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Development and characterization of pumpkin crackers containing live culture of Lactobacillus acidophilus

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ABSTRACT

Aims: Up to date, there is limited heat-treated non-dairy probiotic food products in the market. Therefore, this study aims to develop a new functional cracker containing microencapsulated pumpkin-probiotics with acceptable sensory characteristics.

Methodology and results: The sample crackers (crackers with beads containing pumpkin and probiotic) have achieved a minimum required viable count of $6.26 \pm 0.05 \log_{10}$ CFU/g and showed significantly (p<0.05) lower viability loss compared to control crackers (crackers with beads containing probiotic only). Sample crackers were perceived as acceptable (score 6.61) by 93 untrained panelists when tested using 9-point hedonic test. The ash content of sample pumpkin crackers was significantly higher (p<0.05) than the control. Both sample and control pumpkin crackers exhibit high level of total dietary fiber (>6%).

Conclusion, significance and impact of study: In summary, development of pumpkin crackers with microencapsulated *L. acidophilus* could be an alternative healthy option cracker for consumers. In addition, the results also suggested a new technique to deliver live culture in baked goods.

Keywords: Microencapsulation, pumpkin, Lactobacillus, crackers, probiotics

INTRODUCTION

With the rapid urbanization and modernization lifestyle, eating habits of majority population have changed to convenient and time saving food products. Biscuits or crackers that fall under ready-to-eat or convenience food categories are commonly consumed among Malaysians. They are also the most popular ready-to-eat snack food in the world that act as a substitution for proper meal due to its conveniences and ability to cater for current busy lifestyle (Norhayati et al., 2015). Although biscuits are one of the most commonly consumed convenience foods, there are certain drawbacks including their hydrogenated fat composition which may lead to health concerns (Boobier et al., 2006). Consequently, healthier options such as low-fat, cereal-based, and vegetable-based biscuits with nutritional improvement were developed and produced by the industry.

Probiotics are defined as 'live microorganisms that provide health benefits to the host when consumed in adequate amount' (FAO/WHO, 2002). Due to the demand for overall health improvement, products containing probiotics are becoming more widely available and

comprise between 60 to 70% of the entire functional food market (Tripathy and Giri, 2014). Lactobacilli is the most common bacterial genera to be incorporated into food, mainly dairy products such as yogurt and fermented milk (Iravani et al., 2015). However, consumption of excessive dairy products has been correlated to some health issues such as lactose intolerance and hypercholesterolemic. Therefore, there is an increased demand for non-dairy probiotic products such as soy-based drinks, fruit, and vegetable-based products as well as other cereal-based products (Granato et al., 2010).

As recommended by the United State Food and Drug Administration, the minimum probiotic count in probiotic food products should be at least 10⁶ CFU/mL to exert health benefits. The survivability of probiotics in food is highly influenced by factors like pH, storage temperature, oxygen levels, and manufacturing process. To overcome these, microencapsulation technique is introduced to protect the cells from hostile conditions such as high acidity conditions, thermal processing condition, and others (Malmo *et al.*, 2013). Past studies have shown that

microencapsulation can improve heat tolerance and increase the probiotics survival rate during heat processing (Seyedain-Ardabili *et al.*, 2016). The authors showed that the viability of probiotic in high heat treatment products such as bread was enhanced by encapsulation technique during baking process (at 180 °C for 15 min). In contrast, Malmo *et al.* (2013) demonstrated that chitosan-alginate microencapsulation system achieved only 10% survival rate of probiotic population in chocolate soufflé (baking at 180 °C for 10 min). This contradicting finding showed that there is still lack of study on utilizing encapsulation techniques effectively in developing high heat treatment probiotic goods leading to limited probiotics containing baked goods in the current market

Fruits and vegetables are good source of fibers, vitamins, and minerals. They are mainly incorporated into a balanced diet due to the presence of antioxidants that provide protective effect against various diseases (Dhandevi and Rajesh, 2015). Pumpkin is a fruit vegetable that has an excellent source of carotenoids and contain significant amount of proteins and fibres (Norshazila et al., 2017). Given the good nutritional value, pumpkins can be used in the form of flour for production of ready-to-eat food such as snack and breakfast cereal products. Furthermore, the fiber content, especially the soluble fiber such as pectin found in pumpkin has prebiotic effects to support the growth of probiotics (de Souza et al., 2017). Therefore, it is postulated that using pumpkin extract in microencapsulation of probiotic may improve the viability of probiotic. Thus, the aim of this study is to develop a functional cracker that contain probiotics and to evaluate the effect of pumpkin puree supplementation in probiotic microencapsulation on the physicochemical and sensory characteristics of the crackers.

MATERIALS AND METHODS

Bacteria culture preparation

Lactobacillus acidophilus ATCC 314 culture was activated for three successive times in sterilized de Mann Rogosa Sharpe (MRS) broth (Merck KGaA, Darmstadt, Germany) at 37 °C for 24 h. The consistency of the cultures concentration was standardized at optical density (OD $_{600}$) = 1.20. The activated cultures were then washed three times using 0.9 % (w/v) sterilized saline water by centrifugation at 4000 rpm at 4 °C for 10 min. The cell pellet was then harvested for further analyses.

Preparation of pumpkin puree

Fresh pumpkin was purchased from local supermarket (Selangor, Malaysia). Pumpkin was peeled and sliced prior to heating in microwave for 3 min and subsequently mashed. The mashed pumpkin was blended for 1 min in order to smoothen the puree. The resultant blended pumpkin puree was stored at 4 °C prior to formulation of cracker and microencapsulation.

Microencapsulation

Microencapsulation of culture was done using extrusion method (Rathore et al., 2013). The microencapsulated beads were prepared by mixing the bacteria cultures of $OD_{600} = 1.20$ with 2% (w/v) sodium alginate (Modernist Pantry, Eliot, ME, USA) solution in 1:1 ratio (v:v). Pumpkin puree (10 g) was then added into the mixture. Pumpkin puree was replaced with distilled water to prepare control beads. The mixtures were then added into 50 mL syringe (Becton Dickinson and Company, Temse, Belgium) with needle of 23G × 1 1/4" size (Terumo Neolus, London, UK) that was fixed to the encapsulator (Chemopharm Sdn Bhd, Selangor, Malaysia). The mixture was extruded at a constant rate of 15 mL/min from the syringe directly to 0.3 M calcium chloride solution (Modernist Pantry, Eliot, ME, USA) to form gel-like beads. The beads were collected and froze at -20 °C overnight prior to freeze-drying process.

Production of pumpkin cracker with freeze dried beads

All the ingredients for crackers were purchased from local supermarket in Selangor, Malaysia. All ingredients including all-purpose flour (51%, w/w), pumpkin puree (23%, w/w), olive oil (14%, w/w), cornstarch (7%, w/w), fine granulated sugar (4%, w/w), baking powder (0.7%, w/w), and fine salt (0.3%, w/w) were mixed and kneaded to form cracker dough. Both 0.05% (w/w) of control and pumpkin freeze dried beads were added into respective cracker dough. The crackers were molded into square shape with length of 2.7 cm and 0.15 cm height. The crackers were baked (C&N United, Selangor, Malaysia) at 150 °C for 20 min.

Sensory evaluation

Ninety-three untrained panelists (18 – 45 years old) were recruited for the sensory evaluation. Sensory properties such as appearance, aroma, texture, taste, and overall acceptance were evaluated using 9-points hedonic scale. Panelists were served with three type of crackers, blank (without probiotics beads), control (with probiotics beads), and sample (with pumpkin-probiotics beads). Each type of cracker was served with three-digit coding at random order. Panelists were required to rinse their palate in between samples with plain water.

Viability of probiotics in freeze dried beads and crackers

The viability of probiotics was determined according to the method of D'Orazio *et al.* (2015), with slight modification. Finely ground control and sample crackers (10 g) were mix vigorously with 20 mL phosphate buffer saline solution of pH 7.4, 0.1 M (Sigma-Aldrich®, St. Loius, MO, USA) for 30 ± 5 min to release the cells from the encapsulated materials. Pour plate method with MRS agar (Merck KGaA, Darmstadt, Germany) was used to

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enumerate the viability of probiotics. Triplicate plate was incubated at 37 °C for 48 h.

Proximate Analysis

Moisture analysis

The moisture content of cracker was determined using oven drying method (AOAC 925.10) (AOAC, 2000). Finely grinded cracker (5 g) was dried at 120 °C until constant weight was achieved. The moisture content of cracker was determined based on the weight loss after drying.

Protein analysis

Nitrogen content of cracker was determined based on Kjeldahl method (AOAC 950.36), using Kjeldatherm digestion unit (Gerhardt, Konigswinter, Germany) and Vapodest Kjeldahl Distillation System (Gerhardt, Konigswinter, Germany) (AOAC, 2000). The nitrogen content was converted to protein content by multiplying with a nitrogen-protein conversion factor of 6.25 (Jeon, 1994).

Fat analysis

Fat content of cracker was determined based on Soxhlet Extraction Method (AOAC 920.39), using SOXTHERM rapid extraction system (Gerhardt, Konigswinter, Germany) (AOAC, 2000).

Ash analysis

The ash content of crackers was analysed based on dry ashing method (AOAC Method 923.03) (AOAC, 2000). Approximately 10 g of dried homogenized cracker was placed in porcelain crucible and inserted into muffle furnace (Nabertherm, Lilienthal, Germany) at 600 °C for overnight.

Carbohydrate determination

The carbohydrate content of cracker was determined by difference method (Jeon, 1994).

Total dietary fiber determination

Moisture and fat free samples were analysed for their total dietary fiber by enzymatic and gravimetric method according to AOAC 985.29 method (AOAC, 2000) using TDF-100 kit (Sigma, St. Loius, MO, USA). Samples (1.0 \pm 0.1 g) were digested with α -amylase, protease, and amyloglucosidase according to manufacturer suggestion (Sigma, St. Loius, MO, USA). Heated (60 °C) 95% ethanol was added and the solution was left to precipitate at room temperature for 1 h. Digested samples were filtered using Fibertec 1023 system (FOSS, Hillerod, Denmark). Crucibles containing residues from filtration was dried and weighed. Sample containing dietary fiber residues were then later used for protein and ash content

determination to calculate total dietary fiber values, protein and ash content.

Texture analysis

Hardness of crackers was determined by using Brookfield CT3 texture analyser (Middleboro, MA, USA) according to Yilmaz *et al.* (2014), with slight modification. The texture analyser was attached with Knife edge probe (TA7, 60 mm W) and set at pretest speed 1 mm/s, test speed 5 mm/s, and 1000 g trigger load.

Statistical analysis

The results obtained were analyzed statistically using Microsoft Excel and IBM SPSS Statistics 20 (SPSS Inc., Armonk, NY, USA). All data obtained were analyzed at a 5% level of significance (α = 0.05). Independent t-test was performed for all analysis except sensory evaluation. One-way analysis of variance ANOVA was applied for the sensory evaluation and statistical difference was analyzed with post-hoc Tukey HSD. All data was presented as mean \pm standard deviation from duplicate of three independent runs unless stated otherwise.

RESULTS AND DISCUSSION

Sensory evaluation

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In the food industry, sensory evaluation is used as a primary parameter to determine the success of a new product development, while other forms of quality parameters such as wholesomeness as well as nutrition are mainly considered secondary (Singh-Ackbarali and Maharaj, 2014). Hence, it is important to ensure the food quality is appealing and highly acceptable by the consumer in terms of appearance, aroma, taste, texture and others. In this study, sensory evaluation was carried out with 9-point hedonic scale to analyze the consumers' acceptance on sample (beads containing pumpkin and probiotic) in comparison to blank (without beads) and control (beads containing probiotic) pumpkin crackers. Based on the results obtained, presence of encapsulated beads in sample crackers did not affect the perception of participants on the crackers' appearance compared to the blank. Since pumpkin puree imparts the desirable colour in all three types of crackers, all crackers exhibited similar colour (Table 1) as similar amount of pumpkin puree was used for the crackers.

There was also no significant difference (*p*>0.05) between all the crackers in terms of aroma, gritiness, crunchiness, taste and overall acceptance. This showed that the microencapsulation techniques produced beads that are sufficiently small and not perceivable by the consumers compared to blank (without the micro-beads). Based on Table 1, all the mean scores obtained were between 6 (like slightly) to 7 (like moderately) which indicated that all the pumpkin crackers were generally accepted by the panelists. Considering that all the crackers showed similar acceptance by consumer, both

Table 1: Hedonic scores of sensory attributes for control, sample and blank pumpkin crackers.

Attailereta		Type of Pumpkin Crackers	S
Attribute	Control	Sample	Blank
Colour	6.49 ± 1.14 ^a	6.55 ± 1.23 ^a	6.87 ± 1.27 ^a
Thickness	6.18 ± 1.29^{a}	6.29 ± 1.34^{ab}	6.70 ± 1.57^{b}
Overall appearance	6.52 ± 1.15^{a}	6.58 ± 1.16 ^{ab}	6.95 ± 1.19^{b}
Overall aroma	6.61 ± 1.42^{a}	6.73 ± 1.30^{a}	6.26 ± 1.48^a
Crunchiness	5.95 ± 1.53^{a}	6.20 ± 1.56^{a}	6.35 ± 1.59^a
Grittiness	5.73 ± 1.75 ^a	5.80 ± 1.53^{a}	6.05 ± 1.58^{a}
Overall texture	6.24 ± 1.52^{a}	6.25 ± 1.33^{a}	6.66 ± 1.38^{a}
Sweetness	6.11 ± 1.62^{a}	6.02 ± 1.53^{a}	6.08 ± 1.65^{a}
After taste	6.17 ± 1.38^{a}	6.42 ± 1.46^{a}	6.43 ± 1.57^{a}
Overall taste	6.49 ± 1.30^{a}	6.54 ± 1.32^{a}	6.64 ± 1.40^{a}
Overall acceptance	6.37 ± 1.40^{a}	6.61 ± 1.10^{a}	6.76 ± 1.41 ^a

^{*}Results are expressed as means ± standard deviations with n = 93

Viability Test

Viability of *L. acidophilus* ATCC 314 was evaluated in this research to provide information about the effects of pumpkin puree supplementation on the survivability of probiotics after baking process. High baking temperature at 150 °C for 20 min may provide a harsh condition that may lead to a significant loss of viable cells. Therefore, microencapsulation technique was carried out to improve the survivability of probiotics. Microencapsulation can protect cells from harsh conditions such as high temperature where past researchers has proven that the survival of *L. acidophilus* encapsulated in starch was improved with a high viable count of 10⁶ CFU per bread after baking at 180 °C for 16 min (Altamirano-Fortoul *et al.*, 2012).

The final viability and total viability loss of L. acidophilus in both control and sample crackers were shown in Table 2. The initial viability of probiotics was $8.14 \pm 0.08 \text{ Log}_{10} \text{ CFU/g (OD}_{600} = 1.20)$. The final viability of sample pumpkin cracker (106 CFU/g) was significantly (p < 0.05) higher than the control pumpkin cracker. The viability loss of sample crackers was 1 Log lower compared to control crackers. This might be due to the extra nutrients provided by the pumpkin in the microcapsules as pumpkin is a good source of carbohydrate, pectin, mineral salts, and vitamins (Yok et al., 2015). Previous studies have demonstrated that the addition of protectants such as prebiotics, carbohydrate, and protein can minimize the viability loss of probiotics during heat processing and storage (Savedboworn et al., 2017). Therefore, it is likely that the carbohydrate and pectin in pumpkin may act as extra protectant towards probiotic cells. Pectins also exhibits prebiotic activities that can act as a growth stimulator for probiotics (de Souza et al., 2017).

According to Sarvari et al. (2014), a minimum dose of 10⁶ CFU/g in food product is required for different species

and strains of probiotic to confer health benefits. Hence, probiotic food product should contain high initial cell counts during food production to ensure sufficient number of cells at the point of consumption (Farnworth, 2008). Our results showed that the sample crackers have achieved the minimum bacterial count for probiotic-based food products.

Genevois *et al.* (2016) reported that lactobacilli strains could metabolize and adhere to pumpkin's polysaccharides. Moreover, the oligosaccharides from pumpkin pulp has shown to stimulate growth of lactobacilli and showed a good resistance to hydrolysis in artificial human gastric juice (Du *et al.*, 2011). Hence, this suggested that pumpkin could serve as a carrier to ensure there was sufficient viable probiotics to exert health benefits to host. This may explain our results of lesser viability loss in sample crackers containing pumpkin microencapsulated compared to the control.

Table 2: Viability of *L. acidophilus* ATCC 314 in control and sample pumpkin crackers.

	Control (Log ₁₀ CFU/g)	Sample (Log ₁₀ CFU/g))
Final Viability	5.30 ± 0.30^{a}	6.26 ± 0.05^{b}
Viability Loss	2.84 ± 0.30^{a}	1.88 ± 0.05^{b}

^{*} The initial viable counts ($OD_{600} = 1.20$) was $8.14 \pm 0.079 \text{ Log}_{10}$ (CFU/g). Results are expressed as means \pm standard deviations (n=3).

Proximate and total dietary fiber analysis

Table 3 shows the comparison of percentage of proximate compositions of control and sample pumpkin

ab Means in the same column with different lowercase superscript letters are significantly different (p<0.05).

^{ab} Means in the same row with different lowercase superscript letters are significantly different (p<0.05). control and samples crackers were then used for viability test and other analyses.

crackers. Baked products specifically biscuits and crackers, are highly affected by the amount of moisture content present in the food. In order to have a longer shelf life, the moisture content of biscuit should be low (1-5%), excluding any moisture from fillings or icings. Moreover, low moisture content in the food system is also required to ensure maximum survival of probiotics in a dry glassy matrix. This is because survival of probiotic in a solid matrix is affected when exposed to different temperature. An increased in moisture content, will decrease the glass transition temperature and affect the viability of probiotics (Zhang et al., 2018). Thus, moisture content of the pumpkin crackers was evaluated in this study. Based on Table 3, the moisture contents for control and sample crackers were 3.73% and 4.49%, respectively. Sample cracker was found to have significantly (p<0.05) higher moisture content compared to control cracker. This might be due to the moisture content contributed by the pumpkin puree in the beads. Fresh pumpkin is known to have 80.0-96.0 g/100 g of moisture content (Guiné et al., 2011). The moisture content of shelf stable commercial biscuits ranges from 1.98 to 4.67 g/100 g (Norhayati et al., 2015) which is in tandem with the results obtained in this study. Hence, this suggested that pumpkin crackers produced in this study are shelf stable.

There were no significant differences (p>0.05) observed in fat, protein, and carbohydrate content of both pumpkin crackers (Table 3). The protein content in both pumpkin crackers were approximately 10%. The protein content was mostly contributed by pumpkin which is a good source of amino acids with 12.9% of crude protein in dry basis as well as all-purpose flour with 9 g/100 g of protein (Gurung et al., 2016). The fat contents of both control and sample crackers were 3.88% and 3.51%, respectively which found to be lower compared to the commercial biscuits which ranged from 16.89 to 25.75 g/100 g (Norhayati et al. 2015). The carbohydrate content of pumpkin crackers could be contributed by carbohydrate content in ingredients such as all-purpose flour (74.0 g/100 g), corn starch (90.3 g/100 g), and pumpkin. As pumpkin puree contains a total of 68.3% of carbohydrate in dry basis (Gurung et al., 2016), it can be denoted that pumpkin puree can be a good source of energy. Moreover, the carbohydrate content in the pumpkin crackers are higher compared to commercial biscuits with a range of 56.80 g to 74.61 g/ 100 g (Passos et al., 2013) and may serve as breakfast alternatives. Breakfast is one of the critical meals which help to replenish the body's fuel stores after an overnight fasting. A carbohydrate-rich diet or food product is preferred for breakfast as it able to boost the energy, enhanced alertness, and increase satiety level as well as controlling the appetite throughout the rest of the day (Holt et al., 1999). It is one of the preferred fuels for muscle and nerve functions as it is able to increase the blood glucose level when ingested.

The sample crackers showed significantly (p<0.05) higher ash content than the control crackers. Fresh pumpkin is known to have around 0.8-1.4 g/100 g or about 1.1g/100 g of ash (Guiné *et al.*, 2011). According to Gurung *et al.* (2016), the ash content in pumpkins include

potassium, copper, zinc, iron, and magnesium of pumpkin puree. Therefore, the increased in the ash content of sample crackers is most likely contributed by the additional pumpkin puree in the beads.

Total dietary fiber is the measurement of soluble and insoluble fiber in food. Based on Table 3, the total dietary fiber content for control and sample pumpkin crackers were comparable at 10.78% and 9.92%, respectively. In a study conducted by Norhayati *et al.* (2015), 7.03% was observed for total dietary fiber in a commercial oatmeal biscuit. The comparison between the pumpkin crackers and commercial biscuit showed that pumpkin crackers exhibits a higher amount of dietary fiber. This could be attributed to natural dietary fiber content of pumpkin puree.

Table 3: Percentage of proximate and total dietary fiber content of control and sample pumpkin crackers.

	Control (%)	Sample (%)
Moisture	3.73 ± 0.09^{a}	4.49 ± 0.21 ^b
Protein	9.93 ± 0.03^{a}	9.91 ± 0.05^{a}
Fat	3.88 ± 0.29^{a}	3.51 ± 0.36^{a}
Ash	1.80 ± 0.02^{a}	2.03 ± 0.12^{b}
Carbohydrate	80.67 ± 0.19^{a}	80.06 ± 0.20^{a}
Total Dietary Fiber	10.78 ± 0.56 ^a	9.92 ± 0.56 ^a

*Results are expressed as means ± standard deviations (n=3).

ab Means in the same row with different lowercase superscript letters are significantly different (*p*<0.05).

Physical Analysis

Texture profile analysis

Texture is the critical physical attribute that dominate the quality and sensory acceptability of a product. Therefore, instrumental measurement such as texture profile analyzer was used to measure the food quality and for quality control in industries. Texture profile analysis is a rapid evaluation method for food texture with various parameters such as hardness, cohesiveness, gumminess, and others. Among these, hardness is the most frequently measured textural property for evaluation of baked goods due to its close association with human perception for freshness.

In this study, the hardness of both crackers was comparable (p>0.05; Table 4). This indicates that incorporation of pumpkin beads will not affect the hardness of the crackers. According to study by Yilmaz et al. (2014), the hardness of a plain cracker is approximately 3561 \pm 297 g. Besides that, Galla et al. (2017) found that a plain biscuit exhibit hardness of 2138 \pm 4.04 g. Thus, the pumpkin crackers that was produced in this study were less hard compared to the previous studies. This is desirable as excessive hardness will lead to brittleness that may affect the acceptability of the products. Furthermore, hardness of crackers is also affected by the application of dietary fiber from the pumpkin puree (Dokic et al., 2015). A study from Galla et

al. (2017) found that increased in the supplementation of vegetable (spinach) in biscuit will increase hardness of the biscuits. Although dietary fibers from pumpkin puree might affect the hardness of the crackers, this was not prevalent in this study.

Table 4: Texture analysis of control and sample pumpkin crackers.

Crackers	Hardness (g)	
Control	1906.67 ± 217.72 ^a	
Sample	1732.00 ± 410.77 ^a	

^{*}Results are expressed as means ± standard deviation (n=3)

CONCLUSION

Cracker with live culture can be successfully develop by microencapsulating the cells with pumpkin puree. These crackers can serve as an alternative probiotic product with acceptable physicochemical properties.

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