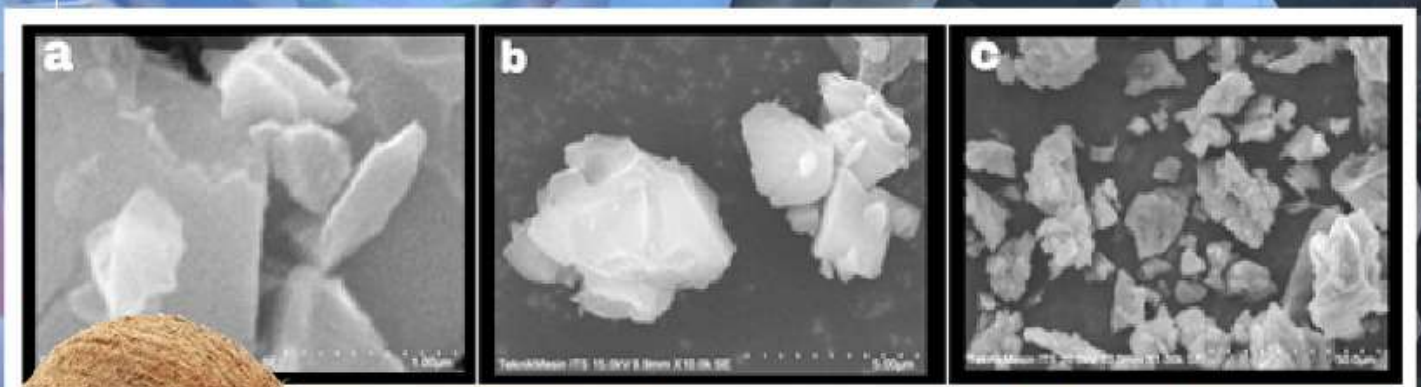


Journal of Marine and Coastal Science

JURNAL PUBLISHED VOL. 11 NO. 2 JUNI 2022

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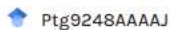
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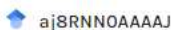
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Pengaruh Penambahan Tepung Jagung terhadap Karakteristik Kimia *Flavor* Pasta dari Cangkang Kerang Hijau

The Effect of Adding Cornstarch to The Chemical Characteristics of Green Mussel Shell Paste Flavor

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Article Info

Received: 2022-06-02

Revised: 2022-06-27

Accepted: 2022-06-28

Online: 2022-06-29

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Abstrak

Kerang hijau merupakan salah satu komoditas perikanan Indonesia yang memiliki nilai ekspor tinggi. Tingginya kegiatan ekspor produk kerang akan berdampak terhadap lingkungan karena limbah cangkang yang dihasilkan. Pemanfaatan limbah cangkang kerang merupakan solusi untuk mengatasi pencemaran lingkungan antara lain sebagai bahan baku perisa makanan. Pembuatan perisa makanan atau *flavor* alami dibutuhkan *emulsifier*. Salah satu bahan yang dapat dijadikan *emulsifier* yaitu tepung jagung. Tujuan dari penelitian ini adalah untuk mengetahui pengaruh penambahan tepung jagung terhadap karakteristik kimia *flavor* pasta dari cangkang kerang hijau. Penelitian ini menggunakan Rancangan Acak Lengkap (RAL) yang terdiri dari 5 perlakuan dengan konsentrasi tepung jagung 0%, 2,5%, 5%, 7,5%, dan 10% dengan 4 kali ulangan. Kandungan protein dan lemak merupakan komponen utama dari pembuatan *flavor*. Parameter utama yang digunakan dalam penelitian ini adalah pasta *flavor* dengan *emulsifier* memiliki kadar proksimat (protein, kadar air, dan kadar lemak) dan parameter penunjang adalah rendemen, kadar VRS, daya larut air, dan kadar pH. Hasil penelitian ini menunjukkan bahwa penambahan tepung jagung berpengaruh nyata ($p < 0,05$) terhadap nilai karakteristik kimia protein, lemak, kadar air, rendemen, daya larut air, VRS, dan pH. *Flavor* pasta dengan konsentrasi 10% memiliki kadar protein, lemak, kadar air, nilai rendemen, daya larut air, dan nilai VRS yang baik.

Kata kunci: karakteristik kimia, tepung jagung, perisa pasta kerang hijau

Abstract

Green mussels is one of Indonesia's fisheries commodities that has a high export value. The high export activity of shellfish products will have an impact on the environment due to shell waste produced. Utilization of shellfish waste is a solution to overcome environmental pollution, among others, as a raw material for food flavours. Making a food flavor or natural flavor requires an emulsifier. One ingredient that can be used as an emulsifier is cornstarch. The purpose of this study was to determine the effect of adding cornstarch to the chemical characteristics of the green mussel paste flavor. This study used a Completely Randomized Design (CRD) consisting of 5 treatments with cornstarch concentration of 0%, 2.5%, 5%, 7.5%, and 10% with 4 replications. Protein and fat content are the main components of making flavors. The main parameters used in this study are flavor paste with emulsifier having proximate levels (protein, water content, and fat content) and supporting parameters are yield, VRS levels, water solubility, and pH levels. The results of this study indicate that the addition of gelatin has a significant effect ($P < 0.05$) on the chemical characteristics of protein, fat, water content, yield, water solubility, VRS, and pH. Pasta flavor with a concentration of 10% has good levels of protein, fat, water content, yield value, water solubility, and VRS.

Keywords: chemical characteristics, cornstarch, green mussel paste flavor

1. Pendahuluan

Perisa (*flavor*) merupakan bahan tambahan pangan yang dapat memberikan dan mempertegas rasa suatu makanan. Perkembangan industri perisa makanan dari hasil laut (seafood) semakin banyak diminati oleh masyarakat di Indonesia. Sampai saat ini *flavor* yang beredar merupakan *flavor* sintesis. Penggunaan *flavor* sintetis yang berlebihan dapat menimbulkan efek buruk bagi kesehatan. Salah satu bahaya yang ditimbulkan dari *flavor* sintetis adalah *Chinese Restaurant Syndrom* yang disebabkan oleh pemakaian monosodium glutamat (MSG) (Cahyadi, 2009). Kerang hijau merupakan salah satu komoditas perikanan Indonesia yang memiliki nilai ekspor tinggi. Badan Pusat Statistik mencatat nilai ekspor produk kerang-kerangan mencapai 54.961,4 ton yang setara dengan 105.799,1 ribu US dollar pada tahun 2018. Tingginya kegiatan ekspor produk kerang akan berdampak terhadap lingkungan karena limbah cangkang yang dihasilkan. Pemanfaatan limbah cangkang kerang merupakan solusi untuk mengatasi pencemaran lingkungan dan sebagai upaya untuk mengurangi limbah cangkang kerang. Cangkang kerang memiliki kandungan protein sebesar 4,14% dan lemak 14,5% (Permana, 2006). Kandungan protein dan lemak merupakan komponen utama dari pembuatan *flavor*. Protein akan

mempengaruhi rasa manis (Zuhra, 2006). Lemak akan menimbulkan *flavor* yang unik (Susilawati, 2001). Pembuatan *flavor* alami dengan menggunakan cangkang kerang, dan pengemulsi dari tepung jagung.

Tepung jagung merupakan homopolimer glukosa yang memiliki kandungan amilosa dan amilopektin. Amilosa merupakan fraksi terlarut dan amilopektin merupakan fraksi tidak terlarut yang dapat dipisahkan menggunakan air panas (Sari, 2016). Tepung jagung memiliki fraksi terlarut dan tidak terlarut yang merupakan ciri suatu bahan yang dapat digunakan sebagai *emulsifier*. *Emulsifier* berfungsi untuk mempertahankan bentuk dan konsistensi makanan dan pada pembuatan *flavor* berfungsi untuk pengkapsulasi aroma (Cassiday, 2016). Oleh karena itu diperlukan penelitian pembuatan *flavor* berbahan dasar cangkang kerang hijau dengan penambahan tepung jagung sebagai *emulsifier*. Diharapkan penambahan tepung jagung dapat meningkatkan karakteristik kimia *flavor* pasta cangkang kerang hijau.

2. Material dan Metode

Material

Bahan penelitian yang diperlukan untuk pasta *flavour* adalah cangkang kerang hijau segar yang diperoleh dari

pasar Pabean Surabaya, tepung aren, dan tepung jagung. Bahan kimia untuk uji proksimat: H₂SO₄ pekat, tablet Kjeldahl, NaOH 40%, asam borat, dan HCl 0,1 N.

Metode

Air Rebusan Cangkang Kerang Hijau

Cangkang kerang hijau dibersihkan dari kotoran yang menempel pada cangkang, kemudian ditumbuk untuk memperkecil ukuran. Setelah ditumbuk dilakukan pencucian menggunakan air mengalir pada cangkang. Selanjutnya ditambahkan air dengan perbandingan cangkang : air yaitu 1:1, direbus pada 80°C selama 30 menit, kemudian didiamkan didinginkan. Selanjutnya untuk mendapatkan air rebusan cangkang kerang hijau sebagai bahan baku, dilakukan penyaringan dengan kertas saring (Mulyadi *et al.*, 2013).

Pasta Flavor Cangkang Kerang Hijau

Sebanyak 100 gram air rebusan cangkang kerang hijau dipanaskan dan ditambah dengan tepung jagung sebagai pengemulsi sesuai dengan perlakuan, yaitu: 2,5% (P1), 5% (P2), 7,5% (P3), dan 10% (P4). Selanjutnya dipanaskan sambal diaduk hingga menjadi kental (Mulyadi *et al.*, 2013). Sebagai kontrol, pasta *flavor* tidak ditambah tepung jagung (P0).

Karakteristiki Kimia Pasta Flavor Cangkang Kerang Hijau

a. Kadar Protein

Pengujian kadar protein dilakukan sesuai dengan SNI 01-2354.4-2006 dengan menggunakan metode Kjeldahl. Tahapan metode *Kjeldahl* meliputi destruksi, destilasi dan titrasi. Sebanyak 1 gram sampel dimasukkan ke dalam labu *Kjeldahl*, ditambahkan 2,5 mL H₂SO₄ pekat dan tablet Kjeldahl. Selanjutnya dididihkan selama 1-1,5 jam hingga jernih, kemudian didinginkan dan dipindahkan ke alat destilasi. Proses selanjutnya, sampel hasil destruksi dicuci dan dibilas hingga 5-6 kali dengan akuades 20 ml. Pada tahap destilasi, sampel ditambah larutan NaOH 40% sebanyak 5 ml. Cairan hasil destilasi ditampung menggunakan Erlenmeyer berisi larutan H₃BO₃ (15 ml) dan 3 tetes indikator yang diletakkan di bawah kondensor. Indikator yang digunakan merupakan campuran dua tetes metil red 0,2% dalam alkohol dan satu tetes metilen blue 0,2% dalam alkohol. Sebelum destilasi dimulai ujung kondensor harus terendam di bawah larutan asam borat (H₃BO₃). Destilasi dilakukan sampai diperoleh ± 200 ml destilat yang bercampur dengan H₃BO₃ dan indikator. Hasil destilasi dititrasi menggunakan larutan HCL 0,01 N hingga larutan berwarna merah muda. Kadar protein dihitung menggunakan rumus:

$$\% \text{ Nitrogen} = \frac{(\text{Volume HCL} - \text{Volume blanko}) \times N \text{ HCL} \times 14.007 \times fp}{\text{bobot sampel (mg)}}$$

Keterangan:

Kadar protein (%) = % N × Faktor konveksi

Faktor konveksi = 6,25

fp (faktor pengencer) = 20

b. Kadar Air

Pengujian kadar air mengacu pada SNI 2354.2-2015. Langkah awal mengeringkan cawan kosong dengan

oven pada suhu 105-110°C selama 10 menit, didinginkan dalam desikator selama 30 menit, kemudian ditimbang. Sebanyak 2 gram sampel diletakkan pada cawan yang sudah dikeringkan kemudian dioven selama 3-4 jam pada suhu 105-110°C. Selanjutnya didinginkan dalam desikator kemudian ditimbang. Kadar air dihitung menggunakan rumus :

$$\% \text{ Kadar air} = \frac{(\text{berat sebelum} - \text{berat kering})}{\text{berat sampel}} \times 100\%$$

c. *Kadar Lemak*

Pengujian kadar lemak berdasarkan SNI 01-2354.3-2006. Sampel pasta sebanyak 0,5 gram dibungkus dengan kertas saring kemudian diletakkan pada alat ekstraksi Soxhlet dengan labu lemak di bagian bawah. Pelarut heksana dimasukkan ke dalam labu lemak, dan selanjutnya dilakukan refluks selama 16 jam sampai pelarut turun. Labu lemak yang berisi lemak hasil ekstraksi dikeringkan dengan oven pada suhu 105°C selama 5 jam kemudian dikeringkan dalam desikator selama 20-30 menit dan ditimbang berat lemak tersebut. Kadar lemak dapat

dihitung menggunakan rumus :

$$\% \text{ Lemak} = \frac{ww-ww1}{ww2} \times 100\%$$

d. *Rendemen*

Analisa rendemen bertujuan untuk mengetahui berapa persen produk yang dihasilkan dibandingkan dengan bahan baku yang digunakan dalam pembuatan produk (Barokah, 2014). Rendemen *flavor* diperoleh dari perbandingan berat *flavor* yang dihasilkan dengan berat air rebusan cangkang kerang hijau yang digunakan.

$$\% \text{ Rendemen Rebusan Cangkang} = \frac{\text{Berat akhir rebusan (g)}}{\text{Berat cangkang kerang (g)}} \times 100$$
$$\% \text{ Rendemen flavor} = \frac{\text{Berat sesudah dipanaskan (g)}}{\text{Berat sebelum dipanaskan (g)}} \times 100$$

e. *Daya Larut Air*

Penentuan daya larut mengacu pada SNI 7612.1.2011. Sebanyak 2 gram sampel dimasukkan ke dalam labu takar 100 ml kemudian ditambahkan air hingga tanda tera, dikocok selama 1 menit dan didiamkan selama 30 menit. Selanjutnya disaring menggunakan kertas saring. Sebanyak 10 ml filtrat dituang ke dalam cawan porselin yang sudah ditimbang beratnya, dimasukkan ke dalam oven dengan suhu pertama 80°C selama 1 jam pertama, kemudian dinaikkan suhunya menjadi 90°C selama 1 jam kedua dan dinaikkan lagi menjadi 100°C untuk satu jam ketiga. Sampel dikeluarkan dari oven dan ditimbang beratnya. Sampel tersebut dimasukkan kembali ke dalam oven selama 30 menit, selanjutnya ditimbang kembali. Perlakuan ini diulangi sampai diperoleh berat yang konstan.

$$\text{Daya Larut} = 100\% - \frac{10(A-B)}{C} \times 100\%$$

Keterangan :

A = Berat akhir

B = Berat cawan porselin

C = Berat sampel

f. *Volatile Reducing Substance (VRS)*

Sampel sebanyak 1 gram dimasukkan ke dalam labu aerasi VRS *apparatus*, ditambahkan 10 ml air destilat dan 10 ml KMNO₄. Setelah 40 menit, segera ditambahkan 5 ml H₂SO₄ 6 N dan 3 ml KI 20%. Titrasi larutan sampel tersebut dengan natrium tiosulfat 0.02 N hingga terbentuk warna kuning. Indikator kanji ditambahkan pada akhir penetrasi. Titrasi dihentikan apabila warna biru hilang. Hal yang sama juga dilakukan terhadap blanko, kemudian kadar VRS dihitung dengan persamaan:

$$\text{VRS (m Eq/gram)} = ((a-b) \times N \times 1000) : \text{sampel(g)}$$

Keterangan :

A = volume titran yang digunakan untuk mentitrasi blanko

b = volume titran yang yang digunakan sampel

g. *Nilai pH*

Pengukuran nilai pH menggunakan pH meter. Sebelum penggunaan pH meter, dilakukan

standarisasi pH meter dengan larutan buffer pH 4, kemudian buffer pH 7. Pencucian elektroda menggunakan air suling. Sebanyak 10 gram sampel dilarutkan ke dalam 50 ml akuades, kemudian ditambah akuades hingga 100 ml, dan diaduk hingga homogen. Bagian elektroda dari pH meter dimasukkan ke dalam larutan sampel, diangkat, dibilas dengan akuades, kemudian dikeringkan dengan tisu.

Analisis Data

Penelitian menggunakan Rancangan Acak Lengkap (RAL) dengan lima perlakuan dan empat ulangan. Data hasil pengujian protein, lemak, air, kadar VRS, daya larut air dan pH dianalisis dengan ANOVA. Analisis data dilanjutkan dengan Uji Jarak Berganda Duncan apabila hasil analisis menunjukkan pengaruh yang berbeda nyata atau

sangat berbeda nyata untuk membandingkan perlakuan mana yang menghasilkan hasil yang terbaik (Kusriningrum, 2008).

3. Hasil dan Pembahasan

Rendemen Pasta Flavor Cangkang Kerang Hijau

Rendemen pasta flavour yang diberi tepung jagung berkisar antara 84,88-85,71% dengan nilai rendemen tertinggi terdapat pada P4 (penambahan tepung jagung 10%). Semakin tinggi konsentrasi tepung jagung yang diberikan, semakin tinggi pula rendemen yang dihasilkan (Tabel 1). Menurut Wijaya and Sadikin (2000), tepung jagung sebagai bahan pengemulsi dapat meningkatkan kandungan total padatan dalam larutan.

Tabel 1. Rendemen pasta *flavor* cangkang kerang hijau

Perlakuan	Rendemen <i>flavor</i> (%)
P0	79,1
P1	84,88
P2	85,45
P3	85,58
P4	85,71

Keterangan: P0 (tanpa penambahan tepung jagung), P1 (penambahan tepung jagung 2,5%), P2 (penambahan tepung jagung 5%), P3 (penambahan tepung jagung 7,5%), dan P4 (penambahan tepung jagung 10%).

Kadar Protein

Kadar protein dari pasta cangkang kerang hijau berkisar antara 1,03-1,80% (Tabel 2). Nilai tertinggi kadar protein *flavor* pasta terdapat pada P4, namun tidak berbeda nyata ($p > 0,05$)

dengan P3 (penambahan tepung jagung 7,5%). Kadar protein terendah terdapat pada perlakuan kontrol (P0) yang tidak berbeda nyata ($p > 0,05$) dengan P1 (penambahan tepung jagung 2,5 %).

Tabel 2. Kadar protein pasta *flavor* cangkang kerang hijau

Perlakuan	Kadar Protein (%) Rata-rata \pm SD
P0	1,03 ^d \pm 0,06
P1	1,20 ^{cd} \pm 0,00
P2	1,43 ^{bc} \pm 0,40
P3	1,61 ^{ab} \pm 0,24
P4	1,80 ^a \pm 0,15

Keterangan: P0 (tanpa penambahan tepung jagung), P1 (penambahan tepung jagung 2,5%), P2 (penambahan tepung jagung 5%), P3 (penambahan tepung jagung 7,5%), dan P4 (penambahan tepung jagung 10%). Notasi huruf yang berbeda menunjukkan perbedaan nyata ($p < 0,05$).

Kandungan protein dengan hasil terendah pada perlakuan P0 yakni sebesar 1,03%. Hal tersebut disebabkan karena penambahan tepung jagung pada perlakuan. Tepung jagung memiliki kandungan protein juga, sehingga ketika ditambahkan akan meningkatkan kandungan protein. Protein merupakan kandungan yang terpenting dalam produk *flavor*.

Kadar Lemak

Kadar lemak dalam *flavor* merupakan komponen penting setelah protein dalam *flavor*. Kadar lemak tertinggi pada *flavor* pada P0 sebesar 0,29% yang tidak berbeda nyata ($p > 0,05$)

dengan P1 (penambahan tepung jagung 2,5%). Kadar lemak terendah terdapat pada P4 (penambahan tepung jagung 10%) dan berbeda nyata ($p < 0,05$) dengan perlakuan lainnya (Tabel 3). Kadar lemak *flavor* cangkang kerang hijau semakin menurun dengan meningkatnya konsentrasi tepung jagung sebagai pengemulsi. Penggunaan tepung jagung dapat mengurangi penyerapan minyak karena granula pati yang tergelatinisasi dapat menahan air dalam adonan sehingga menghindari penguapan air dalam bahan pangan dari minyak menuju bahan pangan berkurang (Shih *et al.*, 2001).

Tabel 3. Kadar lemak pasta *flavor* cangkang kerang hijau

Perlakuan	Kadar Lemak (%) Rata-rata±SD
P0	0,29 ^a ±0,12
P1	0,27 ^{ab} ±0,22
P2	0,26 ^{bc} ±0,08
P3	0,25 ^c ±0,17
P4	0,22 ^d ±0,12

Keterangan: P0 (tanpa penambahan tepung jagung), P1 (penambahan tepung jagung 2,5%), P2 (penambahan tepung jagung 5%), P3 (penambahan tepung jagung 7,5%), dan P4 (penambahan tepung jagung 10%). Notasi huruf yang berbeda menunjukkan perbedaan nyata ($p < 0,05$).

Kadar Air

Kadar air dalam sampel perlakuan semakin rendah dengan bertambahnya konsentrasi tepung jagung (Tabel 4). Penambahan tepung dapat mengikat molekul air dalam *flavor* membentuk gel.

Hasil kadar air terendah diperoleh perlakuan P4 yakni 68,22% yang berbeda nyata ($p < 0,05$) dengan perlakuan lainnya. Sedangkan kadar air tertinggi terdapat pada kontrol P0 yang berbeda nyata ($p < 0,05$) dengan perlakuan lainnya.

Tabel 4. Kadar air pasta *flavor* cangkang kerang hijau

Perlakuan	Kadar Air (%) Rata-rata±SD
P0	99,86 ^a ±0,01
P1	89,63 ^b ±0,45
P2	81,96 ^c ±0,70
P3	72,49 ^d ±0,63
P4	68,22 ^e ±0,34

Keterangan: P0 (tanpa penambahan tepung jagung), P1 (penambahan tepung jagung 2,5%), P2 (penambahan tepung jagung 5%), P3 (penambahan tepung jagung 7,5%), dan P4 (penambahan tepung jagung 10%). Notasi huruf yang berbeda menunjukkan perbedaan nyata ($p < 0,05$).

Kandungan kadar air produk penyedap rasa alami yang baik sebesar 63,5-74,5% selama penyimpanan di suhu ruang (Rahmi, 2018). Kadar air pada

perlakuan penambahan tepung jagung 7,5% (P3) dan 10% (P4) berada pada kisaran tersebut. Sedangkan perlakuan penambahan tepung jagung 2,5% dan 5%

serta perlakuan kontrol mengandung kadar air yang tinggi, karena bahan baku untuk mempuat pasta adalah air rebusan cangkang kerang. Peningkatan konsentrasi bahan pengisi meningkatkan kandungan total padatan dalam larutan yang berarti penurunan konsentrasi air dalam larutan, sehingga setelah penambahan tepung jagung, kadar air bahan akan semakin kecil pula.

Daya Larut Air

Daya larut air dari *flavor* pasta dengan penambahan tepung jagung berkisar antara 98,33-99,45% (Tabel 5). Nilai tertinggi daya larut air *flavor* pasta dengan penambahan tepung jagung pada P4 (penambahan tepung jagung 10%) yang berbeda nyata ($p < 0,05$) dengan perlakuan lainnya.

Tabel 5. Daya larut air pasta *flavor* cangkang kerang hijau

Perlakuan	Daya Larut Air (%) Rata-rata \pm SD
P0	99,99 ^a \pm 0,00
P1	98,33 ^e \pm 0,52
P2	98,58 ^d \pm 0,42
P3	99,00 ^c \pm 0,26
P4	99,45 ^b \pm 0,00

Keterangan: P0 (tanpa penambahan tepung jagung), P1 (penambahan tepung jagung 2,5%), P2 (penambahan tepung jagung 5%), P3 (penambahan tepung jagung 7,5%), dan P4 (penambahan tepung jagung 10%). Notasi huruf yang berbeda menunjukkan perbedaan nyata ($p < 0,05$).

Besarnya nilai kelarutan akan menentukan mutu dari produk *flavor* yang dihasilkan. Semakin besar kelarutan maka diharapkan akan semakin banyak pula komponen *flavor* yang dilepaskan. Tepung jagung memiliki kemampuan mudah larut dalam air sehingga semakin tinggi konsentrasi tepung jagung, nilai kelarutan produk akan semakin tinggi pula. Penelitian Wijaya dan Sadikin (2000) menunjukkan daya larut *flavor*

pandan bekisar antara 94-96%.

Volatile Reducing Substance (VRS)

Nilai VRS dari *flavor* pasta berkisar antara 3,48-12,41 meq/g (Tabel 6). Nilai VRS *flavor* tertinggi terdapat pada pasta dengan penambahan tepung jagung 10% (P4) yang berbeda nyata ($p < 0,05$) dengan perlakuan lainnya.

Tabel 6. Nilai VRS pasta *flavor* cangkang kerang hijau

Perlakuan	Nilai VRS Rata-rata \pm SD
P0	3,48 ^e \pm 0,91
P1	7,13 ^d \pm 0,57
P2	8,54 ^c \pm 0,59
P3	10,32 ^b \pm 0,46
P4	12,41 ^a \pm 0,38

Keterangan: P0 (tanpa penambahan tepung jagung), P1 (penambahan tepung jagung 2,5%), P2 (penambahan tepung jagung 5%), P3 (penambahan tepung jagung 7,5%), dan P4 (penambahan tepung jagung 10%). Notasi huruf yang berbeda menunjukkan perbedaan nyata ($p < 0,05$).

Penambahan tepung jagung 10% memungkinkan jumlah senyawa volatil yang terkapsulasi semakin besar. Hal ini berkaitan dengan fungsi tepung jagung sebagai bahan pengkapsul yaitu semakin tinggi jumlah kandungan senyawa pengkapsul akan semakin banyak pula

jumlah senyawa aroma yang terkapsulasi (Wijaya dan Sadikin, 2000). Semakin tinggi kandungan padatan, semakin singkat waktu yang dibutuhkan untuk pembuatan *film permeabel* sehingga jumlah senyawa volatil yang hilang dapat dikurangi (Bhandari *et al.*, 1992).

Nilai pH

Nilai pH dari *flavor* berkisar antara 5,8-6,9. Nilai yang mendekati pH normal

terdapat pada pasta tanpa penambahan tepung jagung yang berbeda nyata ($p < 0,05$) dengan perlakuan lainnya.

Tabel 7. Nilai pH pasta *flavor* cangkang kerang hijau

Perlakuan	Nilai pH Rata-rata \pm SD
P0	6,9 ^a \pm 0,00
P1	6,2 ^b \pm 0,00
P2	6,0 ^c \pm 0,00
P3	5,9 ^d \pm 0,00
P4	5,8 ^e \pm 0,00

Keterangan: P0 (tanpa penambahan tepung jagung), P1 (penambahan tepung jagung 2,5%), P2 (penambahan tepung jagung 5%), P3 (penambahan tepung jagung 7,5%), dan P4 (penambahan tepung jagung 10%). Notasi huruf yang berbeda menunjukkan perbedaan nyata ($p < 0,05$).

Semakin banyak penambahan tepung jagung menunjukkan nilai pH semakin menurun (Tabel 7). Hal ini disebabkan oleh proses gelatinisasi pada pemasakan *flavor*, karena pada kondisi asam yang tinggi terjadi hidrolisis ikatan glukosida pada granula pati. Hidrolisis ikatan glukosida menyebabkan fragmentasi dan pembentukan polimer berantai pendek (Franco *et al.*, 2002).

Daftar Pustaka

- Barokah, G. R. (2014). Karakteristik mikroenkapsulat pepton berbahan dasar ikan hasil tangkapan sampingan (HTS) multispecies busuk dengan metode spray drying dan bahan penyalut maltodekstrin. Skripsi. Bogor: Fakultas Perikanan dan Ilmu Kelautan. Institut Pertanian Bogor.
- Bhandari, B. R., Dumolin, E.D., Richard, H.M.J., Noleau, I., & Lebert, A.M. (1992). Flavor encapsulation by spray drying: Application to citral and linalyl acetate. *Journal of Food Science*, 57(1):217-221.
- Cahyadi, W. (2009). Bahan tambahan pangan. Edisi 2. Jakarta: Penerbit Bumi Aksara.
- Cassiday, L. (2016). Food emulsifier fundamentals. *Inform*, 27(10):10-16.

4. Kesimpulan

Penambahan tepung jagung berpengaruh terhadap karakteristik kimia *flavor* pasta kerang hijau yaitu kadar protein, kadar lemak, kadar air, nilai rendemen, kelarutan dalam air, VRS, dan pH. Air rebusan cangkang kerang hijau dapat digunakan sebagai bahan baku untuk membuat pasta *flavor* dengan penambahan tepung jagung 10% sebagai pengemulsi.

- Franco, C. M. L., Cabral, R.A.F., & Tavares, D.Q. (2002). Structural and physicochemical characteristics of lintnerized native and sour cassavastarches. *Starch*, 54(10):469-475.

- Kusriningrum, R. S. (2008). Perancangan Percobaan. Surabaya: Airlangga University Press.

- Mulyadi, A. F., Maligan, J. M., Wignyanto, & Hermansyah, R. (2013). Organoleptic characteristics of natural flavour powder from waste of swimming blue crabs (*Portunus pelagicus*) processing: Study on dextrin concentration and drying temperature. *Jurnal Teknologi Pertanian*, 14(3):183-192.

- Permana, H. (2006). Optimalisasi pemanfaatan cangkang kerang hijau (*Perna viridis*) dalam pembuatan kerupuk. Skripsi.



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Telah melakukan penelitian yang dipublikasi pada bulan Juni tahun 2022 dengan judul sebagai berikut:

The Effect of Adding Cornstarch to The Chemical Characteristics of Green Mussel Paste Flavor

Adapun penelitian ini sudah mengacu pada prosedur pertimbangan etik dari:

1. *American Fisheries Society* (AFS, 2014) yang berjudul *Guideline for the Use of Fishes in Research* yang menyebutkan bahwa: penelitian dalam kondisi laboratorium baru mengatur tentang hewan percobaan berupa ikan hidup (hal 43 ; terlampir), dan
2. *Canadian Council on Animal Care* (CCAC, 2005) yang berjudul *Guideline on the Care and Use of Fish in Research, Teaching and Testing* yang menyebutkan bahwa: pedoman tersebut hanya digunakan untuk hewan uji berupa ikan hidup (Kelas: Chondrichthyes, Agnatha, dan Osteichthyes) dan Avertebrata (Kelas: Cephalopoda) (hal 13,14 ; terlampir).

Sedangkan dalam penelitian tersebut tidak menggunakan hewan hidup sebagai bahan penelitiannya. Sehingga penelitian tersebut tidak perlu dilakukan ***Uji Ethical Clearance***.

Demikian Surat Keterangan ini kami buat untuk dapat dipergunakan sebagai persyaratan pengusulan Jabatan Fungsional **Guru Besar** atas nama Dr. Endang Dewi Masithah, Ir., MP.

Surabaya, 27 April 2023

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American Fisheries Society
Bethesda, Maryland
2014

A suggested citation format for this book follows.

Use of Fishes in Research Committee (joint committee of the American Fisheries Society, the American Institute of Fishery Research Biologists, and the American Society of Ichthyologists and Herpetologists). 2014. Guidelines for the use of fishes in research. American Fisheries Society, Bethesda, Maryland.

Cover art: Close-up photograph of Brown Trout, *Salmo trutta*, from the South Fork of the Cache la Poudre River, Colorado, taken by James Rose in 2010.

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Printed in the United States of America on acid-free paper.

Library of Congress Control Number 2014943876
ISBN 978-1-934874-39-4

American Fisheries Society Web site address: www.fisheries.org

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5410 Grosvenor Lane, Suite 100
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7. Laboratory Activities

7.1 General Principles

Working with live fishes under laboratory conditions requires attention to many details concerning the requirements for, and limits of tolerance of, the particular species under study. Acceptable physical facilities and an adequate supply of water with good quality must be provided, even if the fishes are to be held for only short periods of time. Although fish may tolerate marginal facilities and conditions for a few hours or even several days, holding them under less than optimal conditions will affect the results of the research. Standards for humane treatment of animals must also be maintained, regardless of the length of time that the fishes are held.

The reader should note that some content of section 7 is not restricted to laboratory activities, but may be applicable to field situations, as well.

7.2 Confinement, Isolation, and Quarantine

Prior to bringing fishes into a laboratory, facilities and plans should be in place to ensure that the fish cannot escape, especially species not native to the watershed, and that the introduced fishes can be isolated physically from fishes already present. Each holding unit should have its own set of nets and other equipment. Facilities and equipment used for previous studies should be disinfected prior to use in new studies, typically with a chlorinated disinfectant or another disinfectant such as Virkon[®] Aquatic (www.wchemical.com/). If the introduced fishes may carry disease agents, especially pathogens or parasites that are not endemic to the area, quarantine-level facilities should be used. The level of quarantine required will vary with the seriousness of the known or suspected disease agent (see section 2.5 Fish Health Management: Control of Pathogens and Parasites).

Individual fish with suspected ill health should be quarantined from the others so as to negate the potential for spread of potential disease agents. Such fish should be evaluated by an individual with expertise in fish diseases (fish pathologist or veterinarian), and the proper therapeutant should be applied as directed. Providing guidance for the treatment of specific diseases is beyond the scope of this document. The investigator is strongly urged to establish a working relationship with individuals with expertise in fish health with whom they may consult.

Experimentation with nonindigenous fishes, transgenic fishes, or other genetically modified fishes is a special situation that requires additional precautions to preclude their escape. Permitting with site visits by state wildlife agencies may be required for holding nonindigenous species (see section 3.4 Permits and Certificates). The specific barriers may be similar to those used to prevent the escape of disease agents but must be developed to fit the physical characteristics of the laboratory or experimental facility. The USDA has developed

Canadian Council on Animal Care



guidelines on:

***the care and use of
fish in research,
teaching and
testing***

This document, the CCAC *guidelines on: the care and use of fish in research, teaching and testing*, has been developed by the *ad hoc* subcommittee on fish of the Canadian Council on Animal Care (CCAC) Guidelines Committee.

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In addition, the CCAC is grateful to former members of CCAC Council: Ms Susan Waddy, Fisheries and Oceans Canada; Dr Jack Miller, University of Western Ontario; and Dr Choong Foong, Dalhousie University; and to Dr David Noakes, University of Guelph who provided considerable assistance in preliminary phases of this project. CCAC thanks the many individuals, organizations and associations that provided comments on earlier drafts of this guidelines document. In particular, thanks are extended to representatives of Fisheries and Oceans Canada, Environment Canada, the Canadian Aquaculture Institute, the Canadian Food Inspection Agency and the Canadian Society of Zoologists.

© Canadian Council on Animal Care, 2005

ISBN: 0-919087-43-4

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the care and use of fish in research, teaching and testing



A. PREFACE

The Canadian Council on Animal Care (CCAC) is the national peer review agency responsible for setting and maintaining standards for the care and use of animals used in research, teaching and testing throughout Canada. In addition to the *Guide to the Care and Use of Experimental Animals*, vol. 1, 2nd ed., 1993 and vol. 2, 1984, which provide the general principles for the care and use of animals, the CCAC also publishes detailed guidelines on issues of current and emerging concerns. The CCAC *guidelines on: the care and use of fish in research, teaching and testing* is the seventh of this series. This document supersedes Chapter I - Fish, *Guide to the Care and Use of Experimental Animals*, vol. 2 (CCAC, 1984).

These guidelines aim to provide information for investigators, animal care committees, facility managers and animal care staff that will assist in improving both the care given to fishes and the manner in which experimental procedures are carried out.

The present document has drawn substantially from the work of organizations listed in Appendix A. Their contributions to the development of these guidelines are gratefully acknowledged.

The guidelines have been developed by the CCAC subcommittee on fish and were reviewed by a total of 69 experts. A preliminary first draft was agreed on by the subcommittee and circulated to experts in June 2002 (including representatives of the organizations listed in Appendix A), and a second draft was circulated for widespread comment in June 2003. A final review was carried out in August 2004 involving all individuals who had previously provided significant input to the development process. The development of these guidelines also involved consultation with the Canadian Association for Laboratory Animal Science (CALAS) and the Canadian Society of Zoologists (CSZ) through workshops held at annual meetings in Québec City (June 2003), Acadia University (May 2004), and Hamilton (June 2004). Consultations were also held at the Aquaculture Association of Canada and AquaNet annual meetings in Québec City (October 2004), and at the CCAC Workshop on the Fish Guidelines in Vancouver (April 2005).

The guidelines have been organized in a format that should facilitate easy access to relevant sections. Early sections provide an ethical overview relevant to the use of fishes in research, teaching and testing. This is followed

by a brief overview of regulations and responsibilities relevant to the care and use of fishes in science in Canada. The remainder of the document provides guidelines to assist in caring for fishes in laboratory facilities, followed by guidelines to help in the development and review of experimental protocols. An overview of the CCAC *guidelines on: the care and use of fish in research, teaching and testing* is provided through a summary of the guidelines listed in

this document prior to the beginning of the main text.

The refinement of animal care and use guidelines is a continuous process. These guidelines are intended to provide assistance in the implementation of best practices, and should not be viewed as regulations. Where regulatory requirements are involved or where it is absolutely imperative to adhere to a particular guideline, the term *must* has been used.

B. INTRODUCTION

The greatest challenge in providing *guidelines on: the care and use of fish* is the wide variety of fishes used in Canada and the diversity of their habits, behavior, life history, and environmental and husbandry requirements. In addition, the scientific information required to define the preferred conditions for fish well-being is limited. While considerable research has been conducted on culture strategies and environmental and water quality requirements, such studies have generally been aimed at determining conditions that optimize production in aquaculture systems, rather than improving the welfare of fishes, and have not usually addressed the difference between *tolerance* and *preference* (Fisher, 2000).

An important consideration in these guidelines is the naturally high mortality rates of juveniles in species whose ecological strategies include the generation of large numbers of progeny to ensure adequate survival in the wild. In addition, many experimental populations of species with usually high survival contain individuals that will not thrive to adulthood even under the best environmental conditions. In some situations, a population-based (or a group of study fish) approach to well-being may be appropriate, but individuals that are not likely to thrive should be euthanized as soon as they are identified.

Another consideration for these guidelines is the general acceptance by the public of the current killing methods used in harvesting wild fishes or in recreational angling. In general, the public appears to be willing to accept these killing methods for food production but not when fishes are used for research. These guidelines accept that for research, teaching, and testing use of any animal, including fishes, more emphasis will be placed on individual well-being than is generally accepted for the commercial harvesting or production of animals for food. It is recognized, however, that in some instances investigators may obtain fishes from people involved in commercial or recreational harvesting and have little influence over the capture methods.

These guidelines apply to fishes held in facilities for research, teaching and testing, as well as to fishes that are studied in their natural habitats.

1. Definition of Fish

For the purpose of these guidelines, fishes are defined as all bony and cartilaginous fish genera (classes Chondrichthyes [cartilaginous fishes], Agnatha, and Osteichthyes [bony fishes]). Fish eggs, embryos or larvae that have not developed beyond exclusive reliance on their own yolk nutrients are not covered by these guidelines. Similarly, invertebrates (except cephalopods) are not covered under the CCAC system of surveillance, but institutions are encouraged to foster respect for these animals by ensuring that holding facilities and levels of husbandry meet standards equivalent to those used for fishes.

2. Rationale for Guidelines on the Care and Use of Fish

The use of fishes as experimental subjects has increased substantially over the past two decades. This increase in use is a result of the rapid development of the aquaculture industry, requirements for testing involving fishes as indicators of environmental change, and the use of fishes as a replacement for mammals in biomedical, pharmacological and genetic research (DeTolla *et al.*, 1995; Fabacher & Little, 2000). The trend toward the use of fishes as a replacement for studies that would previously have used mammals as experimental subjects is not discouraged. However, it must also be recognized that fishes have the capacity to perceive noxious stimuli. Noxious stimuli are those stimuli that are damaging or potentially damaging to normal tissue (e.g., mechanical pressure, extremes of temperature and corrosive chemicals). Whether or not fishes have the capacity to experience any of the adverse states usually associated with pain in mammals is subject to a great deal of debate in the scientific literature (FAWC, 1996; FSBI, 2002; Rose, 2002; Braithwaite & Huntingford, 2004). Nonetheless, fishes are capable of behavioral,

physiological and hormonal responses to stressors (including noxious stimuli) which can be detrimental to their well-being. These CCAC guidelines both support the leadership role that Canadians play in fish research, and ensure that the welfare of fishes is carefully considered during the use of fishes for research, teaching and testing, recognizing that better welfare will result in better science.

3. Ethical Overview

Guideline 1:

Fishes used in research, teaching and testing must be treated with the respect accorded to other vertebrate species.

The CCAC's surveillance system for animals used in research, teaching and testing is based on the principles of humane science, i.e. the Three Rs of Russell and Burch (Russell & Burch, 1959) - Reduction, Replacement and Refinement. For the CCAC, these principles are laid out in its *policy statement on: ethics of animal investigation* (CCAC, 1989). The *ethics of animal investigation* applies to all species covered by the CCAC system, i.e. all vertebrates and cephalopods.

In addition, the CCAC system takes a "moral stewardship" approach to the use of animals in science as explained in the CCAC Experimental Animal User Training Core Topics - Module 2, Ethics in Animal Experimentation (http://www.ccac.ca/en/CCAC_Programs/ETCC/Module02/toc.html).

The first guideline statement in the CCAC *guidelines on: institutional animal user training* (CCAC, 1999a) states, "Institutions must strive through their training programs to sustain an institutional culture of respect for animal life".

3.1 Principles of the Three Rs

According to the CCAC *policy statement on: ethics of animal investigation* (CCAC, 1989), it is the responsibility of the local animal care committee (ACC) to ensure that fishes are used only if the investigator's best efforts to find a non-animal model have failed.

As for any other species covered by the CCAC system, investigators using fishes are required to use the most humane methods on the smallest

number of animals necessary to obtain valid information. This requires the use of a sound research strategy, including: identification of key experiments that determine whether a particular line of enquiry is worth pursuing; use of pilot studies; staging of *in vitro* to *in vivo* experiments where possible; and implementation of staged increase in test stimuli where possible (Balls *et al.*, 1995). The numbers and species of animals required depend on the questions to be explored. Field studies, aquaculture studies and laboratory studies require different statistical designs; field studies and aquaculture production typically require the use of larger numbers of animals. The life stage of the fishes used in each study will also affect the numbers of animals needed. Studies of early life stages typically require large numbers of individuals. In all cases, studies should be designed to use the fewest animals necessary. Heffner *et al.* (1996) and Festing *et al.* (2002) provide discussions on the appropriate treatment of samples and experimental units. Investigators are encouraged to consult with a statistician to develop study designs that have the appropriate statistical power to accomplish the research objectives (Nickum *et al.*, 2004).

The CCAC *policy statement on: ethics of animal investigation* (CCAC, 1989) also requires adherence to the following principles:

- animals must be maintained in a manner that provides for their optimal health and well-being, consistent with the demands imposed by the experimental protocol;
- animals must not be subjected to pain and/or distress that is avoidable and that is not required by the nature of the relevant protocol;
- expert opinion must attest to the potential value of studies with all animals, including fishes (e.g., scientific merit for research, see CCAC *policy statement on: the importance of independent scientific merit of animal based research projects* [CCAC, 2000a]; pedagogical value for teaching; and the appropriateness of the method to provide data for testing according to current regulatory requirements);
- if pain or distress is a justified component of

- Bogor: Fakultas Perikanan dan Ilmu Kelautan. Institut Pertanian Bogor.
- Sari, M. (2016). Maizena sebagai alternatif pengganti pektin dalam pembuatan selai belimbing (*Averrhoa carambola* L.). *Sainstek: Jurnal Sains dan Teknologi*, 3(1):44-51.
- Shih, F. F., Daigle, K.W., & Clawson, E. L. (2001). Development of low oil uptake donut. *Journal of Food Science*, 66(1):141-144.
- SNI 01- 2354.3-2006. (2006). Cara uji kimia-Bagian 3: Penentuan kadar lemaktotal pada produk perikanan. Jakarta: Badan Standarisasi Nasional.
- SNI 2354.4-2006. (2006). Cara uji kimia-Bagian 4: Penentuan kadar protein dengan metode total nitrogen pada produk perikanan. Jakarta: Badan Standarisasi Nasional.
- SNI 7612-2011. 2011. Penentuan daya larut. Jakarta: Badan Standarisasi Nasional.
- SNI 2354-2015. (2015). Cara uji kimia-Bagian 2: Penentuan kadar air pada produk perikanan. Jakarta: Badan Standarisasi Nasional.
- Susilawati. (2001). Pengetahuan bahan hasil hewani daging. Buku Ajar. Bandar Lampung: Universitas Lampung.
- Wijaya, C. H., & A. C. Sadikin. (2000). Pembuatan *flavor* bubuk dari pandan wangi (*Pandanus amarylifolius*) dengan metode mikroenkapsulasi. *Buletin Penelitian Ilmu Teknologi Pangan*, 4(2):1-6.
- Zuhra, C. F. (2006). *Flavor* (cita rasa). Karya Ilmiah. Repository. Medan: Universitas Sumatera Utara.