new nonthermal processes that could bring about large-scale improvements in different processes of the food industry, including the dairy industry. It has been observed that high-intensity ultrasound induces changes in the functional properties of food. Ultrasound is considered as an alternative method for reducing the size of fat globules and can be applied effectively to homogenize milk [48]. Increases in

exposure times and power levels increase the efficiency of homogenization [68], with significant reductions in the diameter of fat globules [69]. Ultrasound treatment causes alterations in the secondary structure of milk proteins, aggregation of protein particles, and denaturation [70]. In addition, ultrasound produces alterations in the composition and structure of the membrane of fat globules, which improves the efficiency of homogenization in casein gel, compared to conventional methods [71]. It has also been shown that ultrasound depolarizes the particles of gamma-carrageenan and reduces their size, allowing for better homogenization of nanoparticles in a dispersion mixed with beta-lactoglobulin. Thus, ultrasound could have a significant potential in the enrichment of acidified milk drinks [72]. The interaction between β -lactoglobulin and sodium alginate, before and after ultrasound treatment, generates biopolymer nanoparticles that may be used to enrich transparent liquid food products [73]. The changes induced by high-intensity ultrasound depend on the nature of the proteins and their degree of denaturation and aggregation [74]. The most important factor in the application of ultrasound in processes of emulsification and homogenization of milk is to control possible negative effects such as oxidation of fats, inactivation of enzymes, and protein denaturation [75]. Ultrasonic waves propagate faster in milk with low percentage of fat, generating a greater number of small cavitation bubbles, the implosion of which produces thermal energy that causes an immediate increase in temperature and changes the physical properties of milk. Serum proteins are widely used to improve the functional properties of milk such as emulsification, thickening, and foaming [67]. Sonication is used to generate foam in the fluid-fluid interface; the air bubbles produced by sonication are entrained in the mixture [76]. This approach has been used to create aerated β -lactoglobulin gelatin and gels for use in food applications [77].

Another application of ultrasound is the crystallization of lactose. A recovery of 92% lactose was obtained from milk serum solutions after 5 min sonication, compared with 15% using conventional stirring [78]. The cause of the improvement in crystal nucleation is that acoustic cavitation bubbles provide a heterogeneous surface for nucleation; the effects on crystal size and morphology may be related to the shear forces associated with ultrasound and the breaking up of nascent agglomerates [79].

It has also been found that ultrasonic homogenization of milk before inoculation of the starter improves viscosity in yogurt [80]. Vercet et al. [81] showed that the simultaneous application of heat (40°C) and ultrasound (12 s at 20 kHz) under moderate pressure (2 kg cm²) improves the rheological properties of yogurt. These changes can be attributed to the denaturation of milk serum proteins and their association with caseins by effect of ultrasound; denatured serum proteins associated with casein micelles can act as bridging material between casein micelles, facilitating the formation of bonds in the yogurt matrix, which results in firmer yogurt gels [82]. Kartalska et al. [83] suggest that substituting thermal pasteurization with ultrasonic treatment provides optimal conditions for the development of lactic acid bacteria. Ultrasonic treatment has a positive effect on the

fermentation process, probably due to the homogenization of the colloidal system of milk under sonication. Riener et al. [84] found that milk yogurt containing 1.5 or 3.5% fat and treated with thermosonication (45°C, 10 min, 24 kHz) had almost twice the water-holding capacity and good texture properties. Electron microscopy showed differences in the microstructure; that of thermosonicated yogurt was similar to a honeycomb and more porous. Furthermore, the average particle size in thermosonicated yogurt was less than one micron, significantly smaller than in conventional yogurts. Gursoy et al. [85] prepared yogurt drinks using milk samples processed by thermosonication or conventional heating (10 min at 90°C) and observed that thermosonication (70°C, 100, 125, and 150 W) caused a significant decrease in serum separation values, while the apparent viscosity increased with ultrasound power.

The effect of ultrasound on the rheological and foaming properties of ice cream has also been studied [86]. The ice cream mixes treated with ultrasound alone had a minimal increase in foam volume; the greatest increase in foam volume was observed in ice cream mixes subjected to a combined mechanical and ultrasonic treatment. Moreover, the foam of ice cream mixes with higher protein content was more stable. It was concluded that the optimal treatment time was 10 min.

The combination of ultrasound with other technologies reduces processing time and increases efficiency in industrial production processes [87]. Shanmugam et al. [88] emphasize that the minor changes in milk caused by the shear forces of acoustic cavitation suggest the potential for optimizing this technique for industrial applications. The optimization can be achieved by fine adjustments of power density, temperature, processing time, and so on.

Ultrasound, used in combination with high hydrostatic pressures, can be useful to preserve various food properties such as texture, as well as sensory, organoleptic, and microbiological characteristics. Karlović et al. [89] applied ultrasound and high hydrostatic pressures to goat milk. The milk was exposed to ultrasound at 100 W of nominal power and to high pressures of up to 600 MPa. The maximum treatment time was 9 min. They reported an improvement in the homogenization of fat globules, the diameter of which was significantly influenced by the high pressure. The application of both processes improved the stability and quality of the emulsions.

Ultrasound is a nonthermal alternative for pasteurization that allows obtaining a final product of higher quality. High-amplitude ultrasound reduces the microbial content of milk. The effectiveness of ultrasound as a decontamination technique can be enhanced by combining it with other treatments such as pressure, heat, and antimicrobial solutions [4]. Ultrasound has been studied as an alternative to heat pasteurization. Cameron et al. [90] observed a reduction in the presence of potential pathogens to negligible or acceptable levels. The viable cell count of *E. coli*, *Pseudomonas fluorescens*, and *Listeria monocytogenes* decreased by almost 100% after 10 min of ultrasound treatment, without detrimental effects on the total protein or case content of pasteurized milk. However, ultrasonication was ineffective in deactivating the alkaline

phosphatase and lactoperoxidase enzymes regularly used by the dairy industry as indicators of the efficiency of thermal processes.

Treatment with high-intensity ultrasound causes milk to release volatile compounds, which leads to an unpleasant taste. This was reported by Riener et al. [91], who found that when milk is subjected to ultrasonic treatment, it releases benzene, toluene, 1,3-butadiene, 5-methyl-1,3-cyclopentadiene, 1-hexene, 1-octene, 1-nonene, p-xylene, n-hexanal, n-heptanal, 2-butanone, acetone, dimethyl sulfide, and chloroform. Aldehydes can be produced by the decomposition of hydroperoxides generated by ultrasound-induced photooxidation, whereas the series of C6–C9 1-alkenes might be generated by the pyrolytic cleavage of fatty acid chains. The formation of benzene can be attributed to the cleavage of the side chains of amino acids such as phenylalanine. The release of these volatile compounds produces a scent of burning rubber [91].

It could be concluded that ultrasound has a very good milk homogenization effect at high-amplitude levels compared with conventional homogenization. The acoustic cavitation is responsible for some structural changes, particularly in the protein particles. Thermosonication treatment could be successfully used in the production of yogurt drink and improve its major quality parameters such as delayed serum separation and increased apparent viscosity. Ultrasound has also the potential to facilitate the production of commercial yogurt in which supplementation with milk solids can be substantially reduced. Ultrasound treatment after inoculation results in a decreased fermentation time and increased water-holding capacity. However controlled and optimized application of ultrasound demands application of specific ultrasound frequency and optimal treatment time. In general, high-intensity ultrasound seems to be a potential alternative to the conventional processing to obtain good quality products.

3.3. Fruits and Vegetables. The increased consumption of fruit and vegetables worldwide has increased the need to have greater control of the nutritional, sensory, and microbiological qualities of these foods [92]. Emerging technologies have been developed to preserve food as long as possible without the use of additives and without affecting its nutritional value and sensory attributes; these technologies must also be cost-efficient and should use environmentally friendly products [93]. Many studies have focused on the effect of ultrasound as an alternative to washing methods that prevent the adhesion of microorganisms to the tissues of fruits and vegetables [94–98]. This technology is often combined with other sanitizing agents in the washing fruits and vegetables, as in the case of chlorine dioxide in plums, apples, and lettuce [33, 41]; exogenous polyamines (putrescine) in peach [99]; and sodium hypochlorite in lettuce [31]. Ultrasound is useful for decontaminating surfaces when applied in combination with other methods. High temperature, high pressure, ultraviolet radiation, pulsed electric fields, or chemical methods are often used for cleaning and disinfection and can be applied in combination with ultrasound; in the case of fruits and vegetables, heat and pressure are not recommended because they can damage the tissues [93]. Generally, ultrasound is

combined with chemicals such as commercial sanitizers, organic acids, and other antimicrobials.

Bacterial inactivation by ultrasound has also been tested in food products derived from the processing of fruits and vegetables; in this case ultrasound is combined with antimicrobial agents such as vanillin and citral [100]. Although treatment with ultrasound can by itself cause a reduction in microorganism counts, it cannot be efficiently used in industrial applications because of its poor sterilization effect. It requires long treatment times and/or high acoustic energy, damaging fresh tissues and making them more susceptible to infestation and attacks by microorganisms. During ultrasonic treatment, microorganisms are released into the wash water, creating the risk of cross contamination; thus, this technology should be combined with other technologies to ensure sterilization [101]. Many studies show that the physical and chemical effects of ultrasound treatment are related to the amplitude of the ultrasonic waves, the exposure times, the food volume and composition, and the temperature reached by the tissues [102–104].

Table 4 describes some studies that have used ultrasound in fruits and vegetables; the results differ depending on the experimental conditions employed. Most of the studies reviewed do not provide information on important characteristics of the equipment used, such as the effective ultrasonic power fed into the system (different from the electric power of the equipment), which should be measured by calorimetry according to the method reported by M. A. Margulis and I. M. Margulis [105].

Microbial reduction (\log_{10} CFU/g of sample) seems to be more related to ultrasonic power than to ultrasonic frequency. Low power ultrasound generates greater reductions; in the case of iceberg lettuce (ultrasonic equipment with 10 W/L), the reduction was 1.5 \log_{10} CFU/g in *S. typhimurium* [30]. Ultrasound treatment destroys or removes microorganisms from fruits and vegetables by cavitation, which is a combination of mechanical effects (generation of turbulence, circulation flows, and shear stress), chemical effects (generation of free radicals that attack the chemical structure of the cell wall of the microorganisms), and physical effects (generation of extreme temperature and pressure) [106]. Combining ultrasonic energy with a sanitizer potentiates its microbial reduction effect. Regarding *S. typhimurium*, a reduction of 2.7 \log_{10} CFU/g was observed in lettuce when ultrasound was combined with chlorinated water; ultrasound alone caused a reduction of 1.5 \log_{10} CFU/g. These results are similar to those of Brilhante São José and Dantas Vanetti [32], who reported a reduction of 3.9 \log_{10} CFU/g for *S. enterica* Typhimurium when combining ultrasound with peracetic acid; by contrast, ultrasound alone caused a reduction of only 0.8 \log_{10} CFU/g. These results are relative, since studies in other plant species have not shown differences in microbial reduction between ultrasound alone and combined with antimicrobials.

Higher power ultrasound (350 W/L) induced microbial reduction of 0.6 \log_{10} CFU/g (total viable count of mesophilic microorganisms) in strawberries and of 0.5 \log_{10} CFU/g in molds and yeasts [30]. Alegria et al. [107] reported a reduction of 1.3 \log_{10} CFU/g in total mesophilic counts in shredded

TABLE 4: Application of ultrasound in fruits and vegetables for various purposes.

Food	Conditions	Purpose	References
Alfalfa seeds	US 40 kHz + Ca(OH) ₂ 1%	Reduction of <i>E. coli</i> and <i>Salmonella</i>	Scouten and Beuchat [29]
Strawberries	US 40 kHz 350 W/L, 10 min	Reduction of mesophilic microorganisms, molds, and yeasts	Cao et al. [30]
Lettuce	US 10 W/L, 32–40 kHz, 10 min US + chlorinated water (25 ppm)	Reduction of <i>S. typhimurium</i>	Seymour et al. [31]
Cherry tomato	US 45 kHz, 10 min, 25°C US + peracetic acid (40 mg/L)	Reduction of <i>S. enterica</i> Typhimurium	Brilhante São José and Dantas Vanetti [32]
Lettuce apples	US 170 kHz, 6–10 min + ClO ₂ (5 and 1 ppm)	Reduction of <i>Salmonella</i> and <i>E. coli</i>	Huang et al. [33]
Truffles	US + ethanol (70%)	Reduction of mesophilic microorganisms, molds, yeasts, <i>Pseudomonas</i> spp., Enterobacteriaceae, and LAB	Susana Rivera et al. [34]
Lettuce	US 20 kHz US + 100 mg/L Ca(ClO) ₂	Reduction of aerobic mesophilic bacteria	Ajlouni et al. [35]
Spinach	US 200 W/L, 2 min + various sanitizers	Reduction of <i>E. coli</i> O157:H7	Zhou et al. [36]
Lettuce	US 37 kHz, 30 min	Reduction of <i>E. coli</i> , <i>S. aureus</i> , <i>S. enteritidis</i> , and <i>L. innocua</i>	Birmpa et al. [37]
Cabbage, lettuce, sesame, and spinach	US 40 kHz, 3 min, 23°C + electrolyzed water + washing with water	Reduction of <i>E. coli</i> O157:H7	Forghani and Oh [38]
Cranberry, nectarine, raspberry and watermelon, garlic, artichoke, leek, and onion	US 40 kHz, 20–60°C, 5, 10, 20 or 30 min	2–4-fold increase in the extraction of oligosaccharides	Jovanovic-Malinovska et al. [39]
Lychee	120 W, 10 min	Reduction in the degradation of anthocyanins and delayed browning	Chen et al. [40]
Plum	40 kHz, 100 W, 10 min	Inhibition of respiratory rate (greater firmness), preservation of flavonoids, ascorbic acid, reducing sugars, and titratable acids	Chen and Zhu [41]
Persimmon	50 kHz, 200 W, 1–10 min	Firmness retention, higher content of soluble solids, and titratable acids	Wang et al. [42]

LAB: lactic acid bacteria.

carrot and of 0.9 log₁₀ CFU/g for molds and yeasts, using ultrasound alone (45 kHz, 1 min, 25°C); when ultrasound was used under the same conditions but combined with chlorinated water (200 ppm), they observed a reduction of 1.0 log₁₀ CFU/g in total mesophilic counts and of 0.9 log₁₀ CFU/g for molds and yeasts. Ajlouni et al. [35] demonstrated that temperature is an important factor in microbial reduction in lettuce. The use of ultrasound (20 kHz, 2 min) reduced aerobic mesophilic counts by 0.90 and 0.98 log₁₀ CFU/g at 4°C and 50°C, respectively; combining Ca (ClO)₂ with ultrasound caused a greater reduction in mesophilic counts (1.02 log₁₀ CFU/g at 4°C and 1.35 log₁₀ CFU/g at 50°C).

Other potential applications of ultrasound in fruits and vegetables concern the conservation of quality parameters such as texture, color, and nutrients (Table 4). Pretreatment with ultrasound inhibits physiological activities and slows the decline in quality during storage in unripe fruits [30]. Regarding texture, it possibly delays softening by inhibiting enzymatic activity (pectin methylesterase and polygalacturonase). It has been reported that ultrasound delays the degradation of

pigments (chlorophylls, carotenoids, and anthocyanins) during ripening, maintaining the green color of asparagus [108], and inhibiting the decrease of anthocyanins in strawberries [109]. Fruit senescence is associated with reactive oxygen species and oxidative damage of mitochondrial proteins; Zhao et al. [110] found an increase in the activity of peroxidase and superoxide dismutase (antioxidant enzymes) in pears treated with ultrasound; Li et al. [111] also observed an increase in the activity of superoxide dismutase and catalase in peaches treated with ultrasound (50 kHz, 200 W, 3 min).

It can be concluded that ultrasound has a great potential in the preservation of fruits and vegetables; however, the methods and parameters used have not been standardized. Furthermore, the industrial use of ultrasonic technology in fresh produce requires manufacturing and improvement of ultrasonic equipment.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

A Decision-Making Model for Deterring Food Vendors from Selling Harmless Low-Quality Foods as High-Quality Foods to Consumers

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For certain types of foods, food vendors often label low-quality foods that are harmless to human health as foods of excellent quality and sell these falsely labeled products to consumers. Because this type of food poses no harm to human health, when public health units discover their act of false labeling or food adulteration, vendors are only penalized with a fine rather than having them assume criminal liability. Upon discovering vendors act of falsely labeling food, public health units typically punish the involved parties according to the extent of false labeling. Such static protective measure is ineffective. Instead, the extent of punishment should be based not only on the extent of false labeling, but also on the frequency of food sampling as well as the number of samples obtained for food inspections. Only through this dynamic approach can food adulteration or false labeling be effectively prevented. Adopting the standpoint of the public sector in food safety management, this study developed a mathematical model that facilitates discussion on the aforementioned problems. Furthermore, we discussed how the supply-demand environmental factors of the food market are influenced by the administrative means that the public health units have used to prevent food false labeling.

1. Introduction

Presently, consumers are paying increasing attention to the quality and safety of food products, with particular focus on the direct or potential negative impact of food products manufactured in the biotechnological industry (e.g., genetically modified foods). Food safety is defined in both broad and narrow senses [1]. In the narrow sense, food does not cause any acute, subacute, or chronic hazards to human health. In the broad sense, food products must be assured of their quality, should not be involved in false labeling, adulteration, and counterfeiting, and must conform to relevant health standards. Furthermore, because of advancements in information technology, consumers are exposed to food safety scandals almost every week from newspapers and online sources. These scandals usually involve food safety, quality, and labeling problems causing food products in the market to be recalled or taken off the shelf. Consequently, government health departments worldwide are now forced to pay heightened attention to food quality and safety

problems. Henson and Traill [2] indicated that government units across the world have shown a definitive attitude toward the intervention and involvement of food quality and safety management.

In the past 20 years, public health sectors and private organizations worldwide have been developing regulations for food safety management [3], while economically advanced countries are endeavoring to enhance the traceability and transparency of food manufacturing processes [4]. A review of past studies on food safety management reveals that studies have overly focused on discussing safety management systems and strategies. In addition, the discussed contents differ according to the extent of government involvement in food safety and health management [3, 5–7]. Nevertheless, governments around the world have reinforced their food safety standards and management regulations, paying particular emphasis on not only the vertical and horizontal relationships in the supply chain of the food manufacturing industry, but also the division of labor and integration of supply chains in the food industry [8]. Thus, supply

chain actors (including retailers, wholesalers, manufacturers, suppliers, and logistics providers) are also the driving force for food safety.

Businesses must sustain increased marginal cost of production in order to ensure that their products conform to food safety and management standards; such cost increase suppresses business profits. However, if businesses do not comply with food safety regulations, they must assume the risk of having their reputation being discredited, which also curbs business profits. Therefore, to address this problem, businesses must determine how a balance between “business profit” and “food quality and safety” can be maintained. Accordingly, many scholars have concentrated on assessing the value of willing-to-pay (WTP). Subsequently, they evaluated business profit levels by measuring the effect of WTP on the cognition of consumers regarding food quality and safety.

Hedonic pricing methods [9–13] and mixed multinomial logit approaches [14–16] are typically employed to estimate WTP given that market trading information is accessible. If such information is inaccessible or nonexistent and quality and safety are assumed as capable of increasing WTP, then various other evaluation methods are used such as contingent valuation [17–20], experimental economics [21–23], and conjoint analysis [24–26]. The aforementioned studies have yielded informative results, providing effective and applicable methods for helping businesses strike a balance between “business profit” and “food quality and safety”.

Despite that numerous methods have been proposed for food safety management, the reality is the following question: have food quality and safety problems been effectively controlled and improved? The answer to this question is evident from the incessant occurrence of food safety scandals involving adulterated foods and low-quality foods. First, consumers usually purchase products on the basis of “value for money” or “lowest price.” In general, businesses believe that price is the most crucial parameter affecting consumers’ selection of food products [8]. Second, habitual purchasing behavior is often observed when consumers purchase food products; therefore, consumers’ perception of food quality, safety, and price is affected by their prior experience in purchasing the product. Consumers who demonstrate habitual purchasing have a low sensitivity toward the relationship that food quality and safety have with food price. Moreover, they also process information unconsciously [27, 28]. Third, businesses must exert additional marketing efforts to ensure that consumers know that new, quality and safety-improved products are launched. In addition, they must consider consumers’ perception of the compensatory relationship between “quality and safety” and “price.” Generally, low-quality products are compensated by selling them at a lower price; however, the reason for such compensation might differ according to food types [29]. Nonetheless, these considerations inevitably increase businesses’ production cost. Fourth, businesses have a low level of awareness on the consequences of violating food safety regulations. Furthermore, regardless of business size, the status of a business’s competitive advantage, and the food safety challenges they face, all of these factors exert no influence on compliance with food safety regulations [3]. Based on the aforementioned discussion, we found that

implementing food safety control and management from policy perspectives (e.g., food safety standards) and market perspectives (i.e., consumers’ food safety evaluations) is clearly ineffective and inadequate.

When producers of harmless foods are discovered by the public health unit as being involved in broadly defined food safety problems (e.g., false labeling or food adulteration), these food vendors are only penalized with a fine rather than having them assume criminal liability for incurring risk of injury to others and destroy the food products that have not yet been sold in the market. Consequently, falsely labeled (or adulterated) harmless foods are prevalent in the market. This can be attributed to the lack of a mathematical model for analyzing problems concerning food vendors falsely labeling (or adulterating) food products to gain improper profits. This mathematical model can serve as a valuable reference for establishing a penalty function, which can facilitate determining how penalties should differ according to the extent of food false labeling.

This type of food safety management practice is most commonly associated with the following problem: the actual ingredients of a food product do not accord with those labeled on the product packaging. False labeling misleads consumers into thinking that a product has excellent value for money and, therefore, making them willing to purchase it at a high price. In particular, food vendors tend to deceive consumers by selling low-quality products as excellent-quality products. For example, some vendors adulterate a fixed proportion of cooking oil into olive oil and label such adulterated oil as containing pure olive oil. Other vendors have sold Vietnamese rice (or tea) as Taiwan-produced rice (or tea). The act of deceiving consumers by selling low-quality foods as top-quality foods is prevalent for the following reasons:

- (1) Foods adulterated with those that are similar in property cannot be easily distinguished with the naked eye, particularly foods that are illegally adulterated with a low quantity of other types of foods.
- (2) Because low-quality foods are harmless to human health, food vendors illegally labeling low-quality foods as top-quality foods only face charges of fraud rather than being charged with assault.
- (3) Imposing severe punishment on illegal conduct can inevitably deter the occurrence of food vendors selling low-quality foods as high-quality foods. However, when determining the extent of punishment, there is a specific procedure in legal proceedings, and principles of proportionality must be considered instead of allowing the government to independently determine it.
- (4) If the penalty amount increases according to the proportion of illegal adulteration by food vendors, then the proportion of adulteration that is selected by food vendors for maximizing profit and is permissible under government standards (including the frequency of adulteration in the inspected food and the number of samples per inspection) will approach a constant. If this constant is greater than 0, then

competitor vendors will compete in imitating the food products, thereby causing the prevalence of food false labeling.

This study adopted the government perspective (specifically the health sector), incorporating the aforementioned food safety problems into a mathematical model for discussion. Subsequently, the optimal solution of the mathematical model was derived. We then conducted a sensitivity analysis of how the derived optimal solution is affected by market parameters and government administrative parameters, and the obtained results served as the main findings of this study.

2. Symbols

θ : food vendors deceive consumers by selling low-quality products with a ratio of θ as top-quality products, where θ is the vendor's decision variables and $0 \leq \theta \leq 1$. This means that consumers mistakenly think that ingredients are all 100% quality product, but they are, in fact, only $(1 - \theta)$ ratio quality product.

c_θ : is purchase cost per unit food product corresponding to θ ; c_0 denotes the purchase cost per unit of top-quality product; c_1 denotes the purchase cost per unit of low-quality product; and $c_0 > c_1$. c_θ can be expressed as follows.

$$c_\theta = (1 - \theta)c_0 + \theta c_1. \quad (1)$$

p : is market price per unit of top-quality food.

l : is food expiration date (the maximum period during which the food is edible or valid).

A : is setup cost for vendors to purchase or produce the product.

h_θ : is unit time inventory cost per unit of food corresponding to θ ; h_0 denotes the unit time inventory cost per unit of top-quality product; h_1 represents the unit time inventory cost per unit of low-quality product, where $h_0 > h_1$. h_θ can be expressed as follows.

$$h_\theta = (1 - \theta)h_0 + \theta h_1. \quad (2)$$

M : is number of vendors selling the same type of food in the market.

S : is number of vendors from which food products were randomly sampled by the government (health unit).

$g(\theta)$: is the penalty amount based on θ degree of false labeling by food vendors randomly sampled by the government, where

$$\begin{aligned} g(0) &= 0, \\ g'(0) &\geq 0, \\ g''(0) &\geq 0, \\ \forall \theta &\in [0, 1]. \end{aligned} \quad (3)$$

In the present study, functions conforming to the properties of (3) are referred to as penalty functions conforming to the principle of fair proportionality. Because the government cannot comprehensively determine the amount of food products that have been manufactured but have not yet been sold (unless the vendor is willing to confess the truth), the government calculates the penalty for vendors' dishonest behavior by using the frequency vendors that were discovered as being dishonest (as opposed to using the amount of falsely labeled food products).

This study assumed that the government randomly samples S vendors on average every L time period to inspect their food ingredients.

L : is The time interval at which the government randomly samples food vendors to inspect their food products.

Given L and S , the average time interval at which vendors associated with θ degree of food false labeling were inspected by the government as being involved in false labeling is expressed as follows.

$$\begin{aligned} &\frac{S}{M} \cdot L + \frac{M-S}{M} \cdot \frac{S}{M} \cdot 2L + \dots + \left(\frac{M-S}{M}\right)^{i-1} \cdot \frac{S}{M} \cdot iL \\ &+ \dots = \frac{SL}{M} \frac{d}{dz} \left(\sum_{i=0}^{\infty} z^i \right) \Big|_{z=(M-S)/M} \\ &= \frac{SL}{M} \left(\frac{d}{dz} \frac{1}{1-z} \right) \Big|_{z=(M-S)/M} \\ &= \frac{SL}{M} \left(\frac{1}{(1-z)^2} \right) \Big|_{z=(M-S)/M} = \frac{LM}{S}, \end{aligned} \quad (4)$$

where $((M-S)/M)^{i-1} \cdot (S/M)$ denotes the probability of food vendors being discovered as involving in false labeling on the i the government inspection. Therefore, after vendors select θ value,

the penalty they must pay the government per unit time is

$$\frac{g(\theta)}{LM/S}. \quad (5)$$

T : is vendor inventory cycle.

$x_\theta(t)$: is inventory level at t for food products with health hazard indicator of θ , where $x_\theta(t)$ is a decreasing function of t within $[0, T]$ inventory cycle.

$-x'_\theta(t)$: is food sales rate at t corresponding to food products sold at price p .

$Q_\theta(t)$: is (accumulated) sales volume within $[0, t]$ for food products with health hazard indicator of θ .

$G_\theta(T)$: is profit per unit time corresponding to vendor's decision variable θ and inventory cycle T .

$D(p)$: is potential demand per unit time reflected by consumer perception of price p . In other words,

it denotes the rate of demand as expressed by consumers without considering food expiration date. Assuming that $D(p) = a - bp$, where $a > 0$ represents the maximum potential demand, $-b < 0$ denotes the slope of the demand function, and a/b denotes the maximum price p .

$\gamma(t)$: is the amount of food products purchased by consumers according to their daily demand, which is based on the sales price p and $(l - t)$ time remaining before expiration. In other words, it is the ratio of purchase amount of food product with expiration date l to food product with $(l - t)$ remaining before expiration. Specifically, $\gamma(t)$ is a decreasing function of t , where $\gamma(0) = 1$ and $\gamma(l) = 0$.

Considering the transaction cost, consumers adjust their purchase volume according to the ratio of purchase amount of food product with expiration date l to food product with $(l - t)$ remaining before expiration. When consumers purchase a product, the price they pay includes not only the product itself, but also the transaction costs (i.e., purchase time and delivery costs). Therefore, the larger the purchase volume, the smaller the transaction cost per unit of product.

Assuming that $\gamma(t)$ is the linear function of t , then the aforementioned properties can be expressed as follows.

$$\gamma(t) = \begin{cases} \frac{l-t}{l}, & \text{if } t \in [0, l] \\ 0, & \text{if } t \geq l. \end{cases} \quad (6)$$

Assuming that the food vendors pursue maximizing profit, the profit $p - (c + hT)$ per unit of food sold at T (end of inventory cycle) must be ≥ 0 (if $p - (c + hT) < 0$, then vendors can raise their profit by reducing the initial purchase volume to reduce the sales cycle T). Thus,

$$\begin{aligned} T &\leq l, \\ p - (c + hT) &\geq 0. \end{aligned} \quad (7)$$

From (6), given any θ value, the following can be obtained:

$$\begin{aligned} -x'_\theta(t) &= D(p) \cdot \gamma(t) = (a - bp) \frac{l-t}{l}, \\ t &\in [0, T] \subseteq [0, l]. \end{aligned} \quad (8)$$

Integrating (8),

$$\begin{aligned} x_\theta(t) &= x_\theta(0) + \int_0^t x'_\theta(z) dz \\ &= x_\theta(0) - \left[(a - bp) \left(t - \frac{t^2}{2l} \right) \right], \quad t \in [0, T], \end{aligned} \quad (9)$$

where inventory cycle T satisfies

$$0 = x_\theta(T) = x_\theta(0) - \left[(a - bp) \left(T - \frac{T^2}{2l} \right) \right]. \quad (10)$$

From (10), when T value is determined by vendor, then $x_\theta(0)$ can be obtained, thus, simultaneously deriving the function $x_\theta(t)$ of (9).

3. The Mathematical Model for Optimal Inventory Cycle after Vendors Defined θ

From (8), given θ value, the sales volume $Q_\theta(t)$ within $[0, t]$ is expressed as follows.

$$\begin{aligned} Q_\theta(t) &= \int_0^t (a - bp) \frac{l-z}{l} dz = \frac{(a - bp)}{l} \left(lt - \frac{t^2}{2} \right), \\ t &\in [0, T]. \end{aligned} \quad (11)$$

Furthermore, the inventory level (i.e., sales volume within $[t, T]$) at t during inventory cycle $[0, T]$ is $\int_t^T (a - bp)(l - z)/l dz$. Therefore, after vendors decide θ , without considering the loss resulting from the government's penalty, then the sales profit within $[0, T]$ is

$$\begin{aligned} &(p - c_\theta) \int_0^T (a - bp) \frac{l-t}{l} dt - A \\ &- h_\theta \int_0^T \int_t^T (a - bp) \frac{l-z}{l} dz dt. \end{aligned} \quad (12)$$

Using partial integration, $\int_0^T \int_t^T (l - z) dz dt$ can be written as $\int_0^T (l - t)t dt$. Thus, (12) can be rewritten as

$$\frac{(a - bp)}{l} \int_0^T (l - t)(p - c_\theta - h_\theta t) dt - A. \quad (13)$$

Using (5) and (13), the following model can be obtained: after vendors define θ , the model for maximum profit $G_\theta(T)$ per unit time after determining decision variable T is as follows.

$$\begin{aligned} \max_T \quad &G_\theta(T) \\ &= \frac{a - bp}{l} \cdot \frac{1}{T} \int_0^T (l - t)(p - c_\theta - h_\theta t) dt - \frac{A}{T} \\ &\quad - \frac{g(\theta)}{LM/S} \end{aligned} \quad (14)$$

$$\text{s.t. } T \leq l,$$

$$T \geq \frac{p - c_\theta}{h_\theta}.$$

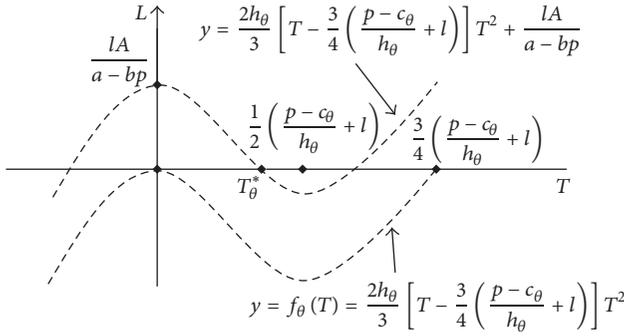


FIGURE 1: Position of T_{θ}^* for given θ .

4. The Optimal Inventory Cycle T_{θ}^* in (14) with Given θ

Differentiating objective function G_{θ} of (14) with respect to T yields

$$\begin{aligned}
 G'_{\theta} &= \frac{a - bp}{l} \left[\frac{(l - T)(p - c_{\theta} - h_{\theta}T)}{T} - \frac{\int_0^T (l - t)(p - c_{\theta} - h_{\theta}t) dt}{T^2} \right] + \frac{A}{T^2} \\
 &= \frac{a - bp}{l} \left[\frac{(l - T)(p - c_{\theta} - h_{\theta}T)}{T} - \left(\frac{l(p - c_{\theta})}{T} - \frac{p - c_{\theta} + lh_{\theta}}{2} + \frac{h_{\theta}T}{3} \right) \right] + \frac{A}{T^2} \\
 &= \frac{a - bp}{lT^2} \left[f_{\theta}(T) + \frac{LA}{a - bp} \right],
 \end{aligned} \tag{15}$$

where,

$$f_{\theta}(T) = \left[\frac{2h_{\theta}}{3}T - \frac{p - c_{\theta} + lh_{\theta}}{2} \right] T^2. \tag{16}$$

Using (15), we obtain

$$\begin{aligned}
 G'_{\theta}(T) &\geq 0 \quad \text{iff } f_{\theta}(T) + \frac{LA}{a - bp} \geq 0 \\
 f'_{\theta}(T) &= 2h_{\theta}T^2 - (p - c_{\theta} + lh_{\theta})T \\
 &= 2h_{\theta} \left[T - \frac{1}{2} \left(\frac{p - c_{\theta}}{h_{\theta}} + l \right) \right] T.
 \end{aligned} \tag{17}$$

Using (7), (15), (16), and (17), the optimal solution T_{θ}^* of (14) can be obtained, as shown in Figure 1.

Proposition 1. *Once a vendor decides θ , the inventory cycle T_{θ}^* that maximizes (expected) profit per unit time must satisfy the following:*

$$(i) \quad \left[\frac{2h_{\theta}}{3}T_{\theta}^* - \frac{p - c_{\theta} + lh_{\theta}}{2} \right] T_{\theta}^{*2} + \frac{LA}{a - bp} = 0. \tag{18}$$

(ii) *When setup cost A is reduced, or maximum demand a is increased, or demand function slope $-b$ is increased (equivalent to consumers being more sensitive to the price changes in the demand function), the vendors' optimal inventory cycle T_{θ}^* will reduce.*

Proof. (i) It can be proven by using (16) and Figure 1.

(ii) From (16), functions f_{θ} are independent of parameters A , a , and b ; therefore, assumption (ii) yields that $A/(a - bp)$ decreases, and hence the upper curve shifts downward (see Figure 1), reducing its intersection point T_{θ}^* with the horizontal axis. \square

Proposition 2. $(d/d_{\theta})T_{\theta}^* < 0, \forall \theta \in [0, 1]$.

Proof. From (i) and (ii), it can be obtained: when θ increases, h_{θ} and c_{θ} decreased, and $(p - c_{\theta})/h_{\theta}$ increases, thus causing the curve $y = (2h_{\theta}/3)[T - (3/4)((p - c_{\theta})/h_{\theta} + l)]T^2 + LA/(a - bp)$ to shift downward, reducing T_{θ}^* (see Figure 1). \square

Proposition 3. *For some $\theta, \theta \in [0, 1]$, the following property is supported:*

$$\begin{aligned}
 (i) \quad & \left[\frac{1}{2} \left(\frac{p - c_{\theta}}{h_{\theta}} + l \right) - T_{\theta}^* \right] \frac{d}{d_{\theta}} T_{\theta}^* \\
 &= \frac{h_1 - h_0}{3h_{\theta}} \left[T_{\theta}^* - \frac{3}{4} \left(l - \frac{c_0 - c_1}{h_0 - h_1} \right) \right] T_{\theta}^*.
 \end{aligned} \tag{19}$$

$$(ii) \quad T_{\theta}^* > \frac{3}{4} \left(l - \frac{c_1 - c_0}{h_1 - h_0} \right) \quad \forall \theta \in [0, 1]. \tag{20}$$

Proof. (i) Differentiating (18) with respect to θ and using (1) and (2) yields the following:

$$\begin{aligned}
 & \frac{2}{3} (h_1 - h_0) T_{\theta}^{*3} + 2h_{\theta} T_{\theta}^{*2} \frac{dT_{\theta}^*}{d\theta} \\
 & - \frac{-(c_1 - c_0) + l(h_1 - h_0)}{2} T_{\theta}^{*2} \\
 & - (p - c_{\theta} + lh_{\theta}) T_{\theta}^* \frac{dT_{\theta}^*}{d\theta} = 0.
 \end{aligned} \tag{21}$$

Eliminating T_{θ}^* from the aforementioned equation yields (19).

(ii) Using the constraint in (14), a positive value is obtained from the left parenthesis terms in (19); from (2), $(h_1 - h_0) < 0$; and using Proposition 2 and (19) proves (20). \square

Proposition 4. *After the vendors decide θ , the optimal inventory cycle T_{θ}^* corresponding to θ and the optimal unit time profit*

$G_\theta(T_\theta^*)$ exhibit the following relationship:

$$G_\theta(T_\theta^*) = \frac{a-bp}{l} \left[(l-T_\theta^*)(p-c_\theta-h_\theta T_\theta^*) - \frac{lS \cdot g(\theta)}{(a-bp)LM} \right]. \quad (22)$$

Proof. Multiply the left and right terms of (14) by T , differentiate them with respect to T , and then substitute T with T_θ^* ; subsequently, Proposition 4 can be proven using $dG_\theta(T_\theta^*)/dT = 0$. \square

5. Obtaining the Optimal Solution θ^* of $\max_\theta G_\theta(T_\theta^*)$

Considering the differentiation of (23) with respect to θ and using (18), the following expression can be obtained:

$$\begin{aligned} \frac{d}{d\theta} G_\theta(T_\theta^*) &= \frac{a-bp}{l} \left[(l-T_\theta^*) \right. \\ &\cdot \left(c_0 - c_1 + (h_0 - h_1)T_\theta^* - h_\theta \frac{dT_\theta^*}{d\theta} \right) \\ &\left. - (p - c_\theta - h_\theta T_\theta^*) \frac{dT_\theta^*}{d\theta} \right] - \frac{Sg'(\theta)}{LM} \\ &= \frac{a-bp}{l} \left[(2h_\theta T_\theta^* - lh_\theta - p + c_\theta) \frac{dT_\theta^*}{d\theta} + l(c_0 - c_1) \right. \\ &\left. - (h_0 - h_1)T_\theta^{*2} - (c_0 - c_1)T_\theta^* + l(h_0 - h_1)T_\theta^* \right] \\ &\quad - \frac{Sg'(\theta)}{LM}; \end{aligned} \quad (23)$$

apply (19) to the first term in the middle parenthesis

$$\begin{aligned} &= \frac{-(a-bp)(h_0-h_1)}{3l} \left[T_\theta^{*2} - \frac{3}{2} \left(l - \frac{c_0-c_1}{h_0-h_1} \right) T_\theta^* \right. \\ &\quad \left. - \frac{3l}{h_0-h_1} \left(c_0 - c_1 - \frac{Sg'(\theta)}{(a-bp)LM} \right) \right] \\ &= \frac{-(a-bp)(h_0-h_1)}{3l} \left[\left(T_\theta^* - \frac{3}{4} \left(l - \frac{c_0-c_1}{h_0-h_1} \right) \right)^2 - \frac{1}{4} \right. \\ &\quad \left. \cdot \delta_\theta \right], \end{aligned} \quad (24)$$

where δ_θ is the discriminant of the T_θ^* quadratic polynomial in the middle parenthesis of (24) and is expressed as follows:

$$\begin{aligned} \delta_\theta &= \left[\frac{3}{2} \left(l - \frac{c_0-c_1}{h_0-h_1} \right) \right]^2 \\ &\quad + \frac{12l}{h_0-h_1} \left[c_0 - c_1 - \frac{Sg'(\theta)}{(a-bp)LM} \right], \end{aligned} \quad (25)$$

$\theta \in [0, 1]$.

Differentiating (25) with respect to θ yields the following:

$$\begin{aligned} \frac{d^2 G_\theta(T_\theta^*)}{d\theta^2} &= \frac{-(a-bp)(h_0-h_1)}{3l} \left[2 \left(T_\theta^* \right. \right. \\ &\quad \left. \left. - \frac{3}{4} \left(l - \frac{c_0-c_1}{h_0-h_1} \right) \right) \frac{dT_\theta^*}{d\theta} + \frac{3l}{h_0-h_1} \right. \\ &\quad \left. \cdot \frac{Sg''(\theta)}{(a-bp)LM} \right]; \text{ using (19)} \\ &= \frac{-(a-bp)(h_0-h_1)}{3l} \left[2 \left(T_\theta^* \right. \right. \\ &\quad \left. \left. - \frac{3}{4} \left(l - \frac{c_0-c_1}{h_0-h_1} \right) \right)^2 \right. \\ &\quad \left. \cdot \frac{(h_1-h_0)T_\theta^*}{((1/2)((p-c_\theta)/h_\theta+1)-T_\theta^*)3h_\theta} + \frac{3l}{h_0-h_1} \right. \\ &\quad \left. \cdot \frac{Sg''(\theta)}{(a-bp)LM} \right]. \end{aligned} \quad (26)$$

Proposition 5. (i) For some θ , $\theta \in (0, 1]$ such that $dG_\theta(T_\theta^*)/d\theta = 0$, then $\delta_\theta \geq 0$ and

$$\begin{aligned} \frac{d^2 G_\theta(T_\theta^*)}{d\theta^2} &= \frac{(a-bp)(h_0-h_1)^2 \delta_\theta}{3l} \\ &\quad \cdot \frac{T_\theta^*}{((1/2)((p-c_\theta)/h_\theta+1)-T_\theta^*)3h_\theta} \\ &\quad - \frac{Sg''(\theta)}{LM}. \end{aligned} \quad (27)$$

(ii) In (i), if (27) is negative, then θ is the maximum point of the function $G_\theta(T_\theta^*)$; and if (27) is positive, then θ is the minimum point of the function $G_\theta(T_\theta^*)$.

Proof. Combining (25) and (26), we obtain (27). \square

Proposition 6. If $\delta_0 \leq 0$, then the optimal solution θ^* of $\max G_\theta(T_\theta^*)$ is $\theta^* = 0$.

Proof. From (3) and (25), δ_θ is a decreasing function of θ ; if $\delta_0 \leq 0$, then $\delta_\theta \leq 0, \forall \theta \in [0, 1]$, thereby obtaining $(d/d\theta)G_\theta(T_\theta^*) \leq 0, \forall \theta \in [0, 1]$ from (25). Therefore, $\theta^* = 0$. \square

Proposition 7. (i) The necessary condition for $\theta^* = 0$ is $[T_\theta^* - (3/4)(l - (c_0 - c_1)/(h_0 - h_1))]^2 \geq (1/4)\delta_0$ (i.e., if $\delta_0 \geq 0$, then $T_\theta^* - (3/4)(l - (c_0 - c_1)/(h_0 - h_1)) \geq (1/2)\sqrt{\delta_0}$) (see (20)).

(ii) The necessary condition for $\theta^* = 1$ is $[T_\theta^* - (3/4)(l - (c_0 - c_1)/(h_0 - h_1))]^2 \leq (1/4)\delta_1$ (i.e., if $\delta_1 \geq 0$, then $T_\theta^* - (3/4)(l - (c_0 - c_1)/(h_0 - h_1)) \leq (1/2)\sqrt{\delta_1}$).

(iii) The necessary condition for $\theta^* \in (0, 1)$ is $[T_\theta^* - (3/4)(l - (c_0 - c_1)/(h_0 - h_1))]^2 = (1/4)\delta_{\theta^*}$ (i.e., if $\delta_{\theta^*} \geq 0$, then $T_\theta^* = (3/4)(l - (c_0 - c_1)/(h_0 - h_1)) + (1/2)\sqrt{\delta_{\theta^*}}$) (see (20)).

Proof. The necessary condition for $\theta^* = 0$ is $(dG_\theta(T_\theta^*)/d_\theta)|_{\theta=0} \leq 0$; the necessary condition for $\theta^* = 1$ is $(dG_\theta(T_\theta^*)/d_\theta)|_{\theta=1} \geq 0$; the necessary condition for $\theta^* \in (0, 1)$ is $(dG_\theta(T_\theta^*)/d_\theta)|_{\theta=\theta^*} = 0$. Therefore, Proposition 7 can be proven by (24). \square

Proposition 8. *If $g(\theta)$ is the linear function of θ , $\theta \in [0, 1]$, then $\theta^* = 0$ or $\theta^* = 1$.*

Proof. The assumption yields that there exists a positive real number satisfying

$$g(\theta) = e\theta, \quad \forall \theta \in [0, 1]; \quad (28)$$

therefore,

$$g''(\theta) = 0, \quad (29)$$

and from (27) it leads to $d^2G_\theta(T_\theta^*)/d\theta^2 \geq 0$, $\forall \theta \in [0, 1]$. This implies that $g(\theta)$ has no maximum point. Thus, $\theta^* = 0$ or $\theta^* = 1$. \square

Proposition 9. *If the unit time adulteration marginal penalty $Sg'(0)/LM$ at $\theta = 0$ is smaller than the price difference $(c_0 - c_1)(a - bp)$ between the purchase costs of top-quality and low-quality products per unit time demand, then $\theta^* \neq 0$.*

Proof. The assumption yields that

$$\frac{Sg'(0)}{LM} \leq (c_0 - c_1)(a - bp); \quad (30)$$

using (25), it leads to

$$\frac{1}{4}\delta_0 \geq \left[\frac{3}{4} \left(l - \frac{c_0 - c_1}{h_0 - h_1} \right) \right]^2. \quad (31)$$

Therefore, $\theta^* \neq 0$ can be proven using (i) in Proposition 7. \square

6. Conclusions

In this study, we incorporated the food expiration factors that influence consumers' purchase intention and the volume they purchase into the conventional EOQ model, establishing the food EOQ model. Subsequently, based on this food EOQ model, we discussed the food safety problems that have attracted the attention and generated intensive response from people of the Taiwanese society. In practice, many food vendors had been discovered to be involved in false labeling of food products such as olive oil adulterated with different proportions of cooking oil. When the government discovers that a vendor sells harmless low-quality food products as top-quality products to consumers, these falsely labeled foods are not destroyed because of their harmless property. Thus, different methods are employed to calculate the inventory risk cost of vendors' food products and that of hazardous food additives. This study developed a mathematical model based on the aforementioned food safety problems. The results revealed the following interesting properties.

Property 1. If the government follows the principle of adopting mild penalty, then regardless of which penalty function

based on the principle of proportionality the government adopts, vendors will not be prompted to produce or sell food products containing no low-quality ingredients (refer to Proposition 9). This indicates that vendors inevitably falsely label their food products. In addition, the government's administrative means can only reduce the optimal ratio of θ^* in vendors' food products containing low-quality ingredient, rather than reduce θ^* to 0. This suggests that, in practice, if the labeled food ingredient is 100% true then it is attributed to the vendor having self-constraint because of his or her morality, instead of to the penalty imposed by the government to deter the act of food false labeling.

Property 2. According to Property 1, if the government adopts an adequately strict penalty of $g'(0)$, then such measure might encourage vendors to select an optimal low-quality ingredient ratio of $\theta^* = 0$. In addition, this study revealed how $g'(0)$ must be related to the model parameters in order to encourage vendors to select $\theta^* = 0$ (refer to Propositions 6 and 7).

Property 3. When the public health department decides the penalty function $g(\theta)$ for vendors who falsely labeled low-quality foods with a ratio of θ as top-quality foods, and the department's inspection frequency and number of vendor samples per inspection are given, this study provided a mathematical model (refer to Proposition 7) for determining the optimal (illegal) adulteration ratio that maximizes vendors' profit. Under special circumstances, if the penalty function $g(\theta)$ is the linear function of the adulteration ratio θ , then the optimal adulteration ratio θ^* for vendors must be either 0 or 1 (refer to Proposition 8).

Competing Interests

The author declares that he has no competing interests.

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