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近江牡蛎冻干组织糖原含量近红外模型的建立*

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摘要 糖原直接影响牡蛎的风味和营养品质, 常作为评价牡蛎品质质量的重要标准。近红外光谱(NIR)模型可以实现糖原含量的快速准确检测。本研究以近江牡蛎(*Crassostrea ariakensis*)为研究对象, 采用微量蒽酮法、近红外技术分别获取了外套膜、鳃、闭壳肌、肝胰腺、唇瓣、性腺等 6 个组织及全软体部混样共 909 份样品的糖原含量及光谱数据, 结合最小二乘法建立了近江牡蛎 6 个组织及全软体部的糖原含量预测模型, 并对该模型进行了外部验证和交叉验证。结果显示, 所测样品光谱数据经一阶求导、乘法散射校正及平滑预处理后, 建立的模型最优; 所建立的 7 个模型的建模相关系数(R_C)为 0.971 6~0.996 3, 其外部验证相关系数(R_{EV})及交叉验证相关系数(R_{CV})分别为 0.949 0~0.990 8 和 0.969 4~0.996 9, 且模型交叉验证和外部验证的 RPD 值均大于 2.5, 表明所建立的模型能准确预测近江牡蛎相应组织样品的糖原含量。本研究建立的近江牡蛎糖原含量 NIR 分析模型, 不仅丰富了牡蛎糖原含量检测方法的研究资料, 还为实现近江牡蛎糖原成分的快速、准确测定提供了技术支撑, 在近江牡蛎品质性状改良等领域具有重要的应用价值。

关键词 近江牡蛎; 近红外模型; 冻干组织; 糖原含量

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糖原是牡蛎体内最直接有效的储能物质, 也是影响牡蛎肉质肥美的主要呈味物质之一, 其含量的高低直接影响到牡蛎的风味和营养品质, 常作为牡蛎肉质性状重要的评判标准。与其他多数双壳贝类相比, 牡蛎拥有较高的糖原成分(Beltrán-Lugo *et al*, 2006; Martínez-Pita *et al*, 2012; Chávez-Villalba *et al*, 2013; Anacleto *et al*, 2013; Vodáková *et al*, 2019), 含量约占其干重的 20%~40% (庾晋等, 2002)。2020 年中华人民共和国农业农村部公告第 324 号公布的长牡蛎(*Crassostrea gigas*)“鲁益 1 号”和长牡蛎“海蛎 1 号”2 个水产新品种均以高糖原为主要优势性状, 高糖原性状成为牡蛎品质性状改良的重点(全国水产技术推广总站, 2020)。

高效、快速、高通量的糖原含量测定方法可为高糖原贝类新种质的培育提供技术支撑。目前, 糖原含量的检测方法多以传统化学检测和试剂盒为主(杨柳

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等, 2018)，虽然这些技术方法成熟，但耗时耗力，成本较高，会产生大量化学废液，不适于大批量糖原含量的快速、批量测定。近红外光谱(Near Infrared, NIR)技术作为现代常见的一种快速高效分析技术，可通过近红外光谱仪记录主要含氢基团的倍频和合频吸收(于征等, 2012)，具有适用范围广、快速高效、便捷准确、对样品无破损等诸多优点(曲艺伟等, 2019)，在水产科学研究领域应用广泛，尤其是在品质元素的检测研究上。例如，2008 年建立的大西洋鲑(*Salmo salar*)脂肪和色素含量 NIR 分析模型，实现了脂肪和色素含量在鲑鱼肉质中的快速无损伤检测(Folkestad *et al*, 2008)。Fluckiger 等(2011)利用 NIR 技术建立了活鲍(*Abalone*)、去壳新鲜鲍和冻干腹足肌样品糖原定量模型，完成了糖原含量绿色高效测定。此后，Miller 等(2019)建立了新西兰绿唇贻贝(*Perna canaliculus*)以及大鳞大马哈鱼(*Oncorhynchus tshawytscha*)中蛋白质、水分、脂肪、灰分和碳水化合物等成分的 NIR 定量模型，进一步实现了多种成分的同步高效检测。在牡蛎研究中，已建立长牡蛎、葡萄牙牡蛎(*C. angulata*)、悉尼岩牡蛎(*Saccostrea glomerata*)和美洲牡蛎(*C. virginica*)水分、脂肪、糖原、总蛋白质、氨基酸、牛磺酸、Zn、Se 和 Ca 等成分的 NIR 定量模型，且模型经验证均具有较高的准确度(王卫军等, 2015; 于颖等, 2016; 黄冠明等, 2020; Brown, 2011; Brown *et al*, 2012; Guévélou *et al*, 2016)。其中，NIR 分析技术已成功应用到了新品种长牡蛎“鲁益 1 号”的选育中，大大提高了牡蛎的育种效率。可见 NIR 测定技术有效克服了化学检测方法的费时费力、成本高等问题，对高品质性状牡蛎的选育具有重要意义。

近江牡蛎(*C. ariakensis*)，又称“赤蛞”，为广温、广盐性的巨牡蛎属贝类，是我国重要的经济养殖种和生态种(Wang *et al*, 2008)。目前，已有的研究主要集中在分子标记开发、群体遗传等方面(Huvet *et al*, 2008; Guo *et al*, 2012; Cong *et al*, 2013、2014; Zhong *et al*, 2014; She *et al*, 2015; 李春燕, 2017; 赵庆等, 2019; 周丽青等, 2020)，糖原含量的快速检测方法尚未建立。本研究以近江牡蛎 6 种冻干组织为建模对象，结合其糖原含量和光谱数据建立各组织糖原含量的 NIR 检测模型，为开展近江牡蛎糖原含量的大规模测定提供方法和技术支撑。

1　材料与方法

1.1　实验仪器与设备

傅里叶变换 NIR 光谱仪(Antaris MX, 美国)，配备 RESULTTM 样本光谱采集的集成软件以及数据处理软件 TQ analyst (Thermo Fisher, 美国)；酶标仪(BIO-RAD, 美国)；真空冷冻干燥机(CHRIST, Alpha 2-4LDplus, 德国)。

1.2　样品的采集与处理

实验用近江牡蛎为性腺不同发育时期的成体，采自山东东营和滨州海区。新鲜近江牡蛎运回实验室后，分别取外套膜、鳃、闭壳肌、肝胰腺、唇瓣、性腺 6 个组织，放入 1.5 mL 离心管中，液氮速冻后置于–80℃冰箱中保存备用。测定前，利用真空冷冻干燥机干燥 48 h 后，研磨成细粉末分管保存，用于后续糖原含量的测定和 NIR 数据的采集。

1.3　糖原含量的化学测定

近江牡蛎糖原含量的化学值测定采用微量蒽酮比色法(陈夕等, 2021)进行。主要步骤如下：称取 20 mg 粉末样品于 10 mL 试管中，加入 30% KOH 溶液(*w*/*w*) 2 mL，沸水浴消化 60 min，期间每隔 5~10 min 摇晃试管，使其消化完全后，蒸馏水定容至 5 mL，12 000 r/min 离心 8 min 后，取上清液获得样品待测液。取稀释 10 倍的牡蛎待测液 100 μL 于 EP 管中，冰浴 5 min，取 0.2%的蒽酮硫酸溶液 200 μL 在冰浴条件下，贴管壁缓慢加至 EP 管中。所有样品统一摇匀之后在沸水浴中加热 8 min，期间迅速颠倒混匀 2 次(每次不超过 5 s)，加热完成后用自来水冷却。室温静置 30 min，移取 200 μL 于微孔板，利用酶标仪测定样品在 620 nm 的吸光值，每个样品设置 3 个重复。

使用浓度为 1 mg/mL 的葡萄糖标准液配置成不同浓度梯度的葡萄糖溶液，替代上述的待测液，按照上述方法进行实验，测定吸光值，绘制浓度与吸光值的标准曲线，建立回归方程。测得吸光值后，由标准曲线计算得到反应体系中葡萄糖的浓度。

1.4　近红外光谱的采集

利用傅里叶变换 NIR 光谱仪(Antaris MX, 美国)获得光谱数据。采集样品光谱前，需使用 RESULT 集成软件编定样品光谱采集工作流，光谱扫描波数范围设定为 10 000~4000 cm^{-1}，扫描次数为 128 次，分辨率为 8 cm^{-1}，用 log(1/R)漫反射方法表示吸收光谱。样品扫描前，先将光谱仪预热 0.5 h，后将研磨好的样品加入光谱采集处的积分球石英杯中(杯直径为 1 cm)，样品高度控制在 1.5 cm 左右。每次扫描样品前，采集背景光谱来消除背景影响，样品扫描选用漫反射光谱。

1.5 模型的建立与验证

利用 TQ Analyst (version 9.1.17, 美国)软件处理采集到的 6 种组织的光谱数据，选用偏最小二乘法(Partial Least Squares, PLS)作为建立定量模型的化学计量方法，根据软件推荐的光谱范围，选用乘法散射矫正、一阶求导、Norris 平滑等预处理方法，并对样本进行异常值的剔除，分别建立了 7 种近红外定量分析模型，其中，全软体部的光谱数据采用的是 6 种组织的光谱数据集。后对建立的定量模型进行外部验证和交叉验证，验证样本量占总样本的 1/9，以确保模型的准确性和可信性。

2 结果

2.1 近江牡蛎 6 种冻干组织糖原化学含量测定结果

利用微量蒽酮法测定了近江牡蛎外套膜 156 个、鳃 155 个、闭壳肌 159 个、肝胰腺 144 个、唇瓣 148 个、性腺 147 个，共 909 个样品的糖原含量。经过优化和验证处理，用于构建近江牡蛎 7 种样品糖原近红外定量模型的建模集和验证集所包含的样品数量，以及样品糖原含量范围值见表 1，其中，全软体部为 6 种组织的混合样品。全软体部、外套膜、鳃、闭壳肌、肝胰腺、唇瓣、性腺的建模集个数分别为 643、76、83、90、69、55 和 85，验证集样本个数分别为 46、12、13、17、9、7 和 13。建模集各组织糖原含量范围分别为全软体部 7.11~602.20 mg/g，外套膜 28.31~509.59 mg/g，鳃 28.83~306.94 mg/g，闭壳肌 7.12~103.09 mg/g，肝胰腺 68.95~516.72 mg/g，唇瓣 345.01~590.39 mg/g，性腺 32.58~602.21 mg/g，各组织糖原含量的标准差均较大。同时，各组织糖原含量的标准差也均较大，其建模集糖原含量的标准差范围在 21.02~186.97，验证集糖原含量的标准差范围在 18.64~182.86。在近红外分析模型建立过程中，要求样品含量分布范围广。

表 1 近江牡蛎 7 个近红外光谱模型建模集和验证集样本数目和糖原含量范围

Tab.1 Number of samples and the glycogen content in the freeze-dried tissue modeling set and validation set of seven NIR models of *C. ariakensis*

组织 Tissue model	建模集 Calibration set			验证集 Validation set		
	样本数 Number of samples	糖原平均值 Mean glycogen content/(mg/g)	糖原范围 Glycogen content range/(mg/g)	样本数 Number of samples	糖原平均值 Mean glycogen content/(mg/g)	糖原范围 Glycogen content range/(mg/g)
全软体部 Soft body	643	220.60±164.16	7.11~602.20	46	256.49±166.04	19.93~563.60
外套膜 Mantle	76	214.11±109.71	28.31~509.59	12	241.39±98.69	75.94~387.10
鳃 Gill	83	120.21±64.58	28.83~306.94	13	153.20±87.28	30.40~275.62
闭壳肌 Adductor muscle	90	53.27±21.02	7.12~103.09	17	46.76±18.64	15.15~75.13
肝胰腺 Hepatopancreas	69	252.20±105.55	68.95~516.72	9	291.31±94.85	153.65~479.46
唇瓣 Labial palps	55	483.95±63.45	345.01~590.39	7	441.15±55.70	348.92~511.35
性腺 Gonad	85	368.39±186.97	32.58~602.21	13	337.86±182.86	52.15~571.97

2.2 光谱数据预处理

采用 NIR 光谱仪扫描近江牡蛎冻干样品 909 份，得到的 NIR 漫反射原始光谱如图 1 所示，所有的光谱曲线走向基本一致。在全软体部、外套膜、鳃、闭壳肌、肝胰腺、唇瓣和性腺的糖原含量 NIR 分析模型的建立过程中，选择软件推荐的最佳光谱范围，光谱数据的最优预处理方法均为一阶求导(图 2)、乘法散射校正及 Norris 平滑，处理过程中的主要参数及最终主因子数见表 2。

2.3 模型建立与外部验证

根据 TQ Analyst 软件推荐的异常值，进行样本剔除。异常值剔除后，建立了 6 种组织及全软体部的近红外定量模型。

建模过程中，相关系数越接近于 1，相应的残差均方根越小，说明该模型越好。在各组织样本糖原含量建模的结果中，其 R_C 均接近于 1，其中，全软体部、外套膜、鳃、肝胰腺、唇瓣、性腺和闭壳肌糖原定量模型 R_C 值范围是 0.971 6~0.996 3，肝胰腺建模相关系数最高，为 0.996 3；闭壳肌糖原定量模型 R_C 值是所有模型中最低的，但也高达 0.971 6，且 7 个模型的建模残差均方根(RMSEC)值在 4.97~26.20 之间，均相对较小，这些数据证明模型的预测值与化学真实值相关度较高。7 个模型交叉验证相关系数(R_{CV})和外部验证的相关系数(R_{EV})较高，均>0.94，最高为 0.996 9，且对应的交叉验证残差均方根(RMSECV)

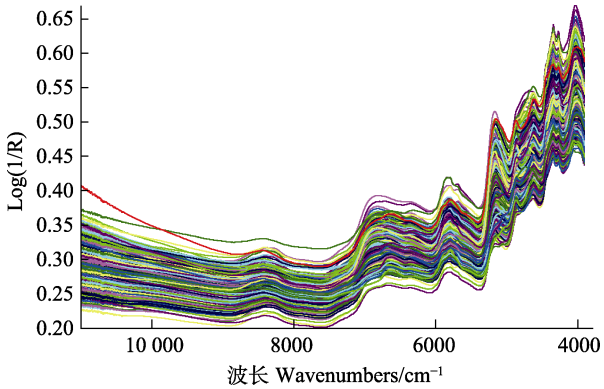

图 1　近江牡蛎冻干组织所有样本的 NIR 漫反射原始光谱

Fig.1　NIR diffuse reflectance original spectra of all samples of freeze-dried tissue of *C. ariakensis*

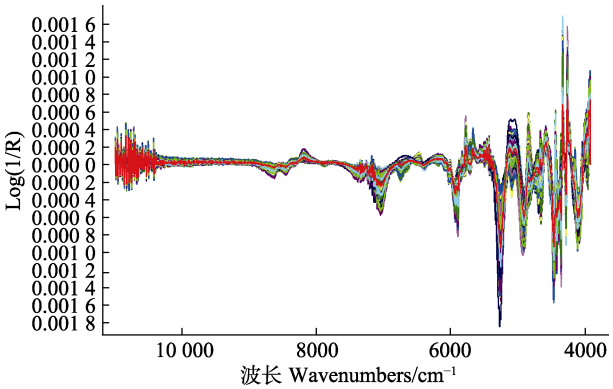

图 2　近江牡蛎冻干组织所有样本 NIR 光谱一阶求导处理

Fig.2　First-order derivation of NIR spectrum for all samples of freeze-dried tissue of *C. ariakensis*

表 2　近江牡蛎糖原含量各样品建模集和验证集光谱数据处理参数

Tab.2　Spectral data processing parameters of the modeling set and verification set of NIR models for the glycogen content of *C. ariakensis*

组织模型 Tissue model	光谱范围 Spectral range/cm	预处理 Pretreatment	主因子数 The main factors
全软体部 Soft body	4092~4061，4227~4173，4987~4948	FD，NDF	8
外套膜 Mantle	4184~3953，4759~4501，6016~5943	FD，NDF	6
鳃 Gill	4015~3976，4292~4252，4624~4454	FD，NDF	7
闭壳肌 Adductor muscle	4312~4265，4728~4593，5314~5172	FD，NDF	9
肝胰腺 Hepatopancreas	4759~4639，6282~6001，6664~6576	FD，NDF	7
唇瓣 Labial palps	4165~3910，4767~4450，6082~6005	FD，NDF	10
性腺 Gonad	4192~3976，7143~6001	FD，NDF	7

注：FD：一阶导数；NDF：导数过滤器。

Note: FD: First derivative; NDF: Norris derivative filter.

值和外部验证残差均方根(RMSEP)值在 4.59~30.30 之间，也相对较小，进一步证明了该模型较高的可信度和准确性；模型验证的重要参数即验证样本真实值标准差与验证残差均方根的比值(RPD 值)变化范围为 4.06~11.60 (表 3)。研究结果表明，全软体部以及 6 种组织的糖原定量模型精确度高，可用于近江牡蛎冻干组织样品的糖原含量预测。

3　讨论

3.1　NIR 技术测定糖原含量的优势

目前，糖原的检测方法主要有传统蒽酮比色法、基于蒽酮比色法基本原理的试剂盒法等(李春燕, 2017; 杨柳等, 2018)以及本实验室已建立的微量蒽酮比色法(陈夕等, 2021)。虽然传统的蒽酮比色法应用广泛，但样品预处理复杂，操作繁琐，不适于大批量样品的快速测定，同时，实验过程中浓硫酸的使用也存在较大的安全隐患。而试剂盒方法虽然在蒽酮硫酸使用量以及样品处理上作了改进等，但因其价格昂贵，极大地增加了实验成本。本实验室建立的微量蒽酮比色法虽降低了实验成本，简化了实验步骤，但在实验操作重仍旧费时费力，实验过程中会产生大量的化学废液，对环境污染严重。而 NIR 技术不仅可以进行大批量样品的测定，还具有快捷高效、省时省力等优点，且在实验过程中无化学废液产生，绿色环保。经过 NIR 技术检测后样品，其化学性质不会发生改变，不会造成样品的大量消耗，可用于后续的回收利用。本实验所用的近红外扫描样品，经短时间的近红外扫描获取光谱数据后，可迅速回收放于–80℃冰箱冷冻保存，不影响后续样品的使用。总的来说，NIR 技术在测定糖原含量方面具有诸多优势，可以应用在牡蛎品质性状的改良上。

3.2　近江牡蛎 NIR 糖原定量模型的重要参数

从图 3 中可以看出，所建模型的 R_C 值、R_{CV} 值以及 R_{EV} 值越接近 1，模型的预测效果就越好。相较于前人在牡蛎上所建定量模型的数据，本研究建立的 7 个 NIR 糖原定量模型，以上 3 个指标值均在 0.94 以上，

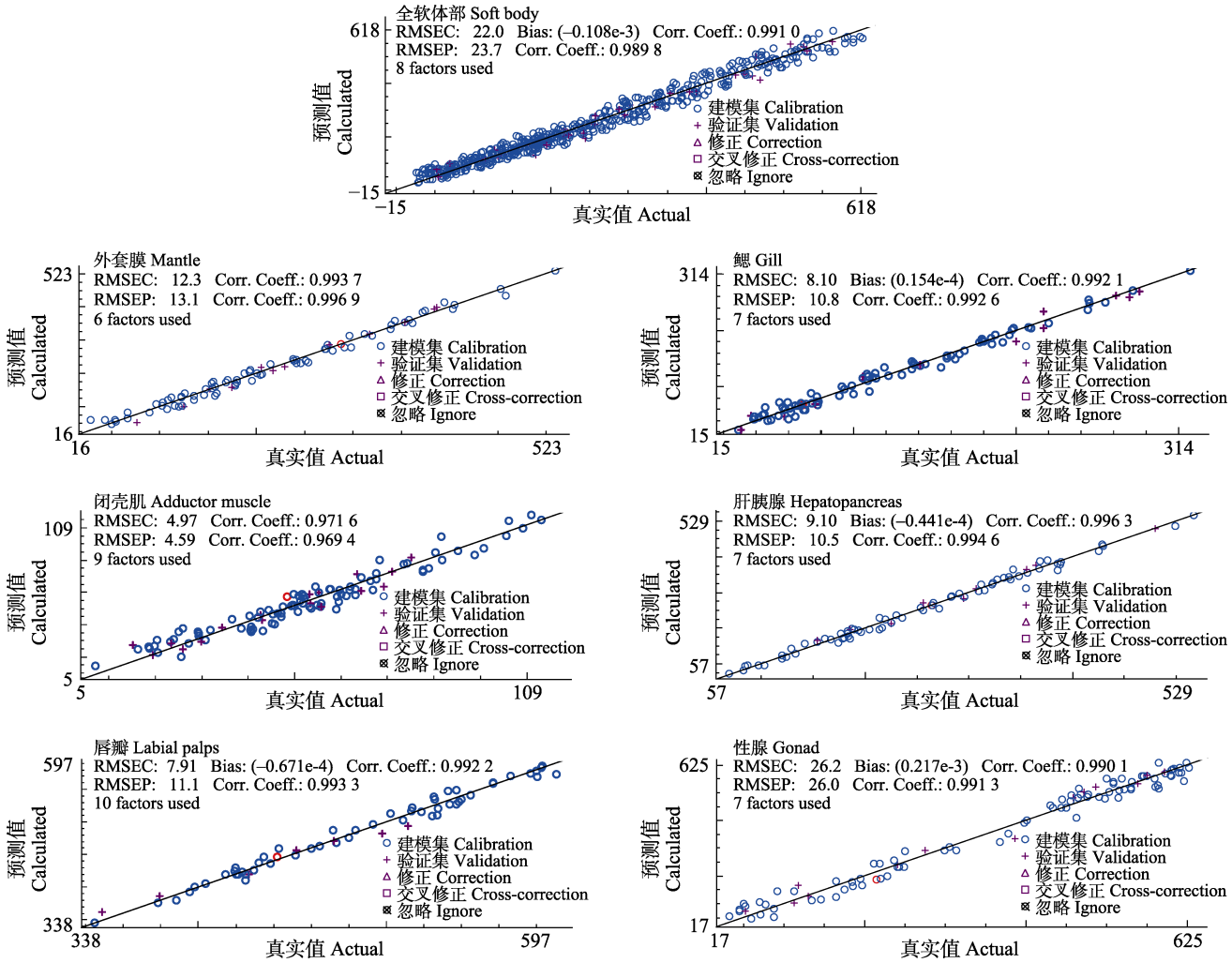

图 3 全软体部、外套膜、鳃、闭壳肌、肝胰腺、唇瓣、性腺糖原含量 NIR 模型主要参数

Fig.3 Main parameters of NIR model for glycogen content of the whole soft body, mantle, gill, adductor muscle, hepatopancreas, labial palps and gonad

表 3 近江牡蛎各组织建模过程中的指标参数

Tab.3 Index parameters in the NIR modeling for each sample of *C. ariakensis*

组织 Tissue	建模集 Calibration set		验证集 Validation set					
			交叉验证 Cross validation			外部验证 External validation		
	RMSEC	R_C	RMSECV	R_{CV}	RPDCV	RMSEP	R_{EV}	RPDEV
全软体部 Soft body	22.00	0.991 0	26.40	0.987 0	7.46	23.70	0.989 8	7.01
外套膜 Mantle	12.30	0.993 7	14.90	0.990 8	8.92	13.10	0.996 9	7.53
鳃 Gill	8.10	0.992 1	10.20	0.987 5	7.97	10.80	0.992 6	8.08
闭壳肌 Adductor muscle	4.97	0.971 6	6.65	0.949 0	4.23	4.59	0.969 4	4.06
肝胰腺 Hepatopancreas	9.10	0.996 3	15.20	0.989 7	11.60	10.50	0.994 6	9.03
唇瓣 Labial palps	7.97	0.992 2	12.10	0.981 8	7.96	11.10	0.993 3	5.02
性腺 Gonad	26.20	0.990 1	30.30	0.986 8	7.14	26.00	0.991 3	7.03

注：RMSEC：建模残差均方根；R_C：建模相关系数；RMSECV：交叉验证残差均方根；R_{CV}：交叉验证相关系数；RPDCV=SD/RMSEC：交叉验证样本真实值标准差(SD)与 RMSECV 的比值；RMSEP：外部验证残差均方根；R_{EV}：外部验证相关系数；RPDEV：交叉验证样本真实值标准差(SD)与 RMSECV 的比值。

Note: RMSEC: The root mean square of modeling residual; R_C: Modeling correlation coefficient; RMSECV: Cross validation root mean square residual; R_{CV}: Cross validation correlation coefficient; RPDCV=SD/RMSEC: The ratio of true standard deviation (SD) of cross-validation samples to RMSECV; RMSEP: Root mean square of external validation residual; R_{EV}: Correlation coefficient of external validation; RPDEV: Cross validation sample true value standard deviation (SD) to RMSECV ratio.

最高可达 0.996 9，说明本实验所建模型的精确性更好。此外，RPD 值也是评判模型准确度的重要指标，优质模型的 RPD 值需>2.5，且 RPD 值越高，模型就越好(Zhou *et al*, 2012)。本研究建立模型的 RPD 值远>2.5，最高可达 11.6，这表明 7 个模型均具有较高的预测准确性。所建 7 个模型的建模集和验证集的 SD 值分别在 21.02~186.97 和 18.64~182.86 之间，可见建模样品糖原含量变化范围广，证明模型预测的精确度高和可信性强。

本研究样本采集于不同季节、2 个不同地理群体，样本量多达 900 多份，样本代表性好。与已建立的长牡蛎、葡萄牙牡蛎等 NIR 品质成分定量模型相比，本研究采用的是分组织样品，与混组织样品相比，准确度会更高，可以满足不同样品糖原测定的需求。这是因为不同组织的糖原含量具有一定的差别，6 种特定组织的 NIR 分析模型可以更准确的检测那些个体大、易于区分组织的样品的糖原含量。而全软体部模型由于其建立是依据 6 种组织的糖原和光谱数据，因此该模型适用于那些个体小、不易于区分组织的样品。本研究分别建立了包括全软体部、外套膜、鳃、闭壳肌、肝胰腺、唇瓣、性腺组织糖原的 NIR 检测模型，可以针对不同现场需求，用于样品糖原含量的检测，具有针对性高、适用性强的优势。

水分对光谱数据质量具有一定的影响。水分吸收区域在 3500~3100 cm^{-1} 和 800~750 cm^{-1}，是光谱中具有非常高吸收值的区域，在该吸收区域会使得仪器设置失去灵敏度，此时产生的噪声会干扰模型的回归(Pedersen *et al*, 2003)。与鲜样模型相比，本研究采用的冻干组织样品，大大降低了水分对光谱质量的影响，使模型预测准确性更高。

3.3　NIR 糖原定量模型在牡蛎品质性状测定中的应用

随着生活水平的不断提高和牡蛎消费群体的不断扩大，人们更加追求高品质的牡蛎，尤其是鲜、甜等口感性状。目前，进入我国牡蛎高端市场的不同风味的牡蛎品种，几乎全部来自于进口，而且价格昂贵。2019 年之前，国内获批的牡蛎新品种中，多数品种的主要优势性状都体现在生长、壳色等性状，牡蛎养殖业也主要靠“以量取胜”，规模、品质之间的矛盾日益突出。可以预见，随着贝类产业的发展，市场对贝类品质的需求更将向高质化和多元化发展，除了对生长速度等传统性状追求的基础上，对高糖原等高品质牡蛎的需求将更为迫切。针对不同牡蛎，国内外已建立了牡蛎多个品质性状成分含量测定的近红外模型，国外有多倍体长牡蛎、悉尼岩牡蛎和美洲牡蛎的水分、脂肪、糖原、总蛋白质 NIR 定量模型(Brown, 2011; Brown *et al*, 2012; Guévélou *et al*, 2016)；国内主要有长牡蛎鲜样组织中水分、糖原、总蛋白质、总脂肪、Zn、Se、牛磺酸和灰分 8 种成分含量的 NIR 分析模型(王卫军等, 2015)；葡萄牙牡蛎蛋白质、糖原、牛磺酸、Zn、Se 和 Ca 6 种成分含量的 NIR 分析模型以及最早建立的牡蛎中氨基酸含量的 NIR 分析模型(于颖等, 2016; 黄冠明等, 2020)，这些模型均具有较高的预测准确度，说明近红外光谱模型可以较好地应用在牡蛎营养元素的测定中。本研究建立的近江牡蛎糖原含量 NIR 分析模型，丰富了牡蛎 NIR 技术检测品质性状的方法，为今后实现快速近江牡蛎糖原成分的大规模测定提供了技术支撑。

4　结论

本研究建立的 7 个糖原含量 NIR 分析模型，可以实现近江牡蛎外套膜、鳃、闭壳肌、肝胰腺、唇瓣、性腺及全软体部冻干组织样品中糖原含量的快速准确、大批量的测定，丰富了近江牡蛎品质性状的检测方法，为今后牡蛎品质性状改良等方面提供技术支撑。

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(编辑　陈　辉)

Establishment of Near-Infrared Model for Glycogen Content in Freeze-Dried Tissues of Jinjiang Oyster *Crassostrea ariakensis*

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Abstract　With the continuous improvement of living standards and expansion of oyster consumer groups, people are pursuing high-quality oysters, especially with fresh and sweet taste traits. At present, almost all varieties of oysters with different flavors entering the upscale oyster market in China are imported, and their prices are high. Before 2019, among the new oyster varieties approved in China, the dominant traits of most varieties were reflected in growth, shell color, etc. The oyster farming industry has also primarily focused on quantity but not quality, and the contradiction between scale and quality has become increasingly prominent. It can be predicted that with the development of the shellfish industry, the market demand for high shellfish quality will be more and diversified. In addition to pursing traditional traits such as growth rate, the demand for high-quality oysters, such as those with high glycogen, will be more urgent. Glycogen directly affects oysters´ flavor and nutritional quality and is often used as an important criterion to evaluate oyster quality. The efficient, rapid, and high-throughput method for determining glycogen content can provide technical support for cultivating new shellfish species with a high glycogen content. At present, the detection methods for glycogen content are mainly traditional chemical detection techniques and kits. Although these methods have been well developed, they are time-consuming and costly, producing a significant amount of chemical waste liquid. Therefore, they are not suitable for rapid and batch determination of glycogen content in large quantities. As a common modern, fast, and efficient analysis technology, near-infrared (NIR) technology can record the frequency doubling and frequency absorption of the main hydrogen-containing groups using an NIR spectrometer. NIR technology can determine large quantities of samples and has the advantages of being fast and efficient, time-saving, and labor-saving, with no chemical waste liquid generation in the experimental process, offering green environmental protection. After testing by NIR technology, the chemical

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properties of the samples do not change and do not require significant sample amounts, which can be used for subsequent recycling. The NIR scanning samples used in this experiment can be quickly recovered and stored in a refrigerator after obtaining spectral data through a short period of NIR scanning without affecting the use of subsequent samples. In general, the NIR technique has many advantages in determining glycogen content, and are applicable for improving oyster quality traits. This method has many advantages, such as a wide application range, and is fast, efficient, convenient, and accurate; it has been widely applied in aquatic science research. This is especially true in quality element detection research. NIR quantitative models of water, fat, glycogen, total protein, amino acid, taurine, zinc, selenium, and calcium in *Crassostrea glomerata*, *C. angulate*, *Saccostrea glomerata*, and *C. virginica* were established, with demonstrably high accuracy. Among them, NIR analysis technology has been successfully applied to breeding a new *Crassostrea gigas* species "Lu Yi 1," significantly improving oyster breeding efficiency. Therefore, NIR assays can effectively overcome problems of chemical detection methods, which are time-consuming, laborious, and expensive; this is highly significant for breeding oysters with high-quality traits. The NIR spectroscopy model can be used to quickly and accurately predict glycogen content. Using *C. ariakensis* as the research object, the glycogen content of 909 samples in seven tissues including mantle, gill, adductor muscle, hepatopancreas, labial palps, gonad, and most soft oyster parts were determined by the micro-reaction system and method of oyster glycogen content. The corresponding spectral data were obtained using a Fourier NIR spectrometer. The spectral data and glycogen content data were analyzed and processed using TQ Analyst software, and NIR quantitative models of six tissues and all the soft parts of oysters near the Jiang River were established, and 1/9 samples of the total sample size were randomly selected for external validation and cross-validation of the models. The results showed that the measured glycogen content ranged from 7.11 to 602.20 mg/g, which had a wide range and was suitable to establish the model. This study aimed to establish a NIR model for the freeze-dried tissues of *C. ariakensis* to realize the rapid and accurate detection of glycogen content. This study obtained the glycogen content and spectral data of 909 samples using the micro-reaction system method and NIR technology. Combined with the least-squares method, the glycogen content prediction model of six tissues and the whole soft body of *C. ariakensis* was established and verified. Results also show that the model is optimal after the first derivative, multiplication scattering, and smoothing pretreatment of the measured spectral data. The modeling correlation coefficients (R_C) of the seven models ranged from 0.971 6 to 0.996 3, indicating that the predicted values of the seven models were highly correlated with the actual chemical values. The correlation coefficients of external validation (R_{EV}) and cross-validation (R_{CV}) were between 0.949 0~0.990 8 and 0.969 4~0.996 9, respectively, indicating that the established model could accurately predict the glycogen content of the corresponding tissue samples of *C. ariakensis*. This method rapidly and accurately determines the glycogen content of oysters and has application value in the field of improvement of oyster characteristics and quality.

Key words *Crassostrea ariakensis*; Near infrared model; Freeze-dried tissue; Glycogen content