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Editorial

COVID-19 pandemic and challenges in delivering laboratory classes for food science courses



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The COVID-19 pandemic had a significant challenge for the educational institutions worldwide, when universities had to abruptly resort to online and remote learning methods. It significantly affects courses that rely largely on laboratory skills, including food science courses. Subjects like Food Chemistry/Biochemistry, Food Analysis, Food Microbiology, Food Processing and Preservation, Food Engineering, Sensory Analysis or New Food Product Development that need skills to be taught directly in-person. On the other hand, the pandemic brought educators worldwide to be exposed to the pedagogic theory of online-learning as well as the technologies needed to deliver course topics that traditionally are taught face-to-face in labs.

There were many options urgently developed in short time in order to cope with the sudden closure, either

partially or fully, of schools and universities worldwide. Educators had to resort to teaching and learning activities using videos of lab demonstrations, for example virtual tours of the equipment followed by detailed description of its operation for Food Engineering labs and recorded videos of analysis process for Food Analysis labs. There were also lecturers who opted for Microsoft PowerPoint slides with voice-over recordings that were prepared as laboratory manuals as well as written manuals for simple kitchen-based experiments that students could perform at home for certain topics in subjects such as Food Commodities, Food Processing, Sensory Analysis and Food Product Development or even for some basic topics involving food chemistry/biochemistry, such as acidity and enzyme activities. Along with the videos and manuals, assessment was also needed to evaluate the effectiveness of the teaching strategies and if the learning



objectives had been met. Evaluation was performed usually via individual quizzes, individual and collective written reports, and individual online exam.

When asked to give opinions regarding the remote teaching of lab sessions, students reported that videos, combined with voiced-over Microsoft PowerPoint slides, helped them to understand important aspects of the labs, while the home-based experiments provided them with a hands-on opportunity to observe and relate to concepts that have been taught in the lecture sessions, although the topics possible to be covered with this method was limited. Thus, those methods were considered relatively sufficient, although flaws existed due to the lack of direct access to labs and equipment, especially for at-home lab sessions. Faculty members also noticed weaknesses in hands-on ability and skills of the students in terms of equipment handling, analytical skills and other general lab skills, which brought suggestion that a face-to-face comprehensive lab session should be arranged specially for the batch of students that went through the remote learning strategy once offline classes were resumed. A special consideration should be taken when the labs involve not only technical skills but also data analysis; thus the remote lab sessions should be followed by tutorial sessions, preferably in smaller groups.

It is also of highly important that food science educators create an environment, where students can form bonds with their peers as what usually happen in offline sessions. Development of group work ethics, trust, respect, and sense of belonging must be considered when innovating asynchronous lab sessions, since communication and teamwork are essential lifelong skills needed by these young food scientists. It is without a doubt that an all-digital approach is not the most ideal strategy for teaching lab sessions in food science courses, but developing educational resources for future hybrid teaching approaches is highly important. Special consideration on infrastructure and digital equality needs to be taken to ensure suitable learning methods are applied to cater for all students. It is also vital to note that no matter what strategy an educator develops to deliver lessons, both in onsite lab sessions as well as in remote labs, they need to ensure that learning objectives are met and social engagement takes place.

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Protein and iron source snack bar made from *Mlanding Tempeh* – A fermented *Lamtoro* (*Leucaena leucocephala*)

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Mlanding tempeh;
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supplementary feeding;
male adolescents.

Mlanding Tempeh is an Indonesian fermented food made from *Lamtoro* (*Leucaena leucocephala*), a tropical legume that contains complete nutrients. The legume has high productivity and adapts well to dry climates thus serving well as a plant-based protein source in arid areas to improve the local food security. This is especially true for Indonesian male adolescents who often suffer from undernutrition. This study aimed to introduce a process to further improve the nutritional value and potential acceptance of the *Mlanding* Tempeh by developing it into a snack bar. A complete food process development and nutrient analysis were performed. Analysis of anti-nutrient content found a reduction of tannins after 24h fermentation of *Lamtoro*, from 52.11 to 4.20 (mg. tannic acid). Frying and oven-baked method was chosen for processing the Tempeh into the snack bar. Formulation with 60% *Mlanding* Tempeh, 35% red kidney beans, and 5% puffed rice had the highest acceptance and preference score. The energy and nutrients such as protein, fat, and carbohydrates content in 100 g of snack bars were 385.44 kcal, 17.62 g, 3.18 g, and 71.58 g, respectively. A 100 g of snack bar provided around 29% of the daily protein and 22.27% of iron needs for male adolescents. Hence, the *Mlanding* Tempeh snack bar can be considered a good source of protein and iron for this demography. In addition, it also has high dietary fibre.

1. Introduction

Indonesia has various legumes, one of which is *Lamtoro* (*Leucaena leucocephala*). This legume contains high protein and complete nutrients (Palupi et al., 2020; Mahmud et al., 2017; Sayudi et al., 2015). It has high productivity and adaptability to high temperatures and dry climates like the arid area of Gunungkidul, Yogyakarta, Indonesia which is prone to food insecurity (Palupi et al., 2019). However, despite its high nutrient contents *Lamtoro* also contains anti-nutritional substances which can compete with the absorption of important nutrients (Orwa, 2009; Hamid et al., 2017). Previous studies showed that the fermentation process reduces these anti-nutritional

substances, increases protein digestibility, optimizes nutritional value, increases the sensory value, and improves the physical properties of the legume-based product (Sayudi et al., 2015; Nkhata et al., 2018).

Acute undernutrition indicated by wasting and severe wasting is prevalent in Indonesia. Adolescent is one of the vulnerable groups at risk of suffering from this type of malnutrition because they need greater nutrition to fulfil their rapid growth and development (Tarwoto et al., 2010). Based on the Indonesian Basic Health Research in 2018, the prevalence of wasting and severe wasting in adolescents aged 13 – 15 years

old was 8.7% while in adolescents aged 16-18 years was 8.1%. For male adolescents, their growth during this period is very rapid and their physical activities including sports are at their peak. They have a more significant peak height velocity (PHV) period (Abaci et al., 2013), twice as much muscle mass gain and a larger skeleton (Buyukgebiz, 2012), a basal metabolic rate about 10% higher than their female counterparts (Traggiai & Stanhope, 2003). Therefore, their nutrition needs as reflected in their Recommended Dietary Allowance (RDA) are greater than that of adolescent girls of the same age (RDA, 2019).

Adequate nutrition intake to fulfil nutritional needs during that accelerated growth and changes in body composition associated with puberty is pivotal (WHO & FAO, 2003). Nutrition deficiency during this phase will lead to growth and development problems (Carrasco-Luna et al., 2018). Easily accessible high-quality supplementary food can help meet the nutritional needs of this vulnerable group. Therefore, this study aimed to develop a supplementary food made from Tempeh *Lamtoro*, a local fermented legume-based food in a form of a snack bar for male adolescents. The drying methods and the ratio combination have been used as the research treatment in this study since those variables has a direct correlation with the sensory quality.

2. Materials and Methods

2.1. The process of making *Mlanding* Tempeh

Mlanding Tempeh was made based on a procedure adopted from *Mlanding* Tempeh craftsmen in Gunungkidul, Yogyakarta. The original process uses wild *Lamtoro* collected from the forest which is then fermented using a traditional yeast derived from cassava flour and dried teak leaf slices (*R. oligosporus*). Whilst the *Mlanding* Tempeh in this study was produced using *Lamtoro* planted in three regions in Bogor, West Java, and fermented using branded yeast "Raprima". The Tempeh was produced through the following stages: sorting the *Lamtoro* seeds, washing, soaking the seed for 24 hours (water : seed = 2:1; ambient temperature), peeling the epidermis, boiling the peeled seed for three hours, draining to room temperature, inoculating the seed with yeast (seed : yeast = 1:1), packing using teak leaves (6 cm x 7 cm x 4 cm), and incubating at 27-29°C for 48 hours (anaerobic,

dark).

2.2. The process of making the snack bar

The main ingredients for the snack bar were *Mlanding* Tempeh, red kidney beans, puffed rice, and binding materials like melted sugar, oil, and water. The Tempeh and red kidney beans were dried prior to the making of the snack bar in order to remove the moisture of Tempeh and prolong its shelf life. Two types of drying methods i.e., deep fat frying (140°C, 7 min, modified from Amalia et al., 2015) and oven baking (90°C, 45 min, Nauli et al., 2006) were applied for this process. While the red kidney bean was dried using roasted-puffing methods (90°C, 45 min, Dwijayanti, 2017). The formulation and process were adapted from a modified snack bar recipe from Ahmad et al. (2017). Previous research showed that the use of puffed rice in the snack bar formulation improves its texture and compactness since it has a better density and bonding ability (Jauhariah & Ayustaningwarno, 2013). Thus, we added puffed rice to our formulation. The ingredients and binding materials were mixed evenly distributed and then formed into a bar shape (printed by using a square mould). The formulations were developed through various trials with different types of Tempeh drying methods (b: baked and f: fried) and different ratios of *Mlanding* Tempeh and red kidney beans used. The final formulations for further evaluation were chosen based on the result of the minimum and maximum use of *Mlanding* Tempeh based on the quantity of limiting amino acids (lysine and sulfur amino acids). The four formulations were further evaluated namely, Fb 50:45, Fb 60:35, Ff 50:45, and Ff 60:35, see Table 1.

2.3. Sensory, nutrient, and anti-nutrient evaluation

The sensory evaluation was performed using an acceptance test (ISO 6658). The content of tannin was measured using the spectrophotometric method (Mukhriani, 2014). While the nutrient content was assessed using proximate analysis such as the following: moisture and ash – gravimetric method (AOAC method 925.10 and 923.03), protein - Foss Tecator Kjeltac 200 method (AOAC method 955.04), fat - Soxhlet method (AOAC method 920.39), carbohydrate - by difference, dietary fibre - enzymatic method (AOAC Method 994.13), and iron - Atomic Absorption Spectroscopy (Sulaeman et al., 1995).

Table 1. Formulations of snack bars made from *Mlanding* Tempeh and red kidney beans

Ingredients	Unit	Fb 50:45	Fb 60:35	Ff 50:45	Ff 60:35
Mlanding Tempeh (Baked)	gram	50	60	-	-
Mlanding Tempeh (Fried)	gram	-	-	50	60
Dry red kidney beans	gram	45	35	45	35
Puffed rice	gram	5	5	5	5
White sugar	gram	15	15	15	15
Water	gram	1	1	1	1
Coconut oil	gram	1	1	1	1

Note: F: Formulation, Fb 50:45, formulation with baked *Mlanding* Tempeh with ratio 50 to 45 between *Mlanding* Tempeh and red kidney beans; Fb 60:35, formulation with baked *Mlanding* Tempeh with ratio 60 to 35 between *Mlanding* Tempeh and red kidney beans; Ff 50:45, formulation with fried *Mlanding* Tempeh with ratio 50 to 45 between *Mlanding* Tempeh and red kidney beans; and Ff 60:35, formulation with fried *Mlanding* Tempeh with ratio 60 to 35 between *Mlanding* Tempeh and red kidney beans.

2.4. Experimental design

This study was performed using a completely randomized design. The number of replications was three batches of the formulation. There was twice an analysis per batch of samples. The data were processed using Microsoft excel for windows 2010 and analysed using SPSS 16. A parametric test using an independent sample t-test and analysis of variance was conducted to evaluate the treatment effect. The data was interpreted as significantly different if the p-value < 0.05.

3. Results

3.1 Anti-nutritional content of tannins

Poor digestibility and utilization of protein from legumes are often associated with its tannins content. There was a significant difference in tannin content between *Lamtoro* seeds and *Mlanding* Tempeh ($p > 0.05$) as shown in Table 2. The tannin content in *Lamtoro* legume reduced by 91.94% after fermentation into Tempeh from 52.11 to 4.20 mg tannic acid/g.

3.2. Sensory evaluation

The acceptance test with 9 hedonic scales showed significant differences between formulations based on the snack bars' six attributes ($p > 0.05$). There were six attributes assessed in this study including colour, tex-

ture, flavour, taste, aftertaste, and overall. The details of sensory evaluation results are presented in Table 3. The level of preference for panellists in snack bar products ranges from neither or dislike to like. The acceptability analysis based on different Tempeh drying methods showed that the attributes of taste, aftertaste, and overall attributes in the baked Tempeh were preferred. The baked *Mlanding* Tempeh when combined with dry red kidney beans and puffed rice had the more preferred taste and a neutral aftertaste compared to the taste of the snack bar made from fried *Mlanding* Tempeh because of the oily taste and aftertaste. This was implied from the open question gathered from the respondents during the sensory evaluation.

3.3. Nutrition content of *Mlanding* Tempeh snack bar

Table 4 presents the result of proximate analysis, dietary fibre, and iron content of selected snack bar formulations namely Fb 60:35 and Ff 60:35. The moisture content in the selected snack bars were 4.85 and 5.76 (%wb), respectively. The difference in moisture content occurred due to different types of drying processes used for the *Mlanding* Tempeh preparation. In this study, *Mlanding* Tempeh was dried using a baking or frying technique. The fat content in the fried formulation was higher than in the baked one. Moreover, the protein content of the baked formulation was higher than the fried formulation, i.e., 18.63 and 15.88 (% db).

Table 2. Tannin content of *Lamtoro* legume and *Mlanding* Tempeh

Sample	Anti-Nutritional Substances (mg tannic acid/g)
<i>Lamtoro</i> legume	52.11±1.99 ^b
<i>Mlanding</i> Tempeh	4.20±0.37 ^a

Note: different superscript indicates significantly different at $p < 0.05$, was evaluated using paired t-test.

Table 3. Sensory acceptance of snack bar products made from *Mlanding* Tempeh

Attribute	Formulation			
	Fb 50:45	Fb 60:35	Ff 50:45	Ff 60:35
Colour	5.11±1.13 ^a	4.91±1.04 ^a	5.42±1.09 ^b	5.20±1.09 ^{ab}
Texture	5.07±1.34 ^a	5.28±1.12 ^a	5.53±1.26 ^{ab}	5.85± 0.97 ^b
Flavour	5.46±1.06 ^a	5.38±1.16 ^a	5.45±0.93 ^a	5.64±0.95 ^a
Taste	5.72±1.10 ^b	6.00±0.84 ^b	5.43±1.20 ^a	5.66±0.83 ^{ab}
Aftertaste	5.26±1.54 ^b	5.47±1.23 ^b	4.91±1.25 ^a	5.38±1.44 ^a
Overall	5.74±1.08 ^b	6.01±0.80 ^b	5.24±1.39 ^a	5.62±1.34 ^a

Note: F: Formulation, Fb 50:45, formulation with baked *Mlanding* Tempeh with ratio 50 to 45 between *Mlanding* Tempeh and red kidney beans; Fb 60:35, formulation with baked *Mlanding* Tempeh with ratio 60 to 35 between *Mlanding* Tempeh and red kidney beans; Ff 50:45, formulation with fried *Mlanding* Tempeh with ratio 50 to 45 between *Mlanding* Tempeh and red kidney beans; and Ff 60:35, formulation with fried *Mlanding* Tempeh with ratio 60 to 35 between *Mlanding* Tempeh and red kidney beans; different superscript in one line indicates significant difference at $p < 0.05$, evaluated using analysis of variance.

3.4. Contribution of *Mlanding* Tempeh snack bar to Recommended Dietary Allowance (RDA) for male adolescent

The study aimed to develop easy-to-access as well as nutritious supplementary food for male adolescents. Previous stages have shown that the selected formulation was Fb 60:35, thus contribution to RDA in this section refers to this product. The serving size of the snack bar, i.e., 35 g, was determined by assuming the ability to consume it at each meal or at snack time. The contribution of nutrient content in the snack bars per serving compared to the Indonesian RDA for male adolescents was divided into 3 age groups. The nutritional claims based on the comparison to the RDA are presented in Table 5.

In regard to macronutrient contribution based on the RDA for Indonesian male adolescents in the three age groups, one serving, or 35 grams of *Mlanding* Tempeh snack bar contributes to 5.09-6.74% of the total daily energy needs and 8.21-12.32% of daily protein

intake. The fat and carbohydrates contribution for one serving ranged from 1.30-1.70% and 6.26-8.34%, respectively. The macronutrient content in 100 g serving size is 385.44 kcal for energy, protein is 17.62 g, fat is 3.18 g, and carbohydrate is 71.58 g. Claims of protein and dietary fibre sources in the *Mlanding* Tempeh snack bar refer to government regulations based on the 2016 nutritional label reference (ALG). According to the percentage of the Nutrition Label Reference Indonesia (ALG), the protein source is not less than 20% ALG per 100 g, the iron source is not less than 15% ALG per 100 g and dietary fibre is not less than 6 g per 100 g (in solid form).

3.5. Comparison of *Mlanding* Tempeh snack bars with other commercial snack bars

The following table presents the comparison of the nutritional content and dietary fibre of the *Mlanding* Tempeh snack bar with other commercial snack bars available in the market. (Table 6)

Table 4. The content of macro-nutrients, dietary red kidney bean and iron in selected snack bar

Nutrient parameters	Unit	Snack bar (Baked)	Snack bar (Fried)
		Fb 60:35	Ff 60:35
Moisture	(%)	5.42±0.06 ^b	4.63±0.03 ^a
Ash	(%db)	2.32 ±0.02 ^a	2.27±0.02 ^a
Fat	(%db)	3.37±0.02 ^a	9.58±0.11 ^b
Protein	(%db)	18.63±0.05 ^b	15.88±0.20 ^a
Carbohydrate	(%db)	75.68±0.06 ^b	72.27±0.15 ^a
Dietary red kidney bean	(%db)	35.68±0.08 ^a	35.84±0.01 ^b
Iron	(mg)	4.90±0.02 ^a	5.65± 0.01 ^b
Digestibility protein in-vitro	(%)	99.76 ^a	99.70 ^a

Note: Different superscript in one line indicates significantly different at $p < 0.05$, was evaluated using paired t-test.

Table 5. Contribution of nutrition content of Mlanding Tempeh snack bar to RDA and nutrition claims

Nutrient	Nutrition content per serving size (35 g)	Nutrition content per 100 g	Contribution of macro nutrients (%) to adolescent male age- (y.o.)			Nutritional Claims
			10-12	13-15	16-19	
Energy (kcal)	134.90	385.44	6.74	5.62	5.09	-
Protein (g)	6.16	17.62	12.32	8.80	8.21	Source of protein
Fat (g)	1.11	3.18	1.70	1.38	1.30	Low fat
Carbohydrate (g)	25.04	71.58	8.34	7.15	6.26	-
Dietary fibre (%)	11.80	22.73	42.14	34.70	31.89	High
Iron (mg)	1.71	4.90	21.37	15.54	15.54	Source of iron

4. Discussion

4.1. Anti-nutritional content of tannins

Tannins are a polyphenol compound commonly found in legumes that can form complex bonds with proteins. The presence of these complex bonds causes the protein to be difficult to break down into amino acids. The presence of tannins also inhibits protease activity (Bakti, 2003). Thus, tannins lower protein digestibility (Suarni, 2012). Our study found that the fermentation process in making the *Mlanding* tempe lowers the tannin content in *Lamtoro* legume by 91.94%, from 52.11 to 4.20 mg tannic acid/g. This result is in line with previous findings from Bakti (2003) on the tannin content in fermented *Lamtoro* legumes. The decrease in tannins could be due to the presence

of the enzyme tanase produced by the yeast *Rhizopus oligosporus* (Koni et al., 2013). The enzyme tanase or tannin acyl hydrolase (EC, 3.1.1.20) is known as a catalyst in the hydrolysis reaction of gallic acid ester bonds (Aguilar et al., 2007). In addition to fermentation, immersion in water, and alkaline, mechanical means of heating can also reduce the effect of tannins (Ma'ruf, 2005). According to Bakti (2003), the dissolution of tannin components in water can be seen in the brownish colour of the immersed water.

4.2. Sensory evaluation

The results from the hedonic test can be taken into consideration to determine the best formulation for the food product (Munir et al., 2018). According to Lestari et al. (2018), the type of drying can affect the

Table 6. Comparison of nutrients and dietary fibre content of *Mlanding* Tempeh snack bar with Commercial Snack Bars

Snack bar products	Weight	Energy	Protein	Fat	Carbohydrate	Dietary fibre	Price
	(g)	(kcal)	(g)	(g)	(g)	(g)	(USD)
Zee Cereal Bar	20	90	2	2.50	15	1	0.24
Fitbar	25	110	3	5.00	16	1	0.29
Milo Chocolate Milk Snack Bar	21	86	1	2.70	14	2.1	0.41
Protein Bar L-Men	22	100	3	3.00	15	1	0.39
Heavenly Blush Tummy Yogurt Bar Berries	25	90	2	2.50	15	4	0.54
Granobar Granola Bars	35	160	3	6.00	24	4	0.64
Soyjoy Almond Chocolate	30	160	5	10.00	12	0	0.85
Soyjoy Crispy	30	120	6	6.00	10	3	0.63
Snack Bar Formulation Fb 60:35	35	134.90	6.16	1.11	25.05	11.80	0.10
Snack Bar Formulation Ff 60:35	35	146.48	5.30	3.19	24.12	11.96	0.11

Note: F: Formulation, Fb 60:35, formulation with baked *Mlanding* Tempeh with ratio 60 to 35 between *Mlanding* Tempeh and red kidney beans; Ff 60:35, formulation with fried *Mlanding* Tempeh with ratio 60 to 35 between *Mlanding* Tempeh and red kidney beans

sensory parameters of the product, especially the taste and flavour produced. There were six attributes assessed in this study namely colour, texture, flavour, taste, aftertaste, and overall. A comparison of attributes acceptance for products with a maximum proportion of 60% *Mlanding* Tempeh and 35% kidney beans showed that the fried Tempeh was preferred for colour and texture compared to the baked formulation. The colour of the fried *Mlanding* Tempeh appeared more homogenous (Kateren, 2008; Wihandini et al., 2012) and the texture was crisper (Zahra et al., 2013) than the baked one. However, based on the attributes of taste, aftertaste, and overall attribute the baked formulation was preferable. Therefore, the baked formulation was chosen for further analysis.

In regard to the proportion of *Mlanding* Tempeh and red kidney beans used, our result showed that the more the use of *Mlanding* Tempeh and the less the red kidney beans produced a more preferable texture, taste, aftertaste, and overall attributes quality. According to Dwijayanti (2017), the use of up to 40% dry red kidney bean content in the snack bar formulation produces good characteristics of taste and

texture. According to Nurlita et al., (2017) red kidney bean flour has a stronger distinctive taste. Therefore, a higher amount of red kidney beans used causes a more distinctive taste of beans which is often disliked by panellists. In addition, red kidney beans produce an unpleasant odour due to the presence of the lipoxigenase enzyme which naturally gives the nuts their special flavour (Pertwi et al., 2017). In this study, the red kidney beans used were dried red kidney beans this was in order to reduce the unpleasant taste and flavour. Moreover, dried kidney beans have a hard and crunchy texture (Kurnianingtyas et al., 2014) The starches content in red kidney beans consists of amylose and amylopectin which is important for gelatinisation process, causing the hard and crunchy texture (Rohma, 2013). While the amylopectin content increases the crispness (Santoso et al., 2007).

4.3. Nutritional content of *Mlanding* Tempeh snack bar

Currently, people are more interested in consuming healthier foods to maintain health (Lin et al., 2010). This leads to the development of healthier snack op-

tions. Snack bars made from legumes are considered a reliable source of high-quality protein, dietary fibre, vitamins, and minerals (da Silva et al., 2014). Champ et al., (2003) reported that an increase in daily dietary fibre consumption is associated with disease prevention, especially digestive diseases, energy balance problems, cancer, heart disease, and diabetes.

Comparison of moisture content from fried and baked methods differed significantly, it was 4.85 (%wb) and 5.76 (%wb) respectively. The difference in moisture content in the snack bar formulation occurs due to different types of processing in *Mlanding* Tempeh. According to Sundari et al., (2015) during frying, there is a mass heat transfer of oil to the surface of the material and propagates inward so that the water content of the material comes out as water vapor and then the material absorbs oil. The presence of water evaporation during heating with the deep-frying method causes the water content to decrease and the concentration of solids increases (Sosa-Morales et al., 2006).

The ash content analysis between the two processing methods was comparable, 2.27 and 2.31 (% db) respectively. Gall et al., (1983) reported no change in mineral composition in fish containing high fat after grilling or frying. According to Jacob et al. (2008) the ash content in fried foodstuffs is depending on the time and temperature of the frying pan used. During frying, the increase in ash content in food is mostly attributed to the loss of moisture caused by high temperatures.

The fat content in the fried formulation was higher than the baked one, this was due to cooking oil being absorbed during the frying process (Susanty et al., 2019). Some of the oil occupies the empty space in foodstuffs that were previously filled with water. In the final stage of frying, oil is also absorbed via capillary action as a vacuum form in the material. Further, during the cooling stage after frying the condensation of moisture accelerates the absorption of oil into the product (Thomas, 2007).

The protein content was found significantly higher in the baked formulation compared to the fried one 18.63 vs 15.88 (% db). According to Sundari et al., (2015) during frying, protein levels can decrease due to the higher temperature used. Similar to the protein

analysis, the carbohydrate content was significantly higher in the baked formulation. The carbohydrate content for each sample was 72.27 and 75.68 (% db) for the fried and baked formulations respectively. Inocent et al., (2011) stated that different types of heating cause different changes in nutrient content including glucose.

Carbohydrate content in the baked formulation was significantly higher, each sample was 72.27 and 75.68 (% db). According to Inocent et al., (2011) differences in carbohydrate content due to different types of drying. Food processing by heating will affect the levels of nutrients including glucose. The reactions that occur in sugar, either with reactions in the form of changes in carbohydrates themselves without the presence of other compounds or changes in carbohydrates (reducing sugars) as their interactions with amino compounds (Maillard reaction). Especially during heating, the higher temperature (650C) will further accelerate the Maillard reaction, thereby reducing the availability of sugars and proteins (amino acids) and consequently decreasing glucose levels (Indradewi 2016).

The red kidney bean content was significantly lower in the baked formulation, 35.84 vs 35.68 (% db). The structure, physicochemical properties, and effects of diet are easily influenced by food processing (Zhang et al., 2011). When Tempeh is fried, the starch changes. Gelatine starch granules rapidly change upon contact with hot oil. The structure of the Tempeh changes and becomes soft after 1-2 minutes of frying. Then upon further heating, the texture of the Tempeh becomes crispy and hard on the surface (Boskou & Elmadafa 2010). Compared to the oven baking process, the resistant starch in fried Tempeh significantly increased in part due to the formation of the amylose lipid complex (Asp & Bjorck 1992). At higher temperatures, drying food red kidney beans can reduce some components of soluble dietary fibre (SDF) and change its hydration properties and fat adsorption capacity (Garau et al., 2007). High temperatures break the glucosidic bonds in polysaccharides which can cause the release of oligosaccharides and thereby increase the amount of SDF (Wolf 2010).

The iron content in the fried formulation was significantly higher compared to the baked formulation,

5.65 vs 4.90 (mg) respectively. The difference in iron levels can be associated with cooking factors, namely the temperature and cooking time (Astuti et al., 2014). The decrease in minerals ranged from 5-40%, for iodine, calcium, selenium, zinc, and iron due to these factors. In particular, a decrease in iron was experienced in foods that were heated at a temperature of 1600C (Omoruyi et al., 2007).

The highest protein digestibility in both formulations was comparable with the value for the oven method treatment was 99.76%, while in the fried formulation the protein digestibility value was 99.70%. According to Wolfe et al., (2016) the higher the digestible protein, the better the quality of the protein in the ingredients. The frying and baked method might cause a decrease in digestibility as the cooking time increases. The protein oxidation and the changes of NH₂ may greatly affect the protein digestibility of cooked food (Tavares et al., 2019).

4.4. Contribution of *Mlanding Tempeh* snack bar to RDA for male adolescents and the nutrition claims

The snack bar can be consumed along with each meal-time or in between meals. According to the Indonesian guidelines for balanced nutrition, it is recommended to consume healthy snacks, three times a day or in a total of around 95 grams of snack bars per day divided into 2 – 3 pieces of snack bars. The energy, protein, fat, and carbohydrate content of the 100 g snack bar were 385.44 kcal, 17.62 g, 3.18 g, and 71.58 g, respectively. Hence, the protein content per 100 g of the snack bar meets about 29% of the RDA for Indonesian male adolescents in the three age groups. Based on the Indonesian National Food and Drug Agency (2016) it can be claimed as a protein source food because it can meet more than 20% of protein needs reflected in the RDA or 12 g protein / 100 g. In addition, the snack bars can also be considered as high in dietary fibre because based on the National Food and Drug Agency in Indonesia (2016) a product is said to be high in dietary fibre if it contains 6 g of dietary fibre in 100 g of product. Based on the iron analysis, the snack bar was also able to meet about 22.27% of the RDA for iron. Thus, it can also be claimed as a food source for iron.

4.5. Comparison of *Mlanding Tempeh* snack bar with other commercial snack bars

In Indonesia, there are many types of snack bars made from various ingredients. *Lamtoro* is similar to soybeans because it contains almost the same nutritional value. *Lamtoro* legumes and kidney beans have a fairly high protein and carbohydrate content of around 25-30% for protein and 50-60% for carbohydrate respectively. In addition to *Lamtoro* legume, the other main ingredient used was puffed rice which is a cereal ingredient. Based on this similarity, several soybeans-based commercial products were chosen as a comparison, The *Mlanding Tempeh* snack bar products had many similarities with commercial products in terms of physical appearance and texture. However, based on the nutritional value, this *Mlanding Tempeh* snack bar contained higher protein, fibre and iron, but lower price compared to other commercial snack bars.

5. Conclusions

This paper presents a study introducing an effort to process the *Lamtoro* legumes into a snack bar which has better acceptance and nutritional value for male adolescents. A complete food process development and nutrient analysis resulted in the best formulation for development was 60% of *Mlanding Tempeh* and 35% red kidney beans. Analysis of anti-nutrient content showed a reduction of tannin after 24h fermentation of *Lamtoro* into *Tempeh* from 52.11 to 4/20 (mg tannic acid/g). Based on the flavour, after taste, and overall attributes, the formulation using *Mlanding Tempeh* dried with the oven-baked method was more acceptable. The energy, protein, fat, and carbohydrate content in 100 g of the selected snack bars were 385.44 kcal, 17.62 g, 3.18 g and 71.58 g, respectively. The protein and iron content per 100 g of the snack bar is able to meet around 29% and 22.27% of the RDA of each nutrient for male adolescents respectively. Thus, it can be claimed as a food source of protein and iron. In addition, the *Mlanding Tempeh* snack bar is also high in dietary fibre content which is considered an added health benefit.

Conflict of Interest

The authors declare no conflict of interest. In addition, the funding organization had no role in the design of the study; in the data collection, analyses, or interpretation of data; in the writing of the manuscript, as well as in the decision to publish the results.

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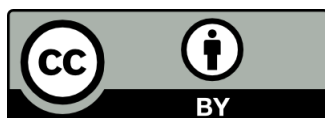
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Rural farmers' learning of weed management methods in Malaysia

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Malim Nawar in Kampar District, Malaysia, is a potential major production site for modern high-technology farms by 2030. To achieve this, a significant increase in intensive agricultural activities and weed management practices is required. To develop strategies and achieve the goals of sustainable agriculture, the present study used a semi-structured questionnaire survey to assess farmers' knowledge and perception of weeds, their sources of information, and their reasons for willingness or unwillingness to adopt non-chemical weed control methods. The survey was conducted from June to October 2018 and included 62 members of the Malim Nawar Vegetable Farmers Association. Descriptive and chi-square statistics were used for the statistical analyses. Of the 62 participants, 50 (80.6%) were over 50 years of age, and 47 (75.7%) spoke the Hakka dialect. Pest infestation and crop diseases were the most important constraints in crop production, followed by weed infestation. Knowledge of weed species led to the anticipation of yield loss and exploration of potential control methods. Social networking and agriculture chemical companies were the main sources of information on weed control methods. Despite knowing the harmful effects of chemical herbicides, farmers' willingness/resistance to adopt non-chemical weed control methods depended on many different factors. The survey results showed that the proactiveness of farmers' associations and relevant government agencies is a prerequisite for achieving agricultural development through education. Moreover, structure and systematic learning using innovative methods adjusted to local socioeconomic conditions could facilitate a paradigm shift from chemical control to environment-friendly weed control methods.

1. Introduction

Agriculture is the backbone of the industrial sector in many countries. In 2019, the contribution of agriculture to the GDP of Malaysia was 7.1%. Despite its small contribution to the economy, it is recognised as one of the most important sectors that provide food and employment to the rural inhabitants of Malaysia. In general, rural farming is a prominent feature of developing countries. For farmers, weeds can hinder crop yield as they compete with the crop for light, wa-

ter, and nutrients, resulting in varying extents of yield loss depending on crop type (Gharde, Singh, Dubey, & Gupta, 2018). Therefore, weed management is critical for ensuring food security and environmental sustainability (Yaduraju & Rao, 2013).

Farmers have been learning weed control methods on a trial-and-error basis in Malaysia; however, their limited knowledge of weed control hinders the improve-

ment of methods, including traditional practices commonly used in agriculture (Obidike, 2011; Agahiu, Baiyeri, Ogbuji, & Udensi, 2012). Moreover, farmers' motivation to accept and incorporate new methods into their practices is greatly influenced by practical (i.e., costs and time), personal (i.e., needs and interests), and local factors (i.e., crops and climatic factors) (Franz, Piercy, Donaldson, & Richard, 2010; Šūmane et al., 2018).

Local socioeconomic factors play a role in information sharing and knowledge acquisition among rural farmers (Pratiwi & Suzuki, 2017; Zossou, Arouna, Diagne, & Agboh-Noameshie, 2019). When an increased number of farmers follow certain practices, it could inspire others within the area to do the same; on the contrary, low participation could discourage other farmers from implementing novel practices. Improving farmers' knowledge of agricultural practices is critical for achieving sustainable agriculture (Šūmane et al., 2018). Information accessibility is important for farmers' continuous learning to improve their practices (Franz et al., 2010; Abdullah, Samah, & Othman, 2012; Azman, D'Silva, Samah, Man, & Mohamed, 2013; Adnan, Md, Rahman, & Noor, 2017; Aku, Mshenga, Afari-Sefa, & Ochieng, 2018; Serebrennikov, Thorne, Kallas, & McCarthy, 2020).

Chemical control is the main weed management control method in Malaysia. Of the total 47,805 tonnes of pesticides used in 2019, 39,692 tonnes (83.0%) were herbicides (FAO, 2021). However, an increasing number of sustainability studies have explained the negative consequences of environmental hazards, food safety issues, and toxicity exposure. The development of herbicide-resistant weed species is one such consequence; there is a total of 263 herbicide-resistant weeds as of June 2021 (Heap, 2021). Herbicides can also affect non-target organisms, usually crops (Herrick, 2017). Studies have shown that herbicide residues in certain foods, such as fruits and vegetables, are major concerns among importing countries and consumers (Amjad, Ahmad, Iqbal, Nawaz, & Jahangir, 2013; Matt, Pehme, Peetsmann, Luik, & Meremäe, 2013). Additionally, the effects of toxicity resulting from herbicide exposure are continuously observed in terrestrial and aquatic organisms (Salvat, Roche, & Ramade, 2016; Diepens et al., 2017; Herrick, 2017) with increasing ecological risks. Therefore, improved weed control methods using non-chemical herbicides

are required; however, their acceptability and applicability are highly dependent on the mindset of farmers regarding the adoption of new technologies. Providing this knowledge to policy makers and practitioners on the factors that hinder sustainable use of environment-friendly strategies could help overcome this obstacle.

The Kampar District in Perak, Malaysia, has approximately 67,980 ha, of which 33% is used for agricultural activities (Perak State Government, 2016). These areas were formerly tin mining areas, but gradually, they have been repurposed for agriculture and diverse uses, such as crop cultivation, aquaculture, and livestock practices (Table 1). Agriculture provides 44.5% of the local income (Kampar District Local Plan 2030, proposed by joint efforts of Kampar District Council, Perak Department of Town and Country Planning, and Peninsular Malaysia Department of Town and Country Planning in 2015). To maintain the economic importance of agriculture in the district, Malim Nawar is included in the agricultural planning to be the main agricultural site for vegetable crop cultivation and the development of modern high-technology farms by 2030. This agricultural planning is expected to result in the development of intensive agricultural activities and weed management practices. Providing information and knowledge to rural farmers is, therefore, essential for rural agricultural development as well as for maintaining productivity and achieving sustainability. However, farmer surveys have rarely been conducted in Malaysia. Few surveys have been carried out in a limited number of areas, and they only involved paddy farmers. They surveyed farmers' attitudes toward precision agriculture (Abdullah, et al., 2012), green fertilisers (Adnan, et al., 2017), sustainable agriculture (Abu Samah, D'Silva, Mohamed, Man, & Azman, 2012), and weedy rice (Dilipkumar, Ahmad-Hamdani, Rahim, Chuah, & Burgos, 2021).

Therefore, the present study attempted to fill the knowledge gap among vegetable farmers by assessing their perceptions and knowledge of weeds, sources of information, and reasons for willingness or unwillingness to adopt non-chemical control methods. The questionnaire used here can help policy makers and practitioners develop strategies to achieve the goals of sustainable agriculture.

Table 1. Land use in Kampar District in 2014

Land use	Area (ha)	Percentage (%)
Forest area	36,484	54.5
Agriculture use	22,128	33.0
Urban development	4,577	6.8
Unused land	2,716	4.1
Water areas (e.g., rivers)	962	1.4
Recreation and park	130	0.2
Total	66,997	100

(Courtesy: Kampar District Local Plan 2030)

2. Materials and Methods

2.1. Study site and participants

The study was conducted in Malim Nawar, situated in Kampar District. Some members of a local farmers' organisation, called Malim Nawar Vegetable Farmers Association (official name in Malay: *Persatuan Pekebun Sayur Malim Nawar*), were recruited for this study. A total of 97 participants were randomly selected from the list. Farmers working on the same farm were excluded. The survey period was scheduled from June to October 2018 because of the non-responsiveness of some respondents and continued persuasion of some participants.

2.2. Sampling procedures

A three-section questionnaire was designed: Section (A) assessed participants' demographic information, Section (B) assessed their perceptions of weeds and constraints affecting crop production, and Section (C) assessed how they learned weed management practices. The semi-structured questionnaire surveys were conducted by face-to-face interviews so that the questionnaire could be explained to the farmers, and they could answer the questions reliably. The interviews were conducted in a familiar environment (e.g., coffee shops, farms), as suggested by the farmers. The answers provided by the participants were written on the sheets by the researcher because the respondents were not very confident in filling out the questionnaire themselves.

2.3. Statistical analysis

Statistical analyses included descriptive statistics and comparative chi-square tests (χ^2) using SPSS 20.0 to study the association between two variables. A *P*-value of < 0.05 was considered significant.

3. Results

3.1. Participants' demographic information

By the end of the survey period, data were collected from 62 of the 97 participants selected initially using convenience sampling. Among the remaining 35 selected participants, 30 refused to participate (most of them did not share their reason for refusal, while a few expressed that the survey questionnaire would not benefit them). The remaining five participants whose data were not collected were family members of the participants who were interviewed (i.e., father-son) or relatives who were also members of the association; they were randomly selected and subsequently excluded as they worked on the same farm as their family members who were already chosen to participate.

Of the 62 participants who were surveyed, 60 (96.8%) were male, and 2 (3.2%) were female (Table 2). There was a significant association between farmers and their backgrounds (grandparents and parents being farmers) ($\chi^2(1) = 13.18, P = 0.000$). The ages of the participants ranged from 20 to 70 years, and 27 (more than 40%) were above 70 years of age. Hakka was the most spoken dialect, followed by Cantonese. Only 3 (4.8%) participants had no formal education, while 37 (59.7%) had secondary school education, and only 2

(3.2%) had tertiary education. One-third of the surveyed farmers had more than 25 years of farming experience. Early dropping out of school was associated with many years of farming experience ($\chi^2(4) = 18.51$, $P = 0.001$).

3.2. Perceptions of weeds and constraints affecting crop production

More than 60% of the farmers considered pests and diseases as major constraints in crop production, followed by weeds (Fig. 1). Herbicides were the main control method used by the farmers. Half of the surveyed farmers agreed that soil fertility was a limiting factor in crop production; while others did not agree. Prolonged seasonal droughts or excessive rain was the main environmental constraint in crop production. Labour shortage due to the declining involvement of locals and foreigners was a cause of concern, whereas farm inputs such as agrochemicals were not a limiting factor if their prices were affordable.

3.3. Participants' perceptions of weeds

Two weed species—*Eleusine indica* and *Cyperus* spp.—were frequently mentioned as the most harmful weeds. The survey showed an association between farmers' knowledge of weed species and perceived economic losses by weeds ($\chi^2(4) = 16.40$, $P = 0.037$) (Table 3). The farmers opined that knowledge of weed species on farms was important, as it helped them select suitable herbicides (e.g., selective or broad-spectrum) for weed control. Moreover, identifying the weeds at an early stage helps remove seedlings before they mature into adult plants. On the contrary, some farmers argued that it was not necessary to learn about weed species to apply herbicides.

Furthermore, an association was found between knowledge of weed species and exploration of the potential uses of weeds ($\chi^2(4) = 20.15$, $P = 0.010$) (Table 4). Less than 10% of the queried farmers agreed that weeds could benefit crop production. Some farmers suggested certain benefits, such as nutrient release, soil improvement, and their function as cover crops. However, none of the farmers had strongly agreed to the potential benefits of weeds. Weed-crop competition, pest harbouring, and increased labour requirement for weeding owing to their root systems were the

major disadvantages of weeds reported by the farmers. The farmers wanted to learn about certain weed species (i.e., *E. indica* and *Cyperus* spp.) to estimate yield losses and explore their potential benefits.

3.4. Perception of weed management learning

The relationship between knowledge of other farmers' weed management practices and knowledge of the said practices, particularly in Malim Nawar, was significant ($\chi^2(3) = 9.01$, $P = 0.029$) (Table 5). None of the participating farmers strongly disagreed with the benefits of learning other farmers' weed management practices. Farmers generally agreed that sharing information on weed management and learning new strategies from the experiences of other farmers are useful strategies for their own farming practices. However, the participating farmers claimed that the practices of farmers in their vicinity would not be different from their own practices. This was the reason most of them did not know about the practices of other farmers in Malim Nawar, although they admitted the importance of such knowledge. They had knowledge of the practices of some farmers outside of Malim Nawar.

Furthermore, the survey demonstrated correlations between farmers' resistance to learning about non-chemical control methods and the reasons behind this attitude (Table 6). A total of 47.8% of the participants stated that "chemical herbicides are harmful to the environment" and that they did not want to learn about non-chemical weed control measures; 39.1% stated that "chemical herbicides are harmful to consumers", that "some weeds became herbicide-resistant", and that they did not want to learn about non-chemical weed control measures; 34.8% of the participants who stated that chemical herbicides are harmful to farmers and workers also stated that they did not want to learn about non-chemical weed control measures. These results showed that despite being aware of the harmful effects of herbicides, the farmers were sceptical of using non-chemical control methods. The reasons behind this resistance and scepticism included possible high costs, the time needed to learn and practice using non-chemical control methods, and the perceived high efficiency of herbicides.

Information on weed management practices was obtained from formal and informal sources. The formal

sources included the association, government agencies, workshops, civil society, and agrochemical companies, whereas the informal sources were friends of the participating farmers. Both agrochemical companies (64.5%) and informal sources (64.5%) were

equally important sources of information on weed control practices (Table 7). Workshops and seminars were not popular options for obtaining this information. Moreover, all farmers had access to at least one of these sources of information.

Table 2. Characteristics of the participants recruited in our survey

Characters	N (%)
Gender	
Female	2 (3.2)
Male	60 (96.8)
Age (years)	
20–30	1 (1.6)
31–40	4 (6.5)
41–50	7 (11.3)
51–60	7 (11.3)
61–70	16 (25.8)
>70 years	27 (43.5)
Speaking dialect	
Hakka	47 (75.7)
Cantonese	7 (11.3)
Others (Teochew, Hokkien)	8 (13)
Level of education	
No formal education	3 (4.8)
Primary education	20 (32.3)
Secondary school (SRP/PMR)*	13 (21.0)
Secondary school (SPM/SPMV)**	24 (38.7)
College degree	2 (3.2)
Years of farming experience	
1–5	8 (12.9)
6–10	11 (17.7)
11–15	9 (14.5)
16–20	12 (19.4)
21–25	1 (1.6)
>25 years	21 (33.9)

*SRP/PMR – Sijil Rendah Pelajaran (SRP) and Penilaian Menengah Rendah (PMR) are public examinations for Form Three students in Malaysia. PMR was formerly known as SRP.

**SPM/SPMV – Sijil Pelajaran Malaysia (SPM) and Sijil Pelajaran Malaysia Vokasional (SPMV) for Form Five students in Malaysia.

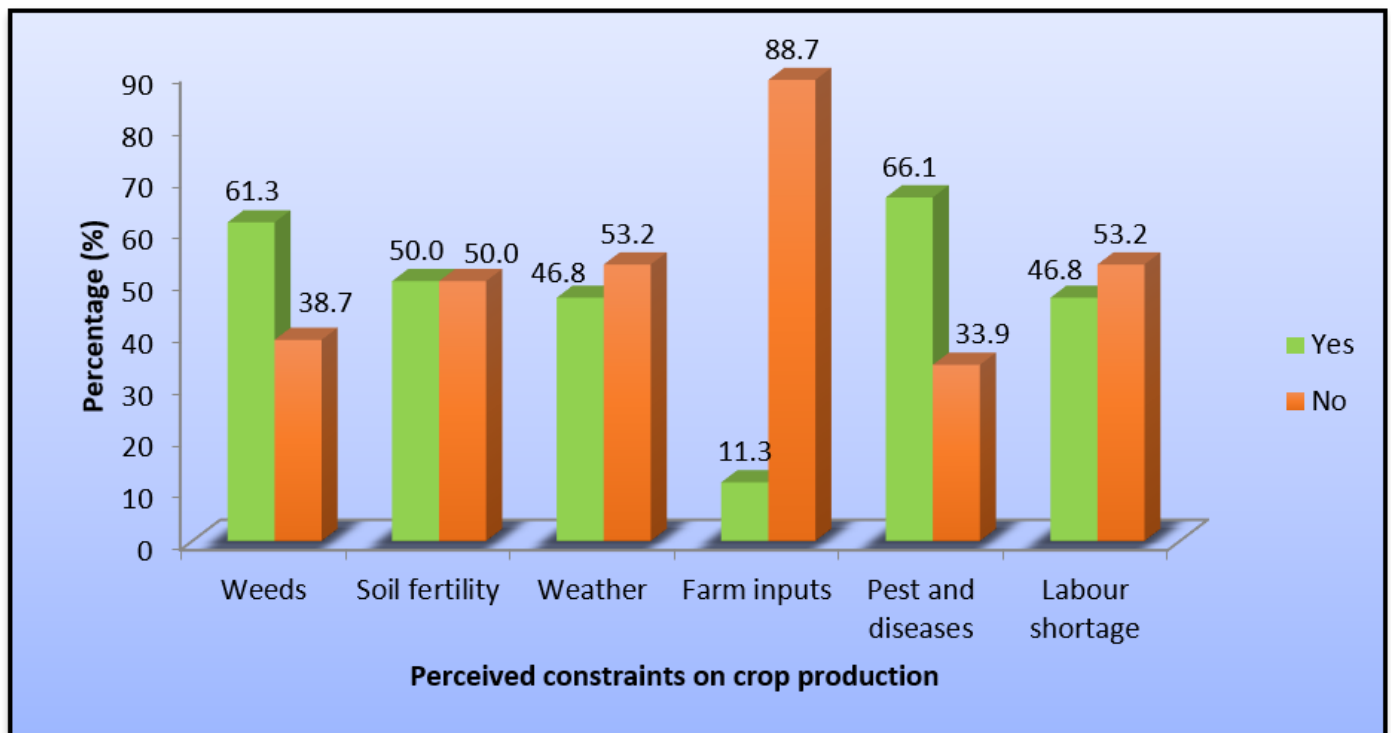


Figure 1. Perceptions of constraints affecting crop production.

Table 3. Association between perceived economic loss by weeds and the importance of knowledge of weed species

Perceived economic loss	Is knowledge of weed species important?			Total	Chi-square (χ^2) value, <i>P</i> -value
	Yes	No	NA		
Very high	11	2	2	15	$\chi^2(4) = 16.40, P = 0.037$
High	17	5	5	27	
Medium	7	2	2	11	
Low	1	5	1	7	
Very low	-	2	-	2	

Note: NA - No answers

Table 4. Association between the importance of knowledge of weed species and potential benefits of weeds

Weed could be beneficial	Is knowledge of weed species important?			Total	Chi-square (χ^2) value, <i>P</i> -value
	Yes	No	NA		
Agree	5	1	-	6	$\chi^2(4) = 20.15, P = 0.010$
Neutral	4	7	-	11	
Disagree	16	5	9	30	
Strongly disagree	10	3	-	13	
NA	1	-	1	2	

Note: NA - No answers

Table 5. Benefits of sharing information on weed management practices

Is knowledge of how other farmers control weeds important?	Knowledge of other farmers' weed management practices in Malim Nawar			Chi-square (χ^2) value, P-value
	Yes	No	Total	
Strongly agree	4	4	8	$\chi^2(3) = 9.01, P = 0.029$
Agree	14	12	26	
Neutral	4	22	26	
Disagree	-	2	2	

Note: NA - No answers

Table 6. Attitudes toward chemical herbicides and learning non-chemical control methods

No.	Reason		Do you want to learn non-chemical weed control?				Chi-square (χ^2) value, P-value
			Yes	No	NA	Total	
1	Chemical herbicides are harmful to the environment	Yes	11	-	-	11	$\chi^2(2) = 22.66, P = 0.000$
		No	12	25	14	51	
2	Chemical herbicides are harmful to consumers	Yes	9	-	-	9	$\chi^2(2) = 17.85, P = 0.000$
		No	14	25	14	53	
3	Chemical herbicides are harmful to farmers and workers	Yes		18		8	$\chi^2(2) = 15.58, P = 0.000$
		No	15	25	14	54	
4	Some weeds can become herbicide-resistant	Yes	9			9	$\chi^2(2) = 17.85, P = 0.000$
		No	14	25	14	53	
5	Chemical herbicides are convenient	Yes		18		19	$\chi^2(2) = 33.92, P = 0.000$
		No	23	7	13	43	
6	Chemical herbicides are cost-effective	Yes		17	1	18	$\chi^2(2) = 31.09, P = 0.000$
		No	23	8	13	44	
7	Other methods are less effective than chemical herbicides	Yes		6		6	$\chi^2(2) = 9.83, P = 0.007$
		No	23	19	14	56	

Note: NA – no answers

Table 7. Sources of information and knowledge on weed management practices

Sources of information	Number of farmers (%)
Malim Nawar Vegetable Farmer Association	24 (38.7)
Workshops and seminars	0 (0)
Government agencies	15 (24.2)
Civil society	3 (0.05)
Friends	40 (64.5)
Agrochemical companies	40 (64.5)
No access	0 (0)

4. Discussion

Agriculture is the main economic activity in Malim Nawar. Farmers in this area are registered with the Malim Nawar Vegetable Farmers Association (official name in Malay: *Persatuan Pekebun Sayur Malim Nawar*), established on November 23, 1992. The mandates of the association are as follows:

- To strengthen networking among farmers,
- To share information and exchange experiences on agricultural practices,
- To contribute to Malaysia's agricultural development, and
- To safeguard the association's members' benefits.

Though the number of registered members has increased over the years, the membership list has not been updated (i.e., deceased members and members who have left farming are still on the list), which is the first challenge for an updated member list. Moreover, there are no specific guidelines to obtain membership in this association—entrepreneurs in aquaculture and oil palm growers were both accepted. This led to the continued addition of new members, resulting in a total of 218 members. Additionally, 123 out of 218 members (56.4%) had registered their names with the association but with no contact details. The actual number of members is unknown, which may have caused a bias in sample selection. In addition, in the present study, there were some limitations related to respondents' literacy levels. Some farmers did not understand the questions even after receiving an explanation or did not know the answer (e.g., scientific names of weed species).

Among the challenges in continuing agricultural activities is the ageing farmer community in Malim Nawar, where 50 (80.6%) farmers in our cohort were over 50 years old, with an average life span of 74.5 years (Department of Statistics, 2019). A similar trend has been reported in the paddy sector, where the average farmers' age was above 50 years old (Alam, Siwar, Murad, Molla, & Toriman, 2010; Abdullah, et al., 2012; Omar, Shaharudin, & Tumin, 2019). This low involvement of the younger generations represents a continuous issue in Malaysia's agriculture. Young people are not interested to work in the agriculture sector even though they have positive perceptions of agriculture

(Abdullah, Ahmad, & Ismail, 2012).

Male farmers are the dominant workforce in Malim Nawar. Their wives are housewives and are only occasional assistants on farms, mostly during harvesting. This is different from paddy planting, where both women and men are involved in farming, from planting to harvesting. For paddy farmers in Malaysia, their farming experience correlates to their age; the elder the age of the farmers, the more is their experience (i.e., number of years) in farming (Dilipkumar et al., 2021).

The findings of the present study are similar to those of Serebrennikov et al. (2020) and Dilipkumar et al. (2021), who showed that the adoption of new practices depends on farmers' age and education level. It was observed that old farmers with low education levels were more resistant than their younger and more educated counterparts. Elsewhere in Malaysia, aged smallholder farmers of paddy, rubber, and oil palm plantations also lack of technical knowledge and support in improving weed management practices (Dilipkumar, Chua, Goh, & Sahid, 2020; Dilipkumar et al., 2021). The key reasons for their resistance were the possible risks of implementing new practices, including uncertainty in yield and increased cost. This scepticism was in contrast to their trust in herbicide efficiency.

Pests and diseases were perceived as more severe constraints than weeds in crop production. Similar findings were reported in a farmer survey in Africa (Laizer, Chacha, & Ndakidemi, 2019). According to the farmers interviewed in the present study, insect populations can increase dramatically depending on the weather. On dry and hot days, *Thrips palmi*, *Tetranychus urticae*, *Polyphagotarsonemus latus*, and *Empoasca fabae* were prevalent, whereas *Helicoverpa armigera*, *Maruca vitrata*, *Maruca testulalis*, and *Plutella xylostella* were prevalent in the rainy season. Herbicides were considered more effective in controlling weeds than pesticides in controlling insects and diseases, which is why pests and diseases were more harmful. Farmers used pre-emergence (before planting) and post-emergence herbicides (after planting); however, they did not have relevant knowledge of weed control methods, including active ingredients and their modes of action. They showed exten-

sive interest in 'effective' herbicides without necessarily knowing the components. Furthermore, negative psychological perceptions were the major obstacles to learning and accepting environment-friendly control methods.

Agricultural education plays a pivotal role in addressing the sustainability goals of rural farmers (Anderson, 1984; Chittoor & Mishra, 2012). Information sources play an important role in knowledge dissemination, as information accessibility and quality determine the adoption of sustainable agricultural practices (Rodriguez, Molnar, Fazio, Sydnor, & Lowe, 2008; Serebrennikov et al., 2020). Although various knowledge sources could complement each other, government agricultural programmes could be the most influential change agent (Rodriguez, et al., 2008), for example, courses and seminars could be used to enhance farmers' knowledge (Ismail, 1995; Samah, D'Silva, Mohamed, Man, & Azman, 2012). If information sources are scarce, this could limit the exposure to new methods and hinder learning. Although television programmes related to agriculture do not provide sufficient information, they were the only information source in some states, such as Kedah and Selangor (Ramli et al., 2013). The present study showed that television programmes were not a source of information in Malim Nawar. Instead, informal sources such as phone calls, farm visits, and social relations served as major information sources.

In addition to informal sources, the farmers also relied on information from agrochemical companies (i.e., a formal source). Farm visits by the representatives of agrochemical companies or organized talks for farmers were the main information dissemination strategies adopted by agrochemical companies. Farmers mainly attended presentations organized by agrochemical companies to learn about new products and application methods; other reasons included the meals provided by the companies and the opportunity to socialise. Although agrochemical companies are considered a formal source of knowledge, according to Šumane et al. (2018), their role in information dissemination should be reviewed carefully, as they are profit-driven entities whose main goal is to meet their sale requirements and sell their products. Smallholders who sought advice from agrochemical retailers have used more pesticides in Cambodia, Laos, and

Vietnam (Schreinemachers, et al., 2017).

5. Conclusion

The survey revealed that weed infestation is one of the most important agricultural constraints after pests and diseases in Malim Nawar. It demonstrated that knowledge of weed species assisted the anticipation of yield loss and exploration of potential control methods. Weed management is a continuous process in agricultural production, and accessibility to knowledge sources can strengthen farmers' expertise and experience. Social networking and agriculture chemical companies were the main sources of information on weed control methods. However, despite knowing the harmful effects of chemical herbicides, farmers' willingness to adopt non-chemical weed control methods is influenced by several factors. Information sources and quality are critical for encouraging farmers to adopt new weed control methods that could replace herbicides, which are currently used predominantly. New management programmes could build on current activities, such as farm visits and social relations, to disseminate information on eco-friendly weed management practices; local farmer organisations could be a good starting point. Farmer organisations are intermediaries between farmers and government agencies and are responsible for transferring quality information. Functions of the Malim Nawar Vegetable Farmer Association include promoting agricultural development and securing the well-being of the members. This farmer organisation and relevant government agencies could play a role in advancing weed management practices through knowledge transfer as their broad objectives are to spur sustainable agricultural growth and development. In collaboration with the Association, relevant government agencies should be proactive in farmer education through small discussion groups, demonstration plots, hands-on workshops, and on-farm demonstrations, as the government agricultural programmes could be the most influential change agent. Together, the study suggests that sequential capacity-building and educational programmes are catalysts of rural agricultural development. Innovative and localised methods that consider environmental sustainability and socioeconomic factors are needed to ensure progressive learning for farmers with mixed literacy and overcome resistance.

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Conflicts of Interest

The authors declare there are no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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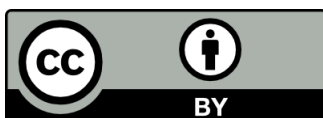
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Future of Cultured Meat Production: Hopes and Hurdles

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Rising environmental issues, animal welfare concerns, and a vulnerable food supply chain demands an effective and long-term solution for food security in the future. All these challenges encourage the researchers to find more reliable and clean ways of food production such as cultured meat. This process involved the production of animal meat in the lab using large bioreactors without raising animals. Cultured meat production is widely accepted among animal rights activists, and it can solve the issues related to conventional farming such as excessive use of the land resource, animal slaughter, foodborne diseases, and antibiotic resistance. Despite all these advantages, it is facing some serious challenges, which include technical, social, and ethical limitations. Extracting specific cell lines, developing animal-free growth media, upgradation of bioreactors, developing desired scaffolds, and changing the public perception towards lab-grown meat are fundamental challenges that need to be discussed. Major technical hindrances include the production of serum-free growth media, the development of economical and sustainable cell lines, and the upgradation of bioreactors to produce meat at the industrial level. Apart from technical issues, social acceptability is another big challenge in the development and marketing of cultured meat. Mass awareness campaigns through electronic and social media along with the provision of incentives to local farmers can address this challenge. This review intends to summarize both technical and social challenges that are halting the availability of cultured meat in the market and suggests some feasible recommendations to overcome these obstacles.

1. Introduction

The human population is increasing continuously and is expected to exceed nine billion by 2050. Ever-increasing human population has created huge pressure on existing food resources that is a looming threat to food security. Animals are the major source of human food after plants, comprised of essential nutrients mandatory for human nourishment. Protein from animal sources is 40% of total protein consumption now and will continue to increase with the increasing population (Stoll-Kleemann & O'Riordan, 2015).

According to the Food and Agriculture Organization (FAO), more than 56 billion domesticated animals are reared and killed for human consumption annually and food demand will be increased by 70% in 2050, so protein consumption will also increase (Tobler et al., 2011). Escalating demand for animal-source products put pressure on the livestock sector, which needs to adjust fast to meet such demand and that would not be achievable without branching out yield and increase of crop agriculture. However, the lack of available new

land for expansion of crop production forbids a 'horizontal' augmentation of current modes of production and forces the meat sector to search for alternative resources (Steinfeld et al., 2017).

On the other side, current livestock production systems are surrounded by a variety of issues including pollution, habitat destruction, biodiversity, animal welfare, and greenhouse gas emissions. If we rely on our livestock farming system to meet that enhanced requirement of protein, then it must produce a huge quantity of high-quality and affordable meat by using an environment-friendly system (Bhat et al., 2017). However, the majority of livestock production is under the factory farming model, where the major focus is the efficiency of the system to produce maximum product rather than its environmental effects, minimizing the use of antibiotics, and animal welfare (Aleksandrowicz et al., 2016). Greenhouse gases (GHG) are not the only factor associated with livestock production systems to affect climate change, but carbon dioxide ("CO₂") emissions also contributed that resulting from fossil fuel burning in tractors for maintenance of crops' lands (Dawson & Hilton, 2011; Gerber et al., 2015; Reisinger & Clark, 2018). The direct effect on human health is also reported by such kinds of intensive livestock farming as both workers and those living in the vicinity of an intensive livestock farm experience high levels of respiratory problems, including asthma (Ilea, 2009). The world is in search of systematized ways of protein production to assist the expanding world population while satisfying current challenges, such as environmental and animal welfare concerns (Aiking, 2014). Among the solutions, cultured meat is proposed as a viable substitute for consumers who do not wish to change the composition of their diet and a source for reducing the pressure on the livestock production system to ensure animal welfare (Kadim et al., 2015; Moritz et al., 2015; Post, 2012).

Cultured meat is the manufacturing of meat in a laboratory by employing tissue culture technology. The basic idea of cultured meat is to produce animal meat without raising the live animal. In this technique, stem cells are taken from live animals and planted into specific culture media having the necessary nutrients required for cell proliferation and growth (Bhat et al., 2015)(Fig. 1). On basis of theoretical knowledge, we have up until now, it is considered that cultured meat

is much better as compared to conventional meat production as it can potentially reduce environmental challenges and can address the issue of animal welfare by reducing intensive livestock farming. Every year, billions of animals are being slaughtered to satisfy the growing human hunger for meat, bringing huge suffering to sentient beings. Thus, developing more feasible and cost-effective methods could help humanity to find more humane and clean ways to produce meat. Cultured meat could transform meat production providing a much more sustainable and environmentally friendly substitute for conventional meat production. Despite its obvious advantage over conventional meat production, there are several challenges and limitations to cultured meat production and its commercialization (Alexander, 2011; Zuhaib Fayaz Bhat et al., 2017; Bryant & Barnett, 2020). The major challenge in the production process of in vitro meat is the use of foetal bovine serum (FBS) as growth media during cell proliferation because FBS has the necessary nutrients and proteins for muscle cell growth. FBS is obtained by slaughtering cattle during pregnancy, which is considered an inhumane act and cruelty to animals. Apart from this, FBS is also expensive, as it constitutes 80% of the total production cost for cultured meat (Mattick et al., 2015). Social and religious limitations are other big challenges to the commercialization of in vitro meat. The first time, cultured meat was produced in 2013 and got much popularity among the media and scientific community followed by a sudden rise in the number of companies investing in the production of cultured meat. At present 32 companies are working on cultured meat with more focus on beef and poultry meat production but still, it is facing many social and ethical barriers that are major hindrances to the acceptability of cultured meat.

It seems that cultured meat will be adopted gradually in near future and it will open new horizons in the meat production industry (Hocquette, 2016). This review intends to address the questions that why there is a need for culturing clean meat in near future and how it can help humanity to cope with environmental challenges and address animal welfare concerns.

2. Cultured Meat: The meat of the future

After the industrial revolution and human-eating habits also revolutionized, human hunger for meat has increased manifold. There is an interesting phe-

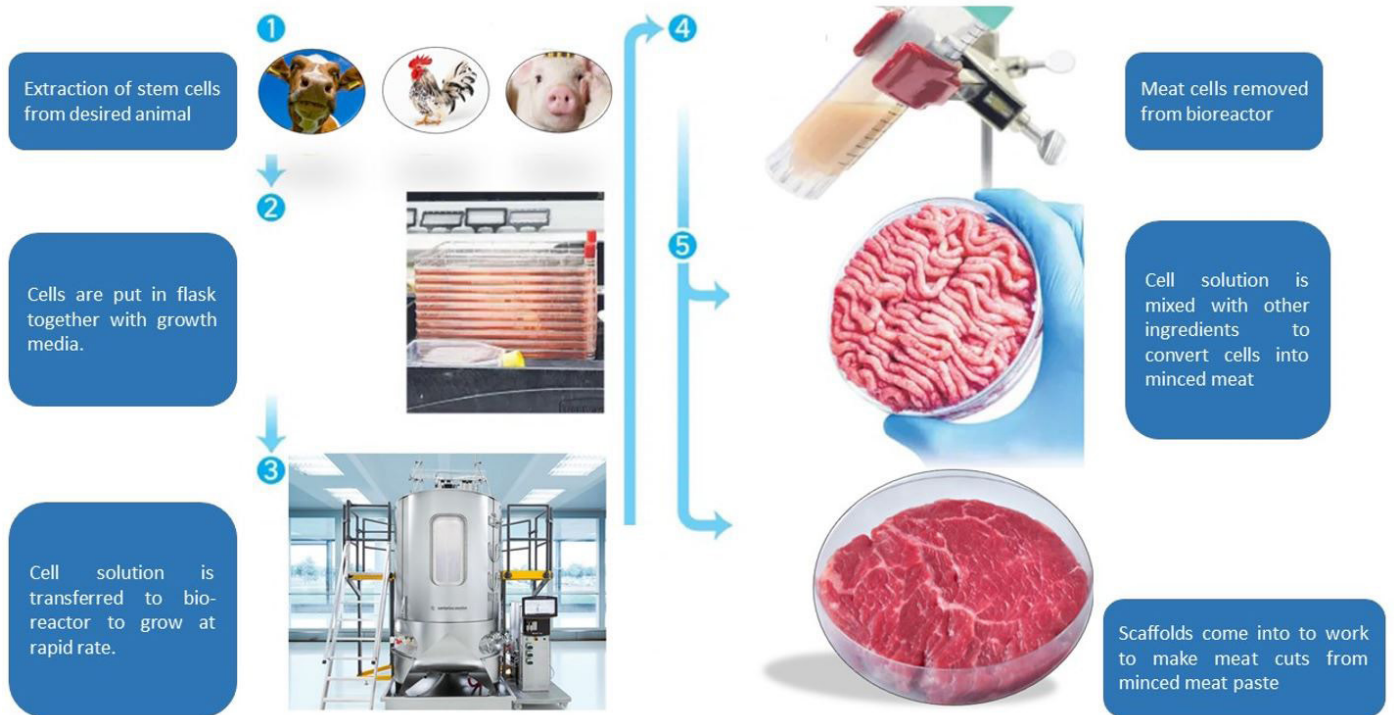


Figure 1. Stepwise illustration of the process involved in the production of cultured meat in the lab.

nomenon related to economic stability and meat consumption. Meat consumption is higher in developed parts of the world and its consumption is gradually increasing with the rise of the middle-income class across the globe. Keeping this trend in mind, developing an efficient meat production system is mandatory to fulfil future meat demands. During the last decades, the intensive factory farming model followed by escalating meat demands is on the rise. Unfortunately, this intensive farming gave birth to several environmental, health, and animal welfare issues. Secondly, the burgeoning human population is putting more pressure on limited land resources that will be insufficient to meet human demands. It is a need of the hour to develop such an efficient mechanism that can potentially help humanity to produce enough food by utilizing minimum resources (Post et al., 2020).

Addressing this growing challenge requires a dramatic change in meat-eating habits by shifting to a vegetarian lifestyle but such a major change in food habits is unlikely to happen in near future. Some plant-based meat substitutes have also been developed to discourage the increasing demand for animal meat, but this strategy too did not work well. The only option left is

to find more effective, humane, and cleaner ways to produce meat (Hocquette, 2016). Producing meat in the lab without harming animals is an innovative technique as compared to conventional methods of meat production. Moreover, conventional meat production is a very lengthy and laborious process involving different stages such as breeding animals, raising them in specific environments, feeding, and then killing the animals. The whole process of conventional animal farming is much laborious, requires more resources, and violates the universal standards of animal welfare. In contrast to this, cultured meat is grown in the laboratory by using desired cell sample under controlled conditions through modern techniques of biotechnology (Laestadius & Caldwell, 2015). This technology reduces time and uses very limited resources to produce a huge quantity of meat. To sum up, lab-grown meat will likely offer huge benefits regarding environmental impact, animal welfare, and human health.

3. Animal Welfare Prospective

Animals provide food to humans including meat. Just as humans, animals deserve benefits. Therefore, humans should have a moral duty to make sure the com-

fortability of animals. In the meat-based production sector of livestock, animal welfare is highly important to improve productivity, quality of meat, and economic returns. Concerns about animal welfare are on the rise globally. In research areas, animal welfare subject has made its significant place; even this subject is now in the media and politics as well. Animal welfare has been included in the major agendas of OIE for more than a decade because of its connection to animal health management and food safety, which are linked to human health. So, animal welfare is of supreme importance and cannot be neglected in any case (Madzingira, 2018). On the other hand, the outcome of conventional production has caused many problems, especially with the high concentration of livestock. Over the past 20 years, world meat production has been increasing mainly in the intensive livestock sector (Ilea, 2009).

There is growing and highly notable concern about animal welfare in meat production (Broom et al., 2019). If we consider the pig production system, which is a huge industry for meat production is surrounded by many welfare concerns. Most of these issues are related to close confinement, lack of enrichment, and breeding for the traits that are responsible for intensive production. Ultimately, the constraint for improving welfare is usually set by certain housing, pen design, feeding method, or genetics. But these factors cannot easily have changed and will grow more with an increase in demand (Pedersen, 2018). Similarly, the broiler production system is full of welfare issues which include their feeding methods, overcrowding, humidity issues, lightning schedule, and capturing method (Filho et al., 2014). In beef production, many welfare issues are present among smallholder and commercial farmers including draught power, poor housing conditions, poor transport conditions, ritual slaughter, tradition, social customs, and beliefs (Ndou et al., 2011). All welfare issues are related to intensive livestock production as demand is increasing no way is found except to increase the number in limited space and focus on traits of intensive production for breeding (Pedersen, 2018). That increasing demand is requiring an alternate way of meat production rather to put the whole burden on animals and damage their welfare (Ilea, 2009). That alternate way of meat production is cultured meat that requires less energy, low GHG, low water, and low land requirement with ex-

cessive production. This will not only provide a clean way of production but also reduce the burden on the animal sector and minimize the welfare concern of domestic animals (Lynch & Pierrehumbert, 2019).

4. Challenges to the production of cultured meat

4.1 Technical challenges

Despite the rapid progress in the technology of in vitro meat production, there are a few challenges in the production of synthetic meat such as procurement of cell lines, high production cost, culture media from animal sources, and limited scale of production. This topic intends to discuss the obstacles in the way to delivering synthetic meat from the lab bench to the dining table (Bhat et al., 2017).

4.1.1 Obtaining specific cell line

In recent days, cell lines are mostly used in the biotech business and research for the production of various biological organisms and products such as viruses and proteins. In the process of cultured meat production, stem cells are used as they can divide, proliferate, and differentiate into different types of organs or meat. In this procedure pluripotent stems are needed that can be obtained from different parts of the animals including muscles, liver, and adipocytes. Apart from this, pluripotent stem cells can also be obtained from embryos or separated from fibroblasts (Z.F. Bhat & Bhat, 2011; Datar & Betti, 2010). The process of developing suitable stem cell lines is very costly and time-consuming, as it requires advanced gene delivery technology. It has been tested to introduce specific genes to obtain desirable traits like fast muscle production. The conventional method of introducing genes through viruses has a low transfection yield and it limits the scalability of meat production (Dilworth et al., 2015). Secondly, poor characterization of a cell line can badly affect meat quality control. During the rapid division of cells, the genetic content of cells becomes highly unstable. For example, during DNA replication, any variation, insertion, or deletion can cause serious structural and functional changes (Dilworth et al., 2015).

4.1.2 Preparation of scaffolds

During cultured meat production, the scaffolds are used to anchorage the cell and support the physical activities of meat including stem cell differentiation. Scaffolds are important during this process as they assist in nutrient, oxygen uptake, and help the cells to diffuse the metabolic waste products. So, different types of scaffolds have been designed by tissue engineers based on the previous knowledge of organ regeneration (Gaydhane et al., 2018; Hocquette, 2016).

Commonly, two types of scaffold materials are being used which include naturally derived or synthetic. Chitosan and cellulose are natural scaffold material, and it has many benefits over synthetic material, as they are biodegradable, safe, and economical. However, they exhibit much variation in their characteristics including the extent of polymerization and molecular weight. While, synthetic scaffolds are synthesized and designed according to desired properties such as porosity and ligand availability (Bhat et al., 2017). As the production of synthesized scaffolds is a complex process, it has a higher production cost and it is harder to get regulatory approval for these kinds of scaffolds (Kadim et al., 2015). The hybrid model of scaffolds including naturally derived scaffolds as the primary material and a small quantity of synthetic material could solve the issue of biomaterial scaffolds. Apart from this, advanced production methods like 3D printing can be implanted to print the desired scaffolds for meat culture (Noor et al., 2016).

4.1.3 Development of animal-free growth media

For large-scale production of cultured meat, cells grow and differentiate rapidly; the growth media should be able to provide enough supply of nutrients such as glucose, amino acids, and vitamins (Godfray et al., 2018). One of the reasons behind the high production cost of cultured meat is expensive commercial growth media. It is widely considered that cultured meat is animal-free meat but in reality, its production involves the animal source growth media (foetal bovine serum). This serum contains all of the vital nutrients and growth factors that are not easy to prepare and find an alternate animal source growth media (Noor et al., 2016; Post, 2012; Tuomisto, 2019). Some researchers are finding ways to increase the production of certain proteins like growth factors at cheaper prices through genetically engineered microorgan-

isms, fungi, and plants to replace the animal source of growth media. Some research groups have successfully developed growth media free from serum such as serum-free media manufactured during a study that supports the growth of bovine myoblast but not as fast as the media with serum content (Z.F. Bhat & Bhat, 2011).

4.1.4 Upgradation of bioreactors

Production-scale bioreactors which are available commercially are typically 1–2 m³ in working volume for cell culturing, although larger reactors up to 10–20 m³ can be custom-built (Flickinger et al., 2010), still, their size is much smaller than microbial reactors, which can be 200–2000 m³. Many reasons are behind the use of smaller reactors for cell culturing as multiple smaller units can provide flexibility and offer proper control and management of contamination. So it is very critical to address these challenges regarding the type of reactors for large-scale cultured meat production (Zhang et al., 2020). Currently, laboratories are the only production sites for cultured meat as it is at a high cost and still facing difficulties to commercialize. A major reason for this is that current artificial meat products do not compete with the quality of real meat in a cost-effective and resource-efficient way. Therefore, it is now important to satisfy these needs with real colour, nutrition, fragrance, and taste e.g., haemoglobin addition in artificial meat is necessary to give natural colour. Haemoglobin sources are animal blood or plant tissue, but extracting from them is time-consuming and not cost-effective (Zhang et al., 2017).

4.2 Lack of financial support for cellular research

Most of the research, to produce cheap cultured meat at a large scale, is still needed to be done which specifically involves the selection of cell lines and the development of animal-free growth media. Until now, there are no specialized institutes or scientific disciplines to entirely focus on research related to cellular agriculture. Most of the studies related to cultured meat are conducted as an isolated projects and not linked to academic interests. Cultured meat can be produced only in lab conditions using expensive materials and techniques adapted from other related fields of biotechnology. To find a long-term solution

to address the challenges related to cultured meat production, separate research funding and opportunities are needed for only the development of cellular agriculture (Dolgin, 2019).

4.3 Animal welfare concerns regarding growth media

The purpose of culture media is to support cell growth and division by feeding them essential nutrients such as amino acids, sugars, vitamins, and minerals. At present, foetal bovine serum (FBS) is a key component of most growth media. This ingredient is obtained from the blood of the foetus by slaughtering the pregnant cow, which is an inhumane practice. Therefore, it raises severe concerns about animal welfare. Although no animals are involved in the production of meat during this process still needs animal source culture media to grow the meat cells. An ideal growth media should not involve animal products. Some studies demonstrated that growth media can be produced from fungi and plants but these growth media are not as effective as FBS (Slade & Bauen, 2013).

4.4 Public perception about cultured meat

It is a common perception, that anything natural is good while anything unnatural is bad for human consumption. It is just an assumption and has nothing to do with reality. Something can be natural and bad, and at the same time, an unnatural thing can also be good (Schneider et al., 2013). There is a strong relationship between natural and unnatural. Everything and every process are already present in the universe, and we just need to discover that. In ancient times, there was no intensive animal farming, in that way animal farming during those times was an unnatural process. So, the term natural and unnatural is very ambiguous, especially in regard to the production of cultured meat (Takala, 2004). Although cultured meat is grown in a lab artificially, the result is just as original as orthodox meat and possesses low health risks as compared to conventional meat. In reality, since it is synthesized in a controlled manner, cultured meat is less likely to comprise harmful by-products, unhealthy fats, and food-borne pathogens than its conventional counterpart (Lynch & Pierrehumbert, 2019; Woll et al., 2019). Secondly, some people raise questions about the ethical standing of cultured meat. As cultured meat re-

quires FBS as growth media which is an animal-derived product and involves the killing of pregnant cows and foetuses, and hence poses a serious ethical challenge. To achieve this goal, the development of animal-free culture media is mandatory. Apart from cultured media, some scientists are afraid that widespread acceptance of cultured meat will promote cannibalism as after the development of this technique any kind of meat can be grown in the lab by using the cell line. This is also a serious ethical challenge that requires proper legislation regarding the production of cultured meat around the globe (Woll, 2019).

5. Strategies to overcome these challenges

In the modern world where the population is increasing due to advancements in medical sciences, the needs of the growing human population are also increasing. In such a situation of competitiveness in life, cultured meat is inevitable. China and India with a massive increase in population appealing for the alternate source of meat because people of these countries are raising from the poverty level rapidly (Beinhocker et al., 2007). When there is a rise in the poverty level due to the betterment of the economy of the country, people become more able to afford better nutrition, which ultimately increases the demand for meat. The conventional agriculture system is unable to fulfil the demands of such growing populations and shortage of food, inflation in prices of food, and uncontrolled damage to the environment could be the consequences. Such destructive effects can be avoided by realizing the inevitability of cultured meat production and if it becomes a source to satiate the global nutrition needs. To make the cultured meat sector a sustainable source there is a need for more aggressive development if we want to avert food and agriculture, and environmental crises.

5.1 Role of media

Consumer interest is being developed in food ethics, which is linked with different cultural values. Therefore, the compatibility of food with the lifestyle of any human being matters a lot in any society. People, who are not choosy about their food, even reject some kinds of food due to social, cultural, and religious unacceptability. Here come the media, which can play a significant role in the opinion-making about any

kind of food including cultured meat. There is a dire need for understanding media coverage about the culture's meat authorities (Goodwin & Shoulders, 2013). To date, the coverage of the media remained about highlighting animal farming issues and telling about the benefits of cultured meat but it can make a huge difference when cultured meat is present at the market for providing not only an alternate option but with proper nutritious value (Post et al., 2014). Media coverage regarding a disease outbreak, antibiotic use, and inhuman handling of animals raised many concerns about intensive livestock farming. Moreover, global warming impact and the increasing human population should be highlighted to spread awareness concerning alternate meat sources like cultured meat (Springer et al., 2014).

A survey of Dutch consumers observed that, when asked the question, if they were ready to try cultured meat once it gets accessible, being given information about its environmental benefits triggered positive answers to increase from 25% to 43%, a near-doubling compared with only basic information regarding the technology itself. Another online survey conducted on social and news media sites has found that 70% of consumers are willing to try cultured meat once it becomes available. Therefore, these surveys highlight the importance of the media's role in spreading awareness and information related to this new technology could help to change the public perception in near future (Tuomisto, 2019).

5.2 Involving conventional farmers in the production process

In many countries, agriculture is the major sector and most of the population in developing countries is directly or indirectly involved in this sector. In East Asia, 62% population is involved in the agriculture sector. Even in Europe, 4.4% of employment is related to agriculture (Eurostat et al., 2017). In the case of the cultured meat sector, no doubt that there will be the creation of new jobs for people, and a completely different skill set will be needed for that kind of job as compared to the conventional agricultural or livestock farming skills. In such a case farmers may end up having a small share in the market and be financially unstable (Bonny et al., 2015). In history, we can see many examples when one sector is revolu-

tionized with some innovation many people having conventional mindsets lost their businesses and jobs. For example, Luddites of the textile sector in England damaged the equipment and machinery to go against the job losses that new technology created in the 19th century. The point about joblessness can be summarized through allegory, according to which an economist once visited the construction place and raised the question. Why are hundreds of workers using shovels instead of modern machinery? The supervisor replied that is how jobs are created, the economist said if the purpose is job creation, then give them even spoons instead of shovels (Tanner et al., 2015). There are no doubt innovations like this create job losses instantly, but that's how betterment comes up as the society in which we are living with great advancement in the technology and many other sectors couldn't be possible without such innovations which have created many jobs for some, and some lost them. However, there is a middle ground in this regard, which is the involvement of local farmers in the cultured meat sector. As the cultured meat sector is not going to be established in a single night, there is a transition time for switching from conventional livestock farming to cultured meat farming; which is more sophisticated and may come up as a more profitable business. There is a dire need to spread awareness among not only the public but also involving the local farmers and attract them by designing a profitable business model in this sector.

5.3 Research funding and legislation

The governments and research organizations should allocate more funds for agricultural biotechnology to accelerate the research progress in the development of cultured meat. Research should emphasize producing optimal cell lines, animal-free culture media, up-gradation of bioreactors, and innovating the existing techniques for producing the intricate muscle tissues. After the sustainable and economic production of lab-grown meat, it will require new legislation and regulatory framework in every region of the world, where production takes place. It also requires the involvement of political and legal organizations to design regulations for the production and consumption of lab-grown meat.

6. Conclusion

Conclusively, cultured meat offers a hope that humanity can become less dependent on animals for meat, thus decreasing the environmental and health impact of animal production. There are still major scientific obstacles to overcome including the development of quality cell lines, cost-effective, and animal-free growth media production, designing bioreactors for producing tissue layers at a large scale before cultured meat can become a common food product. Modern technology in genetic engineering, biomaterial design, and sequencing methods can provide real technical solutions to all these issues. We can fulfil the needs of the increasing population, address animal welfare concerns and make the environment clean and healthy through cultured meat production.

Conflict of interest

The authors declare no conflict of interest.

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Economics of food safety practices among cassava processors in northcentral Nigeria

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Food safety is a critical issue and a growing public health concern. Cassava being one of the staple foods widely consumed by the majority of households in Africa involves several processes. Assessment of the safety practices adopted by the processors is an important approach to enhancing consumers' food safety and avoiding food poisoning. Thus, this research assessed the economics of food safety practices adopted by cassava processors. Data collected from randomly selected 120 cassava processors were analysed using descriptive statistics, costs, and returns analysis, and the Likert-type ranking method. The results show that the majority of the processors were female with an average age of 46.6 years. They sourced food safety processing information through radio, public sanitary officers, extension agents, television, and the internet. All the processors always practised the peeling, washing, fermenting, grating, and pressing of cassava as safety practices to remove hydrogen cyanide. About 95% always applied safety practices in sifting and frying while 74% stored their products. The motivating factors of food safety practices adopted by cassava processors were demand-driven, to make profits, health concerns of their consumers, improve efficiency and reduce wastage. The cassava processors had a net profit of N81, 052.33 (USD 195.75), a return on capital invested of 0.62, an operating ratio of 0.61, and a benefit-cost ratio of 1.52 per two tonnes of cassava processed. Thus, food safety practice in cassava processing was profitable. Inadequate finance, high cost of cassava tubers, time-consuming, poor access to clean water, and lack of modern processing facilities were major constraints to food safety practices in cassava processing. These call for government and NGO support to promote food safety practices through proper education of the processors on food safety practices, provision of modern processing facilities, and credit facilities.

1. Introduction

Food poison is a serious concern, especially in developing countries where local food processors did not use modern facilities to process food which may lead to food-borne diseases. Food-borne disease due to poor food processing contributes significantly to the huge burden of ill-health and death of people (Thomas & Philips, 2015; World Health Organization, 2015).

Four hundred and twenty deaths are recorded globally as a result of food-borne illness (World Health Organization, 2015). There is evidence of unsafe food practices among food producers, handlers in the food chain system, caterers, and processors. Therefore, food-borne illnesses may result from eating contaminated foods containing chemical or biological poisons

and pathogenic microorganisms arising from careless or poor food handling practices at any stage in the food supply chain (Federal Ministry of Health, 2014).

Food safety practices involve measures taken to protect human health from harm arising from consuming food that is not well prepared. Safe foods are food prepared, produced, processed, and stored in such a way that their consumption will not affect consumers negatively. Unsafe foods are contaminated food with a chemical, microbiological or physical hazard which can affect human and animal health negatively (Focker & van der Fels-Klerx, 2020). Due to the vital role food safety plays in human health, governments, and local and international agencies play surveillance and regulatory roles to enhance food safety globally. The United Nations, Food and Agriculture Organization, the United States Department of Agriculture Food Safety Inspection Service, and the Food and Drug Administration assists to achieve food safety at the global level. Nationally (Nigeria), the National Agency for Food and Drugs Administration and Control (NAFDAC), the Federal Ministry of Health, Standards Organization of Nigeria (SON), and the Federal Ministry of Agriculture and Rural Development ensure food safety in Nigeria. Although, most of the food safety interventions in Nigeria were targeted at branded food products. Only branded products request for and issued NAFDAC registration number and SON accreditation. Whereas, the local producers of food such as garri (cassava flakes) which is a common food in Nigeria, especially among the low-income households which are the majority of the population, find it difficult to get a NAFDAC registration number. This posed a serious threat to the consumers as they are not sure of the safety of the food. Garri is, however, the most important product of cassava in Nigeria and some other African countries.

Cassava (*Manihot* species) belongs to the family Euphorbiaceae. It is widely grown in Nigeria, especially in the southern and middle belts part of Nigeria. In Africa, cassava is the second most-consumed staple food crop after maize (Alamu et al., 2019). Despite the great importance of cassava in achieving food security, it contains a poisonous substance known as hydrogen cyanide or simply called cyanide. Because of the presence of hydrogen cyanide in fresh cassava tubers, it requires proper and safe processing. Poor processing of cassava results in health challenges and

may lead to the death of people. This is because poor food processing methods and contamination of food during pick-up and preservation are major sources of food-borne diseases. Proper processing of cassava will reduce the hydrogen cyanide in cassava. Thus, it is vital to understand the safety practice of cassava processors and if it is economical.

Studies have assessed the processing of cassava (e.g., Adeoye et al., 2019; Alamu et al., 2019; Thomas & Philips, 2015). Adeoye et al. (2019) assessed garri production safety practices among cassava processors in Oyo state Nigeria. Alamu et al. (2019) evaluated household-level cassava processing and utilization in Zambia. Thomas and Philips (2015) investigated cassava processing food safety practices in Oyo state, Nigeria. Apart from the fact that none of the studies was conducted in northcentral Nigeria, none of the studies investigated how economical the food safety practices are. This study, therefore, examined the economics of food safety practices among cassava processors in northcentral Nigeria. To have an extensive study on the assessment of food safety in cassava processing, the study specifically (1) examined the motivating factors of the use of safety practices, (2) assessed cassava processors' knowledge of safety practices, (3) identified the source of food safety information, (4) examined various food safety practices adopted in cassava processing, (5) investigated the profitability of food safety practices, (6) assessed the perceptions of cassava processors on the various risks involved in processing cassava, and (7) identified the constraints to cassava processing food safety practices. This would provide relevant information by helping to identify various ways by which cassava processors go about the safe processing of their cassava produce. It will also help in understanding the problem faced by the processor while trying to make the product safe and healthy enough for human consumption.

2. Methodology

2.1 Study area

This research work was carried out in Kwara State, North Central, Nigeria. Kwara State was purposively selected as the study area of this research work because of its considerable socio-economic heterogeneity, location, and a large amount of the total population (about 70%) was engaged in farming and allied

activities such as cassava processing. Considering the location, it is the gateway between the southern and northern regions. The people of the state comprise Yoruba, Fulani, Nupe, and Baruba. Kwara State is located between latitudes 8° 30' N and 8° 50' N and longitude 4° 20' E and 4° 35' E. The state has four Agricultural Development Programme (ADP) zones and sixteen Local Government Areas (LGAs). Each LGA is divided into districts, which are made up of villages. The state shares an international border with the Republic of Benin and a national border with Oyo state in the west, Kogi state in the east, Ondo and Osun states in the south, and Niger state in the north.

2.2 Sampling procedure and sample size

This study employed a four-stage sampling technique to select the respondents. Two ADP zones (Zone C and Zone D) out of the four zones in Kwara state were purposively selected due to the predominant of cassava processors in the zones. Two LGAs were randomly selected from each of the ADP Zones making a total of four LGAs (Asa, Ilorin East, Ifelodun, and Offa). In the third stage, three communities were randomly selected from each of the LGAs, this gave a total of twelve rural communities. Afon, Eiyenkorin, and Laduba were selected from Asa LGA; Ile Apa, Oke Oyi, and Iporin were selected from Ilorin East LGA; Idofian, Jimba Oja, and Ore-ago were selected from Ifelodun LGA; Offa, Balogun and Shawo East were selected from Offa LGA. In the fourth stage which was the last stage of the sampling procedures, ten cassava processors were randomly selected from each community which gave a total of 120 cassava processors that were used for the study.

2.3 Data collection techniques

Primary data were collected using questionnaires that were administered to the cassava processors. The data collected during the field was on demographic data such as gender, age, marital status, household size, farming experience, income, and level of education. Data on food safety in cassava processing, constraints to food safety in cassava processing, safety practices used by cassava processors, and costs and returns associated with safety practices were collected.

2.4 Analytical techniques

The analytical tools that were employed to achieve the objectives of the study include descriptive statistics, costs and return analysis, and the Likert-type scale.

2.4.1 Descriptive statistics

Descriptive statistics such as frequency, percentages, and means were used to describe the demographic features of the cassava processors. The descriptive analysis was also used to identify the safety practices adopted by cassava processors in the study area to guarantee consumer safety. The source of information on cassava processing, the perceptions of cassava processors on the various risks involved, the motivating factors of the use of safety practices, and cassava processors' knowledge of safety practices were also examined using descriptive statistics.

2.4.2 Cost and return analysis

Gross margin analysis: This is the difference between the total revenue accrued from cassava processing and the total variable cost incurred. It is a proxy for the profitability of food safety practices by cassava processors. It is expressed as:

$$\text{Gross margin} = \text{Total revenue} - \text{Total variable cost}$$

Where:

Total revenue is the returns from cassava processing and is calculated as the total output multiplied by the price per unit of produce (i.e., $P * Q$).

Total variable cost is the cost of all variable inputs used for processing cassava.

Net profit: Because gross margin analysis did not consider the fixed cost in its estimation, the study estimated the net profit of food safety practices among cassava processors. Net profit analysis considered the fixed cost in estimating the profit in food safety practice in cassava processing. It is used to ascertain the actual profit after deducting all costs of production. It is expressed as:

$$\text{Net profit} = \text{Gross margin} - \text{Total fixed cost}$$

OR

$$\text{Net profit} = \text{Total revenue} - \text{Total cost}$$

Operating ratio: The operating ratio is directly related to the variable input usage in cassava processing. It measures the ratio of total variable costs to total revenue. The lower the ratio, the higher the profitability of the food safety practice among cassava processors and vice versa. It is expressed as:

$$\text{Operating Ratio} = \frac{\text{Total Variable Cost}}{\text{Total Revenue}}$$

The benefit-cost ratio: The benefit-cost ratio is defined as the total revenue from cassava processing divided by the total cost. It is expressed as:

$$\text{Benefit cost ratio} = \frac{\text{Total revenue}}{\text{Total cost}}$$

Return on capital invested: It measures the proportion derived as profit per unit of currency invested in food safety practices in cassava processing. It is expressed as:

$$\text{Return on capital invested} = \frac{\text{Gross margin}}{\text{Total variable cost}}$$

2.4.3 Likert-type scale rating technique

The Likert-type scale was developed in 1932 by Renzis Likert. It employs the principles of measuring attitude or opinion by asking people to respond to a series of statements about an issue, in terms of the level or extent of their agreement or disagreement with the statement. To examine the various safety practices among the cassava processors, a four-point Likert scale was used which was represented as never (1), rarely (2), sometimes (3), and always (4). The mean score of these four points Likert scale was 2.5. Therefore, any Likert mean score equal to or greater than 2.50 was considered a widely practised food safety measure among the cassava processors while those less than 2.50 were not widely used. To identify the challenges encountered by cassava processors in the

food safety practice, a Likert-type scale was also used which was presented as: Extremely Severe (4), Very Severe (3), Moderately Severe (2), Not Severe (1). The mean score of these four points Likert scale was 2.50. Therefore, any Likert mean score equal to or greater than 2.5 was considered a severe constraint, and those less than 2.50 were not severe problems.

3. Results

3.1 Socio-economic characteristics of the cassava processors

The socioeconomic characteristics of the cassava processors were presented in Table 1. The results revealed that the majority (91.2%) of the cassava processors were females while only 8.3% were males. The majority (about 63%) were below 50 years, 25.8% were between 50 and 60 years of age, while only 10.8% were above 60 years of age. The average age of the cassava processors was 46.6 years. The majority (73.3%) of the cassava processors were married, 22.5% were widowed while only 4.2% were divorced. About 48% of the cassava processor had a household size between two and six persons, 44.2% had a household size between seven and ten persons, and 8.3% of them had a household size above ten persons. Their average household size was eight persons. Regarding the educational level of cassava processors, 31.7%, 28.3%, and 4.2% had primary education, secondary education, and tertiary education respectively while 35.8% had no formal education. The majority (85%) of the cassava processors had cassava processing as their primary occupation, while 9.17%, 3.3%, and 2.5% had farming, civil service, and artisan as their major occupations, respectively but practice cassava processing as a secondary occupation. The majority (52.5%) of the cassava processors had between 10 and 20 years of experience, 21.7% had 20 to 30 years of experience while 25.8% had less than ten years of experience. The mean years of experience was fifteen years. A larger proportion (55.83%) of cassava processors made a monthly income between ₦50,000 (USD 120.76) and ₦100,000 (USD 241.51), 21.67% of them had a monthly income between ₦100,000 (USD 241.51) and ₦150,000 (USD 362.27), 14.17% had above ₦150,000 monthly while 8.3% had between ₦20,000 (USD 48.30) and ₦50,000 (USD 120.76) monthly income. Their average monthly income was ₦72,666.67 (USD 175.50). The majori-

ty (77.5%) of the processors did not belong to any cooperative association while only 22.5% were members of some cooperative associations and organizations.

3.2 Motivating factors for the use of safety practices

Table 2 shows the various motivating factors of food safety practices adoption by cassava processors. About 66% of cassava processors agreed with demand-driven as a motivating factor for the adoption of food

safety practices. 60% opined that they adopt the food safety practice in cassava processing to make a profit. About 84% of the cassava process was motivated to adopt food safety practices due to the health concerns of their consumers. The majority (95.0%) agreed that improving efficiency was a motivating factor in practising food safety in cassava processing. About 56% opined that to reduced wastage was a motivating factor in practising food safety in cassava processing.

Table 1: Socioeconomic characteristics of the respondents

Variables	Category	Frequency	Percentage	Mean
Sex	Male	10	8.3	
	Female	110	91.7	
Age	30 - 40	37	30.8	46.67
	40 - 50	39	32.5	
	50 - 60	31	25.8	
	60 - 70	13	10.8	
Marital status	Married	88	73.3	
	Widowed	27	22.5	
	Divorced	5	4.2	
Household size	2 – 6	57	47.5	8.12
	7 - 10	53	44.2	
	10 - 12	10	8.3	
Highest education level	No formal	43	35.8	
	Primary	38	31.7	
	Secondary	34	28.3	
	Tertiary	5	4.2	
Primary occupation	Farming	11	9.17	
	Cassava Processing	102	85.0	
	Civil service	4	3.33	
	Artisan	3	2.5	
Cassava processing experience	<10	31	25.8	15.05
	10 - 20	63	52.5	
	20 - 30	26	21.7	
Average monthly income (₦)	20,000 - 50,000	10	8.3	72,666.67
	50,000 - 100,000	67	55.83	
	100,000 - 150,000	26	21.67	
	>150,000	17	14.17	
Member of the cooperative society	Yes	27	22.5	
	No	93	77.5	

Source: Research Survey, 2021.

Table 2: Motivation for use of safety practices in cassava processing

Motives of adoption	Yes	No
Demand driven	79 (65.8)	41 (34.2)
To make more profit	72 (60.0)	48 (40.0)
Health concern	101 (84.2)	19 (15.8)
Improve efficiency	114 (95.0)	6 (5.0)
Reduced wastage	67 (55.8)	53 (44.2)

Source: Research Survey, 2021

3.3 Assessment of cassava processors' knowledge of safety practices

Table 3 presents the cassava processors' knowledge of safety practices. Only 15.8% of the cassava processors opined that sorting of cassava was not necessary while the majority (90.8%) agreed that sorting was a good safety practice. The majority (97.5%) disagreed that peeling of cassava leads to the loss of edible tissues. Also, 95.8% of the cassava processors agreed that washing peeled cassava improves garri quality and reduces the health risk associated with unwashed cassava. In the same vein, 80.3% agreed that grating enhances the reduction of hydrogen cyanide in cassava. About 92% of the processors agreed that consuming cassava products that do not pass through fermentation is dangerous to human health. The majority (93.3%) were aware that frying cassava improves the quality and reduces poisonous substances in garri. All the cassava processors were aware that environmental hygiene helps to prevent contamination of cassava products. The majority of the processors (95%) agreed that cassava contains a poisonous substance (hydrogen cyanide). The majority (91.7%) agreed that cassava that does not pass through fermentation is dangerous to human health or for consumption. A larger proportion (64.2%) agreed that cyanide content varies with varieties of cassava.

3.4 Sources of information on food safety practices in Cassava Processing

Table 4 shows the various sources of information on food safety practices in cassava processing among the respondents. The majority (89.2%) of the respondents sourced food safety information through the radio. About 68% of the cassava processors sourced food safety information from their fellow processors who had undergone training or attended food safety

programmes. About 56% sourced information from extension agents and 38.3% obtained food safety practice information through television programmes. Only 3.3% of them used the internet to source information on food safety practices. About 68% sourced food safety information from public sanitary officers.

3.5 Various safety practices by the cassava processors

Table 5 shows the various safety practices among cassava processors. The responses of the respondents were ranked using the Likert-type method. From the result, peeling, washing, grating, fermenting, and pressing were ranked first and always practised by all the processors. Sifting and peeling were ranked second among the safety practices in cassava processing. Storing of cassava products was also practised among the cassava processors. Since the mean value of all the listed safety practices was greater than 2.50, it suggests that all the listed safety practices were major safety practices the respondents have identified to use in their processing activities. This is an indication that cassava processors practised safety measures to a maximum in the course of their operations.

3.6 Profitability of food safety practices in cassava processing

Table 6 presents the profitability of food safety practices in cassava processing. The total revenue accrued from processing two tonnes of cassava to garri was ₦217,431.7 (USD 525.12). The total variable cost was ₦133,820.84 (USD 323.19). The cassava processing had a gross margin of ₦83,610.86 (USD 201.93) and a net profit of ₦81,052.33 (USD 195.75). The return on capital invested in food safety practices in cassava processing was 0.62. Food safety practices in cassava processing had an operating ratio of 0.61. The ben-

Table 3: Assessment of processors’ knowledge of safety practices

Cassava processors knowledge	Yes	No
It is not necessary to sort cassava tuber	19 (15.8)	101(84.2)
Sorting of cassava tuber is a good practice	109 (90.8)	11 (9.2)
Peeling of cassava reduces the edible tissues	3 (2.5)	117(97.5)
Washing of peeled cassava improves cassava products’ quality	115 (95.8)	5 (4.2)
Grating of cassava enhances the reduction of cyanide in cassava	97 (80.3)	23 (19.2)
Frying improves the quality and reduces poisonous substances in garri	112 (93.3)	8 (6.7)
Keeping hygienic environmental prevent contamination	120(100.0)	0 (0.0)
Cassava contains a poisonous substance (cyanide)	114 (95.0)	6 (5.0)
Consuming cassava products that do not pass through fermentation is dangerous to human health	110 (91.7)	10 (8.3)
Content of cyanide varies with varieties of cassava	77 (64.2)	43 (35.8)

Source: Research Survey, 2021.

Table 4: Sources of information on safety practices in cassava processing

Source of information on cassava processing	Frequency	Percentage
Fellow processors	81	67.5
Extension agents	69	57.5
Internet	4	3.3
Radio	107	89.2
Television	46	38.3
Public sanitary officers	82	68.3

Source: Research survey, 2021.

Table 5: Food safety practices by cassava processors

Safety practices	Never	Rarely	Sometimes	Always	WS	MS	Rank
Peeling	0(0.0)	0 (0.0)	0 (0.0)	120(100.0)	480	4	1 st
Washing	0(0.0)	0 (0.0)	0 (0.0)	120(100.0)	480	4	1 st
Grating	0(0.0)	0 (0.0)	0 (0.0)	120(100.0)	480	4	1 st
Fermentation	0(0.0)	0 (0.0)	0 (0.0)	120(100.0)	480	4	1 st
Pressing	0(0.0)	0 (0.0)	0 (0.0)	120(100.0)	480	4	1 st
Sifting	0(0.0)	0 (0.0)	6 (5.0)	114 (95.0)	474	3.95	2 nd
Frying	0(0.0)	0 (0.0)	6 (5.0)	114 (95.0)	474	3.95	2 nd
Storing	0(0.0)	0 (0.0)	31 (25.8)	89 (74.2)	449	3.74	3 rd

Source: Research Survey, 2021.

efit-cost ratio in cassava processing was 1.52. These results show that food safety practices in cassava processing were profitable.

3.7 Perception of the processors on the risk involved in cassava processing

Table 7 shows the perceptions of cassava processors on the various risks involved in food safety practices in the processing of cassava. Fifty percent of the cassava processors agreed that raw cassava spoilage due to pest and disease was a risk in cassava processing while 50% disagreed. The majority of the processors

disagreed that an inadequate market for produce was a risk in cassava processing while 25.8% agreed that it is a risk. About 64.2% agreed that poor fermentation was a risk in cassava processing while 35.8% disagreed. Meanwhile, 20.8% agreed that a communal conflict was a risk in cassava processing while 79.2%

disagreed. Ninety-five percent of the cassava processors agreed that cassava and its product's spoilage was due to inadequate storage facilities was a risk in cassava processing while only five percent disagreed with it.

Table 6: Profitability of food safety practices in cassava processing

Variables	Amount (in Naira) per 2 tonnes
Total Revenue (A)	217,431.7
Raw cassava	102,125.00
Water	450.83
Firewood	4,304.17
Transport	6,125.00
Labour	12,316.67
Grating and pressing	8,500
Total Variable Cost (B)	133,820.84
Rent	723.53
Knife	315
Sieving and frying material	1,520
Total Fixed Cost (C)	2,558.53
Total Cost (D = B + C)	136,379.37
Gross margin (E = A - B)	83,610.86
Net profit (F = E - C)	81,052.33
Benefit-cost ratio (G = A/D)	1.59
Return on capital invested (H = E/B)	0.62
Operating ratio (I = B/A)	0.61

(USD 1 = ₦414.06)

Source: Research Survey, 2021

Table 7: Perception of cassava processors on the risk involved

Risks involved	Yes	No
Spoiled cassava due to pest and diseases	60 (50.0)	60 (50.0)
Marketing risk	31 (25.8)	89 (74.2)
Poor fermentation	77 (64.2)	43 (35.8)
Communal conflict	25 (20.8)	95 (79.2)
Products spoilage due to inadequate storage facilities	114 (95.0)	6 (5.0)

Source: Research Survey, 2021



3.8 Constraints to food safety practices in cassava processing

Table 8 shows the various constraints to food safety practices in cassava processing. The major or severe constraints faced in food safety practises were inadequate finance (\bar{X} = 3.71), high cost of cassava roots (\bar{X} = 3.55), the time-consuming nature of food safety practices (\bar{X} = 3.43), poor access to clean water (\bar{X} = 3.38), cumbersome nature of the safety practices in

cassava processing (\bar{X} = 3.38), fluctuation in market price (\bar{X} = 3.27), lack of access to disposal facilities (\bar{X} = 3.15), non-availability of modern processing facilities (\bar{X} = 3.09), unable to meet demand volume (\bar{X} = 3.03), weak institutional support for extension (\bar{X} = 2.89), and branding of cassava products (\bar{X} = 2.76). While lack of skilled labour (\bar{X} = 2.47) and poor access to public education and information (\bar{X} = 2.11) were not considered major constraints to food safety practices among cassava processors.

Table 8: Constraints to food safety practices in cassava processing

Constraints	ES	VS	MS	NS	WS	MS	Rank
Inadequate finance	85(70.8)	35(29.2)	0 (0.0)	0 (0.0)	445	3.71	1 st
High cost of cassava roots	66(55.0)	54(45.0)	0 (0.0)	0 (0.0)	426	3.55	2 nd
Time consuming nature of food safety	66(57.4)	49(42.6)	0 (0.0)	0 (0.0)	411	3.43	3 rd
Poor access to clean water	62(51.2)	41(34.2)	17(14.2)	0 (0.0)	405	3.38	4 th
Cumbersome nature of the safety practices	55(45.8)	55(45.8)	10(8.3)	0 (0.0)	405	3.38	4 th
Fluctuation in market price	36(30.0)	80(66.7)	4 (3.3)	0 (0.0)	392	3.27	6 th
Lack of access to disposal facilities	51(42.5)	36(30.0)	33(27.5)	0 (0.0)	378	3.15	7 th
Lack of modern processing facilities	48(40.0)	35(29.2)	37(30.8)	0 (0.0)	371	3.09	8 th
Inability to meet demand volume	41(34.2)	42(35.0)	37(30.8)	0 (0.0)	364	3.03	9 th
Weak institutional support for extension	28(23.3)	51(42.5)	41(34.2)	0 (0.0)	347	2.89	10 th
Inability to brand	20(16.7)	51(42.5)	49(40.8)	0 (0.0)	331	2.76	11 th
Unavailability of skilled labour	6 (5.2)	55(47.8)	54(47.0)	0(0.0)	297	2.47	12 th
Poor access to public education and information	0(0.0)	41(34.2)	51(42.5)	28(23.3)	253	2.11	13 th

ES = extremely severe; VS = very severe; MS = moderately severe; NS = not severe; WS = weighted score; MS = mean score

Source: Research Survey, 2021

4. Discussion

Description of the socioeconomic characteristics of the cassava processors is important as it can influence the processors' decision to practise food safety in cassava processing and the profitability of the venture. The majority of the cassava processors were females which imply that females dominate the cassava processing ventures. This is because the males engage in on-farm activities while females engaged in the processing of crops in most rural areas. This finding was in line with Alamu et al. (2019), and Kolawole et al. (2012) who reported that women carried out most cassava processing activities. The average age (46.6 years) of the cassava processors suggests a population that was still in their economic active age where they can practise cassava processing efficiently and in a safe manner. The majority of the cassava processors were married while the minority were widowed and divorced. Previous studies had also shown that rural dwellers were married (Mukaila et al., 2021a; Obetta et al., 2020). This suggests that cassava processing is a means of catering for the households in the study area. Their average household size of eight persons implies a relatively large household size among the cassava processor. Households determined the availability of family labour in rural households which could enhance their productivity (Mukaila et al., 2022). Thus, the cassava processor could be said to have cheap family labour which could assist them in processing cassava.

The majority of the cassava processors had some level of education which could enhance their decision-making process. Akanbi et al. (2020) stated that the level of education enhanced farmers' decision-making process. Farmers' high educational level enhanced their access to and use of information on new technology (Mwang & Kariuki, 2015), and influenced their thoughts and attitudes toward the benefits associated with innovation or new technology (Uematsu & Mishra, 2010; Waller et al., 1998). The majority of the cassava processors had cassava processing as their primary occupation, which suggests that the study targeted the right population and that cassava processing plays a significant role in people's wellbeing. The mean years of cassava processing experience of fifteen years suggest a population with a high level of experience in cassava processing. This is an indica-

tion of technical know-how. This is because people's understanding of a business increase as their years of experience increase (Mukaila et al., 2021b).

The average monthly income of cassava processors was ₦72,666.67 (USD 175.50). This implies that cassava processing plays a vital role in their economic status. The majority of the processors did not belong to any cooperative association or organizations. The implication of this is that the cassava processing population may not be financial inclusive as they may not be able to take full advantage of economies of scale inherent in membership of cooperatives. This is because groups enable farmers to access adequate credit and market information (Adong et al., 2012; Falola et al., 2022), enjoy economic benefits (Wossen et al., 2013) and can join resources to try modern processing facilities. Also, group social capital in rural areas is used for mutual benefits (Alele et al., 2013).

Regarding the various motivating factors for food safety practices adoption by cassava processors, the majority of cassava processors were motivated to engage in food safety practices due to demand-driven for well-processed cassava products. Globally due to health concerns, consumers are now willing to pay for safe food with no foodborne related diseases (Liu et al., 2020; Yin et al., 2020; Vajda et al., 2020; Louw, 2020; Nayga, 2006). Because many consumers are now concerned about the safety of the food they eat, they go for a well-prepared cassava product that will not affect their health status. Nigerian garri consumers taste the product to know if it is well fermented and prepared. Well-processed cassava products had a high demand from consumers. A larger proportion of cassava processors adopt the food safety practice in cassava processing to make a higher profit. This could be due to the high demand for well-prepared cassava products which, in turn, attracts a better price. In the same vein, the majority of the cassava process was motivated to adopt food safety practices due to the health concerns of their consumers. This suggests that they were aware of the negative impact of poorly prepared food (cassava). The majority agreed that improving efficiency and to reduced wastage were also motivating factors for practising food safety in cassava processing.

Regarding the cassava processors' knowledge of safety practices, the majority of the cassava processors

opined that sorting of cassava was necessary and good practice to maintain high-quality cassava products. Sorting of cassava tubers allows the processors to remove the bad or spoiled cassava to avoid food poisoning and affecting consumers' health. The majority of the processors disagreed that the peeling of cassava leads to the loss of edible tissues. This implies that they were aware of the importance of peeling cassava before processing. The cassava processors were aware that washing peeled cassava improves garri quality and reduces the health risk associated with unwashed cassava. This could be because unwashed peeled cassava might harbour pathogenic materials. In the same vein, they agreed that grating enhances the reduction of hydrogen cyanide in cassava. This is because the grating of cassava allows easy removal of hydrogen cyanides as the cassava will be mashed. In the same vein, the processors were aware that cassava that does not pass through fermentation is dangerous to human health or for consumption. This implies that the cassava processors were aware of the health risk associated with consuming cyanides. The majority of the processors were aware that frying cassava improves the quality and reduces poisonous substances in garri. All the cassava processors were aware that environmental hygiene helps to prevent contamination of cassava products. The majority of the processors were aware that cassava contains a poisonous substance (hydrogen cyanide). A larger proportion was aware that cyanide content varies with varieties of cassava. It is a usual practice among the local processors to break a raw cassava tuber to see the inside and sometimes taste it to check if the cassava is sweet or bitter. They were aware that bitter cassava contains a high level of hydrogen cyanide than sweet cassava. These results are indications that the majority of the processors had adept knowledge of various safety and precaution practices among cassava processors. Adeoye et al. (2019) reported a similar finding that the majority of the cassava processors were aware that sorting, washing, grating, pressing and fermentation are good safety practices.

Regarding the various sources of information on food safety practices in cassava processing among the respondents. The majority of the cassava processors sourced information through the radio. The government organised some food safety programmes on the radio where they enlightened the people on how to

prepare and process food in a hygienic way to avoid foodborne diseases. Cassava processors who had undergone training or attended food safety programmes disseminate information on food safety information to other processors. Thus, well-trained processors served as means of food safety practises information to other cassava processors. More than half of the cassava processors sourced food safety practice information from extension agents who visited their processing centres to educate them on food safety practices. A smaller proportion of the cassava processors obtained food safety practice information through television programmes. The internet as a source of food safety information was used by a few of the cassava processors who had a high level of education. A larger proportion of cassava processors sourced food safety information from public sanitary officers who enlightened them on the importance of maintaining a hygienic environment, the use of clean water in the processing activities, fermentation, and proper disposal of waste products. These are indications that cassava processors are not lacking information as they possess various media through which they can obtain food safety information. Thomas and Philips (2015) reported a similar result that cassava processors sourced food safety information from fellow processors and public sanitary officers.

Regarding the various safety practices among the cassava processors, all the cassava processors peeled the cassava tuber to remove the outer part. After peeling the cassava, all the cassava processors washed the peeled cassava to remove all dirt from the cassava to avoid sand or other unwanted dirt. After washing, all the cassava processors proceeded to grate the cassava to a fine texture. All of them fermented the grated cassava to lower the effect of hydrogen cyanide present in cassava and for a better taste as most consumers prefer well-fermented cassava (garri) in Nigeria. After fermentation, all the cassava processors pressed the cassava to remove hydrogen cyanide and the liquid in the cassava. The majority of the cassava processors sifted the cassava after pressing. The few cassava processors that did not sift processed the cassava into cassava flour locally called elubo lafun (a local food prepare to make amala. A food widely consumed by the Yoruba tribe in Nigeria). The majority of the cassava processors fried the cassava to make garri. This further removes the remaining hydrogen cyanide and

moisture content present in it. It also increases the lifespan of garri during storage. The few that did not fry it sundry it to make cassava flour (elubo lafun).

This also removes the moisture content and hydrogen cyanides present in it and further elongate the lifespan of elubo lafun. Oghenechavwuko et al. (2013) also reported that oven drying or sun drying of cassava is to reduce post-harvest loss and improves its shelf life. The majority of the cassava processors stored the cassava products for a short period after production. The short storage period was because they did not have modern storage facilities. Only 25.8% sold cassava products immediately after production. These results imply that the cassava processors widely practised food safety in cassava processing.

Based on the profitability of food safety practices in cassava processing, the cassava processing had a gross margin of ₦83,610.86 and a net profit of ₦81,052.33 from total revenue of ₦217,431.7 accrued from processing two tonnes of cassava. The return on capital invested in cassava processing was 0.62. This implies that for every ₦1 invested in cassava processing, ₦0.62 was earned as a return to cassava processing. Thus, cassava processing had a relatively high investment return. The operating ratio of 0.61 in cassava processing implies that sixty-one percent of the gross revenue was used as operating cost in cassava processing. The positive benefit-cost ratio (1.52) in cassava processing implies a positive benefit to cassava processing. These results imply that food safety practices in cassava processing were profitable and productive. Thus, apart from the safety concern and benefits of cassava processing, it was economical, productive, and profitable.

Regarding the perceptions of cassava processors on the various risks involved in the processing of cassava, half of the cassava processors perceived that raw cassava spoilage due to pests and disease was a risk in cassava processing. This is because infected cassava tuber reduced the quality and quantity of cassava products' output and consequently the profitability of the enterprise. It further affected the safety of cassava products for consumption. The majority of the processors did not perceive an inadequate market for cassava products as a risk in cassava processing. This could be a result of the availability of a market for well-processed cassava. A larger proportion perceived that poor fer-

mentation was a risk in cassava processing. This is because poor fermented cassava produce will attract low demand and price as consumers prefer well-fermented cassava products (garri). This makes the processors undertake and participate in the food safety practices in cassava processing. The majority of the cassava processors agreed that communal conflict was not a risk in cassava processing. This suggests a peaceful environment for cassava processing. The majority of the cassava processors agreed that spoilage of cassava and its products due to inadequate storage facilities was a risk in cassava processing. These results indicate that pests and diseases, poor fermentation, and inadequate storage facilities were the highly considered risk among the cassava processor while marketing and communal clashes were not major risks.

Regarding the various constraints to food safety practices in cassava processing, inadequate finance was a major constraint to food safety practices in cassava processing and was ranked first. This could be due to their inability to access credit among them. The processors disclosed that it was difficult for them to get financial support in form of credit, especially from commercial banks. This could limit their production to a small scale (Falola et al., 2022). The high cost of cassava roots was also a major challenge to cassava processing. The COVID-19 pandemic which affected the 2020 planting season increased the price of cassava tuber. One tonne of cassava which was sold for about ₦30,000 (USD 72.45) in 2019 was sold for ₦80,000 (USD 193.21) to ₦130,000 (USD 313.96) in 2020/2021. This affected the processors as their capital was not enough to purchase a large volume of cassava. This makes most processors to processed two to three tonnes per month. The time-consuming nature of food safety practices was considered a severe constraint by the processors. They spent at least one week for each processing cycle to maintain food safety. Poor access to clean water was also a severe constraint to food safety practices in cassava processing. This could be because water is a major input in cassava processing. They need clean water to wash the cassava after peeling and to clean the processing materials and environments. The cumbersome nature of the safety practices in cassava processing was also a major constraint as it involves several methods to produce a product free from foodborne diseases and safe for human consumption. Fluctuation in market price also

affected their processing activities as cassava tuber prices are highly volatile which consequently affected their plan and decision-making process.

Lack of access to disposal facilities was also a major constraint to the processors. Before food safety practices, they dump their waste very close to the processing centres. But as they practice food safety in cassava processing, they have to move their waste to a far place as they were unable to access modern disposal facilities. The non-availability of modern processing facilities was a severe problem for food safety practices among cassava processors. Due to inadequate financial support, the processors could not afford to get modern processing facilities such as modern fryers, peelers, graters, pressers, washers, and extractors which could have enhanced their food safety practices and reduced their stress and time. The processors who practised food safety were unable to meet demand volume due to low production as a result of poor financing and a lack of modern processing facilities. Nigeria alone has over 200 million consumers of cassava products who are ready and willing to purchase well-processed cassava products. Weak institutional support for the extension also hinders food safety practices among cassava processors. Extension contacts with cassava processors were low due to weak support. Branding of cassava products, especially gari, was a constraint to food safety practices among the cassava processors. The processors lack the technical skills to brand their products to meet export demand.

This also hinders their ability to get NAFDAC registration numbers and SON accreditation. Lack of skilled labour was not considered a major constraint to food safety practices among cassava processors. This could be because the processors did not use modern equipment that requires technical know out and because they supervised the activities of their workers. Poor access to public education and information was also not a constraint to food safety practice. This could be because they had access to several sources of food safety information.

5. Conclusion

This study investigated the economics of food safety practices among cassava processors. The study revealed that the majority of the cassava processors were

females who were married, still in their economically active age, well experienced and employed food safety practices in processing cassava. Their sources of food safety information for cassava processing were radio, public sanitation officers, extension agents, colleagues, television and the internet. The processors made use of all safety practices (such as peeling, washing, grating, fermenting, pressing, sifting, frying or drying and sometimes storing) involved in cassava processing. The processors perceived cassava pests and diseases, poor fermentation and inadequate storage as the major risk involved in cassava processing while marketing risk and communal conflict were not major risks. The motivational factors to embark on food safety practices by the cassava processors were high demand for safe cassava products, high profit, health concerns, to improve the efficiency of production and to reduce wastage of the products. The food safety practices were profitable with a relatively high return on capital invested, low operating ratio, and a positive benefit-cost ratio. This shows that apart from the food safety concern, food safety practices in cassava processing were economical, productive and profitable. Despite the profitability of the ventures, the cassava processors faced some challenges. The major constraints faced in food safety practices in cassava processing were inadequate finance, time-consuming nature of food safety practices, poor access to clean water, cumbersome nature of the safety practices in cassava processing, fluctuation in market price, lack of access to disposal facilities, non-availability of modern processing facilities, unable to meet demand volume, and weak institutional support for the extension agents.

To enhance food safety practices among cassava processors, sufficient extension services should be extended to the processors to educate and encourage the processors to continue and improve the practice of food safety in cassava processing. Also, the use of improved varieties of cassava low in cyanide content should be encouraged among the processors to avoid food poisoning. This can be achieved through the distribution of low or no-cyanide cassava seeds by the government and research institutes to the farmers who sell cassava tubers to the processors. There should be an effective institution established by the government or processors groups or associations to standardize the products from cassava processing

and grade the products according to their safety of consumption. This would further assist and motivate the processors to brand their products. Furthermore, provision of credit facilities to the processors towards ensuring large-scale processing will have positive effects. The provision of automated modern processing facilities by the government is needed to reduce the stress experience and time during cassava processing. These would ensure food safety practices among cassava processors, ensure efficiency in the processing activities and enhance the enterprise's profitability.

Conflict of Interest

The authors declare that there is no conflict of interest.

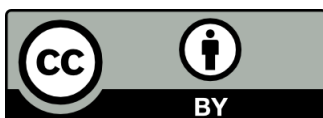
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Comparing herbal phytochemicals in different Pegaga: *Centella asiatica* and *Hydrocotyle verticillata*

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Centella asiatica, *Hydrocotyle verticillata*, pentacyclic triterpenoids, leaf morphology, LC-MS/MS.

This study aimed to reveal the differences between *Centella asiatica* and *Hydrocotyle verticillata*. Both species are known as Pegaga in the local name and are commonly eaten as a salad in Malaysia. The phytochemical differences are important to prevent the misuse of herbs in product development. The key phytochemical groups such as phenolics, flavonoids, and terpenoids were estimated from the calorimetric assays and subsequently identified the intense compounds using LC-MS/MS. The reported triterpenoids (asiatic acid and madecassic acid) and their trisaccharides (asiaticoside and madecassoside) were detected in *C. asiatica*. Glycosylated quercetin and rhamnocitrin were found in *H. verticillata*, but absent in *C. asiatica*. Quercetin and rutin appeared to be the compounds differentiating *H. verticillata* from *C. asiatica* based on unsupervised multivariate data analysis. The leaf images of the herbs were compared using a computational edge detection technique. The leaf morphology based on the leaf shape and vein pattern could clearly differentiate the herbs. Therefore, the application of the herbs in product formulation should be careful, since both herbs have different phytochemical profiles which would contribute to different biological activities.

1. Introduction

Centella asiatica (L.) Urban which is commonly known as Indian pennywort, Asiatic pennywort, or gotu kola is a perennial herb belonging to the plant family Apiaceae (formerly Umbelliferae). It was formerly named *Hydrocotyle asiatica* and then transferred to the genus of *Centella* by Ignatz Urban in 1879 (Urban, 1879). It can usually be found in the temperate and tropical swampy areas in Southeast Asian countries

such as India, Sri Lanka, China, Indonesia, and Malaysia, as well as South Africa and Madagascar (Jamil, et al., 2007). This herb is one of the most commonly used herbs which has been claimed to possess various pharmacological effects, particularly on wound healing, maintenance of connective tissue, inhibition of excessive scar tissue (keloids), and treatment of various skin conditions such as ulcers, eczema,

and psoriasis (Brinkhaus, et al., 2000; Mangas, et al., 2008; Gohil, et al., 2010). The healing effects are mainly due to the presence of active constituents such as pentacyclic triterpenoids (siatic acid and madecassic acid), and their trisaccharides (asiaticoside and madecassoside) (Meeran, et al., 2018). These triterpenoid saponins and their sapogenins are also responsible for memory enhancement, haemostatic and venous hypertension (Gohil, et al., 2010; Chaisawang, et al., 2017; Meeran, et al., 2018). Asiatic acid was proven to be effective against malignant glioma which is one of the most damaging and incurable tumors in the brain (Kavitha, et al., 2011). The other phytochemicals include plant sterols, phenolics, and flavonoids (Srivastava, et al., 1997).

This herb has been widely used as a folk remedy for thousands of years (Diwan, et al., 1991). The recent publication also supports the beneficial use of the herb through scientific studies. Scientists and researchers are getting interested to generate technical data in line with the traditional remedies. The ever-increasing use of the herb has caused the problem of adulteration purposely or unintentionally with cheaper material. The common material that has been mistreated is *Hydrocotyle bonariensis* Comm. ex Lam, which is usually called largeleaf pennywort or coast pennywort from the plant family Araliaceae (Plunkett, et al., 2004). This exotic aquatic macrophyte is also called Ulam Pegaga which means Pegaga salad in Malaysia. A similar phenomenon is happening in Indonesia.

The researchers reported that *C. asiatica* is potentially adulterated with either *Hydrocotyle verticillata* or *Merremia emarginata* which have the same local name as Pegagan (Subositi, et al., 2016; Maruzy, et al., 2020). The misidentification has also happened in the Philippines by local folks (Daminar & Bajo, 2013). *H. bonariensis* is primarily planted in canals and water features for aesthetics and phytoremediation (Strosnider, et al., 2011). The juice of the plant is traditionally prepared to treat fever, colds, coughs, hepatitis, influenza, pruritus, and sore throat, as well as headaches and urinary problems (Sujanapal & Sankaran, 2016). In 2014, a group of researchers from Singapore compared the vegetative differences between *C. asiatica* and *H. verticillata*. *H. verticillata* which is also known as water pennywort or whorled marsh-pennywort, is an exotic aquatic macrophyte that is commonly found in marshes. The difference between both species, in

terms of phytochemicals, is extremely limited in the literature. The difference in phytochemicals in both species is of great importance, especially for herbal product formulation.

Plant recognition is still the specialization of plant taxonomists and botanists with adequate experience to authenticate plant species. The advancement of computing technologies and the invention of digital cameras have supported the works of non-specialists. The approach is known as digital image processing which eases herbal identification in a rapid, simple, and effective manner. The leaf features such as edge or shape, vein, dimension, and colour appear to be reliable inputs being considered in computing. Works have been extensively carried out on leaf image processing and plant classification using different algorithms (Azlah, et al., 2019). To the best of our knowledge, studies have not been performed to relate phytochemicals and leaf morphological observation for plant recognition. Most probably, there are two different fields of studies in which cross-disciplinary collaboration is relatively limited in academia. Therefore, this study was carried out to investigate the differences in phytochemicals and leaf morphology between *C. asiatica* and *H. verticillata* which are commonly mistreated for product formulation in the market.

2. Materials and Methods

2.1. Phytochemical extraction

Phytochemical extraction was conducted using 1 g powdered leaves and stems in 100 mL solvent systems consisting of different concentrations of ethanol ranging from 0-100 %v/v. The mixture was refluxed at the boiling points of the solvent systems for 2 hours. The supernatant was collected after centrifuged and filtered by Whatman cellulose filter paper (Grade 1, 110 mm x 11 µm). The supernatant was then concentrated using a rotary evaporator and dried in an oven at 50 °C until dry. The weight of dried crude extract was recorded. All experiments were carried out in triplicate unless otherwise stated.

2.2. Total Phenolic Content

The total phenolic content of samples was estimated using the colorimetric method according to the pro-

cedures described by Siddiqui et al. (2017) with modification. Different concentrations of samples were reconstituted in 50% methanol. About 1 mL methanolic sample was mixed with 5 mL Folin–Ciocalteu reagent which was previously diluted with deionized water. The mixture was left for 5 min at 25 °C and then added with 5 mL sodium carbonate (7.5%). After incubation for 20 min, the absorbance of the mixture was measured using a UV-Vis spectrophotometer (UV-1800, Shimadzu, Japan) at 760 nm. A calibration curve of standard chemical, gallic acid (0 - 100 µg/mL) was constructed and the results are expressed as milligram gallic acid equivalent per gram sample (mg GAE/g).

2.3. Total Flavonoid Content

The total flavonoid content of samples was also estimated using the colorimetric method (Aryal, et al., 2019). An aliquot of 1 mL sample was mixed with 3 mL methanolic AlCl₃ solution (10 %w/v), 0.2 mL potassium acetate (1 M) and 5.6 mL distilled water. The mixture was incubated at 25 °C for 30 min and followed by the measurement of absorbance at 420 nm using a UV-Vis spectrophotometer. The results are expressed as milligram quercetin equivalent per gram sample (mg QE/g).

2.4. Total triterpenoid content

The total triterpenoid content was estimated spectrophotometrically using a vanillin assay (Chua, et al., 2019). The 1 mg/mL methanolic sample (250 µL) was added into a test tube containing 8g/100 mL vanillin (250 µL) and topped up with 72 % sulfuric acid (2.5 mL). The mixture of the solution was heated for 10 min at 60 °C, and subsequently cooled in an ice-water bath for 5 min. The absorbance of the solution was recorded by a UV-vis spectrophotometer at 544 nm. Diosgenin (5.7–71.4 mg/L) was used as the standard chemical to build a calibration curve. The results are expressed as mg diosgenin equivalent per g sample (mg DE/g).

2.5. Free radical scavenging activity

The antiradical capacity of samples was determined using DPPH (2,2-diphenyl-2-picrylhydrazyl) assay as described by Chu et al. (2000). A 2 mL sample at different concentrations ranging from 100-500 µg/mL was added into 2 mL methanolic DPPH (0.1 mM) solu-

tion. The mixture was kept aside in a dark area for 30 min. The absorbance of the solution was measured at 517 nm spectrophotometrically. BHA was used as the standard chemical for a calibration curve construction. The percentage of radical inhibition was calculated using Equation 1. The results are expressed as the effective concentration at 50% inhibition (IC₅₀).

$$\text{Inhibition (\%)} = \frac{A_o - A_s}{A_o} \times 100 \quad (1)$$

Where A_o = absorbance of control and A_s = absorbance of sample.

2.6. Cation radical scavenging activity

The cation radical inhibition of sample was determined using ABTS (2,2'azinobis(3-ethylbenzothiozoline-6-sulfonic acid) disodium salt) assay according to the method described by Biskup et al. (2013) with some modifications. The ABTS^{•+} solution was prepared by reacting ABTS (7 mM) with potassium persulfate (2.45 mM) at a ratio of 1:1, and incubated overnight in a dark place. The solution was then diluted with 50% methanol to have an absorbance of 1.00 at 734 nm. Samples were also dissolved in 50% methanol in the concentration of 0 to 1,000 mg/mL. Then, 2 mL of the diluted ABTS^{•+} was added with 100 µL sample solution, and incubated for 6 min under subdued light condition. The absorbance was measured at 734 nm using a UV-Vis spectrophotometer.

2.7. Reducing power

The reducing power of samples was determined using ferric reducing antioxidant power (FRAP) assay which was carried out according to the procedures reported by Chua et al. (2013) with modification. FRAP reagent was freshly prepared by mixing 2.5 mL 2,4,6-tripyridyl-s-triazine complex (10 mM, Fe³⁺-TPTZ) in hydrochloric acid (40 mM), 2.5 mL iron (III) chloride (20 mM, FeCl₃) and 25 mL acetate buffer (0.3 M, pH 3.6). The reagent solution was kept in the dark at 37°C before use. Sample (0.2 mL) was mixed with 1.8 mL FRAP reagent, and incubated at room temperature under subdued light condition for 10 min. The absorbance was measured at 593 nm using a UV-Vis spectrophotometer. Ascorbic acid (10 mg/L) was used

as standard chemical.

2.8. Compound screening by LC-MS/MS

A Liquid chromatography (Ultimate 3000; Dionex Corporation; Sunnyvale, CA, USA) integrated with a diode array detector (Dionex Ultimate 3000) and a tandem mass spectrometer (QSTAR Elite; AB Sciex; Foster City, CA, USA) was used for compound screening. Compounds were separated by a C18 XSelect HSS T3 column (2.1 mm × 100 mm, 2.5 μm) at a flow rate of 150 μL/min. A binary solvent system consisted of solvent A (water with 0.1% formic acid) and solvent B (acetonitrile) was used as the mobile phase at the following gradient: 0–10 min, 10% B; 10–20 min, 10–80% B; 20–25 min, 80% B; 25–25.1 min, 80–10% B; and 25.1–30 min, 10% B. The injection volume was 5 μL. Compounds were eluted from the column and detected at the wavelength of 254 nm. Subsequently, compounds were ionized by a turbo ion spray (-4,500 V) before mass detection at the negative ion mode. The mass range was set at the range of 100–1000 m/z. Nitrogen gas was used for curtain gas (25 psi) and nebulizing gas (40 psi). The declustering potential was 40 V, whereas the focusing potential was 200 V. Samples were filtered using a 0.2 μm nylon membrane filter prior to injection.

2.9. Leaf morphological recognition

An in-house leaf image recognition system which was developed using the Java programming language was used to process the leaf images of both herbal species, namely *C. asiatica* and *H. verticillata*. The leaf image of each plant species was uploaded into the system for image processing and feature extraction. The leaf images were pre-processed via segmentation, grayscale conversion, and noise removal. The key features such as leaf edge, vein pattern, and dimension were extracted from the processed images using a series of algorithms. Prewitt and thinning algorithms were used for edge detection. The algorithms of CheckLines, CheckLineLength, paintLines, and paintPoints were used to construct the vein pattern of leaves. An array of tokens was designed to identify the coordinates of lines using cosine and sine angles for the determination of diagonal dimensions.

2.10. Multivariate data analysis

An unsupervised principal component analysis was carried out using a Pareto scaling in the data processing software (MarkerView 1.2.1, Applied Biosystems/MDSSciex, Foster City, CA, USA). The parameters for peak finding and alignment were set as minimum peak width, 0.05 Da; mass tolerance, 0.01 Da, and retention time tolerance, 0.5 min.

3. Results and discussion

3.1. High throughput mass screening

A high throughput mass screening was performed to detect phytochemicals in *C. asiatica* extracts which were prepared using different concentrations of ethanol ranging from 0-100%. The previously reported phytochemicals such as phenolic acids (caffeoylquinic acid, dicaffeoylquinic acid, and dicaffeoyl methoxyoxaloylquinic acid), flavonoids (kaempferol, quercetin, and glucuronyl quercetin) and triterpenoids (asiatic acid, madecassic acid, asiaticoside, and madecassipside) were detected in this study. The intensities of the compound peaks are plotted in Figure 1. The figure shows that madecassic acid has the highest peak intensity, followed by asiatic acid among the detected phytochemicals. The figure also shows that 50% ethanol is likely to be the most effective ethanol composition in the solvent system for phytochemicals extraction.

In the subsequent analysis, 50% ethanolic extracts of the leaves and pericladial petioles of *C. asiatica* were examined for total phenolic, flavonoid, and triterpenoid content spectrophotometrically (Figure 2). The results showed that leaf extract exhibited higher content of phytochemicals such as phenolics, flavonoids, and triterpenoids than pericladial petiole extract. The proximate content of phytochemicals was also compared with its mimicking counterpart, *H. verticillata*. The comparison revealed that both herbal species had different compositions of phytochemicals, and phenolics were the largest phytochemical group in the samples (Figure 2).

Total phenolic content was determined using the widely accepted Folin-Ciocalteu assay. This assay is non-specific phenol oxidation in an alkaline medium catalyzed by two strong inorganic oxidants, namely phosphotungstic and phosphomolibdic acids. The heteropoly acid was reduced from the valence state of

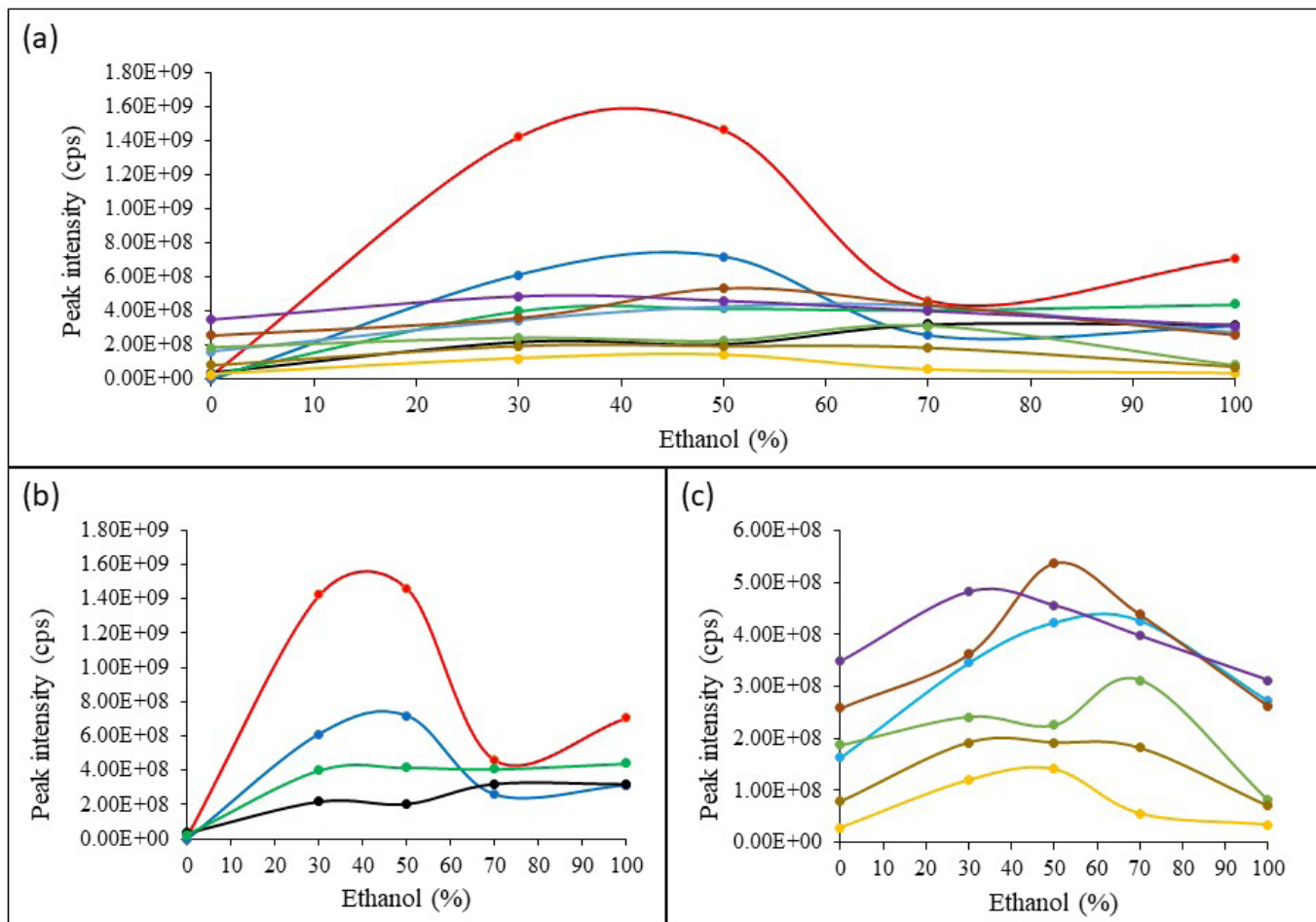


Figure 1. (a) Ten target phytochemicals consisted of (b) two triterpenoids and their trisaccharides and (c) three phenolic acids, two flavonoids and one glycosylated flavonoid in the extracts of *Centella asiatica* prepared using different ethanol concentrations, where —●— asiatic acid, —●— madecassic acid, —●— asiaticoside, —●— madecassoside, —●— caffeoylquinic acid, —●— dicaffeoylquinic acid, —●— dicaffeoylmethoxyoxaloylquinic acid, —●— quercetin, —●— kaempferol and —●— glucuronyl quercetin

+6 to +5 and resulting in the formation of a blue molybdenum-tungsten complex for absorbance measurement. The other non-phenolic organic and inorganic compounds could possibly contribute to an elevated apparent phenolic content. Hence, the assay actually describes the total reducing capacity of a sample which is often correlated to its antioxidant activity.

In the present study, quercetin was used as a standard chemical to build the calibration curve of total flavonoid content. The absorbance was attributed to the formation of acid labile complexes after chelating flavonoids with aluminum ions. Possibly, the C-4 keto, C-3, or C-5 hydroxyl groups and ortho-dihydroxyl groups in the A or B rings of flavonoids may chelate with aluminum ions to produce colored complex for detection (Kasprzak, et al., 2015). The use of alumi-

num ions in the presence of acetate salt was more suitable for flavonols (Pekal & Pyrzyńska, 2014).

The antioxidant capacity of the herbal extracts was also evaluated in terms of scavenging free and cation radicals, as well as reducing ferric ions as presented in Figure 3. In line with the proximate content of phytochemicals, the antioxidant capacity of leaf extract was higher than its pericladial petiole extract. This is because the antioxidant capacity of plant extract is mostly attributed to the presence of phytochemicals, particularly phenolic acids and flavonoids. The figure also clearly shows that the leaf extract of *C. asiatica* could exhibit the highest scavenging activities against free and cation radicals, and reducing power. The 50% ethanolic extract was also found to be an effective radical scavenger compared to its capacity as a reducing

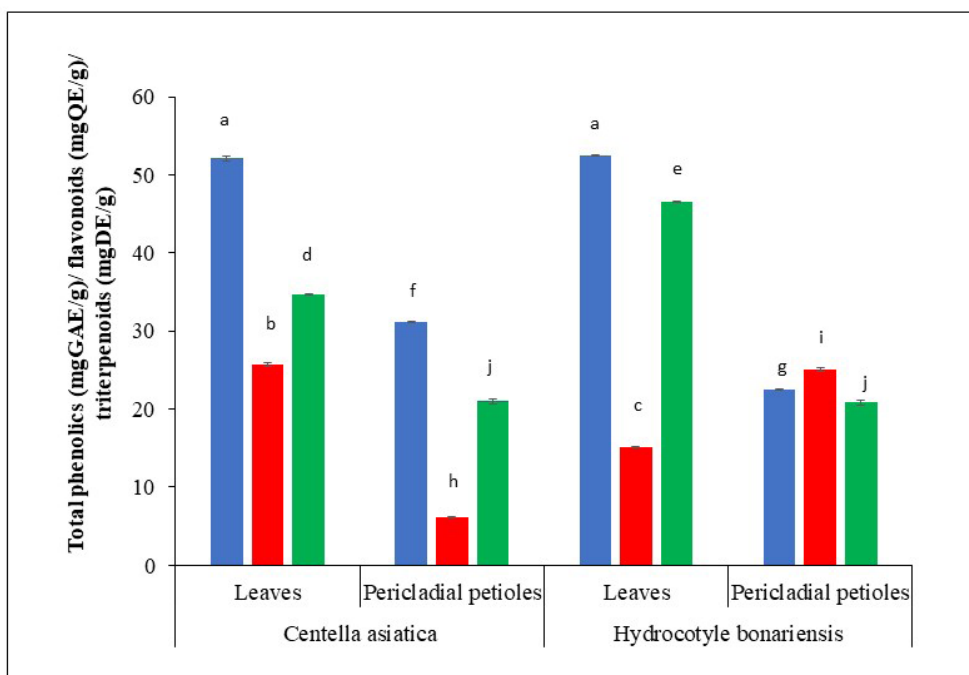


Figure 2. Total phenolics (blue bar), flavonoids (red bar) and triterpenoids (green bar) of the leaf and pericladial petiole extracts from *Centella asiatica* and *Hydrocotyle verticillata*. One-way analysis of variance (ANOVA) followed by T-test paired two samples for means were conducted to determine the significant difference of phytochemical content in the leaf samples of *C. asiatica* and *H. verticillata*, and in the pericladial petiole samples of *C. asiatica* and *H. verticillata*. Different small letters indicate the significant difference at $p < 0.05$.

agent. This was because the concentration of extract which was required to inhibit 50% of radicals was lower than that value to reduce ferric ions. The scavenging activity could achieve more than 80%, whereas the reducing power was about 70% which was about 10% lower than its scavenging capacity.

The antioxidant compounds primarily follow the electron transfer mechanism to inhibit the radicals. The compounds might also involve in hydrogen atom transfer at a slower rate (Gulcin, 2020). Therefore, compounds with bulky rings would have difficulty accessing radicals for electron transfer. On the other hand, compounds with conjugated double bonds and multiple hydroxyl groups would be the dominant chemical characteristics to inhibit radicals. DPPH assay is considered to be more selective because aromatic acid with a single hydroxyl group does not react with DPPH radicals (Cerretani & Bendini, 2010). This also indicates that the leaf extract of *C. asiatica* may have many polyol phenolics either from the group of phenolic acids or polyphenols.

Compounds that react with ABTS radicals would also respond to the FRAP assay because of similarity in

redox potentials (Gulcin, 2020). However, the results showed to have a higher concentration of samples to inhibit 50% of ferric ions. The lower reducing power could only be contributed by water-soluble antioxidative compounds (Apak, et al., 2007). The acidic medium of the FRAP assay was used to promote ferric ion solubility which indirectly increased the redox potential. Pulido et al. (2000) reported that the absorbance of compounds such as caffeic acid, quercetin, and tannic acid was not stabilized even after several hours of reaction time in FRAP assay. The observation was in good agreement with previous researchers that antioxidant activity measured in the FRAP assay was lower than that in the ABTS assay (Gulcin, 2020).

The variance of phytochemicals in both herbs could be clustered into 3 major principal components. The unsupervised multivariate analysis indicated that the phytochemicals in both herbs could achieve up to 78.4 % of the total variance for the first principal component (PC1). Figure 4 shows the phytochemicals in *C. asiatica* are prone to be located in the positive region, whereas the phytochemicals in *H. hydrocotyle* are mostly located in the negative region of PC1. The phytochemicals such as m/z 301 (quercetin), 353

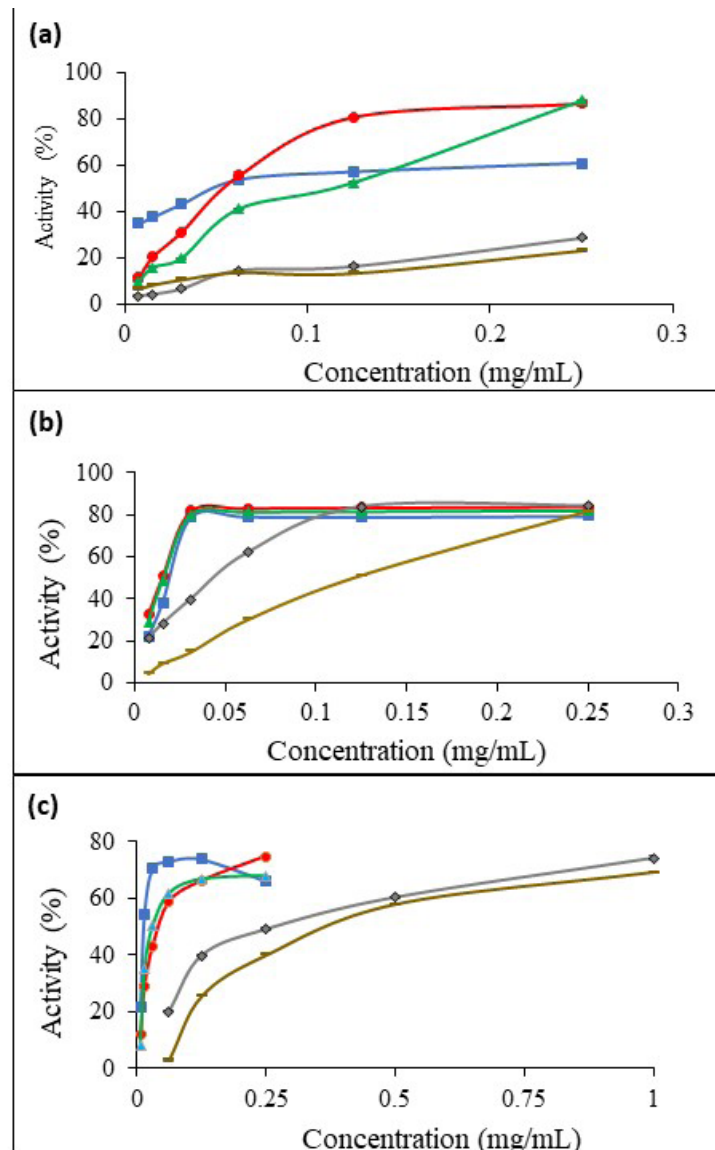


Figure 3. Antioxidant capacity of extracts based on the scavenging activities of (a) free radicals and (b) cation radicals, as well as (c) reducing power of ferric ions for (■) ascorbic acid, (●) the leaf extract of *Centella asiatica*, (▲) the leaf extract of *Hydrocotyle verticillata*, (◆) the pericladial petiole of *C. asiatica* and (–) the pericladial petiole of *H. verticillata*.

(caffeoylquinic acid), 609 (glucosylrhamnosyl quercetin or rutin), 721 (tricafeoyl-2,7-anhydro-2-octulopyranosonic acid) and 1101 (saponin) are likely to be the dominant compounds differentiating *H. hydrocotyle* from *C. asiatica* (Figure 4(c)). Although *m/z* 461 (unknown), 477 (glucuronyl quercetin), 515 (glycosyl caffeoylquinic acid), and 601 (dicafeoyl methoxyoxaloylquinic acid) were found in both plant species, they were present in higher amount in *C. asiatica* (Figure 4(d)). The pentacyclic triterpenoids and their trisaccharides were located near the center of the axis as indicated in Figure 4.

3.2. Comparison of target phytochemicals

The presence of selected phytochemicals was then compared in both 50% ethanolic extracts of *C. asiatica* and *H. verticillata*. The comparison is made in term of its peak intensity as presented in Figure 5 (supplementary). The figure clearly illustrates that *C. asiatica* has higher content of the target phytochemicals, except for caffeoylquinic acid and quercetin. This could support the belief that *C. asiatica* is more active for ethnomedicine, especially for gastrointestinal disorders like dysentery, constipation, stomach problems,

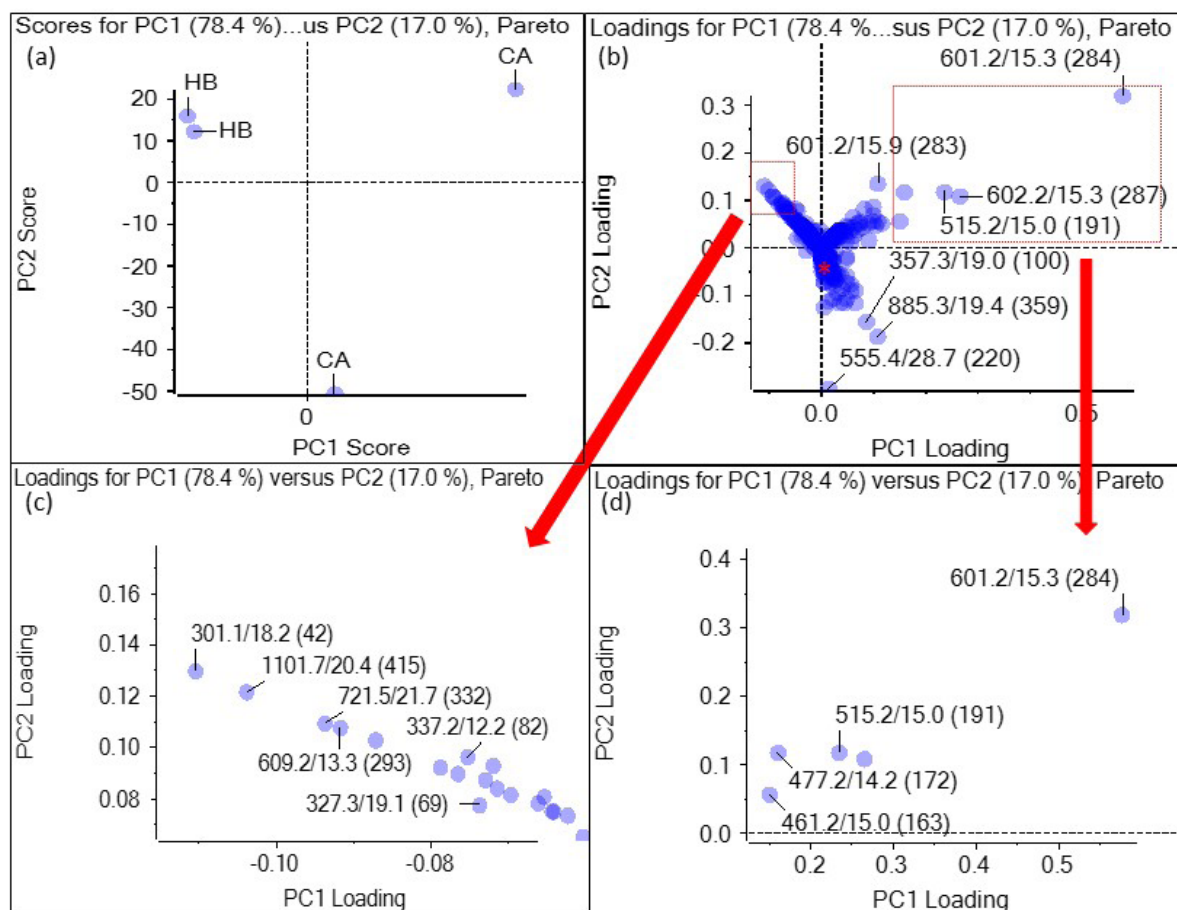


Figure 4. (a) Score and (b) loading plots of *Centella asiatica* (CA) and *Hydrocotyle verticillata* (HB) with the zoom-in area of masses, specifically for (c) HB in the negative region and (d) CA in the positive region of first principal component. * is the location of the pentacyclic triterpenoids and their trisaccharides in *C. asiatica*.

indigestion and loss of appetite, and for memory enhancement (Jahan, et al., 2012). Interestingly, there were a few of glycosylated polyphenols detected only in the extract of *H. verticillata* as listed in Table 1. The quick mass screening results indicated that *C. asiatica* had higher triterpenoids and their glycosides, whereas *H. verticillata* contained more polyphenols and their glycosides. Previous researchers from Taiwan also reported the detection of quercetin, isorhamnetin and rutin in *Hydrocotyle* species (Huang, et al., 2008; Yang, et al., 2008). The results revealed that both species are totally different in phytochemical profile, even they are locally called as Pegaga. The difference in phytochemical profile most possibly will contribute to pharmacological variance.

3.3. Differentiation of leaf morphology

The leaf images of both plant species were also processed using the established computing system for

comparison. This is one of the non-destructive and rapid recognition techniques for plant recognition. The leaf edge including shape, vein pattern, and dimension are selected as the dominant leaf features for the differentiation of plant species (Ehsani Rad, 2010; Lee & Hong, 2013). The leaf colour was not considered because this feature might be changed due to seasonal and environmental factors.

The edge of plant leaves is the most obvious and easily recognised feature for identification. Prewitt algorithm was used to detect the edge of leaves in this study. This algorithm has been proven for its reliability for leaf classification and plant disease detection in previous studies. (Navarajan, et al., 2015; Vilasini Ramamoorthy, 2020). The detected edge points produced pixels forming the leaf edge and vein as presented in Figure 6. From the pixels produced by the Prewitt algorithm, it is clearly indicated that both species of plants have different shapes and vein patterns

morphologically. The leaves of *C. asiatica* show to have a kidney shape with second-order veins branched off at the intervals of several first-order veins, and reticulate meshes could also be observed between the third-order veins and minor veins. On the other hand, the round-shaped leaf of *H. verticillata* displays multiple first-order veins.

Vein patterns could be the fingerprint of plants which is sometimes not easily observed without the assistance of a pattern recognition tool (Scoffoni, et al., 2008). Therefore, the use of a high-performance computing system would be the method of choice. Besides phytochemical identification, leaf morphology including the vein pattern has been recognized as a reliable tool in identifying plant species. In the present study, both *C. asiatica* and *H. verticillata* belong to palmately veined species with multiple first-order veins branching from the petiole (Sack, et al., 2008). The venation architecture is important to determine the sensitivity hydraulic conductance of leaves. A clear correlation has been established between the vein characteristics

and properties of leaves, particularly on the aspects of leaf damage and drought tolerance (Scoffoni, et al., 2011; Sack, et al., 2008).

5. Conclusions

It is important to highlight the difference between phytochemicals in *C. asiatica* and *H. verticillata*, even though both species are known as Pegaga in Malaysia. The findings of the study proved that *C. asiatica* contained pentacyclic triterpenoids (asiatic acid and madecassic acid) and their trisaccharides (asiaticoside and madecassoside), whereas *H. verticillata* contained a high amount of quercetin and its glycosylated derivatives. The different venation of the plant leaves has also explained the variance of phytochemical profiles which would contribute to different biological activities.

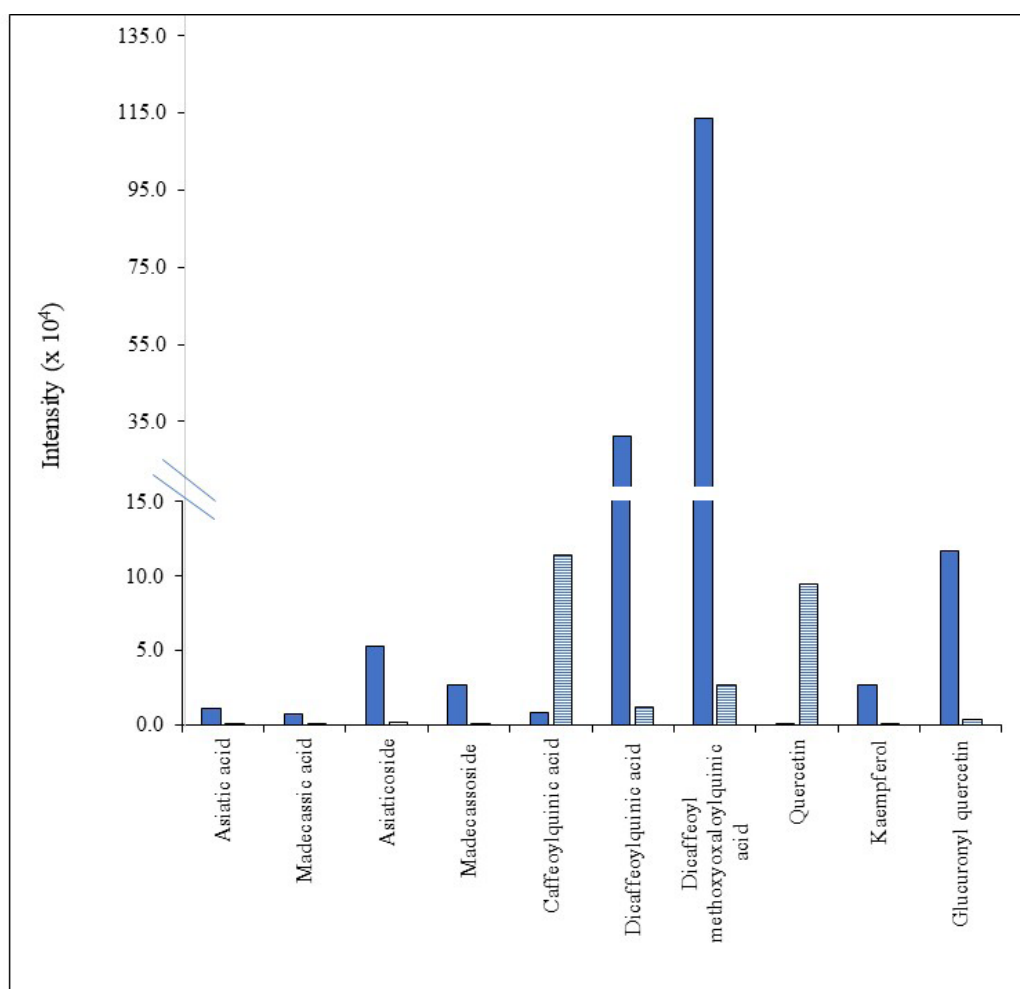


Figure 5. Target phytochemicals detected in *Centella asiatica* (solid blue bar) and *Hydrocotyle verticillata* (line blue bar) extracts prepared using 50% ethanol.

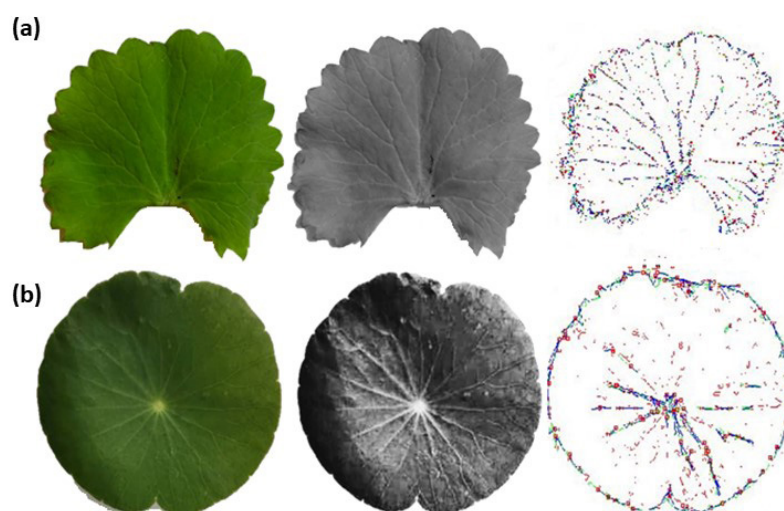


Figure 6. Original leaf morphological images of (a) *Centella asiatica* and (b) *Hydrocotyle verticillata* which are converted to gray scale and pixel images

Table 1. Phytochemicals detected in *Hydrocotyle verticillata* extract

<i>Hydrocotyle verticillata</i>	Putative compounds	References
301/273/179/151	quercetin	(Maulidiani, et al., 2014)
433/300(-133)/299/271	quercetin pentoside	(Maulidiani, et al., 2014)
447/300(-147)/283/271(-176)/255	isorhamnetin pentoside	(Li, et al., 2016)
463/300(-163)/271(-192)	quercetin glucoside	(Li, et al., 2016)
593/564/531(-62)/491/449/429/284/283(-310)/255/227	luteolin rutinoside	(Brito, et al., 2014)
609/507/361/300/271	rutin	(Maulidiani, et al., 2014)
639/463(-176)/300(-163)/269/255	caffeoyl rhamnocitrin glucuronide	(Chen, et al., 2016)
653/299(-354)/284	caffeoylquinoyl rhamnocitrin	(Chen, et al., 2016)
669/463(-206)/300(-369)/271(-398)/255	feruloyl rhamnocitrin glucuronide	(Chen, et al., 2016)
695/300/299(-396)	rhamnocitrin tripentosides	(Chen, et al., 2016)
755/299bp(-456)/271(-484)	rhamnocitrin diglysoypentoside	(Chen, et al., 2016)
1187/581/285(-296)	[2M-H] ⁻ , luteolin rutinoside	(Brito, et al., 2014)

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Conflict of interest

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

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Terra Madre Salone del Gusto 2022



Terra Madre Salone del Gusto is the largest international event dedicated to good, clean and fair food and to food politics.

This huge event takes place from September 22-26, 2022 in Parco Dora, Turin.

The program will be enriched with 100 more events, including taste experiences, areas for young activists, taste education tours, cooking classes, community meals and new exhibition areas. In addition to the major conferences scheduled from September 22nd to 26th in the Sala Kyoto in the Environment Park and in the Parco Dora in Turin, there are also two new areas to exchange and discuss the important topics: the arenas of the event, named after two great activists of our time: Gino Strada and Berta Càceres.

Besides, this year, food and health will be presented by the collaboration between Slow Food and the insurance company Reale Mutua, one of the official supporters and main partners of the event. In this space there are forums and food talks to explore the relationship between our food and our health from different points of view. Also, the events organized by the University of Gastronomic Sciences, between themed aperitifs and breakfasts with producers, tastings and meetings, as well as the RegenerActions and events where you can learn about recipes from Italy and around the world and cook them with guidance. Not forgetting that Terra Madre events will help people to get to know the city of Turin and the theme of regeneration better, through festivals, book launches, urban kitchen gardens, visits and much more.

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A gene responsible for triggering plants to develop fruits and seeds could help face the increased global temperature and decreased pollinators

One of the many impacts of climate change is the rising global temperatures and diminishing pollinator populations. These negative impacts are making food production more and more difficult for the world's growers.

Therefore, understanding the process of how flowering plants, such as peanuts, corn and rice, develop fruits and seeds becomes very important for researchers. A research team at the University of Maryland's Department of Cell Biology and Molecular Genetics has addressed this issue.

The researchers studied the influence of two factors; fertilization and pollination. They believed that there is an internal communication system that is responsible for signalling the plant to develop fruit, however, they wanted to know which factor was responsible for this communication.

To understand the mechanism, the team used strawberry plants. Strawberry is an inside-out fruit that has a unique structure and seed location, therefore, it is considered very suitable for this study. With the strawberry plant, it is easy to observe the seeds and extract genetic information during different development stages.

The researchers uncovered a relationship between a gene and a hormone. A gene called AGL62 stimulates the production of an essential hormone called auxin. The auxin has a significant impact on the size of the grain and enlargement of the fruit. In nature, pollination is the factor that prompts the AGL62 gene to trigger the production of auxin. Climate change is becoming a big challenge in this regard as the rising temperature is killing both pollinators and pollen itself.

Thus, the results of this study are important as they could give a potential solution to increase the productivity of agricultural crops.

1. Lei Guo, Xi Luo, Muzi Li, Dirk Joldersma, Madison Plunkert, Zhongchi Liu. **Mechanism of fertilization-induced auxin synthesis in the endosperm for seed and fruit development.** Nature Communications, 2022; 13 (1) DOI: [10.1038/s41467-022-31656-y](https://doi.org/10.1038/s41467-022-31656-y)

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Replanting Agricultural Biodiversity in the UN Convention on Biological Diversity (CBD)

<https://www.foei.org/publication/replanting-agricultural-biodiversity-in-the-cbd/>



Biodiversity represents the enormous variety of life on Earth. Agricultural biodiversity is a vital part of overall biodiversity. However, agricultural biodiversity has been decreasing continuously over the years. As negotiations on the Global Biodiversity Framework are ongoing, agriculture must be back centre stage.

The CBD report deals with the considerable importance of agricultural biodiversity as an essential part of saving overall biodiversity, as well as the possible ways in which the CBD and the international community can make agricultural biodiversity a priority to the world.

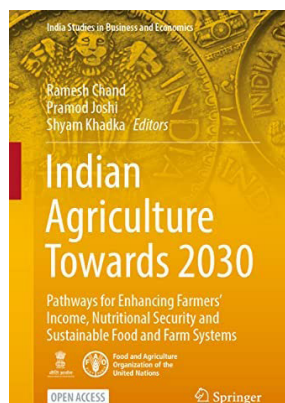
CBD took place in Nairobi in March 2022; in addition to COP15, there is hope that this report will be helpful and encourage you to share it widely.

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Indian Agriculture towards 2030; Pathways for Enhancing Farmers' Income, Nutritional Security and Sustainable Food and Farm Systems

A review by Nayram Ama Doe

Authors (Eds.): Ramesh Chand, Pramod Joshi and Shyam Khadka

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The records of most countries' economies depict that the agricultural sector plays a significant role in employment, national income, and economic development. It is undisputed that agriculture provides food for human survival, adequate nutrition, and a healthy lifestyle. Further, agriculture significantly supports and improves rural livelihood and serves as a foundation of raw material supplies for economic activities extending from industrial production to trade and business. On the other hand, agriculture is a significant contributor to climate change. At the same time suffers the consequences and must therefore adjust to the penalties of this change by reducing emissions of greenhouse gases.

This educative book enlightens readers on various topics such as dietary diversity, nutrition and food safety, management of climatic risks in agriculture, the need for a transformative vision in Indian agriculture towards 2030, pests, pandemics, preparedness and biosecurity, science, technology and innovation, and finally, structural reforms and governance issues in Indian agriculture.

This book begins with an introduction, discussing agriculture's global and national challenges. Eradicating poverty and hunger for a long time has been a primary goal, and numerous activities and initiatives have been developed to address them. For example, the most recent initiative, the Sustainable Development Goal (SDGs) 2030, provides a platform to solve issues on climate change, hunger, poverty, nutrition, inclusive development, and sustainability, which are directly or indirectly related to agriculture. However, research shows that approximately 2 billion people are food insecure, with less frequent access to safe, nutritious, and sufficient food. Another challenge experienced is a drastic increase in agrochemicals utilization in the pre and post-harvest phases of plant and animal food.

The first chapter of this book discusses India's commitment to SDGs and climate change. The NITI Aayog, appointed by the Indian government as the nodal institution for coordinating all forces geared towards achieving the SDG at national and sub-national levels. Climate change is now a focus in our environment, and the recognition of its impact has led to severe measures taken by environmentalists to combat these impacts. Some goals set by the party toward combating climate change include; reducing the emission intensity of the country's gross domestic product (GDP) by 33-35% by 2030. Also, increasing the share of non-fossil-based energy to 40%, and creating an additional carbon sink of 2.5-3 billion tonnes of CO₂ equivalence through planting more trees and forests.

The second chapter of this book discusses dietary diversity, nutrition, and food safety. India continued to face malnutrition and recorded 68.2% deaths in children under age five due to malnutrition in 2017. The SDG 2 is geared toward ending hunger, improving nutrition, achieving food security and promoting sustainable agriculture. Further, dietary diversity and pattern are dependent on the monthly income of individuals and families. There was an observation of a precipitous decline in food expenditure in urban areas than in rural areas, which is in line with Engel's Law that as household income increases, food expenditure decreases in a household budget.

The final chapter of this book focuses on the structural reforms and governance issues in Indian agriculture. India's agricultural and structural development has significantly improved since its independence. Some advancements include public investment in agriculture, investment in irrigation systems, regulated marketing of agricultural produce, and enlistment of agrarian companies to guarantee a con-



tinuous supply of pesticides, seeds, fertilizers, and other Ag-ric products to farmers at prices below current market rates. Overall, this book was informative and educative as it dis-cusses and enlightens readers on dietary diversity, nutri-tion, and food safety. Additionally, it discusses the need for a transformative vision in Indian agriculture towards 2030. Finally, advancements and structural reforms India has un-dertaken to ensure food and nutrition security.

About the author:

Nayram Ama Doe is a master's student at the University of Kassel and Fulda University of Applied Sciences, Germany, studying International Food Business and Consumer Stud-ies. Her research focuses on food sustainability, international food legislation, agriculture, and food systems, and she is very passionate about food security and food supply chain issues.

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